Indexed in Web of Science - ESCI journals

ISSN: 0976-5921

International Journal of Ayurvedic Medicine

Volume 16, Supplement 2, 2025

SPECIAL ISSUE

On

NATIONAL CONFERENCE

Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine

Sindhu Education Society's

K. C. Bajaj College of Pharmacy & Research

Sindhu Education Society Campus, Jaripatka, Nagpur, Dist. Nagpur, (MS). India.

Published online at
https://ijam.co.in

Official Publication of
Ayurveda Sahiti Prabha's
IJAM

2 DAYS NATIONAL CONFERENCE

"Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine"

(Oral/Poster Presentation)

ORGANIZED BY

Sindhu Education Society's

K. C. Bajaj College of Pharmacy & Research

Sindhu Education Society Campus,
Jaripatka, Nagpur,
Dist. Nagpur, (MS) India

10th March to 11th March- 2025

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ABOUT COLLEGE

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At the very dawn of India's rise, Sindhu Education Society a pioneer in the field of education from Nagpur District came to surface giving scope to aspiring scholars in the city of Nagpur and vicinity of Vidharbha region. Sir Khubchand Bajaj has served for years as Director of the society since 1952. The organization which started modestly is now a group of elite institutions. New golden feather was added to the wings of Sindhu Education Society, in the form of K.C. Bajaj College of Pharmacy & Research in the centre of Nagpur City. The College has been approved by Pharmacy Council of India, New Delhi, recognized by Govt. of Maharashtra, DTE Mumbai, affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Raigad (DBATU) & Maharashtra State Board of Technical Education (MSBTE), Mumbai. The College offers high quality education to the students enrolled in B.Pharm and D.Pharm courses.

The institution aims at recognizing, respecting and nurturing the creative potential of each student. Our institute encourages and promotes modern teaching, learning process with the help of innovative tools. The college has magnificent ground plus three floors building including well-designed, automated classrooms, well equipped laboratories, machine room, central instrument room, multimedia centre, with Wi-Fi facility, grand library with huge number of books and journals inbuilt E-library, training and placement cell, spacious auditorium, sports room with multiple number of sports accessories.

We also have huge compound ground with the facilities of Volleyball court, foot-ball, cricket net, associated with a big Swimming pool. We envision our college a pioneering force, setting new benchmarks in education, research and community engagement. Our aim is to shape the future of healthcare by fostering highly skilled and compassionate pharmacists who positively impact individuals and communities.

ABOUT CONFERENCE

The worldwide healthcare sector is experiencing a significant transformation as traditional knowledge converges with contemporary advancements. The conference titled "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" aims to explore this intersection, fostering dialogue and collaboration among thought leaders, practitioners, researchers, and policymakers from diverse disciplines. This event highlights the need of integrating traditional therapeutic methods with synthetic innovations, fostering a comprehensive approach to future healthcare issues. Traditional medicine, based on millennia of empirical knowledge and cultural customs, has historically served as the foundation of healthcare systems throughout. Traditional medicine, including herbal medicines, acupuncture, Ayurveda, and Indigenous healing practices, has offered successful answers for many health concerns. Conversely, contemporary medicine, grounded on rigorous scientific inquiry, advanced technology, and evidence-based methodologies, has transformed our comprehension and management of illnesses. The integration of these two fields presents exceptional prospects to create novel medicines and improve global health results. This conference will provide a multidisciplinary forum to explore the synergies and difficulties of merging traditional and contemporary medicine. Participants will explore the scientific confirmation of traditional treatments, the creation of synthetic analogs derived from natural chemicals, and the influence of new technologies, including artificial intelligence, genomics, and biotechnology, in this evolution. Keynote addresses, panel discussions, and workshops will concentrate on converting historic medical knowledge into contemporary pharmaceutical uses, while also addressing ethical issues, sustainability, and fair healthcare access.

Vol. 16 No. S2 (2025)
Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine 2025
DOI: https://doi.org/10.47552/ijam.v16iS2

Index

Review Articles

https://doi.org/10.47552/ijam.v16iS2.6197 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Psychedelic Herbal Medicines: Exploring the Renaissance of a Therapeutic Frontier

Review Article

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Abstract

A revolutionary change in contemporary therapeutic paradigms may be seen in the renewed interest in psychedelic plant remedies. Psychedelic plants like psilocybe mushrooms, ayahuasca, iboga, and salvia divinorum, which have long been valued for their spiritual and therapeutic qualities in a variety of cultures, are now the subject of intense scientific research. According to recent clinical research, they have great promise for treating a variety of illnesses, such as addiction, PTSD, anxiety, and depression. This resurgence is fuelled by mounting evidence that these drugs may support profound psychological healing, emotional resilience, and neuroplasticity when used under careful supervision. Technological developments in ethnobotany, neurology, and psychopharmacology are revealing the intricate processes by which these herbal psychedelics work, particularly via serotonergic pathway regulation and the amplification of introspective experiences. The regulatory environment is gradually changing, indicating a cautious sense of hope about the incorporation of these traditional medicines into contemporary medical treatment. But there are still issues to be resolved, such as standardising botanical formulations, guaranteeing patient safety, and tackling the social stigma attached to psychedelics. This study highlights the need for multidisciplinary cooperation in furthering this exciting field by examining the historical origins, molecular foundations, therapeutic uses, and potential future developments of psychedelic plant medicines. Psychedelic herbal treatments provide a rare chance to rethink mental health therapy by fusing ancient wisdom with modern research, paving the door for more individualised and holistic approaches to recovery.

Keywords: Psychedelic Herbal Medicines, Neuroplasticity, Mental Health, Ethnobotany, Therapeutic Applications.

Introduction

History and Traditional Use of Psychedelic Herbal Medicines

Indigenous societies have been using hallucinogenic plants for thousands of years; throughout this time, they were essential to religious rites, healing methods, and rituals that bonded communities. Psilocybe mushrooms and other hallucinogenic plants have been utilized by people in Mesoamerica for at least 3,000 years, according to archeological data (1). They were employed in religious rites to converse with the spiritual world by the Aztecs, who called them "divine flesh," or "teonanácatl" (2).

Similarly, Amazonian tribes have been using Ayahuasca, a hallucinogenic beverage made mostly of Psychotria viridis and Banisteriopsis caapi, to elicit visionary experiences for divination and healing for millennia (3). The Bwiti religion in Central Africa emphasizes the plant's ability to promote reflection and ancestry by including Tabernanthe iboga into spiritual rites and initiation procedures (4).

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Additionally, Salvia divinorum has long been used for divination and spiritual healing by the Mazatec people of Oaxaca, who often chew the leaves or make infusions for religious rituals (5). These customs demonstrate the intricate ethnomedical systems in which hallucinogenic substances served as instruments for psychological, emotional, and social well-being in addition to physical healing (6).

Modern Research

Driven by increasing interest in these compounds' psychopharmacological qualities, modern scientific study on psychedelics started in the early-to-mid 20th century. Early research in the 1950s and 1960s revealed encouraging therapeutic uses for psilocybin and LSD in the treatment of existential anguish, depression, and alcoholism (7).

Political and cultural changes in the late 1960s, however, caused a general ban on psychedelic research. Classified as Schedule I drugs, psychedelics greatly limited scientific research (8). Beginning in the 1990s, the resurgence of psychedelic research intensified into the 21st century. Recent research has shown that psilocybin-assisted therapy may lead to quick and lasting decreases in treatment-resistant depression and major depressive disorder (9,10).

Studies on Ayahuasca have shown its possible efficacy in treating treatment-resistant depression, drug

misuse, and post-traumatic stress disorder (PTSD) via processes including improved emotional control and neuroplasticity (11). Research on ibogaine has shown strong anti-addictive properties, particularly for opioid use disorders (12). Salvia divinorum and its active component salvinorin A are being investigated, meanwhile, for their unusual interactions with the kappa-opioid receptor, maybe providing knowledge on how to treat mood and cognitive problems (13).

Modern clinical investigations are defined by strict methods including randomized controlled trials, brain imaging studies (fMRI, PET), and multidisciplinary approaches combining psychiatry, neurology, and ethnopharmacology. These developments are opening the way for the reintegration of psychedelic treatments into conventional medicine under carefully controlled settings (14).

Table 1: Comparison of Traditional and Modern Uses of Psychedelic Plants

Psychedelic Plant	Traditional Use	Modern Research Application
***Psilocybe* mushrooms**	Spiritual rituals, healing ceremonies	Depression, anxiety, addiction therapy
Ayahuasca	Shamanic visions, communal healing	PTSD, depression, emotional regulation therapies
Tabernanthe iboga	Initiation rites, spiritual communion	Opioid addiction treatment
Salvia divinorum	Divination, religious ceremonies	Mood disorders, cognitive flexibility research

Types of Psychedelic Herbal Medicines

Ayahuasca

One of the traditional ingredients in the Amazonian drink known as ayahuasca is the plant Psychotria viridis, which contains the hallucinogenic compound N, N-Dimethyltryptamine, or DMT, and Banisteriopsis caapi, which contains monoamine oxidase inhibitors (15). Although DMT is inert when taken orally, the synergy of these plants makes it capable of producing strong psychedelic effects, such as altered states of consciousness, emotional introspection, and vivid visions (16).

Spiritual healing, communicating with spirits, and illness diagnosis are the traditional applications of Ayahuasca among Amazonian communities (17). Through enhancing emotional control and neuroplasticity, Ayahuasca has been shown in recent clinical trials to considerably alleviate PTSD, anxiety, and depression symptoms (18). Ayahuasca alters brain areas including the default mode network that are involved in self-referential processing and emotional regulation, according to neuroimaging research (19).

Psilocybin Mushrooms

Popularly known as "magic mushrooms," psilocybin mushrooms are really fungi that contain the prodrug psilocybin, which is converted into the hallucinogenic molecule psilocin (20). Many of the more than 180 kinds of Psilocybe mushrooms utilized in ancient Mayan rituals are still in use today (21).

Recent clinical trials have shown that psilocybin may help with a variety of mental health issues, including major depressive disorder, anxiety about dying, and addiction (22, 23). According to neuroscientific research, psilocybin mainly changes brain connection patterns by acting as an agonist at the serotonin 2A receptor, which in turn reduces inflexible activity in the default mode network (24). Positive effects on mental health have been associated with psilocybin's ability to induce deep sensations of connection, ego disintegration, and spiritual importance (25).

Peyote and San Pedro Cacti

Two psychotropic cacti, one native to South America (Echinopsis pachanoi) and the other to North America (Lophophora williamsii), are found in the Americas. The phenethylamine alkaloid mescaline is present in both plants and is responsible for eliciting strong emotions and dazzling hallucinations (26).

For more than five thousand years, indigenous communities in the American Southwest and northern Mexico have utilized peyote in religious rituals as a means of healing and connecting with the divine (27). The San Pedro cactus, which is widely utilized for holistic treatment and divination, also has a long history in Andean shamanic activities (28).

According to recent research, mescaline shows promise as a therapeutic tool for treating addiction and mood disorders, and it also has a decreased potential for misuse (29). Researchers have shown that mescaline may promote mystical experiences, emotional breakthroughs, and heightened openness, all of which are psychological variables linked to better treatment results (30).

Table 2: Summary of Key Psychedelic Herbal Medicines

Plant/Source	Active Compound(s)	Traditional Use	Modern Research Focus
Banisteriopsis caapi + *Psychotria viridis*	DMT, Harmine	Amazonian healing, spiritual communication	Depression, PTSD, emotional regulation
Psilocybe spp.	Psilocybin, Psilocin	Mesoamerican rituals, spiritual guidance	Depression, anxiety, addiction treatment
Lophophora williamsii (Peyote) / *Echinopsis pachanoi* (San Pedro)	Mescaline	Healing rituals, divine communion	Mood disorders, addiction therapy

Therapeutic Potential of Psychedelic Herbal Medicines

Mental Health Disorders

There is encouraging evidence that psychedelic drugs produced from herbal medicines, such psilocybin, Ayahuasca, and cacti containing mescaline, may be used to treat a range of mental health issues. One or two sessions of psilocybin have been shown to significantly alleviate symptoms of major depressive disorder and treatment-resistant depression in clinical studies (31). Psilocybin improves emotional processing and causes neuroplastic alterations by acting on serotonin 2A receptors (32).

In a similar vein, Ayahuasca has been associated with quick and long-lasting antidepressant benefits in those with anxiety and depression. Research indicates that the beverage improves emotional control by influencing brain networks such as the limbic system and the default mode network (33). Anxieties can be alleviated with the use of psychedelics, according to a meta-analysis that suggests the mystical-type experiences that are a key component of these treatments (34).

Addiction Treatment

One new way to treat addiction is via psychedelic-assisted therapy. One of the distinctive anti-addictive effects of the alkaloid ibogaine identified in Tabernanthe iboga is its effectiveness against opioid and cocaine dependency (35). The actions of ibogaine on the dopaminergic and glutamatergic circuits may explain why it alleviates cravings and withdrawal symptoms (36).

Research on psilocybin's potential as a treatment for alcohol and nicotine addiction has also been extensive. Eighty percent of smokers who took part in a seminal pilot research that used psilocybin-assisted treatment continued to abstain from smoking six months later (37). The results of this study provide evidence that psychedelic-induced states of heightened awareness, emotional liberation, and connectivity may promote substantial behavioral modification and sustained abstinence (38).

End-of-life Anxiety

Anxiety and profound existential crisis are common among terminally sick individuals. One potential solution to these serious mental health issues is psilocybin treatment. A single psilocybin session may considerably decrease anxiety and despair associated with end-of-life, according to controlled clinical investigations (39). The benefits of the session can continue up to six months.

Psychedelic users often report better spiritual health, less anxiety about dying, and a more accepting attitude toward mortality following their trips (40). Brain imaging investigations corroborate these findings by demonstrating that psilocybin inhibits activity in regions linked to fixation on one's own thoughts and feelings (41). Results from preliminary investigations into the use of ayahuasca for comparable purposes have been positive, suggesting that it may help terminally ill patients achieve emotional reconciliation and tranquility (42).

Table 3: Summary of Therapeutic Applications of Psychedelic Herbal Medicines

Condition	Substance	Proposed Mechanism	Clinical Outcomes
Major Depressive Disorder	Psilocybin	5-HT2A receptor activation, neuroplasticity	Rapid and sustained symptom reduction (39).
Opioid and Cocaine Addiction	Ibogaine	Dopamine system modulation, craving reduction	Decreased withdrawal symptoms and relapse rates (40).
Smoking and Alcohol Addiction	Psilocybin	Mystical experience, behavior change facilitation	High long-term abstinence rates (41).
End-of-life Anxiety	Psilocybin, Ayahuasca	Emotional processing, existential acceptance	Reduced depression and anxiety at 6-month follow-up (42).

Traditional Uses of Psychedelic Herbal Medicines

Many different civilizations have a long history of using psychedelic botanical medicines for ceremonial, medicinal, and spiritual reasons. Mesoamerican, South American, and African ancient cultures recognized the medicinal, hallucinogenic, and enlightening properties of these plants and used them in rituals for healing, initiation, and other life transitions.

The Aztecs held Psilocybin mushrooms (of the genus Psilocybe) in the highest regard; they called them teonanácatl, meaning "flesh of the gods," and used them as part of their sacred ceremonies to invoke healing and divine contact (43). Indigenous Amazonian communities have employed Ayahuasca, a mixture of Banisteriopsis caapi and Psychotria viridis, for shamanic purposes, including illness diagnosis, spiritual insight, and healing, for generations (44).

Native American spiritual traditions in North America revolved around the Peyote cactus (Lophophora williamsii). In order to promote spiritual connection, moral reflection, and physical healing, the peyote cactus is consumed during rites that are being practiced by the Native American Church today (45). Traditionally, the Bwiti people of Gabon used iboga, scientifically known as Tabernanthe iboga, for initiation ceremonies and psycho-spiritual healing trips. The psychedelic effects of ibogaine were thought to provide direction and a link to one's ancestors (46).

Traditional applications declined due to colonial persecution and religious prohibition from the 1600s to the 1900s, but there has been a recent upturn of interest, often combining indigenous wisdom with contemporary therapeutic frameworks (47).

Table 6: Traditional Psychedelic Herbal Medicines and Their Cultural Roles

Plant	Region/Culture	Traditional Use	Spiritual/Healing Purpose
Psilocybin Mushrooms	Aztecs, Mesoamerica	Religious rituals, healing ceremonies	Divine communication, soul purification
Ayahuasca	Amazonian tribes	Shamanic healing, spiritual journeys	Diagnosis, spiritual insight
Peyote	Native American Church	Communal prayer meetings	Spiritual communion, moral introspection
Iboga	Bwiti religion (Gabon)	Initiation rites, psycho-spiritual healing	Ancestral connection, personal healing

Safety and Risks Associated with Psychedelic Herbal Medicines

The therapeutic advantages of psychedelic plant medicines are encouraging, but there are significant hazards and safety concerns associated with their usage. Strict ceremonial frameworks were often a part of traditional usage in indigenous contexts, which served to reduce the likelihood of negative consequences. There are more valid safety concerns when utilized outside of these circumstances.

Psychological Risks

The majority of the negative side effects associated with psychedelic usage are thought to be psychological. Some of these symptoms include sudden, severe anxiety, panic attacks, paranoia, and, very rarely, psychosis in susceptible people (48).

In the absence of adequate psychological preparation or support, users may be overwhelmed by the strong emotional experiences triggered by psilocybin and ayahuasca, for instance (49).

On top of that, you might have hallucinogen persistent perception disorder (HPPD), an extremely uncommon medical disease where you keep seeing hallucinations even after the medication has stopped working (50). People who have a history of psychotic illnesses, including schizophrenia, either in themselves or their families are usually not allowed to participate in psychedelic treatment studies (51).

Physiological Risks

There are nevertheless hazards, even if physiological toxicity is often lower than that of synthetic medications. Some foods and drugs may have harmful interactions with the monoamine oxidase inhibitors (MAOIs) included in ayahuasca, which might cause hypertensive crises or serotonin syndrome (52). Mescaline, found in peyote and San Pedro cactus, has the potential to induce vomiting and nausea as well as cardiovascular side effects such as rapid heartbeat and elevated blood pressure (53).

Significant cardiotoxic hazards, such as QT interval prolongation and potentially lethal arrhythmias, are associated with the alkaloid ibogaine, which is produced from Tabernanthe iboga (54). The significance of medical screening and monitoring should be considered when contemplating the therapeutic use of ibogaine, since cases of sudden cardiac death have been linked to its usage.

Dependence and Abuse Potential

There is no evidence of physiological reliance on traditional psychedelics like psilocybin, DMT (in ayahuasca), and mescaline, in contrast to many other psychoactive drugs (55). The psychological pull of strong events, however, might cause susceptible people to develop unhealthy routines.

Current Approaches to Risk Mitigation

Modern psychedelic treatment methods include thorough preparation sessions, medical tests, in-session monitoring, and integration therapy post-experience. Such systems greatly lower the frequency of negative occurrences (56).

Table 7: Common Risks Associated with Major Psychedelic Herbal Medicines

Substance	Main Risks	Preventive Measures
Psilocybin Mushrooms	Anxiety, panic, psychosis	Psychological screening, therapeutic support
Ayahuasca	Hypertensive crisis, serotonin syndrome	Dietary restrictions, medication review
Peyote/San Pedro	Nausea, tachycardia	Medical supervision for cardiovascular health
Ibogaine	Cardiac arrhythmias, death	ECG monitoring, hospitalization

Pharmacology and Mechanism of Action

Primarily via altering the serotonin system in the brain, especially the 5-hydroxytryptamine 2A (5-HT2A) receptor, psychedelic plant drugs produce their effects. This receptor's activation starts a cascade of intracellular signaling changes thought to underlie the dramatic changes in perception, emotion, and cognition typical of the psychedelic experience (57).

Serotonergic System

Structurally comparable to serotonin are compounds include mescaline (from peyote and San Pedro cactus), DMT (from ayahuasca), and psilocybin. Psilocybin binds strongly to the 5-HT2A receptor upon consumption; it is quickly dephosphorylated to psilocin, its active form. This receptor activation leads to enhanced excitatory neurotransmission, primarily in the prefrontal cortex and default mode network (DMN), producing heightened sensory perception, emotional release, and a dissolution of ego boundaries (59).

Table 8: Key Psychedelic Compounds and Their Primary Receptors

Compound	Primary Active Metabolite	Main Target Receptor	Additional Effects
Psilocybin	Psilocin	5-HT2A	Mood regulation, cognitive flexibility
DMT (Ayahuasca)	DMT	5-HT2A	Visual imagery, emotional insights
Mescaline	Mescaline	5-HT2A, adrenergic	Emotional arousal, enhanced perception
Ibogaine	Noribogaine	NMDA, kappa-opioid, 5-HT2A	Addiction modulation, dream-like states

Other Neurotransmitter Systems

Some psychedelic substances interact with opioid systems, glutamate, dopamine, sigma-1 receptors, and the serotonergic system, all of which contribute to the hallucinogenic and therapeutic effects they produce. It is believed that ibogaine's anti-addictive effects are based on its complicated pharmacology, which it displays via regulating opioid and NMDA receptors (60).

Functional magnetic resonance imaging (fMRI) research also shows that psychedelics cause a discordance in brain networks, which loosens up previously set patterns of connection and makes room for more malleable neural pathways (61). When it comes to addressing mental health issues like depression and post-traumatic stress disorder (PTSD), this occurrence is thought to be vital.

Pharmacokinetics Overview

- **Psilocybin:** Oral bioavailability is around 50%, it starts working after 20-40 minutes, and it lasts for around 4-6 hours (62).
- **DMT:** Oral delivery necessitates MAO inhibition, similar to ayahuasca, and has a rapid onset when inhaled (seconds to minutes).
- **Mescaline:** A gradual start (1-2 hours) and a long-lasting impact (up to 12 hours).
- **Ibogaine:** Ibogaine and its metabolite noribogaine have a lengthy half-life of around 7 to 10 hours.

Psychedelic Herbal Medicines in Spiritual Practices

Psychedelic plant remedies have long been an integral part of many religions' and spiritual traditions. These herbs had other purposes beyond physical treatment, including induction into altered states of consciousness, communication with the divine, and the promotion of social cohesion and cultural identity.

Indigenous and Traditional Spiritual Use

Traditional indigenous communities typically saw psilocybin mushrooms, ayahuasca, and peyote as holy sacraments rather than just recreational drugs. Shamanic rituals in the Amazon basin revolve upon ayahuasca ceremonies, which have long been used for spiritual cleansing, divination, and making a link with the spirits of the natural world (63). Native American church rites in North America have also used peyote for millennia, with the goals of fostering spiritual direction, healing, and community unity (64).

Among the ancient Mesoamerican cultures, the Aztecs and others honored psilocybin mushrooms as "teonanácatl" or "flesh of the gods" (65). To optimize spiritual insight and safety, these traditions centered on ritualized, regulated use of psychedelics, often under the tutelage of a seasoned shaman or spiritual leader.

Contemporary Revival of Spiritual Practices

The potential spiritual benefits of psychedelics have seen a significant upturn in attention throughout the last few decades. Ayahuasca is included into Christian-based spiritual rites by legitimate religious organizations in Brazil, including the Santo Daime and the União do Vegetal (UDV), who merge indigenous traditions with contemporary theological structures (66).

Also, the spiritual aspect is becoming more recognized as an important component of healing processes in the worldwide psychedelic-assisted therapy movement, particularly in the treatment of existential crisis, loss, and despair (67).

Table 9: Major Psychedelic Herbal Medicines Used in Spiritual Practices

Plant/ Compound	Traditional Spiritual Context	Contemporary Application
Ayahuasca (DMT)	Amazonian shamanism for healing and spirit contact	Santo Daime, UDV religious ceremonies
Peyote (Mescaline)	Native American Church rituals	Ceremonial use in indigenous and non-indigenous groups
Psilocybin Mushrooms	Aztec and Mayan religious rites	Psychedelic churches, mindfulness retreats
Iboga (Ibogaine)	Bwiti religion in Central Africa	Psycho-spiritual addiction recovery ceremonies

Potential for Addiction Treatment

Psychedelic herbal drugs have recently gained attention as potential therapy options for a range of addictions, such as amphetamine usage, alcoholism, cigarette addiction, and opioid dependency. Psychedelics, in contrast to traditional pharmaceutical treatments, may get to the bottom of addiction by addressing its existential and psychological aspects; they can help with emotional release, neuroplasticity, and long-term recovery (68).

Mechanisms Supporting Addiction Treatment

Psychedelics may have anti-addictive effects via many pathways:

- **Neuroplasticity enhancement:** Psychedelics promote the reconfiguration of brain networks, which is essential for breaking addictive behaviors, by

stimulating growth factors such as BDNF (Brain-Derived Neurotrophic Factor) (69).
- **Resetting reward circuits:** By altering the serotonergic, dopaminergic, and glutamatergic systems, drugs like psilocybin and ibogaine prevent maladaptive reward learning (70).
- **Psychospiritual insights:** Substance usage and desires are significantly reduced in those who have profound spiritual or ego-dissolving experiences during psychedelic sessions (71).

Key Psychedelics in Addiction Treatment
- **Ibogaine:** Its active ingredient, Tabernanthe iboga, has been successful in treating stimulant and opiate addiction by preventing withdrawal and cravings (72).
- **Psilocybin:** There is evidence from clinical trials that it may help people quit smoking and lessen their reliance on alcohol (73).
- **Ayahuasca:** There is evidence from both clinical and traditional use that it may help with trauma and depression, two of the psychological components that contribute to drug addiction (74).

Table 10: Summary of Psychedelic Compounds and Evidence in Addiction Treatment

Compound	Addiction Target	Key Mechanisms	Clinical Evidence
Ibogaine	Opioids, stimulants	NMDA antagonism, dopamine modulation	Open-label trials, observational studies (72).
Psilocybin	Tobacco, alcohol	5-HT2A agonism, psychological restructuring	Pilot clinical trials (73).
Ayahuasca	Alcohol, polysubstance abuse	MAOI action, emotional processing	Observational studies, qualitative reports (74).

Future Scopes in Psychedelic Herbal Medicine Research

Numerous interesting study pathways have been opened by the growing scientific interest in psychedelic plant medications, although there are still considerable gaps. Integration of psychedelics into larger healthcare frameworks, development of new formulations, comprehension of long-term safety profiles, and improvement of clinical trial procedures should all be priorities for future research (75).

Clinical Trials and Regulatory Advances

Optimal dosage techniques, confirmation of effectiveness, and identification of patient categories most likely to benefit need bigger randomized controlled trials, notwithstanding promising early-phase outcomes for addiction, depression, and post-traumatic stress disorder (76). In order to guarantee similar therapy results across trials, it is necessary to codify uniform criteria for setup and optimization, therapeutic preparation, and integration techniques (77).

Novel Delivery Systems and Formulations

Improving bioavailability, decreasing adverse effects, and prolonging therapeutic effects are the goals of research into sublingual formulations, transdermal patches, and nanoencapsulation of hallucinogenic chemicals (78). Another approach being studied for cognitive improvement and mood stability is microdosing, which involves using very tiny dosages that do not produce hallucinations (79).

Personalized Psychedelic Medicine

New biomarkers, genetics, and neuroimaging techniques will pave the way for more targeted psychedelic treatments. A better understanding of how each person's neurobiology and genetic markers work could lead to more effective and safer psychedelic therapies (80).

Ethical, Legal, and Cultural Considerations

Researchers of the future will face formidable cultural, legal, and ethical obstacles. We must honor the indigenous wisdom and practices that have preserved these medicines for generations and incorporate them into modern medicine without exploiting them (81). Another important objective is the guarantee of equal access to psychedelic treatments.

Table 11: Key Challenges and Research Opportunities in Psychedelic Herbal Medicine

Research Area	Current Challenge	Future Opportunity
Clinical efficacy	Small sample sizes, lack of diversity	Large, multi-center trials
Pharmacokinetics	Short half-life of some compounds	Novel delivery technologies
Personalization	Interindividual variability in response	Biomarker-driven, customized protocols
Ethical considerations	Cultural appropriation risks	Collaborative models with indigenous groups
Regulatory frameworks	Schedule I legal barriers	FDA breakthrough therapy designations

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https://doi.org/10.47552/ijam.v16iS2.6209 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Cissus quadrangularis: An Herbal Remedy Climbing Towards Health & Wellness

Review Article

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Abstract

Traditional medical systems, such as Ayurveda and Siddha, have long acknowledged the many medicinal uses of the succulent plant *Cissus quadrangularis* (CQ), which is a member of the Vitaceae family. Popularly known as "Harjor" or "Bone Setter," CQ has attracted the attention of scientists due to its powerful ability to reduce inflammation, act as an antioxidant, alleviate pain, and rebuild bone. Plants with a high concentration of bioactive components, such as flavonoids, triterpenoids, ketosteroids, and phenolic compounds, have a wide range of potential medicinal uses. It is a potential natural option for osteoporosis and bone injuries, as shown in preclinical and clinical research that it speeds up fracture healing, increases bone mineral density, and modulates osteoblastic activity. In addition to its effects on bone health, CQ shows promise in the treatment of metabolic diseases like diabetes, obesity, and hyperlipidemia by influencing insulin sensitivity, suppressing hunger, and lipid metabolism. Its multifunctional botanical properties are further supported by its gastroprotective, antibacterial, and hepatoprotective activities. Based on molecular research, CQ is thought to exert its therapeutic effects by lowering oxidative stress and inflammatory cytokines and by influencing important cellular pathways such as NF-κB, MAPK, and PPARγ. Integration into evidence-based medicine is hindered by the lack of extensive clinical validation and standardized formulations, notwithstanding the therapeutic potential. By outlining current findings and pointing out areas where more study is needed, this review offers a comprehensive overview of *Cissus quadrangularis*' phytochemistry, pharmacology, and medicinal uses. The purpose of this paper is to encourage further research into this adaptable plant and its potential use in contemporary health and wellness programs.

Keywords: *Cissus quadrangularis*, Bone regeneration, Phytochemicals, Metabolic disorders, Anti-inflammatory activity.

Introduction

Historical Significance of Herbal Remedies

Herbal therapy has constituted the cornerstone of treatment for millennia, especially within traditional systems such as Ayurveda, Traditional Chinese therapy (TCM), and African ethnomedicine. These methods use plant-based medicines to tackle a range of conditions, from infections to chronic disorders, often using a holistic approach (1, 2). The worldwide revival of interest in phytotherapeutics arises from their comparatively low toxicity, cultural acceptance, and abundance of bioactive compounds, particularly when synthetic pharmaceuticals encounter issues such as resistance and adverse effects (3).

Rationale for Focusing on *Cissus quadrangularis*

Cissus quadrangularis, a succulent vine belonging to the Vitaceae family, has attracted interest for its historical use in the treatment of bone fractures, joint discomfort, obesity, and gastrointestinal disorders. In Ayurvedic and Siddha traditions, it is often referred to as the "Bone Setter" plant. Scientific research has substantiated several traditional assertions, emphasising its osteogenic, antioxidant, anti-inflammatory, and anti-obesity characteristics (5, 6). Its varied phytochemical composition and advantageous safety margin provide it a compelling prospect for integrative therapeutic advancement.

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Scope and Structure of the Review

This study compiles the most up-to-date information on *Cissus quadrangularis's* pharmacological properties, ethnomedicinal applications, phytochemistry, and therapeutic prospects. It provides an in-depth analysis of unanswered questions, regulatory concerns, and potential medicinal and nutraceutical uses.

Botanical Description and Phytochemistry

Botanical Classification and Morphology

- **Kingdom:** Plantae
- **Order:** Vitales
- **Family:** Vitaceae
- **Genus:** *Cissus*
- **Species:** *C. quadrangularis* L.

The plant is a perennial, fleshy climber with quadrangular, winged stems and tendrils. Its leaves are ovate to cordate, and it bears small greenish flowers and berries. The plant thrives in arid and semi-arid regions and is easily propagated via stem cuttings (7).

Geographical Distribution and Habitat

Cissus quadrangularis is widely distributed across tropical and subtropical zones of Asia and Africa. It is native to India and Sri Lanka but is also found in Myanmar, Thailand, and parts of Africa (8).

Phytochemical Profile

The plant is a reservoir of biologically active compounds, including ketosteroids, flavonoids, triterpenoids, stilbenes, phytosterols, ascorbic acid, and carotenoids (9). These constituents are distributed in various parts: stem, leaves, and roots.

Table 1: Active Constituents and Their Roles (9)

Phytochemical	Reported Activity
Ketosteroids	Bone healing, anabolic effect
Quercetin	Antioxidant, anti-inflammatory
β-Sitosterol	Anti-inflammatory, cholesterol-lowering
Ascorbic acid	Antioxidant, immune support
Calcium	Bone mineralization
Carotene	Antioxidant, vision support
Triterpenoids	Anti-inflammatory, hepatoprotective
Flavonoids	Antioxidant, cardioprotective

Traditional and Ethnomedicinal Uses

Applications in Ayurveda, Siddha, and Folk Medicine

In Ayurveda, *C. quadrangularis* is used under names like "Asthisamharaka" (bone protector) for treating fractures and sprains. Siddha medicine utilizes it for hemorrhoids, bone strengthening, and metabolic disorders. Folk healers employ stem paste or decoctions for wound healing, hemorrhoids, and menstrual disorders (10,11).

Cultural Relevance and Regional Practices

The plant holds ethnobotanical significance in Indian tribal medicine, where its stem is chewed fresh or prepared into poultices. In parts of Africa, it is administered orally for gastric ulcers and topically for wounds. Its wide usage across continents signifies its cultural integration into traditional health systems (12).

Pharmacological Properties and Mechanisms of Action

Anti-inflammatory and Analgesic Activity

Cissus quadrangularis exhibits potent anti-inflammatory effects through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways. Its steroidal constituents suppress pro-inflammatory cytokines such as TNF-α and IL-6, alleviating pain and swelling in models of arthritis and inflammation (13, 14).

Bone Healing and Osteoprotective Mechanisms

The plant promotes bone regeneration by enhancing osteoblast proliferation and mineral deposition. Key mechanisms include increased alkaline phosphatase (ALP) activity and collagen matrix formation. Animal models show faster fracture healing and increased callus formation (15, 16).

Antioxidant and Cytoprotective Effects

Rich in flavonoids, ascorbic acid, and carotenoids, *C. quadrangularis* effectively neutralizes reactive oxygen species (ROS). It elevates endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), thus protecting cells from oxidative damage (17, 18).

Antimicrobial and Anthelmintic Properties

Ethanolic and aqueous extracts of the stem exhibit antibacterial activity against *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These effects are mediated by membrane disruption and enzyme inhibition in microbes (19).

Anti-obesity and Metabolic Effects

The plant reduces body weight, LDL-cholesterol, and leptin levels in obese models, potentially via appetite suppression and enhanced lipid metabolism. It has been suggested to modulate peroxisome proliferator-activated receptors (PPARs) and AMPK pathways (20, 21).

Other Therapeutic Activities

Gastroprotective: Enhances mucin secretion and reduces gastric acidity, aiding ulcer healing.

Hepatoprotective: Reduces hepatic transaminases and improves liver histology in toxin-induced damage models (22, 23).

Preclinical and Clinical Evidence

In Vitro Studies

Numerous in vitro assays show antioxidant, anti-inflammatory, and osteogenic effects. The stem extract enhances osteoblast differentiation and reduces lipid accumulation in adipocytes (24, 25).

In Vivo Animal Studies

Animal models confirm fracture healing, anti-ulcer, hepatoprotective, and hypolipidemic effects. Rat models of bone fractures treated with extracts show significantly faster union and increased bone density (15, 16)

Human Clinical Trials

Several small-scale trials in India and Africa reported improvements in fracture healing, lipid profiles, and body weight. However, most studies are limited by small sample sizes, lack of blinding, or short durations (26, 27).

Safety, Efficacy, and Dosing

C. quadrangularis is considered safe at doses up to 3 g/day in adults. Mild gastrointestinal discomfort has been reported in some cases. Long-term toxicity remains under-researched (28).

Formulations and Delivery Systems

Traditional Preparations

Used as powdered stem, decoctions, or pastes in Ayurveda and Siddha medicine, often administered with milk or ghee for enhanced absorption (27).

Modern Herbal Formulations

Available as capsules, tablets, and syrups marketed for bone health, weight management, and joint support. Standardized extracts with quantified ketosteroid content are gaining popularity (28).

Nanoformulations and Bioavailability Enhancement

Recent innovations include nanoemulsions and liposomal formulations to enhance the solubility and intestinal uptake of hydrophobic phytoconstituents. These systems show promise in improving systemic availability and targeted delivery (29, 30).

Toxicology and Safety Profile

Acute and Chronic Toxicity Studies

Cissus quadrangularis has demonstrated a high safety margin in preclinical studies. Acute oral toxicity testing in albino rats revealed no signs of mortality or behavioral toxicity at doses up to 5000 mg/kg (31). Sub-chronic administration (1000 mg/kg/day for 90 days) caused only mild hepatic changes at high doses in Wistar rats (32). Chronic exposure (up to 6 months) did not induce significant hematological or histopathological alterations (33).

Genotoxicity assays, including Ames tests and micronucleus tests, reported no mutagenic potential (34). Reproductive toxicity studies showed no teratogenic or embryotoxic effects in rodent models (35).

Herb-Drug Interactions

Studies on human liver microsomes suggest *C. quadrangularis* may inhibit CYP3A4, potentially affecting the metabolism of drugs like simvastatin and warfarin (36). Co-administration with NSAIDs like ibuprofen occasionally caused mild gastrointestinal discomfort, likely due to additive mucosal effects (37).

Regulatory Status and Quality Control

C. quadrangularis is listed in several traditional pharmacopeias and approved in India as a dietary supplement. However, standardization remains a major concern, with variability in active ketosteroid content across preparations. Quality control is typically performed via TLC, HPTLC, or HPLC methods (38, 39).

Challenges and Limitations in Research

Gaps in Mechanistic Understanding

While multiple pharmacological benefits have been documented, the precise molecular targets of *C. quadrangularis* constituents remain unclear. Most studies focus on gross outcomes rather than mechanistic pathways like receptor modulation or gene expression.

Standardization Issues

Variability in plant chemotypes, harvesting conditions, and extraction methods contribute to inconsistencies in bioactivity. Lack of standardized biomarkers for quality assurance limits reproducibility and clinical translation (40).

Clinical Trial Shortcomings

Human clinical studies are limited in number, often underpowered, and lack rigorous randomization, blinding, or placebo control. Many are conducted in regional settings without long-term follow-up or adverse event monitoring (41, 42).

Future Perspectives and Potential Applications

Role in Modern Integrative Medicine

Cissus quadrangularis is gaining recognition as a complementary remedy for bone health, obesity, and inflammatory conditions. Integration into orthopedic and metabolic care protocols is feasible, provided evidence-based dosing guidelines are developed.

Commercial and Nutraceutical Potential

The plant's use in sports nutrition, anti-obesity, and joint health supplements continues to rise. Patent activity around nanoformulations and standardized extracts has increased in the past decade (43). Market acceptance hinges on validated health claims and regulatory approval.

Research Directions and Innovations

Key future directions include:

- Pharmacokinetic profiling of active compounds
- Multi-omics approaches (transcriptomics, metabolomics) to elucidate mechanisms
- Large-scale, multicentric, randomized clinical trials
- Novel formulations (e.g., nanocarriers, bioadhesive gels)

Conclusion

Cissus quadrangularis, a climbing perennial herb deeply embedded in the pharmacopeias of Ayurveda, Siddha, and folk medicine, has emerged as a prominent candidate in the realm of evidence-based herbal therapeutics. Its historical use in fracture healing, inflammation, and gastrointestinal ailments has prompted an upsurge in scientific investigations aimed at validating and expanding its traditional applications.

The pharmacological breadth of *C. quadrangularis* is attributed to a diverse array of bioactive constituents, including ketosteroids, flavonoids (quercetin, kaempferol), triterpenoids, stilbene derivatives like resveratrol, and β-sitosterol. These compounds contribute to a wide range of therapeutic activities such as anti-inflammatory, analgesic, antioxidant, bone regenerative, hepatoprotective, antimicrobial, and anti-obesity effects. Mechanistic studies have demonstrated that these effects are mediated via multiple pathways, including COX-2 inhibition, NF-κB downregulation, and enhancement of osteoblastogenesis through upregulation of alkaline phosphatase and collagen synthesis. The herb also shows promise in modulating metabolic parameters such as lipid profiles and blood glucose levels, highlighting its potential role in metabolic syndrome management.

The safety profile of *C. quadrangularis* is generally favorable, with acute and sub-chronic toxicity studies in animal models reporting minimal adverse effects at high doses. Clinical trials, though limited in number and scale, have demonstrated efficacy in promoting bone healing, reducing weight, and alleviating joint pain with minimal side effects. However, concerns about herb-drug interactions, especially via cytochrome P450 inhibition, warrant more rigorous pharmacokinetic and toxicovigilance studies.

From a translational perspective, the herb has been incorporated into a variety of formulations, including powders, capsules, decoctions, and more recently, nanoformulations designed to improve bioavailability and target-specific delivery. These advancements open new avenues for its integration into mainstream pharmaceutical and nutraceutical sectors. However, lack of standardized extracts, poor regulation of commercial products, and inconsistencies in active constituent concentrations remain significant barriers to clinical and commercial success.

Despite its promise, several limitations persist. The majority of existing studies are preclinical, and those involving humans often suffer from methodological flaws such as small sample sizes, lack of blinding, and inadequate follow-up. Moreover, the absence of universal quality standards and authentication procedures undermines the reproducibility and scalability of research findings. There is also a paucity of mechanistic insights at the molecular and genomic levels, which could further solidify the herb's therapeutic legitimacy.

Looking forward, *Cissus quadrangularis* presents a compelling opportunity for integration into modern integrative and personalized medicine frameworks. To fully harness its therapeutic potential, future efforts should focus on conducting large-scale, randomized, placebo-controlled clinical trials with robust methodological designs. Standardization of extracts using modern analytical tools such as HPLC, LC-MS/MS, and NMR should be prioritized. Furthermore, exploration of gene-expression modulation, signal transduction pathways, and interaction with the human microbiome will provide a deeper understanding of its multifaceted pharmacology.

In conclusion, *Cissus quadrangularis* exemplifies the synergy between traditional wisdom and modern science. With continued research, quality control, and innovation, it is poised to become a cornerstone in the evolving landscape of botanical therapeutics for bone health, metabolic disorders, and beyond.

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https://doi.org/10.47552/ijam.v16iS2.6202 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Advancements and Innovations in Herbal and non-herbal, Self-Micro emulsifying Drug Delivery Systems (SMEDDS): A Comprehensive Review

Review Article

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Abstract

Self-microemulsifying drug delivery systems (SMEDDS) formulations have emerged as crucial methods for improving the bioavailability of poorly water-soluble drugs. However, they exhibit several limitations, including oxidation of unsaturated fatty acids, restricted lymphatic absorption, handling challenges, in vivo drug precipitation, a lack of predictive in vitro research—and their high surfactant content can cause gastrointestinal irritation. These factors can impede their broader application. Incorporating precipitation inhibitors or polymers within lipid-based formulations helps maintain drug supersaturation after dispersion, thereby reducing exposure variability and enhancing bioavailability. Converting liquid SMEDDS into solid forms also addresses issues related to liquid handling and stability. This review highlights recent developments, such as the use of nanotechnology, innovative excipients, and solidification techniques in the formulation of herbal SMEDDS. It also evaluates their advantages and drawbacks in drug delivery, with a particular focus on key performance parameters like droplet size, zeta potential, and stability. Additionally, self-nanoemulsifying drug delivery systems (SNEDDS) show considerable promise for improving the bioavailability and solubility of poorly water-soluble herbal extracts by encapsulating them in nanoemulsions. In summary, SMEDDS offer a viable oral platform for administering poorly soluble medications and herbal extracts, with the potential to significantly enhance therapeutic outcomes. For wider clinical adoption, however, challenges related to formulation stability, scalable manufacturing, and regulatory compliance must be addressed. Future research should aim to overcome these barriers and expand SMEDDS applications across diverse therapeutic areas.

Keywords: Emulsion, Herbal SMEDDS Formulation, Stability, Factors, Composition, Medication.

Introduction

Several strategies have been explored to increase the oral bioavailability of poorly water-soluble medicines (1–3). Oral administration is the most common route for continuous therapy due to its excellent patient compliance. However, the high lipophilicity of many drugs hinders the oral administration of nearly half of all active pharmaceutical ingredients. In fact, approximately 40% of new drug candidates exhibit poor water solubility, which complicates the development of effective oral solid dosage forms in terms of formulation and bioavailability.

Various approaches have been employed to address these challenges, such as enhancing solubility or maintaining the drug in a liquid state throughout gastric transit (4, 5). These include the use of surfactants, cyclodextrins, micronization, liquid–solid conversion techniques, salt formation, pH modification, nanoscale delivery systems, solid dispersions, and penetration enhancers (6).

Among these methods, lipid-based solutions—including emulsions and pre-emulsion concentrates—have attracted significant attention, as they offer physically stable formulations capable of encapsulating poorly soluble drugs (7). Nevertheless, emulsion

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systems present inherent challenges, such as stability concerns and manufacturing complexities during commercialization (8). Self-emulsifying systems offer a promising formulation technology to address these issues.

Self-emulsifying drug delivery systems (SEDDS) are effective tools for enhancing the bioavailability of weakly water-soluble drugs (9). SEDDS are isotropic mixtures of drugs, lipids, and surfactants, often supplemented with hydrophilic co-solvents or co-emulsifiers (10). Upon mild agitation and dilution with aqueous media, they rapidly form fine oil-in-water emulsions, typically with droplet sizes ranging from 100 to 300 nm. In contrast, self-micro-emulsifying drug delivery systems (SMEDDS) form clear microemulsions with droplets smaller than 50 nm (11). In recent years, significant attention has focused on lipid-based formulations—especially SMEDDS.

Poor water solubility remains a major obstacle in oral drug formulation, since drugs must be in solution to be absorbed through the gastrointestinal tract (12). Many pharmacologically promising substances suffer from poor aqueous solubility. Furthermore, approximately 30% of marketed drugs and nearly 50% of novel compounds exhibit high lipophilicity and low aqueous solubility. According to the Biopharmaceutical Classification System (BCS), Class II drugs are highly permeable but poorly soluble, while Class IV drugs are low in both solubility and permeability (13). Both classes face variable absorption and poor oral bioavailability.

To improve solubility and absorption, various strategies have been proposed, including solid dispersions, crystal habit modification, particle size reduction, solid solution techniques, and salt formation (14). Recently, lipid-based carrier systems have regained interest for enhancing the bioavailability of poorly soluble drugs. The primary aim of these formulations is to maintain lipophilic molecules in a solubilized state throughout the gastrointestinal tract.

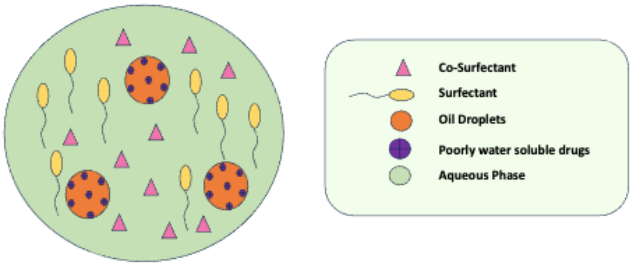
Lipid-based carriers take several forms: emulsions, microemulsions, solutions, suspensions, SEDDS, and dry emulsions (15). SEDDS, in particular, have demonstrated efficacy for the absorption of lipophilic drugs (e.g., cyclosporine A) in microemulsions. They have received more attention for oral drug development than conventional formulations of lipophilic compounds.

The Lipid Formulation Classification System (LFCS) categorizes lipid-based formulations. According to LFCS, SEDDS (Type II) are isotropic mixtures of oils and surfactants that form oil-in-water emulsions upon contact with gastric fluids. The terminology has evolved: SEDDS now often refers to Self-Nano Emulsifying Drug Delivery Systems (SNEDDS) or falls under Type IIIA or IIIB, which include co-surfactants and sometimes co-solvents. Their optimization commonly involves Response Surface Methodology (RSM) (16).

RSM reduces the experimental burden required to develop SEDDS with desirable characteristics. Multiple research groups have investigated lipid-based systems—including macroemulsions, SMEDDS, microemulsions, nanoemulsions, lipoplexes, solid lipid nanoparticles, and liposomes. This progress has led to SMEDDS featuring smaller droplets and improved thermodynamic stability. Emulsions are composed of microscopic droplets dispersed in another liquid phase containing surfactants; they are often thermodynamically unstable, transparent (or sometimes opaque), and exhibit viscous, liquid-like properties (17).

The various components of SMEDDS are shown in Figure 1.

Figure 1: Different components of the SMEDDS.



In self-emulsifying formulations, the resulting emulsion enhances membrane permeability through the action of surfactants and improves lymphatic transport when medium- and long-chain oils are used. These factors can significantly enhance the performance of the formulation (18). In recent years, there has been growing interest in SMEDDS in particular. This is primarily because SMEDDS are physically stable, easy to manufacture, suitable for encapsulation in soft gelatin capsules, and capable of producing drug-loaded microemulsions with a large surface area upon dispersion in the gastrointestinal system. These microemulsions not only facilitate the rapid breakdown of the formulation by gastrointestinal enzymes but also promote drug partitioning into mixed micelles and enable direct absorption from emulsion droplets into the aqueous phase of intestinal fluid.

Here, we present SMEDDS as a leading approach for formulating lipophilic drugs and improving their oral bioavailability (19).

Composition of SMEDDS (20,21,22)

The self-emulsification process is unique to the characteristics of the oil-surfactant pair. The method is dependent on:

1. Oils
2. Surfactant
3. Co-Surfactant
4. Co-Solvents
5. Consistency Builder
6. Polymer

1. Oil (Lipid)

Lipids are a crucial component of SMEDDS, as the quantity of oil in the formulation influences how effectively drugs are solubilized and reach the lymphatic system. Typically, lipids are soluble in non-polar organic solvents but insoluble in water; they are characterized by their fatty acid content, melting point, hydrophilic-lipophilic balance (HLB), and solubility in

these solvents. Lipids with a high melting point and a low HLB value are particularly suitable for sustained-release applications, while semi-solid excipients with high HLB values promote rapid drug release and increased bioavailability.

SMEDDS have been developed using both long-chain triglycerides (e.g., soy oil) and medium-chain triglycerides (e.g., Capmul MCM) with varying levels of saturation. Due to their biocompatibility, modified or hydrolyzed vegetable oils have significantly contributed to the success of SMEDDS (23). Recently, innovative semi-synthetic medium-chain triglyceride-based excipients, such as Gelucire, have been developed to replace traditional medium-chain triglycerides. Other suitable oils and fats include olive oil, maize oil, soy oil, and certain animal fats (25).

Examples of Oils: Corn oil, peanut oil, and beeswax oil.

2. Surfactant

In the creation of SMEDDS, non-ionic surfactants with high HLB values are used, such as Common surfactants used in SMEDDS include ethoxylated polyglycolysed glycerides, Tween-80, LABRAFAC CM10, a mixture of saturated compounds with eight carbon atoms, polyglycosylated glycosides (with an HLB of 10), and long-chain alkyl sulfonate surfactants such as sodium dodecyl benzene sulfonate, sodium lauryl sulfate, and dialkyl sulfonates. Surfactant concentrations in SMEDDS formulations typically range from 30% to 40% by weight (w/w) (24, 25).

A high HLB value and resulting hydrophilicity are essential for the rapid formation of oil-in-water (o/w) droplets and for ensuring effective dispersion and self-emulsifying behavior in aqueous environments. The amphiphilic nature of these surfactants allows them to solubilize and stabilize relatively large amounts of hydrophobic drugs, which is crucial for maintaining the drugs in a soluble form that facilitates efficient absorption and prevents precipitation within the gastrointestinal lumen (26, 27).

A surfactant molecule consists of two components with different solvent affinities—one that is more attracted to water (the polar head group) and another that is more attracted to oils (the non-polar tail). By forming an interfacial film between these two liquids, the surfactant reduces interfacial tension and facilitates the creation of stable emulsions.

Examples Of Surfactant: Polysorbate 80, Polysorbate 20, Sorbitan mono oleate

Types of surfactants

(a) Anionic Surfactant: where the hydrophilic group carries negative charge, such as carboxyl, sulphate, and sulphonate. Ex-Potassium laurate

(b) Cationic Surfactants: where the hydrophilic group carries positive charge, such as ammonium. Ex-Quaternary ammonium halides.

(c) Ampholytic Surfactants (Zwitter Ionic Surfactants): where the hydrophilic group carries both positive and negative charge. Ex-Sulfobetaines.

(d) Non-Ionic Surfactants: where the hydrophilic group does not carry any charge but its water solubility is forming highly polar group such as hydroxyl or polyoxyethylene. Non-ionic surfactant is most widely recommended as they possess relatively high HLB value.

3. Co-Surfactant

A co-surfactant with an HLB value between 10 and 14 is typically employed in SMEDDS. The preferred hydrophilic cosurfactant is an alcohol with an intermediate chain length, such as hexanol, pentanol, or octanol, which is known to lower the oil/water contact and enable the spontaneous production of microemulsion (24, 25).

Production of optimum SMEDDS requires a high concentration of surfactant in order to sufficiently reduce interfacial tension, which can be harmful. So cosurfactant is needed to reduce the concentration of surfactant. Surfactants and cosurfactants give the interfacial film enough flexibility to accept the various curvatures required to form micro-emulsion in a wide range of compositions. Cosurfactants that are suitable for use in medicine include ethanol, propylene glycol, and polyethylene glycol 400. Lipid-soluble solvents are employed in the creation of SMEDDS because they make it possible to dissolve significant amounts of hydrophilic surfactants.

4. Co-Solvents

Organic solvents suitable for oral administration —such as ethanol, propylene glycol, and polyethylene glycol—are frequently incorporated into SMEDDS. They enhance the dissolution of hydrophilic drugs or support greater quantities of hydrophilic surfactants within the lipid base. To further aid solubilization, co-solvents like triacetin (glyceryl triacetate) are added; triacetin is particularly effective due to its compatibility with lipid phases and ability to solubilize hydrophobic drugs.

For optimal performance, SMEDDS formulations typically include a high surfactant concentration—generally over 30% w/w—which promotes efficient self-emulsification and ensures the rapid formation of fine oil-in-water micro- or nano-emulsions upon dilution. (24,25).

5. Consistency Builder

The consistency of the emulsion can be changed by adding more material, such as acetyl alcohol, tragacanth, stearic acid, and beeswax (28).

6. Polymer

Inert polymer matrix that makes up between 5% and 40% of the composition by weight, is not ionizable at physiological pH, and can create matrices. Examples include ethyl cellulose, hydroxypropyl methyl cellulose, etc. (29).

Emulsion

Emulsions are mixtures of two or more liquids, with one liquid dispersed as small or ultra-small

droplets within the other. These emulsions typically form through mechanical agitation, provided the liquids are immiscible.

Types of Emulsions

1. **Water-in-Oil Microemulsion (W/O)**
2. **Oil-in-Water Microemulsion (O/W)**
3. **Multiple Emulsions**
   a. Oil-in-Water-in-Oil (O/W/O)
   b. Water-in-Oil-in-Water (W/O/W)

1. Water-in-Oil Emulsions (W/O)

The continuous phase in a W/O emulsion is hydrophobic (oil), with water serving as the dispersed phase (30). In crude oil contexts, over 95% of emulsions are the W/O type (31). Stability is critical in W/O emulsions, and they are typically stabilized using natural surfactants like resins and asphaltenes (32, 33). Fingas and Fieldhouse classified W/O emulsions into four categories: stable, mesostable, unstable, and entrained water. Stable emulsions, which are brown, contain 60–80% water (34).

2. Oil-in-Water Emulsions (O/W)

In O/W emulsions, water is the continuous phase and oil is the dispersed phase. If poorly managed in petroleum operations, either W/O or O/W emulsions can result in considerable financial losses (35). O/W emulsions, often termed reverse emulsions, are less common than W/O and typically classified as mesostable—brown or black emulsions with properties intermediate between stable and unstable types. Unstable emulsions rapidly separate into two phases, while entrained water emulsions (initially 30–40% water) settle to about 10% over a week. Only the mesostable and stable categories are generally recognized as emulsions.

3. Multiple Emulsions

Multiple emulsions include O/W/O and W/O/W forms. These complex systems are stabilized by both hydrophobic and hydrophilic surfactants. They feature small droplets nested within larger droplets, all suspended in a continuous phase. For example, W/O/W emulsions consist of water droplets within oil droplets, themselves dispersed in a continuous aqueous phase. Stabilization typically requires two surfactants: one with low HLB and another with high HLB (36–39).

Emulsion Formation

Emulsification refers to the process by which emulsions form—a dynamic, energy-driven process. Mechanical energy (e.g., shaking, rotor-stator mixing, membrane injection, high-pressure homogenization, or ultrasound) is used to disperse one liquid into finely sized droplets within another phase (40, 41). Deformation under shear or agitation breaks droplets into smaller units (40, 41).

In crude oil processing, stabilizing emulsions with waxes, resins, and asphaltenes is a primary challenge. The highly stable W/O emulsions in this industry owe their stability to these natural surfactants (42). The formation of a stable emulsion requires:

- Immiscibility of phases
- Agitation to disperse one liquid into another
- Sufficient surfactant

An emulsion's properties will evolve post-formation based on variables like time, mixing speed, temperature, and pressure. A stabilizing agent is essential for maintaining a stable emulsion.

Stability of Emulsion

Emulsion stability is governed by both the type and concentration of surfactants, which form interfacial films around water droplets, reducing interfacial tension and increasing interfacial viscosity (43). Temperature, water content, and mixing speed also influence stability (44, 45). Increasing energy input yields smaller droplets and greater stability. However, higher temperatures can alter interfacial film properties, surfactant solubilities, and reduce emulsion viscosity—especially in the oil phase (46). Although emulsions are thermodynamically unstable and prone to changes over time, understanding both kinetic and thermodynamic stability is key to controlling their behavior.

Kinetic vs. Thermodynamic Stability

Thermodynamic stability refers to the inherent tendency of emulsions to separate due to unfavorable oil-water interactions. Over time, emulsions may break down unless stabilized by surfactants (47). Kinetic stability, on the other hand, is achieved by adding stabilizing agents that inhibit droplet coalescence, delaying separation (48).

Types of SMEDDS

1. Herbal SMEDDS

Definition: SMEDDS that incorporate plant-derived (phytochemical) active ingredients.

Examples:

Herbal Drug	Application	Benefit via SMEDDS
Curcumin (from turmeric)	Anti-inflammatory, anticancer	Poor water solubility improved significantly
Thymoquinone (from Nigella sativa)	Antioxidant, anticancer	Enhanced oral bioavailability
Berberine	Antidiabetic, antimicrobial	Enhanced intestinal permeability
Resveratrol	Antioxidant	Better stability and systemic availability

Challenges:
Herbal compounds are often chemically unstable.
Standardization and reproducibility are difficult.

2. **Non-Herbal SMEDDS**

Definition: SMEDDS used for synthetic or semi-synthetic pharmaceutical compounds.

https://doi.org/10.47552/ijam.v16iS2.6202 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Examples

Drug	Class	SMEDDS Benefit
Cyclosporine A	Immuno-suppressant	Marketed SMEDDS product (Neoral®) for enhanced bioavailability
Fenofibrate	Anti-hyperlipidemic	Poor water solubility; improved systemic absorption
Tacrolimus	Immuno-suppressant	Improved solubility and reduced variability
Ritonavir	Antiviral (HIV)	Enhanced oral bioavailability in lipid-based formulations

3. COMPARISON: HERBAL VS. NON-HERBAL SMEDDS

Feature	Herbal SMEDDS	Non-Herbal SMEDDS
Active Ingredients	Phytochemicals	Synthetic drugs
Regulatory complexity	Higher (due to natural variability)	More standardized
Clinical data availability	Limited	Well-established for many drugs
Appeal	"Natural" appeal for consumers	Widely accepted in pharmaceuticals
Solubility challenges	Often extreme	Variable but better characterized

Preparation of SMEDDS

A glass vial is charged with a precisely weighed drug, followed by oil and co-surfactant. The mixture is gently stirred and vortexed for 30 minutes, then heated at 40 °C on a magnetic stirrer until the drug dissolves. The solution is stored at room temperature until use.

Methods for Preparing Solid SMEDDS (S-SMEDDS)

1. **Capsule Encapsulation**
   - Liquid SMEDDS can be encapsulated directly or sealed via banding or micro-spraying.
   - For semisolid SMEDDS: heat excipients ~20 °C above melting point, dissolve drug in the molten blend, fill capsules, and cool to room temperature. Ideal for high-potency, low-dose drugs (49).
2. **Spray Drying**
   - Liquid SMEDDS mixed with a solid carrier and solvent, then atomized into fine droplets. These are dried to yield solid particles (50).
3. **Melt Granulation**
   - Similar to spray drying, involving atomized droplets in a controlled drying setup (50).
4. **Extrusion–Spheronization**
   - Liquid SMEDDS mixed with extrusion aids and water to form a wet mass, extruded, spheronized, dried, and sized pellets (51, 52).

Mechanism of SMEDDS

Self-emulsification occurs when the entropy gain from dispersion exceeds the energy required to increase surface area. The free energy of emulsion formation is given by:

$$\Delta G = \Sigma N \pi r 2 \sigma$$

where ΔG is the free energy change (ignoring mixing energy), N is the number of droplets, r is droplet radius, and σ is interfacial tension (53).

As emulsion phases separate to reduce free energy and interfacial area, traditional emulsifiers stabilize droplets by forming monolayers that lower interfacial tension and prevent coalescence. With sufficiently low or negative ΔG, self-emulsification becomes spontaneous with minimal energy input. During this process, non-ionic surfactants and aqueous interfaces interact, leading to phase inversion behaviors related to emulsifier properties (54).

On gentle agitation, water infiltrates the surfactant-oil mixture, disturbing the interface and generating droplets. Microemulsions remain thermodynamically stable, maintaining equilibrium through a dynamic balance of droplet coalescence and fragmentation (55).

Evaluation of SMEDDS

- **Visual Assessment:** Indicates self-emulsifying capacity and dispersion behavior (56, 57).
- **Emulsification Efficiency:** Measured via rate of emulsification and particle size distribution. Poulton used turbidity measurements to evaluate equilibrium achievement (58).
- **Droplet Polarity:** Influenced by oil HLB, fatty acid properties, emulsifier characteristics, and correlating to drug release (59).
- **Droplet Size:** Critical for drug release and absorption; measured using dynamic light scattering instruments for 10–200 nm particles (60, 61).
- **Dissolution Studies:** Assess sustained-release characteristics, particularly for drugs insoluble at acidic pH (62).
- **Zeta Potential:** Used to determine droplet charge and stability (62).

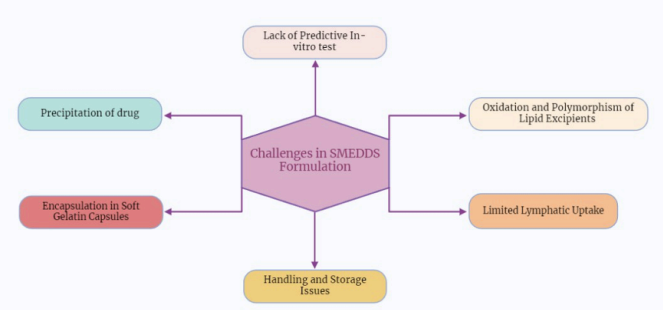
Factors Affecting SMEDDS

- **Drug Dose:** Drugs with poor water and lipid solubility, especially at high doses, are less suitable unless highly soluble in at least one component (63).
- **Drug Solubility in Oil:** Influences the ability to maintain the drug in solution; dilution may cause precipitation if surfactants solubilize the drug more than oil.
- **Equilibrium Solubility:** Predicts GI precipitation; some formulations remain supersaturated for up to 24 hours post-emulsification (64).
- **Oil Droplet Polarity:** Governs drug release rate; higher polarity facilitates faster drug transfer to the aqueous phase (64).

SMEDDS's Limitations

Despite the SMEDDS formulation's many benefits, there are certain drawbacks to this system, which are illustrated in the below image.

Figure 2: Limitations of SMEDDS formulation



Precipitation of Drug upon Dilution:

SMEDDS, once diluted in digestive fluids, often experience drug precipitation. Maintaining the drug in a solubilized form throughout the GI tract is a fundamental requirement for lipid-based formulations; otherwise, the benefits are nullified. The increased water content from dilution raises the risk of precipitation, so polymers are typically added to minimize this effect in vivo (65, 66).

Soft-Gelatin Capsule Encapsulation

Most SMEDDS formulations on the market are delivered in soft-gelatin capsules, but gelatin presents some drawbacks. Animal-derived gelatin may carry risks such as transmissible spongiform encephalopathy (TSE) and raises concerns related to consumer preference and religious beliefs (67). Moreover, volatile co-solvents in self-microemulsifying formulations can permeate gelatin capsules—both hard and soft—resulting in the precipitation of lipophilic drugs (68). Consequently, HPMC capsules have emerged as a preferred alternative (69).

Storage and Handling

Liquid SMEDDS face practical challenges in handling, stability, and storage, making the development of solid SMEDDS a logical solution (49).

Limited Lymphatic Targeting

Lymphatic delivery has two main advantages over traditional portal absorption: it bypasses pre-systemic hepatic metabolism, enhancing systemic drug levels, and facilitates targeted delivery to lymphatic tissues. However, effective lymphatic transport typically requires drugs with high log P values and triglyceride solubility, and uptake varies by drug. Thus, more accurate predictive models are needed, along with a better understanding of how lipophilicity and lipid solubility affect lymphatic transport (70, 71).

Insufficient In Vitro Models

The development of SMEDDS and other lipid-based formulations is hindered by the lack of reliable in vitro models for evaluation (72). Conventional dissolution tests fail to capture the lipid digestion process necessary for drug release. Although in vitro models that simulate duodenal digestion have been developed (73), they require further validation. Many prototype formulations still need in vivo testing to establish credible in vitro–in vivo correlations (74).

Oxidation and Lipid Polymorphism

Unsaturated fatty acid excipients in SEDDS or SMEDDS are prone to oxidation (75), necessitating the inclusion of lipid-soluble antioxidants in the formulation. Additionally, process controls are essential to prevent polymorphic changes in the lipid matrix caused by thermal softening during production (76).

Table 1: List of recently used oral drugs in SMEDDS formulation

S. No	Drug	Route of Administratio	Potential	Delivery System	Excipients Used	Outcomes Achieved	References
1	Agomelatine	Oral	Antidepressant	Solid-SMEDDS	Capmul MCM, KolliphorEL, PEG 400,	Enhanced drug	Priyankae 2023 (77)
2	Azilsartan medoxo	Oral	In hypertension	Solid-SMEDDS	Soya lecithin comp clove oil, tween20, glycol	Improved dissol	Madanet al (78)
3	Curcumin	Oral	Antidepressant	SMEDDS	Oleicacid, tween 80 glycol	Increased brain p and impro pharmacologica	Manojet al. (79)
4	Ferulic ac	Oral	In insomnia	FA-SMEDDS	Glyceryl triacetate, OP-10and Labrasol, PEG 400	Enhanced hypno	Liuetal., (80)
5	Hydrochlorothiazide (HCTZ)	Oral	In hypertension and edema	Self-micro emulsifying tablet (SMET)	Oleicacid, tween 20, propylene glycol, neusilinUS2,	Enhanced drug	Arpanaet al (81)
6	Licochalcor	Oral	Anti- hyper uricemic activity	SMEDDS	Ethyl oleate, Cremophor, EL 35, n-	Increase solub release rate of lic A	Zhongane 2021 (82)
7	Loratadine and Su	Oral	In pancreatic	SMEDDS	CapmulC8, Tw PEG 40(	Enhanced chemo preventive	Desai et al. (83)

8	Myricitrin	Oral	Anti-inflammatory, hypoglycemia,	SMEDDS	Ethyl oleate, Cremophor EL35, dimethyl carbinol	Enhanced drug r oral bioavail	Namanet al (84)
9	Nilotinib	Oral	In chronic myel leukemia (C	SMEDDS	Capryol90, Tra HP and Twe	Improved solub oral bioavail	Zakkulaet a (85)
10	Phillygenin	Oral	Antioxidant, hypc inhibition of tyrosi and antihypert effects	SMEDDS	Labrafil M1944CS, PEG-400, Cremophor EL	Improved oral a and enhanc bioavailab	Lingzhiet al (86)
11	Raloxifene hydrochloride	Oral	In breast canc osteoporis	Liquid-SMEDDS	Capryol 90 (oil),T Labrasol, PE	Enhanced therapeutic	Ansariet al. (87)
12	Resveratrol	Oral	Anticancer, antiox inflammatory and a	SMEDDS	Isopropyl myristate, Cremophor RH40, PEG 400	Enhanced solubility and oral	Hongwei 2019 (88)
13	Rosuvastatin	Oral	Antihyperlipidemi c	Solid-SMEDDS	Capryol90, KolliphorEL, TranscutolHP	Enhanceme physiochemical a biologic attribute	Suparnaet a (89)
14	Saquinavir	Oral	Antiretroviral	Supersaturated - SMEDDS	Capryol90, Labrasol, propylene glycol, HPMC	Enhanced lymphatic absorption	Kangheee 2020 (90)
15	Zingerone	Oral	Antioxidant, anticancer,	SMEDDS	Ethyl oleate, tween80,	Improved oral bioavailability	Xiaetal., (91)
16	**Commiphora Wightti extract**	Oral	Obesity	SMEDDS	CapryolPropylene GlycolCremophore	Improved oral bioavailability	Singh et al, 2022
17	***Beta vulgaris L.*** l	Oral	hepatoprotective activity	SMEDDS	linseed oil or olive oil, Tw80 and DMSO at two SA/	Improved oral bioavailability	Kassem et al., 2020

Conclusion

SMEDDS are being actively investigated for delivering poorly water-soluble drugs. However, the demand for lipid-based drug delivery systems exceeds the availability of commercially formulated SMEDDS. Most marketed SMEDDS are packaged in soft gelatin capsules, which complicates handling and increases costs. Developing solid SMEDDS can resolve these handling challenges, reduce production expenses, and improve the stability issues associated with liquid formulations. Additionally, these formulations should be designed to suit physiological conditions, ensuring SMEDDS reach their full potential—especially for poorly soluble drugs

Future prospectives

Self-Microemulsifying Drug Delivery Systems (SMEDDS) hold great promise in the pharmaceutical field, offering innovative solutions for poorly water-soluble drugs. Although many formulations exist, commercially available SMEDDS remain limited compared to the demand. The majority of marketed SMEDDS are soft-gelatin capsules, which present handling difficulties and higher costs. Solid SMEDDS, by contrast, can address these issues by improving stability, simplifying handling, and lowering production costs. To fully harness their potential, these systems must also be tailored to physiological conditions, ensuring their safe and effective application—especially for poorly soluble medications.

With continued research and development, SMEDDS could revolutionize drug delivery by enhancing solubility, bioavailability, and enabling targeted therapeutic strategies. Their adaptability supports a diverse range of compounds, from lipophilic drugs to biologics, paving the way for breakthroughs across multiple therapeutic areas. As regulatory frameworks evolve and industry standards mature, the integration of SMEDDS into pharmaceutical pipelines is expected to accelerate. Overall, SMEDDS offer a compelling future in drug development, with the capacity to address unmet medical needs and significantly improve patient outcomes.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgment

The authors would like to thank the Deanship of scientific research at shaqra university for supporting this work.

Funding

The Authors did not receive any funding.

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https://doi.org/10.47552/ijam.v16iS2.6198 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Bergamot unleashed: Exploring the multifaceted traits and healing powers of *Citrus bergamia*

Review Article

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Abstract

In this comprehensive review article, the focus is on Bergamot plant, elaborating on its various properties and applications. This article provides a detailed examination of the botanical aspects, significance, and characteristics of Bergamot plant, including its anatomy and chemistry. Additionally, it delves into the pharmacological activity exhibited by Bergamot plant, highlighting its diverse range of effects such as anti-anxiety, wound healing, neuroprotection, anti-depressant, antiproliferative, antibacterial, anticancer, antioxidant, anti-inflammatory, antinociceptive, anti-mycoplasmal, anti-fungal, and antiallodynic properties. The scientific designation of Bergamot, *Citrus bergamia* Risso, indicates its botanical lineage as a hybrid of Citrus limon L and Citrus aurantium, categorising it under the Rutaceae family and Citrus genus. This plant, is known for its unique combination of volatile and non-volatile fractions, each contributing to its therapeutic potential and aromatic profile. Through an exploration of its pharmacological activity and chemical composition, this article offers a comprehensive overview of the multifaceted nature of Bergamot plant, shedding light on its rich botanical heritage and promising therapeutic applications.

Keywords: Botanical aspects, Volatile, Neuroprotection, Pharmacological Activity, *Citrus bergamia Risso*.

Introduction

Citrus bergamia Risso, an intriguing hybrid of *Citrus limon L.* and *Citrus aurantium*, is technically identified as the bergamot citrus fruit. This unique citrus species is classified under the *Rutaceae* family and the Citrus genus. Easily distinguished by its vibrant greenish-yellow peel, bergamot boasts a notable acidity and emits a subtle yet delightful aroma. The principal derivative of this fruit is the esteemed bergamot chemical compounds, obtained by careful cold pressing or exact steam distillation of the peel area inside the fresh fruit's mesocarp. Due to its versatile nature, the bergamot chemical components, commonly referred to as BEO, plays a pivotal role in the global fragrance industry, particularly in solidifying the aromatic compositions of various perfumes. Beyond its fragrance applications, BEO finds extensive use in the culinary realm, where it imparts its distinct flavor profile to an array of treats, ranging from confectionery to teas and carbonated beverages. Notably, the refreshing and flavorful essence of BEO, along with its concentrated extracts, is often blended into the classic earl grey tea (EGT), enriching this beloved beverage with its invigorating citrus notes (1, 2).

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Fig. 1: Bergamot plant with its fruits

The flavor and quality of EGT are influenced by the tea blend used and the caliber and quantity of bergamot chemical components incorporated. Bergamot chemical components are used in aromatherapy to help with stress-related symptoms, as well as in dermatology, gynaecology, dentistry, and ophthalmology.

Italy is renowned in the global trade community for its high-quality bergamot chemical components, with the harvest season playing a significant role in determining its chemical composition. The volatile and nonvolatile fractions of bergamot chemical components contain compounds such as psoralens, coumarins, and bergamottin, contributing to its unique characteristics. Bergamot, a name that encompasses both the fragrant herbs belonging to the genus Monarda in the Lamiaceae family and the luscious fruit harvested from the bergamot orange tree, Citrus × aurantium. These distinct variations of bergamot, whether derived from plant or borne from citrus, boast a shared characteristic in their enchanting floral aroma. Revered for centuries, bergamot's captivating scent has found its way into the

realms of perfumery and culinary arts alike. The aromatic allure of bergamot infuses fragrances with a bright, uplifting note, adding depth and complexity to a myriad of scents. In the culinary world, the zest of bergamot imparts a refreshing citrusy touch, elevating dishes with its unique flavor profile. Among its varied applications, bergamot stands as a versatile ingredient that inspires creativity and sensory delight. From classic perfumes to delectable dishes, bergamot's essence weaves a thread of sophistication and allure through each creation it touches. Whether in the subtle hint of a perfume or the bold tang of a dish, bergamot's floral notes and citrus undertones beckon the senses, inviting exploration and appreciation of its multifaceted charm. Through its enduring presence in diverse fields, bergamot continues to captivate hearts and tantalize taste buds, a true testament to its timeless appeal and allure (3, 4).

Bergamot herbs, including perennial species indigenous to North America, are aesthetically pleasing as ornamentals and are vital for attracting significant pollinators such as bees, butterflies, and hummingbirds. The leaves of bee balm are flexible and are used to enhance the flavours of numerous beverages, including punches, lemonades, and other pleasant cold drinks. Moreover, lemon bergamot, also known as lemon bee balm (M. citriodora), and wild bergamot (M. fistulosa) provide further possibilities for enhancing beverages and infusing teas with their unique fragrant characteristics.

Shifting the focus to another type of bergamot, the bergamot orange stands out as a prized citrus fruit mainly cultivated in Italy. This fruit is particularly renowned for its association with Earl Grey tea, where the essence of the bergamot orange infuses a unique and captivating flavor into the tea blend. The tree producing this citrus fruit has unique yellow-green, pear-shaped fruits sought after by the flavouring and perfume industries for the valuable essential oil derived from the peel. The essential oil derived from bergamot orange peel enhances culinary dishes and plays a vital role in the fragrance industry, making it a highly desired component in both culinary and perfumery applications.

Key components like linalool, linalyl acetate, terpinene, limonene, and pinene are present in bergamot chemical components, along with aroma chemicals like geraniol, decanal, and limonene oxide that mimic its fragrance. Bergamot's chemical composition is distinguished from other citrus oils by a higher concentration of linalyl acetate and linalool relative to limonene. The ratio of linalyl acetate to linalool, referred to as the "essence degree," profoundly influences the aromatic character of bergamot's chemical constituents. Beyond its aromatic properties, bergamot chemical components exhibit a range of pharmacological effects, including melanogenic, antinociceptive, antioxidant, and antibacterial properties, as well as cytotoxic, wound-healing, anxiolytic, and anti-tumor effects. Additionally, it possesses sedative, calming, and soothing qualities, making it a versatile natural remedy for various conditions such as allergies, fungal infections, and bacterial issues (5, 6).

Pharmacognostical features of bergamot

Bergamot, a citrus fruit, was first introduced in 1818 by Joseph Antoine Risso and Pierre Antoine Poiteau. It is said to be a hybrid of sour orange and lemon. Bergamot trees may reach a height of 12 meters, with an erect, dark grayish-brown trunk and slender, irregular roots. They are known for their resilient, enduring trees that withstand inclement weather. The fruit, classified as a hesperidium or subglobose to pyriform berry, with many glands and a peel that is 3 to 6 mm thick, containing chemical component cavities. The degradation of chlorophyll and an elevation in carotenoid levels result in alterations in rind colouration. The fruit's fragrance originates from aromatic compounds. The word "bergamot" may have derived from the Turkish phrase "beg-a-mudi," which translates to "pears of the prince," due to the resemblance of the pears to that expression (7, 8, 9).

Table 1: Biological classification of bergamot

Sr. no.	Type of classification	Classification
1	Kingdom	Plantae
2	Clade	Tracheophytes
3	Clade	Angiosperms
4	Clade	Eudicots
5	Clade	Asterids
6	Order	Lamiales
7	Family	Lamiaceae
8	Genus	Monarda
9	Species	M. didyma

Chemical constituents and important features

The ISO characterises a chemical component as a product derived from naturally existing raw materials sourced from plants, using techniques such as dry distillation, steam distillation, or mechanical extraction from the epicarp of many plants and fruits. These processes aim to separate any aqueous phases and yield chemical components distinct in their chemical composition and manufacturing techniques. Specifically, Citrus components stand out due to a production method exclusive to them among the three processes employed. This exclusivity facilitates easy differentiation of Citrus components from other chemical components. Furthermore, a notable disparity lies in the compositions of distilled chemical components and pressed cold chemical components. Distilled chemical components predominantly comprise unstable compounds, whereas pressed cold chemical components contain larger chemical compounds and molecules absent in their distilled counterparts (10).

Citrus fruits are multifunctional, containing chemical components, by-products, and fruit juice. Popular varieties include mandarins, lemons, sweet oranges, and grapefruits. Bergamots and bitter oranges, though not widely produced, have higher costs due to their limited production. This economic aspect sheds

https://doi.org/10.47552/ijam.v16iS2.6198 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

light on the pricing disparity between citrus chemical components derived from different fruit types (11).

Anatomy of citrus fruits

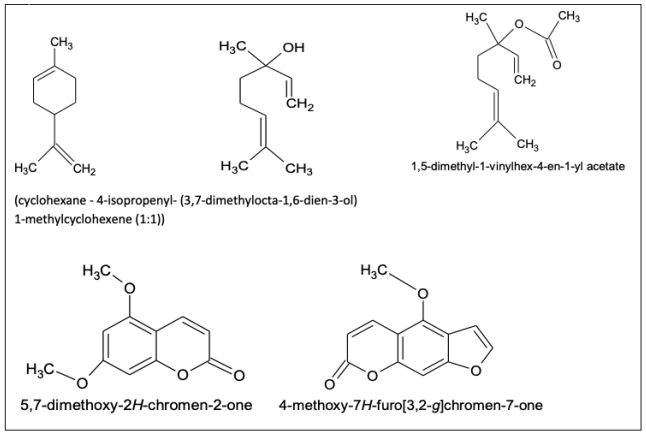
The hesperidium, a citrus berry characterised by a robust outer rind, albedo mesocarp, and flavedo exocarp, is spherical in shape, including lacunae, essential oil-filled cavities, and dense cellular structures. Their sizes range from 0.4 to 0.6 mm and are often categorised into primary, secondary, and tertiary cavities. The optimal technique for extracting citrus chemical constituents is attributed to the surface dispersion of oil glands, cold pressing, or mechanical processing (12).

Chemical constituents of bergamot

Bergamot Chemical components is a unique citrus chemical component with higher levels of oxygenated terpenes like linalyl acetate and linalool, contrasting with the high limonene content found in other oils (13, 14).

Bergamot Chemical components, classified as volatile or non-volatile, are susceptible to fraudulent activities due to their high market value and complexity, making detection challenging. Compared to other citrus chemical constituents, the primary components of Bergamot—limonene, linalool, pinene, linalyl acetate, and terpinene—constitute less than 90% of the total composition, underscoring the complex and varied nature of these valued chemical constituents (15, 16, 17). The chemical components of bergamot shown in Fig. 2 are as follows: 3,5-dimethyl-1-vinylhex-4-en-1-yl acetate, 3,7-dimethoxy-2H-chromen-2-one, 4-methoxy-7H-furo[3,2-g]chromen-7-one, and 3,7-dimethoxy-2H-chromen-2-one.

Figure 2: Major chemical composition of bergamot plant



Fraction of volatility

93–96% of BEO's total content consists of volatile components, mostly including linalool, linalyl acetate, and limonene. Despite these prevalent compounds, the scientific literature reveals a comprehensive array of over 100 substances that have been thoroughly discovered and recorded. BEO has a much-reduced hydrocarbon concentration relative to its citrus equivalents, however prominent monoterpene hydrocarbons such as α-pinene (4–11%), limonene (25–55%), and γ-terpinene (5–11%) dominate its volatile composition. Furthermore, the presence of sesquiterpene hydrocarbons such as -bisabolene and caryophyllene, along with notable elements like (E)-bergamotene, adds depth to the aromatic bouquet of BEO (18). Noteworthy distinctions from other citrus chemical components emerge when considering the oxygenated derivatives of mono and sesquiterpene hydrocarbons, which can account for over Half of the volatile compound, far surpassing the levels found in most extra citrus EOs which tend to average between 1 to 6%. Among the array of compounds, terpenic alcohols and esters emerge as prominent players, with linalool standing as the most significant monoterpene alcohol due to its prevalent presence ranging from 2–20%. Additionally, the rare sesquiterpene alcohol, bisabolol, known to be exclusive to Bergamot, can be detected in minute quantities, adding a unique flair to the overall composition (19, 20, 21).

Fraction of non-volatile content

In addition to inert substances like as pigments and waxes, which constitute 4–7% of the overall essential oil content, this particular fraction mostly comprises oxygen-containing heterocyclic compounds. The main components within this fraction are coumarins, psoralens, and polymethoxyflavones such as tetra-O-methyl scutellarin and sinensetin. Specifically, it is dominated by four key compounds: the psoralens bergapten and bergamottin, along with the coumarins geranyloxy-7-methoxycoumarin and citropten, which are commonly associated with Candida bergamia (22, 23, 24). Genuine essential oils include many psoralens, including akangelicin, bergaptol, epoxybergamottin, isoimperatorin, biakangelicol, oxypeucedanin, and oxypeucedanin hydrate. This particular fraction showcases a diverse array of compounds that collectively contribute to the characteristic composition of chemical components (25, 26, 27, 28).

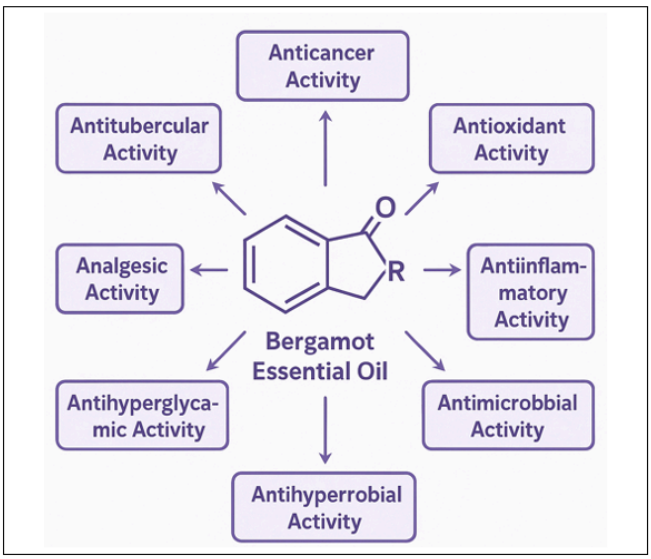
Pharmacological activities

The chemical components of bergamot are extensively used in the pharmacological, culinary, and cosmetics sectors because to their melanogenic qualities. It is used in suntan products and supplementary medicine to alleviate neuropathic and nociceptive pain. BEO has shown cytotoxicity against human neuroblastoma cells, inhibiting their proliferation by more than 70%. The bioactive chemicals 5-geranyloxy-7-methoxycoumarin and bergamottin are accountable for these actions. BEO and d-limonene have shown the ability to alter autophagic pathways in SH-SY5Y cells, with lipidomal BEO exhibiting enhanced anticancer activity against these cells (29, 30, 31).

The agreeable scent of bergamot has shown efficacy in reducing behavioural sadness and stress-induced anxiety in rats subjected to prolonged stress. It also exhibits broad antifungal activity against various bacteria and fungi, including Fusarium solani, Penicillium italicum, and Trichophyton, Microsporum, and Epidermophyton dermatophytes. The antibacterial

and antifungal properties are believed to be facilitated by enhanced generation of reactive oxygen species (ROS) in human polymorphonuclear leukocytes. The powerful anti-mycoplasmal actions of bergamot chemical components have been identified against Mycoplasma fermentans, Mycoplasma hominis, and Mycoplasma pneumoniae. Overall, BEO's potential benefits in various industries make it a valuable addition to the natural remedies and treatments for various health conditions (32, 33, 34). Fig. 3. Biological activities of primary chemical components of bergamot, emphasizing their contributions to antioxidant, anti-inflammatory, antibacterial, cardiovascular, and neuroprotective properties.

Figure 3: Biological activities of chemical components present in bergamot



Neuroprotective activity

In developed countries, it is common to utilize a botanical extract known as BEO to address various mood-related issues and alleviate mild symptoms of stress-related conditions such as depression, anxiety, behavioral abnormalities linked to dementia, and chronic pain. This natural remedy is frequently employed due to its potential therapeutic benefits. BEO comprises multiple constituents, each of which plays a crucial role in its efficacy. Upon delivery via many routes, the constituents of BEO may traverse the blood-brain barrier, therefore accessing the central nervous system. This unique characteristic of BEO enhances its effectiveness in targeting the underlying causes of these conditions and providing relief to individuals suffering from these health concerns. The process through which the constituents of BEO cross the blood-brain barrier underscores the intricate mechanisms by which this botanical extract exerts its positive effects on mental health and overall well-being, making it a valuable option in the treatment and management of a diverse range of psychological and physiological symptoms (35, 36, 37).

Behavioral activity

BEO, has shown a consistent impact on the rat central nervous system when supplied at escalating doses. It induces both hypnotic and stimulating effects on EEG spectra, resulting in enhanced movement and behaviour.

Following the administration of 250 l/kg of the Plant complex, rats demonstrated behavioural arousal, marked by stereotypical movements and heightened energy levels in the theta and alpha frequency bands in the cortex, as well as beta and alpha rhythms in the hippocampus. The substantial dosage of BEO elicited behavioural excitement and EEG desynchronisation, accompanied by a significant increase in cortical theta band amplitudes and hippocampus beta band strength. Recent findings indicate that BEO selectively influences essential synaptic neurotransmission pathways (38, 39, 40, 41).

Effect on synapses' regular transmission

Research using Microdialysis indicates that in freely moving rats, BEO enhances the levels of extracellular amino acids, including glycine, taurine, and aspartate, especially within the hippocampus area. Interestingly, this effect seems to be modulated by the absence of Ca2+ in the cerebrospinal fluid as studies have shown that in experiments where calcium ions are lacking, the ability of BEO to increase these particular amino acids is hindered. This highlights the intricate interplay between BEO and the presence of calcium ions in influencing amino acid levels in the hippocampus of rats (42, 43).

Neuroprotection from excitotoxicity

Under ischaemic circumstances, anoxic depolarisation causes the loss of excitatory amino acids from nerve terminals, impairing the essential driving force for the proper operation of membrane transporters. This disturbance induces the production of reactive oxygen species (ROS), leading to the aberrant functioning of glutamate transporters and elevated levels of excitatory amino acids at synapses. As a result, neurones sustain damage from the ischaemic insult. Intraperitoneal administration of BEO in rats has shown encouraging outcomes in mitigating brain damage resulting from localised cerebral ischaemia in a dose-dependent fashion. Microdialysis tests have shown that BEO significantly reduces excitatory amino acid outflow, opposing the usual rise in efflux seen in the brain area quickly after middle cerebral artery closure (44).

Anxiolytic activity

This research examined the anxiolytic and sedative-like effects of BEO in rats. The results unequivocally indicated that in animal behavioural evaluations when DZP could not be directly contrasted, BEO had anxiolytic and sedative effects. The rats were intraperitoneally injected with BEO thirty minutes prior to each behavioral test, highlighting the timing of administration. Overall, the results of this particular research point towards a distinction in how BEO interacts in terms of its calming and anxiolytic properties when compared to DZP, shedding light on potential differences in their pharmacological effects (45).

Antidepressant activity

The inquiry into the antidepressant effects of olfactory stimulation using diverse odorants was rigorously executed using the validated forced swimming test, a recognised method for evaluating antidepressant qualities. In the experiment, the addition of a lemon aroma significantly reduced the overall length of immobility while concurrently amplifying the decrease in total motionlessness caused by imipramine treatment. The observed synergistic impact of imipramine and lemon fragrance should not be prematurely ascribed to the potential interference of lemon scent with the metabolism of imipramine. The lemon smell was seen to reduce locomotor activity in open space, suggesting that its mechanisms of action resemble those of conventional antidepressants rather than psychostimulants. Significantly, the primary component of lemon scent, citral, demonstrates characteristics that closely mirror those inherent in lemon odour itself (45, 46).

Anti-nociceptive activity

The research examined the effects of BEO, a chemical abundant in linalyl acetate and linalool, its principal volatile constituents, on the capsaicin test. In the capsaicin test, after an intraplantar injection of 1.6 g of capsaicin, the paw that received the injection had a transient reflexive reaction characterised by licking and biting. A significant finding was observed: a dose-dependent reduction in nociceptive behavioural response induced by capsaicin occurred after the intraplantar administration of BEO. The research only included male ddY (SD) mice weighing between 22 and 26 g, obtained from the Shizuoka Laboratory Centre in Japan. The administration of artificial or saline cerebrospinal fluid (CSF) did not provide statistically significant effects on capsaicin-induced pain when administered intraplantarly at dosages similar to those of BEO (5 g) and linalool (1.25 g). Notably, the concurrent administration of morphine, benzophenone, and linalool resulted in a significantly enhanced effect on the nociceptive response elicited by capsaicin (46).

Wound healing activity

Traditional medicine use BEO (subsp. bergamia C. aurantium L) as an anthelmintic and antibacterial agent, as well as to facilitate wound healing. Research on BEO's influence on immunity is few; nonetheless, it seems to have significant antibacterial properties. Research investigated the effect of BEO on the formation of reactive oxygen species (ROS) by human polymorphonuclear leukocytes (PMN) and the role of Ca2+ in functional responses. The results indicate that BEO may enhance intracellular ROS generation in human PMN, corroborating its inherent proinflammatory capacity and facilitating infection combat and tissue repair (47).

Antiproliferative activity

The research used the human neuroblastoma cell line SH-SY5Y to assess the cytotoxic effects of blue-eyed octahedral structures (BEOs). The preliminary test indicated that the introduction of coloured BEOs to SH-SY5Y cells considerably increased the proportion of cell mortality. The cell count assay and MTT assay were used to quantify cell proliferation. The pharmacotoxicological profile indicates that 5-geranyloxy-7-methoxycoumarin and bergamottin may significantly contribute to the antiproliferative effects of BEO (47).

Neuroprotection against ischemic conditions

This research systematically evaluated the influence of BEO on the brains of rats experiencing chronic localised cerebral ischaemia. After a 24-hour middle cerebral artery occlusion (MCAo) period, various doses of BEO (from 0.1 to 0.5 mL/kg, except 1 mL/kg) were delivered intraperitoneally, indicating a potential decrease in infarct size. The therapeutic dosage of 0.5 mL/kg demonstrated considerable efficiency in reducing cell death throughout the brain, particularly in the striatum and motor cortex, as seen by tissue slice staining with TTC. Results from Little Dialysis experiments also indicated that BEO at 0.5 mL/kg significantly reduced the efflux of excitatory amino acids, including glutamate, in the frontal parietal cortex, while without altering baseline amino acid concentrations. Furthermore, during 24 hours of continuous MCAo, there was a significant elevation in the phosphorylation and activation of Akt, a pro-survival kinase, as shown by Western blotting experiments. BEO demonstrated an elevated degree of GSK-3 phosphorylation, a significant kinase whose activity is inhibited by Akt phosphorylation (48, 49).

Anti-inflammatory and antioxidant activity

The research examined the anti-inflammatory effects of D-limonene in a rat model of ulcerative colitis (UC). The rats received several treatments, with D-limonene markedly decreasing disease activity and intestinal mucosal injury. The therapy elevated antioxidant protein levels in UC rats and regulated the expression of MMP2 and MMP9 mRNA in the UC rat model, suggesting possible antioxidant and anti-inflammatory capabilities (49).

Antibacterial and antifungal activity

The research revealed that bergamot oil had significant efficacy, with linalool identified as the most efficient antibacterial agent. The in vitro trials demonstrated increased susceptibility of both gram-positive and gram-negative bacteria to these compounds. The vapour of linalool and bergamot oils significantly inhibited E. coli O157 and Campylobacter jejuni. This study highlighted the significant effectiveness of Bergamot Chemical components (BEO) against several clinical isolates from diverse pathogenic dermatophytes. The research included ninety-two isolates from seven unique types of dermatophytes. Twelve isolates were obtained from the clinical isolate collection at the Mycology Section of the Catholic University Medical Centre. This collection included specimens of Epidermophyton floccosum, Trichophyton rubrum, Trichophyton interdigitale, Trichophyton

tonsurans, Microsporum canis, and Microsporum gypseum (50, 51).

Major components of bergamot

Limonene, a monoterpene hydrocarbon, has anxiolytic properties in male Swiss albino mice. The EPM test revealed substantial alterations in parameters after the inhalation of 0.5% and 1.0% (+)-limonene. Mice treated with limonene exhibited mechanical hyperalgesia resulting from spared nerve injury (SNI), which may be alleviated by limonene. Intraperitoneal injection of limonene enhanced the survival rate of mice experiencing provoked seizures and postponed convulsions. It also inhibited ischemia-related cerebral damage in stroke-prone animals (51).

Linalool, a monoterpene alcohol, has anticonvulsant effects due to NMDA receptor antagonistic activity and decreased potassium-stimulated glutate release in cortical synaptosomes. It exhibits proliferative and anti-inflammatory properties. The antinociceptive properties of linalyl acetate and linalool are dependent on the quantity of linalool it contains. Behavioral testing showed that a local linalool intraplantar injection decreased pain perception in mice with hypersensitive neuropathy caused by partial sciatic nerve ligation (51).

Linalool may assist in alleviating sadness and anxiety. Additional study is required to establish techniques for antiepileptic pharmacotherapy (51).

Toxicity assessment of bergamot oil

The European Medicines Agency indicates that significant concerns about BEO toxicity pertain to its melanogenic and photosensitive constituents. These traits are historically linked to the presence of furocoumarins, particularly psoralens like bergapten, in BEO. Notably, personal characteristics such as age, gender, and UV sensitivity seem to have little influence on skin response to BEO. The emphasis transitions to certain components such as the ethanol and psoralens concentration in the formulation, the degree of skin moisture, and the individual's inherent skin pigmentation. This pattern may indicate a probable decrease in reported instances for several causes. Alternatively, it may also indicate that the market is progressively providing safer options via psoralen-free bergamot derivatives. This transition to safer alternatives indicates a continuous endeavour to reduce possible hazards linked to the conventional usage of BEO, signifying a constructive progression in product safety within the industry (51, 52, 53).

The International Fragrance Association (IFRA) recommends capping the BEO concentration in skincare products at 0.4%, in addition to restricting psoralen levels. Light-induced dermal responses may manifest 2 to 72 hours post-BEO treatment, mimicking bullous dermatitis. The predominant minor chemicals and principal components are safe for systemic use. BEO should be contraindicated in breastfeeding or pregnant individuals, those with allergy diathesis, youngsters, and the elderly owing to insufficient safety evidence. Prolonged and excessive use of BEO may induce neurological symptoms and photosensitivity of the skin. The safety profile of BEO is under evaluation; nevertheless, using formulations devoid of psoralen and limiting intake to brief durations may alleviate significant adverse effects (54).

Table 2. Citrus bergamia (bergamot) safety guidelines, revised in light of suggestions from the IFRA and the EMA, including a review of toxicity data, an explanation of contraindications, and suggested dosage limits for both topical and oral use. In especially for those who are photosensitive or otherwise susceptible, these recommendations stress the need of managing the risk of phototoxicity, establishing safe dose levels, and identifying and avoiding contraindications.

Table 2: Updated Safety Guidelines for Citrus bergamia (Bergamot) Based on IFRA and EMA Recommendations

Parameter	Details	Source/Guideline
Phototoxicity	Bergamot essential oil contains bergapten (5-MOP), a furanocoumarin known to cause phototoxic reactions upon UV exposure.	IFRA, EMA
Recommended Usage Limit (Leave-on Products)	≤ 0.4% (maximum concentration in finished leave-on cosmetic products to avoid phototoxicity)	IFRA Amendment 49 (2020)
Recommended Usage Limit (Rinse-off Products)	≤ 2.0% (lower risk due to reduced skin contact time)	IFRA Amendment 49 (2020)
Toxicological Concerns	High doses linked to photosensitivity, neurotoxicity (in rare cases of excessive intake), and hepatotoxicity (with prolonged or excessive use of unmodified oil)	EMA, EFSA
Contraindications	- Avoid during pregnancy and lactation (due to lack of safety data) - Not recommended for individuals with known photosensitivity disorders - Avoid concurrent use with other photosensitizing agents (e.g., tetracyclines)	EMA monograph on herbal medicinal products
Systemic Exposure Limit (SEL)	15 mg/kg/day (based on bergapten content)	IFRA Safety Assessment Reports
Sensitization Potential	Low to moderate; patch testing advised for sensitive individuals	IFRA, European SCCS

Drug Interactions	Potential inhibition of CYP450 enzymes (particularly CYP3A4), which may alter metabolism of medications like statins, benzodiazepines, and calcium channel blockers	EMA, PubChem Toxicology Data
Recommended Daily Oral Dose (Standardized Extract)	≤ 1000 mg/day of flavonoid-rich extract (under medical supervision)	EMA Assessment Reports, Clinical Guidelines
Children and Infants	Use not recommended due to insufficient safety data	EMA, IFRA

Conclusion

The distinctiveness of Bergamot Chemical components (BEO) is in its composition among citrus chemical constituents. A notable characteristic of BEO is its potential to contain elevated concentrations of limonene, ranging from 80% to 95%. Limonene, representing around 4% to 7% of the entire chemical composition, is a significant percentage mostly consisting of oxygen-rich heterocyclic molecules. The chemicals include coumarins, psoralens, and minimal quantities of polymethoxy flavones, in addition to inert materials such as pigments and waxes. BEO demonstrates varied biological actions that significantly impact many body systems, particularly the central neurological and cardiovascular systems. Research has validated its antibacterial, anti-inflammatory, antiproliferative, and analgesic characteristics. Although clinical investigations have mostly examined the benefits of aromatherapy using BEO, these results indicate possible future therapeutic uses of this chemical component. These investigations have specifically aimed at modulating stress responses and enhancing anxiolytic effects with aromatherapy treatments utilising BEO. The findings suggest that aromatherapy therapies using BEO may relieve symptoms of stress and anxiety, indicating a therapeutic route worthy of future exploration.

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https://doi.org/10.47552/ijam.v16iS2.6227 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Indian Spices: An Insightful Review on Reported Antipsychotic, Antidepression, Neuroprotective and Anti-Anxiety Activities

Review Article

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Abstract

The potential of traditional medicinal herbs to cure neurodegenerative diseases like Alzheimer's disease (AD) has attracted more and more attention from researchers. Notably, a number of plants have long been used to enhance memory and cognitive performance, including *Crocus sativus, Nigella sativa, Coriandrum sativum, Ferula assafoetida, Thymus vulgaris, Zataria multiflora*, and *Curcuma longa*. By lowering oxidative stress, increasing antioxidant levels, and blocking acetylcholinesterase activity, their bioactive substances—carotenoids, monoterpenes, and polyphenols—have neuroprotective benefits. Additionally, by reducing levels of pro-inflammatory cytokines like IL-6, IL-1β, TNF-α, and total nitrite, these herbs control neuroinflammation. Taken together, these results provide credence to the medicinal potential of these herbs and their active ingredients in the treatment of depression, anxiety, and AD. Traditional medicinal potential in these findings, when combined, support the therapeutic potential of these plants and their active components in the treatment of AD, anxiety, and depression.

Keywords: Anxiety, Depression, Inflammation, Spices, Anti-Alzheimer activity.

Introduction

Cognitive decline and sensory impairment in neurodegenerative diseases like Alzheimer's, Parkinson's, and multiple sclerosis are brought on by the slow loss of neurons (1). Researchers have recently connected these diseases to intricate social and economic variables (2, 3). Anti-inflammatory drugs have also been suggested to slow the progression of neurodegenerative diseases like Alzheimer's. Numerous studies have connected non-steroidal anti-inflammatory drugs (NSAIDs) to a lower risk of developing Alzheimer's disease (4,5). Parkinson's disease (PD) is characterized by neuronal degeneration that is influenced by oxidative stress, inflammation, apoptosis, mitochondrial dysfunction, and genetic factors (6). In AD (7), cholinergic neurons may be destroyed by excessive lipid peroxidation, while in PD, dopaminergic neurons may be destroyed by oxidative stress. (8) The brain contains a variety of antioxidants, both enzymatic (like superoxide dismutase, or SOD) and non-enzymatic (100% thiol groups) (9). The polyunsaturated fatty acids found in the CNS make it more vulnerable to peroxidation processes (10). Because it contains very little antioxidant activity compared to other tissues, brain tissue is particularly vulnerable to oxidative damage. (11) The leaves, stems, roots, flowers, fruits, and seeds of plants were employed as a kind of supplementary and alternative medicine in ancient practices. Herbal extracts with neuroprotective properties have been discovered. These include resveratrol, curcumin, ginsenoside, polyphenols, triptolide, and others. (12) Phytochemicals found in herbs, such as flavonoids, alkaloids, and isoprenoids, are complex active components. Because of this, it is sometimes hard to tell which part of the plant is responsible for the majority of its biological effects (13,14). This review aimed to clarify the advantages of many medicinal plants that have been used historically for induced neurotoxicity as food additives, spices, and other medicinal purposes.

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Methods

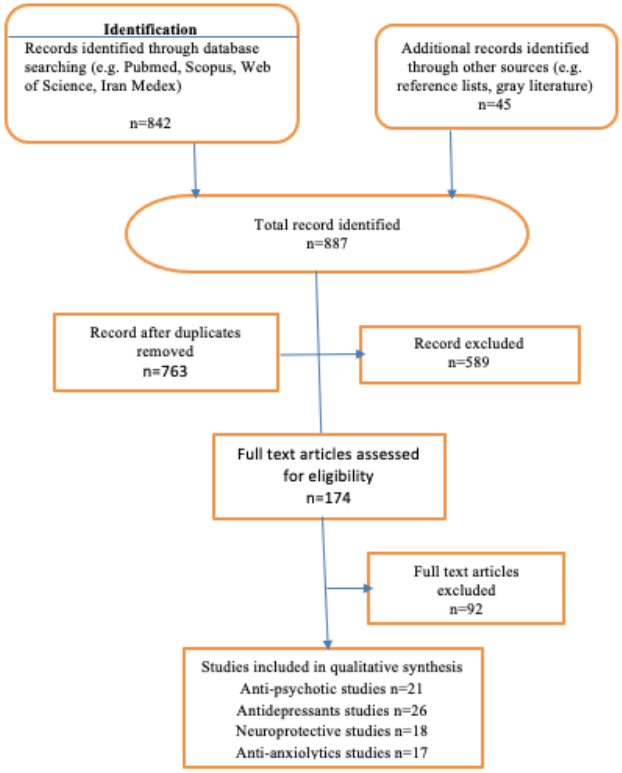
To gather the information for this study, we searched databases such as PubMed, Web of Science, Scopus, and IranMedex through the end of May 2025. All forms of research (in vitro, animal, review, and clinical) included neurotransmitter release, behavioral changes, oxidant/antioxidant parameters, and pro-inflammatory cytokines as outcomes. Both previously unpublished data and letters to the editor were excluded.

Neuroprotective effects of Indian Spices

An increasing worldwide health burden is attributed to psychiatric and neurodegenerative diseases like Alzheimer's disease (AD), depression, and anxiety. The World Health Organisation (2023) estimates that AD accounts for 60–70% of dementia diagnoses, which affect approximately 55 million individuals globally. By 2050, this number is expected to increase as a result of population ageing. Present-day pharmaceutical

therapies, such as cholinesterase inhibitors (donepezil, rivastigmine) and NMDA receptor antagonists (memantine), Tricyclic antidepressants or SSRI (Selective Serotonin Reuptake inhibitors) provide only a limited alleviation of symptoms and are frequently linked to side effects such nausea, vertigo, and sleeplessness. Similar hazards include reliance, withdrawal symptoms, and diminished effectiveness over time associated with many antidepressants and anxiolytics.

Figure 1: PRISMA style flow diagram showing inclusion/exclusion criterion



These drawbacks highlight the critical need for adjunct therapies or safer, more accessible alternatives. In this regard, chemicals originating from plants, especially from culinary spices, have drawn attention from researchers because of their diverse pharmacological profiles, which include neuroprotective, anti-inflammatory, and antioxidant qualities. Bioactive chemicals found in Indian spices including Crocus sativus, Nigella sativa, and Coriandrum sativum etc., alter neurotransmitter systems, lower oxidative stress, and affect neuroinflammatory indicators. Nevertheless, these therapies continue to be underutilised in standard medical protocols despite encouraging preclinical and early clinical findings, indicating a substantial gap in translational research and therapeutic use.

Crocus sativus

Grown commercially in a number of nations, saffron, or *Crocus sativus* L. (C. sativus), is a flowering plant belonging to the Iridaceae family and Crocoideae superfamily (15). The dried, dark-red stigma and a small portion of the style, which is frequently yellow, are the reasons *C. sativus* is harvested. In many regions of the world, it is primarily used as a natural medication. (16). Saffron contains more than 150 different compounds, including water, lipids, carbohydrates, polypeptides, minerals, and vitamins. The active biological components in saffron are called crocins, which are a class of crocetin glycosides. Crocins are water-soluble, red carotenoids. The four main bioactive substances found in saffron are crocin, crocetin, picrocrocin, and safranal. The bitter-tasting substance picrocrocin is also found in saffron (17).

Medicinal properties of *C. sativus*

Traditional Iranian medicine uses *C. sativus* to treat brain disorders. Recently, parts of the *C. sativus* plant have been used to relax smooth muscle and treat certain neurological conditions (18–20). Studies on humans and animals have demonstrated the anti-Alzheimer's and anti-convulsant properties of saffron extract (18). The effects of *C. sativus* on brain neurotransmitter levels and its interaction with the opioid system were assessed, as was clinical trial research on its efficacy in treating mild to moderate depression (18). The main antioxidant in *C. sativus*, crocin, is what gives the plant its potent antioxidant qualities (21, 22). While pretreatment with *C. sativus* extract (100 mg/kg, p.o.) reduces glutamate and aspartate concentrations as well as superoxide dismutase (SOD), catalase, and kinase (K-ATPase) activities, middle cerebral artery occlusion (MCAO) causes cerebral ischemia in rats. (23). Additionally, mice's neurotoxicity from aluminum chloride was lessened by *C. sativus* extract (200 mg/kg) and honey syrup (given over a 45-day period) (24).

When used to treat mild-to-moderate Alzheimer's disease in people aged 55 and over, saffron extract (30 mg/day) was just as effective as donepezil. Its side effects, with the exception of vomiting, were as common as those of donepezil (26). Similarly, 46 individuals with mild to severe AD showed improved cognitive abilities after receiving saffron for 16 weeks (26). In treating mild to severe depression, the effects of 30 mg of saffron extract per day for six weeks were comparable to those of 100 mg of imipramine and fluoxetine (27). In a double-blind study, the efficacy of fluoxetine (30 mg/day) and hydro-alcoholic extract of C. sativus (40 and 80 mg) was assessed over a six-week period. The results showed that fluoxetine (30 mg/day) and *C. sativus* (80 mg) together were more effective than either medication alone in treating mild to moderate depression (28). Around the world, dried saffron stigma (Crocus sativus) is used for both medical and culinary uses. Colour, taste, and aroma are all attributed to the most potent biological constituents, crocetin, crocin, picrocrocin, and safranal, respectively. These are essential to the central nervous system, which is linked to sadness and anxiety. In addition to being neuroprotective and anxiolytic, these bioactive substances can help people with memory and learning disabilities. Physicians most frequently prescribe tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and selective serotonin

noradrenaline reuptake inhibitors (SSNRIs) as antidepressants. (296)

Nigella sativa

Nigella sativa L. (*N. sativa*) is an annual herbaceous plant belonging to the Ranunculaceae family that grows widely throughout the Mediterranean region, Western Asia, the Middle East, and Eastern Europe. N. sativa seeds are a common seasoning for Persian bread, pickles, sauces, and salads. They are nutty, savory, and slightly bitter (29). In addition to other substances, *N. sativa* seeds contain oil, protein, carbs, and fiber. In its fixed oil, *N. sativa* contains linoleic acid, oleic acid, palmitic acid, arachidic acid, eicosadienoic acid, stearic acid, and myristic acid (30). Among the several phenolic chemicals found in *N. sativa* seeds, p-cymene accounts for 37.3%, followed by Thymoquinone (TQ) at 13.7%, carvacrol at 11.7%, and thymol at 0.3%.(29,31,32)

Medicinal properties of *N. sativa*

The medicinal plant *N. sativa* is well known for its potent antioxidant properties. According to a study, *N. sativa* decreased kidney damage by 33% (34). Consuming *N. sativa* seeds may significantly lessen the spatial learning deficits caused by chronic cerebral hypoperfusion in rats. (35) *N. sativa* also helped restore memory and learning that had been hampered by scopolamine by reducing oxidative stress and AChE activity in the rats' brains (36). Because of its antioxidant properties, *N. sativa* oil was used to lower serum levels of IL-10, MDA, and NO in patients with rheumatoid arthritis (RA). In RA patients, *N. sativa* also improved anti-inflammatory responses and reduced oxidative stress (37). In another study, forty healthy subjects were administered 500 mg capsules of *N. sativa* or a placebo twice a day for nine weeks (38). When compared to a placebo group, *N. sativa* enhanced memory, focus, and overall mental acuity. After four weeks, 500 mg of N. sativa decreased anxiety, stabilized mood, and altered cognition in the human model (39). The potential neuroprotective effects of N. sativa and one of its primary constituents, thymoquinone (TQ), on neurological disorders such as Alzheimer's disease, epilepsy, and neurotoxicity have been investigated (40). A pilot polysomnography study by Shyam Das et al. found that black cumin oil, which is high in thymoquinone, enhances sleep quality and reduces stress and anxiety in healthy participants who have sleep issues. (297)

Coriandrum sativum

The annual plant Coriandrum sativum L., commonly referred to as coriander, belongs to the Apiaceae family (41). This plant is commonly known as Geshniz in Persian. Although C. sativum originated in the Mediterranean, it is now widely grown throughout the world (42, 43). While oil extracted from fresh herbs is rich in aliphatic aldehydes (mostly C10-C16 aldehydes) with a fetidlike scent, oil extracted from coriander fruit is rich in linalool and other oxygenated monoterpenes and monoterpene hydrocarbons (44,45). Coriander may be a good source of lipids (petroselinic acid) and essential oils (EO), both of which are important for brain development. The majority of coriander's essential oil is composed of linalool, linoleic acid, and linolenic acid (46). Although coriander seed oil contains 20% hydrocarbons and 60–70% linalool, the composition of the herb oil differs significantly from that of the seed oil (47).

Medicinal properties of C. sativum

Folk medicine has long utilized *Coriandrum sativum* (*C. sativum*) as a digestive aid. Because of its antibacterial and antirheumatoid qualities, C. sativum seed extract was utilized in cosmetics such as shampoos and lotions (48). In traditional Iranian medicine, C. sativum has been used to treat insomnia (49, 50). Some people have found that taking a single dosage of crushed plant seeds or an extract of fresh leaves before bed helps them relax and fall asleep. (49) *C. sativum* seed has been shown to have similar benefits in various traditional treatments. (51) Increases in open-arm duration and open-arm percentage entries suggested an anxiolytic effect from *C. sativum* leaf extract (200 mg/kg). (52) When C. sativum fruit extract was given, both the duration and the number of entrances into the open arms increased. Less locomotion activity and less frequent rearing were observed in groups administered 200 mg/kg (i.p.) of the extract. At doses of 100 and 200 mg/kg, C. sativum extract also extended the amount of time spent socializing. (53) Using both aqueous (0.5 g/kg, i.p.) and ethenolic (3.5 and 5 g/kg, i.p.) extracts, the anticonvulsant effect of coriander seeds was examined in the pentylenetetrazole (PTZ) and maximum electroshock seizure models. In the maximum electroshock test, these extracts showed significant anticonvulsant action, reducing the duration of tonic-clonic seizures. Furthermore, phenobarbital-induced clonic convulsion onset latencies were prolonged by both extracts, but notably by ethenolic extract (54). Swati Sahoo explored that CSE treatment brought monoamines and GABA levels back to their baseline levels and enhanced exploratory activity in animal models of anxiety. In the hippocampal area, CSE also decreased excitotoxic glutamate levels. (298)

Ferula assafoetida

Harvested from the taproot or rhizome of a living plant, Asafoetida (*F. assafoetida L.*) belongs to the Apiaceae family. Gum-resin, or *F. asafoetida*, is referred to by the Persian terms "Anghouzeh," "Khorakoma," and "Anguzakoma." (55). It has long been used as a culinary spice and medicinal herb in Nepal and India (55). E-1-propyl sec-butyl disulfide is one of the primary components. After analysis, it was discovered that hydrodistilled oil contained between 25 and 56 distinct components. Germacrene B (7.8%) and e-1-propenyl sec-butyl disulfide (40.0%) are the main constituents of F. assafoetida (56).

Medicinal properties of *F. assafoetida*

F. asafoetida (Apiaceae) is considered by researchers due to its nutritional and pharmacological

properties. The roots, young stems, and leaves of the plant are all considered vegetables. The plant's roots are used as an antipyretic, and its leaves have diaphoretic, carminative, and anthelmintic properties (57). In traditional medicine, *F. asafoetida* has been used to treat a variety of ailments, including influenza, intestinal parasites, sluggish digestion, stomachaches, flatulence, asthma, and epilepsy (58). The oleo-gum resin of *F. asafoetida* has been linked to a wide range of medicinal effects, such as sedative, expectorant, analgesic, carminative, stimulant, antiperiodic, ant-diabetic, antispasmodic, emmenagogue, vermifuge, laxative, anti-inflammatory, contraceptive, and anti-epileptic (59). Research has been done on muscarinic receptors in the tracheal smooth muscle of guinea pigs as well as possible pathways for the functional antagonistic effects of F. asafoetida. Research has been done on potential mechanisms for F. assafoetida's muscle-relaxing effects (60,61,62). According to pharmacological and biological studies, Ferula asafoetida's ole-gum-resin has antiviral, antifungal, antioxidant, anti-diabetic, molluscicidal, antispasmodic, and antihypertensive qualities. A study assessing the acute and sub-chronic toxicity of F. asafoetida found no increase in mortality or detectable toxicological signs in rats given oral administration of 500 mg/kg or repeated doses of 250 mg/kg over a 28-day period (63). Oleo gum resin of F. asafoetida has been demonstrated to decrease lymphocyte infiltration in the neuropathic tissue in mice and to encourage regeneration and re-myelination, in addition to its well-known neuroprotective and nerve-stimulative effects in peripheral neuropathy (64)

Additionally, it has been demonstrated that *F. asafoetida* resin inhibits monoamine oxidase B (MAO-B), which suggests that it could be helpful in treating neurodegenerative diseases like Parkinson's and Alzheimer's (65). *F. asafoetida* has been shown to have acetylcholinesterase (AChE) inhibitory effects on snails' neurological systems both in vitro and in vivo. Researchers think that the herb F. asafoetida may improve memory because it inhibits AChE in the rat brain (66). The plant extract improved memory performance in a dose-dependent manner in behavioral models such as the raised plus maze (67). In a passive avoidance test, the extract enhanced memory at a higher dosage (400 mg) but not at a lower dose (200 mg). Other behavioral paradigm rodents treated with PTZ and amygdala-activated rodents demonstrate the strong anticonvulsant effect of *F. asafoetida* extract. When researchers compared two doses of F. asafoetida (50 and 100 mg/kg), they discovered that the higher dose had a stronger anticonvulsant effect (59). Research on FA's antidepressant effects indicates that it may work in a number of ways. These include the control of monoamine and non-monoamine neurotransmitter levels, the suppression of neuroinflammation and hyperfunction of the hypothalamic-pituitary-adrenal axis, the stimulation of hippocampus neurogenesis and the upregulation of brain-derived neurotrophic factor, neuroprotection (inhibition of oxidative stress, neuroinflammation, mitochondrial dysfunction, and apoptosis), and the downregulation of oxidative stress. (299)

Thymus vulgaris

Thymus vulgaris (*T. vulgaris*) is a plant of the intensely scented Lamiaceae family. About 38 distinct species make up this plant genus, all of which may be found in subtropical regions. (68) TV's primary constituents are the phenols carvacrol (15%) and thymol (40%). Phenol levels were lower in the winter. In addition, the essential oil contains thymol methyl ether (2%), cineol, cymen, pinene, borneol, and esters (68).

Medicinal properties of *T. vulgaris*

Native to the western Mediterranean, the subshrub *Thymus vulgaris*, commonly referred to as thyme, is used as a spice worldwide. Traditional medicine makes use of thyme-based herbal teas and infusions. (69). Thyme bioactive compounds, including thyme essential oil (TEO) constituents like flavonoids and phenolic acids, natural terpenoid thymol, and phenol isomer carvacrol, have well-established antibacterial, antitussive, antispasmodic, and expectorant qualities (70,71). Studies have shown that thymus vulgaris oil (TO) contain phenolic and tocols that may directly interact with free radicals to stop lipid peroxidation (72). Antioxidant levels in the brains of rats have been shown to rise after receiving thymol therapy. (73) Moreover, behavioural investigations have shown that a 1-week course of oral administration of thyme extract might have anxiolytic effects in rats. This conclusion is corroborated by data indicating that thyme extract lengthens the time spent in the safe haven of the labyrinth and increases the number of successful maze completions (74). In an animal model of anxiety testing using the elevated plus maze (EPM) in mice, kaempferol, a component of thyme extract, was demonstrated to have anxiolytic effects. In the plus maze test, carvacrol, which was isolated from this plant, demonstrated anxiolytic effects with a remarkable score (75,76). Animal studies have shown that bioactive monoterpenes found in thyme extract, including linalool, may help reduce anxiety. (77) Additionally, thyme essential oil may provide protection against alfatoxin toxicity in a dose-dependent manner. (78) Additionally, thymol has been shown to imitate or facilitate GABA activity and alter the $GABA^A$ receptor, suggesting that it exerts its effects centrally. (79) As a result, its considerable anticonvulsant and antiepileptogenic actions may be used. Recent research has demonstrated the neuroprotective and therapeutic effects of thymol, a bioactive monoterpene derived from T. vulgaris, on rats with amyloid or scopolamine-induced cognitive impairment (80) The possible impact of thymol on GABA-mediated regulation of synaptic transmission has been linked to its neuroprotective benefits. (81) Meanwhile, it was shown that TO might enhance synaptic acetylcholine (Ach) and nicotinic Ach receptor activation, suggesting that it may be used to control cholinergic function. (82) It was also shown that thymol has antidepressant properties. Deng et al. found that depressed mice subjected to chronic unpredictable

mild stress (CUMS) had their immobility time significantly reduced after receiving thymol and that their hippocampal levels of serotonin (5-HT) and norepinephrine (NE) were restored. (80) When it comes to anxiety, the essential oil from Foeniculum vulgare seeds is more effective than that from depression, although the essential oil from the aerial portions of the plant only little alters anxiety (300).

Zataria multiflora

The family Lamiaceae includes the genus Zataria (*Z. multiflora*). (83) P-cymene derivatives include dihydroxyaromadendrane, luteolin, α-tocopherolquinone, Multiflotriol, and Multiflorol, a novel aromatic ester of p-hydroxy benzoic acid (84–86). The main constituents of the plant oil were beta-caryophyllene (2.06%), gamma-terpinene (3.88%), PARA-cymene (7.72%), carvacrol (33.65%), and thymol (37.59%) (87).

Medicinal properties of *Z. multiflora*

The chemical profile of *Z. multiflora*, which contains terpens, luteolin, 6-hydroxyluteolin glycosides, and di-, tri-, and tetraethoxylated compounds, may be responsible for its therapeutic qualities. (88) Although *Z. multiflora* Boiss essential oil (ZEO) has preservation benefits, its potent flavor and odor have hindered its widespread use as a food preservative. (89) Traditional Iranian medicine makes use of the plant's analgesic, antiseptic, and carminative qualities. (88) In vitro studies have demonstrated the antimicrobial, antifungal, and antioxidant properties of *Z. multiflora* essential oil. (89, 90) Research has demonstrated that ZEO has a stronger antioxidative effect than pomegranate juice. (89) Studies have demonstrated the plant's antibacterial (90), immunoregulatory (91,117), and anti-inflammatory (92,118) qualities. Additionally, it has been demonstrated that intraperitoneal injections of Z. multiflora essential oil can reverse the memory and learning deficits caused by Aβ in rats. Researchers discovered that the zataria multiflora plant's essential oil effectively reduced the cognitive symptoms linked to Alzheimer's disease (AD) (93).

Curcuma longa

Curcuma longa (C. longa), a plant in the Zingiberaceae family, is grown in countries in Southeast Asia. (94) The flavonoid curcumin (diferuloylmethane) and volatile oils such as tumerone, atlantone, and zingiberone are among the active components of turmeric. Additionally, there are carbohydrates, proteins, and resins. The most thoroughly researched active ingredient is curcumin, which is present in fresh turmeric at concentrations ranging from 0.3% to 5.4% (95).

Medicinal properties of *C. longa*

Plants such as C. longa naturally contain curcumin, a polyphenol and non-flavonoid compound. Due to its anti-inflammatory, antioxidant, and other qualities, curcumin has been researched for its potential in a range of biological and medical applications. Curcumin has garnered a lot of attention lately as a potential treatment for neurodegenerative diseases. (6) Kulkarni demonstrated that the water soluble extract of curcumin raised dopamine, norepinephrine, and 5-HT levels in the central nervous system (96). In animal and cell culture models, extracts from the *C. longa* plant—scientifically known as curcumin—have demonstrated neuroprotective benefits against oxidative brain damage, memory loss, Parkinson's disease (PD), reactive oxygen species (ROS) generation, apoptosis, platelet aggregation, cytokine release, and cyclooxygenase enzyme activity. (97, 98) It has been demonstrated that *C. longa* extract (1000 mg/kg, body weight, per mouth) guards against kidney damage and oxidative (99,100).

It has been demonstrated that treating rats with curcumin at doses of 50, 100, or 200 mg/kg improves their mitochondrial dysfunction and cognitive deficits (101). Curcumin has also been demonstrated to have neuroprotective effects in cases of cerebral ischemia and neuronal degenerative diseases (102,103). Curcumin protects the rat brain against localised ischemia, according to scientific research. It does this via increasing the expression of the antioxidant enzyme HO-1 and the transcription factor Nrf2. (104) Curcumin inhibits glutamate neurotoxicity in the rat hippocampus, according to the study's authors, likely via blocking the activation of inflammatory genes TXNIP and NLRP3 in response to ER stress (105). As suggested by Linlin et al., curcumin has been demonstrated to shield the rat brain from ischemia-reperfusion damage. They also discovered that curcumin activated the JAK2/STAT3 signaling pathway, enhanced neuron survival rate, and inflammatory cytokine activity (106). Curcumin has been demonstrated to decrease oxidative stress and neurotoxicity brought on by oxyhemoglobin in an in vitro model of subarachnoid hemorrhage (SAH) (Xia LI) (107).

Curcumin's neuroprotective benefits in Parkinson's disease are associated with its antioxidant properties. In the human cell line SH-SY5Y exposed to 6-OHDA, curcumin prevents ROS intracellular accumulation (108), per a study by Wang. (109). Over a three-week period, curcumin (60 mg/kg body weight, taken orally) reduced neuronal degeneration in the striatum of rats with 6-OHDA lesions (110). By raising GSH levels, which had been depleted by ROS, curcumin rescued the neurons. (111) The neurotoxic 6-OHDA was generated in MES23.5 cells, and curcumin elevated SOD levels in the lesioned striatum of 6-OHDA mice (112.108) There is evidence that curcumin may prevent LPS from damaging axons (113). The neuroprotective effects of curcumin may be mediated by overexpression of BCl-2, an inducible nitric oxide synthase (iNOS) blocker. Curcumin is therefore helpful in lessening the harm brought on by NO-mediated aging (114). When administered orally for a week at a dose of 150 mg/kg, curcumin reduced the striatal production of proinflammatory cytokines like IL-6, IL-1beta, TNF-alpha, and total nitrite in MPTP-induced animals (115). Additionally, curcumin prevented NF-kB activation in

inflammatory reactions triggered by 6-OHDA and LPS116 (108).

Antipsychotic effects of Indian Spices

Turmeric (*Curcuma longa*)

Curcumin, a polyphenol with significant neuroprotective qualities, is found in turmeric (116). Curcumin influences neurotrophic factors like BDNF, modifies neurotransmitter levels (dopamine and serotonin), and has anti-inflammatory and antioxidant properties (117,118). Studies suggest curcumin's potential in alleviating symptoms associated with schizophrenia and other psychotic disorders (119,120). Turmeric (*Curcuma longa*), a staple spice in Indian traditional medicine and cuisine, has garnered significant attention in recent years for its potential role in neuropsychiatric disorders, particularly those with psychotic features (121).

The primary active constituent of turmeric, curcumin (diferuloylmethane), along with its derivatives such as demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone, demonstrates a wide range of pharmacological actions relevant to the management of psychosis and schizophrenia (122). In terms of mechanism, curcumin has potent anti-inflammatory and antioxidant properties, both of which are essential for neuroprotection (123). Curcumin reduces oxidative stress, a major factor in the pathophysiology of schizophrenia, by scavenging reactive oxygen species (ROS), upregulating the expression of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, and inhibiting lipid peroxidation (124). Additionally, it reduces neuroinflammation by suppressing the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in the brain, as well as by downregulating pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6 (125,126). Notably, curcumin also inhibits microglial activation, which is a central mechanism associated with neuroinflammation in psychotic disorders (127).

In terms of neurotransmitter modulation, curcumin has shown the ability to increase brain levels of dopamine and serotonin, both of which are crucial for mood regulation and psychotic symptom control (128). Additionally, it helps balance glutamatergic neurotransmission by reducing glutamate-induced excitotoxicity and enhancing GABAergic tone, offering a stabilizing effect on brain circuits often dysregulated in schizophrenia and related disorders (129).

Several preclinical studies have validated the antipsychotic potential of curcumin. For instance, Kandhare et al. (2014), in a study published in *Biomed Research International*, demonstrated that curcumin significantly reversed ketamine-induced schizophrenia-like behavior in rats by modulating oxidative stress and inflammatory markers (130). Similarly, Kulkarni et al. (2008) reported that curcumin improved behavioral parameters and cognitive function in rodent models of psychosis (131). These findings suggest curcumin may mimic or augment the effects of atypical antipsychotics through multifaceted mechanisms (132).

Clinically, Lopresti et al. (2014) reported in the *Journal of Affective Disorders* that curcumin supplementation in humans improved depressive symptoms in individuals with mild-to-moderate depression, including those with psychotic features (133). Curcumin has shown promise in lowering Positive and Negative Syndrome Scale (PANSS) scores in patients with schizophrenia when used as an adjuvant therapy in conjunction with atypical antipsychotics (134). However, there is still a lack of human data, which calls for more investigation in larger, regulated clinical trials (135).

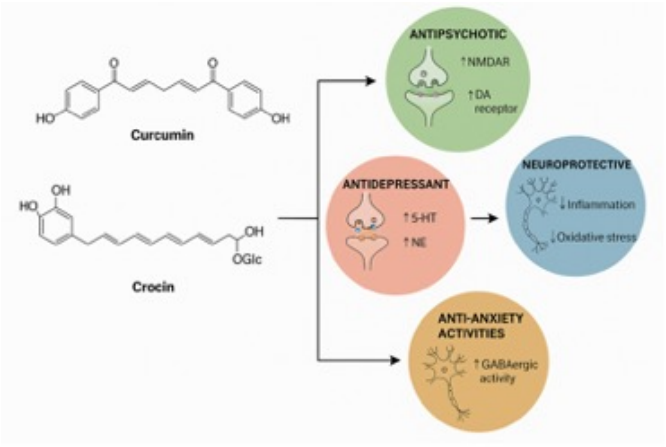
The low bioavailability of curcumin is one of the major problems with it, attributed to its rapid metabolism and low absorption (136). This limitation is often overcome through bioenhancement strategies, such as co-administration with piperine (from *Piper nigrum*), which has been shown to increase curcumin's bioavailability by nearly 2000% (137). Moreover, the development of nanocurcumin, liposomal curcumin, and curcumin–piperine complexes is underway to enhance central nervous system (CNS) penetration and pharmacological efficacy (138). Hussian et. al. in 2022 investigated antistress and antidepressant activities of synthetic curcumin analogues by behavioral and biomarker. The study revealed that these compounds significantly enhanced hippocampus CAT, SOD and GSH, and reduced MDA levels in the scopolamine-induced stress mice model (301). Subsequent research done by Jinglin Chen et. al. revealed that the antidepressant properties of CACN136 were linked to a decrease in the metabolism of 5-HT and the modulation of oxidative stress levels in vivo. Chen concluded, CACN136 showed potent antidepressant activity and could be an effective antidepressant (302).

In terms of safety, curcumin is generally well-tolerated even at higher doses (up to 1500 mg/day), with minor gastrointestinal disturbances being the most common adverse effect (139). Its synergistic potential with other adaptogenic and neuroprotective herbs such as *Withania somnifera* (ashwagandha) and *Bacopa monnieri* (brahmi) is also under exploration for integrative mental health management (140).

Despite the encouraging preclinical and limited clinical evidence, the lack of large-scale, randomized controlled trials specifically targeting psychotic disorders remains a limitation. Therefore, future directions include long-term studies evaluating standardized, bioavailable formulations of curcumin, especially in patients with schizophrenia or bipolar disorder with psychotic features (141). The incorporation of turmeric-based nutraceuticals into complementary and integrative psychiatric treatment models holds great potential for improving outcomes in mental healthcare (142). The GABAergic and nitrergic systems are influenced by curcuma longa, giving it hypnotic and anxiolytic properties. On the other hand, combined administration of C. longa and midazolam intensifies hypnosis induced by barbiturate.(303)

https://doi.org/10.47552/ijam.v16iS2.6227 ISSN No: 0976-5921

Figure 1: Mechanism of curcumin and crocin



Black Pepper (*Piper nigrum*)

Black pepper is rich in piperine, which enhances the bioavailability of curcumin. Piperine also exhibits monoamine oxidase (MAO) inhibitory activity and modulates neurotransmitter systems, potentially contributing to antipsychotic effects. Its synergistic use with curcumin may amplify therapeutic outcomes in neuropsychiatric conditions (143).

One of the most common spices in India, black pepper (*Piper nigrum*), has demonstrated great promise in the treatment of neuropsychiatric disorders, including psychotic disorders. Piperine, an alkaloid with a wide range of pharmacological characteristics, such as neuroprotective, antioxidant, anti-inflammatory, and bioenhancer effects, is the main active ingredient in black pepper (144). According to research, piperine can penetrate the blood-brain barrier and have effects on the central nervous system (CNS) that are useful in treating diseases like schizophrenia and psychosis (145).

The mechanisms underlying the antipsychotic effects of piperine involve modulation of several neurotransmitter systems. Notably, piperine increases the brain levels of dopamine and serotonin, two key neurotransmitters involved in the pathophysiology of schizophrenia and mood disorders (146). Furthermore, it enhances GABAergic tone while reducing glutamatergic excitotoxicity, creating a balanced neural environment conducive to symptom reduction in psychosis (147). Inhibiting monoamine oxidase is one of its other neuropharmacological actions. This helps sustain higher levels of serotonin and dopamine in the synaptic cleft, a function that is shared by a number of atypical antipsychotics (148).

Preclinical studies have consistently highlighted the potential of black pepper in modulating behavior related to psychosis. Srinivasan (2007) demonstrated the cognitive-enhancing and anxiolytic properties of piperine in rodent models (149). Likewise, Bukhari et al. (2013) reported that piperine exerted significant antidepressant-like and anti-anxiety effects in behavioral tests (150). By neutralizing reactive oxygen species (ROS), piperine's antioxidant qualities help prevent oxidative damage to neuronal tissue. Its anti-inflammatory effects are achieved by downregulating inflammatory cytokines like TNF-α and IL-6 (151). Additionally, it has been demonstrated that piperine increases brain-derived neurotrophic factor (BDNF), a vital neurotrophin implicated in cognitive function and synaptic plasticity (152).

Importantly, piperine is also recognized as a bioavailability enhancer—a unique feature that makes it invaluable in combination therapies. It inhibits glucuronidation in the liver and intestine, thereby prolonging the plasma half-life of co-administered drugs and phytochemicals. For instance, when used in combination with curcumin, piperine increases its bioavailability by over 2000%, enhancing its therapeutic potential in psychiatric disorders (153). Clinical evidence supports this synergy; Ghosh et al. (2016) showed that patients with major depressive disorder and psychotic features experienced better symptom resolution when treated with a piperine-curcumin combination, compared to curcumin alone (154).

While black pepper is generally considered safe and is widely consumed, high doses of isolated piperine may cause gastrointestinal discomfort. However, dosages between 5 to 20 mg/day in clinical settings have shown no significant adverse effects (155). Current innovations include the development of piperine-loaded nanoparticles, nanoemulsions, and bioenhanced CNS formulations, which are being explored to improve delivery and efficacy in psychiatric therapy (156).

Despite these promising results, a notable limitation is the lack of large-scale, dedicated clinical trials targeting psychotic disorders such as schizophrenia. Most existing human studies focus on piperine's adjunctive benefits rather than its standalone antipsychotic potential. Thus, future research should prioritize randomized controlled trials in psychotic populations, explore multi-target formulations, and develop piperine-based nanocarriers to improve CNS-specific delivery and efficacy (157).

Black pepper (*Piper nigrum*), through its active compound piperine, exhibits potent antipsychotic-like effects by modulating neurotransmitters, suppressing neuroinflammation, enhancing neurotrophin levels, and improving the bioavailability of therapeutic agents. These multi-dimensional actions position it as a valuable candidate for complementary and integrative approaches to psychiatric care, particularly in adjunct treatment of schizophrenia and related psychotic disorders.

Ginger (*Zingiber officinale*)

Bioactive substances with anti-inflammatory and antioxidant qualities, such as shogaol and gingerol, are found in ginger (158). These ingredients may have anxiolytic and cognitive-enhancing effects by modulating GABAergic neurotransmission, which may help control psychotic symptoms (159).

Ginger (*Zingiber officinale*), a widely used Indian spice and medicinal herb, has gained increasing recognition in the neuroscientific and psychiatric research domains for its neuroprotective and antipsychotic-like properties (160). Ginger is a potential

adjunct or complementary therapy for the treatment of psychotic disorders like schizophrenia and bipolar disorder with psychotic features because its bioactive constituents, particularly 6-gingerol, 6-shogaol, and zingerone, have demonstrated a variety of effects on the central nervous system (CNS) (161).

Ginger's strong anti-inflammatory and antioxidant properties are the main ways it works in the brain (162). Ginger inhibits the NF-κB signaling pathways and microglial activation, which are both linked to neuroinflammation linked to psychotic disorders (163). It also lowers the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). Ginger also dramatically boosts the activity of antioxidant enzymes like catalase and superoxide dismutase (SOD), which protects neurons from oxidative damage and preserves cognitive function (164).

Of particular importance is ginger's ability to modulate neurotransmitter levels. Preclinical findings reveal an increase in dopamine and serotonin levels following administration of ginger extracts—two neurotransmitters that play critical roles in the development and management of schizophrenia and mood disorders (165). Furthermore, ginger promotes GABAergic transmission and reduces excessive glutamatergic activity, resulting in a calming effect on the brain and reduction of excitotoxicity—both vital in psychotic symptom management (166). Brain-derived neurotrophic factor (BDNF), a protein essential for synaptic plasticity, learning, and memory, is upregulated in conjunction with these neurochemical alterations (167).

In terms of behavioral effects, studies in rodent models have shown that ginger extract can reduce psychosis-like symptoms. Hasanein and Riahi (2019) reported that 6-gingerol attenuated behavioral disturbances and reversed oxidative stress markers in ketamine-induced psychosis models (168). Similarly, Ghayur et al. (2008) observed significant anxiolytic and mood-enhancing effects, further validating the therapeutic relevance of ginger in psychiatric conditions (169).

Clinical evidence also supports ginger's neuropsychiatric benefits. For instance, Khandouzi et al. (2015) demonstrated that ginger supplementation significantly reduced depressive symptoms in type-2 diabetic patients—conditions often comorbid with psychosis (170). Mahluji et al. (2013) found that elderly individuals with mild cognitive impairment showed improved memory and processing speed following ginger administration, indicating potential benefits in schizophrenia-related cognitive dysfunction (171).

Although high dosages of ginger may result in mild gastrointestinal side effects like heartburn, it is generally regarded as safe at therapeutic doses (500–1000 mg/day in human studies) (172). Innovations such as gingerol-loaded nanoparticles and polyherbal CNS-targeting formulations are currently being explored to enhance bioavailability and CNS penetration (173). Synergistic combinations with compounds like curcumin, piperine, and *Bacopa monnieri* may further augment its therapeutic efficacy (174).

However, despite promising preclinical and limited clinical results, a major limitation remains the scarcity of direct clinical trials examining ginger's effects specifically on schizophrenia or psychosis (175). As such, the future scope of research should focus on well-designed randomized controlled trials, particularly in populations diagnosed with schizophrenia, schizoaffective disorder, or bipolar disorder with psychosis. Ginger's safety, affordability, and wide cultural acceptance make it a strong candidate for integrative psychiatric therapies in the Indian context and globally (176).

Ashwagandha (*Withania somnifera*)

Ashwagandha, an adaptogenic herb, contains withanolides that exhibit GABA-mimetic activity, reduce cortisol levels, and modulate dopaminergic function. Clinical studies have demonstrated its efficacy in reducing schizophrenia symptoms and improving cognitive functions (177).

One of the most valued herbs in Ayurvedic medicine, ashwagandha (Withania somnifera), also referred to as "Indian ginseng" or "Winter cherry," has recently attracted a lot of interest in neuropsychiatric research because of its neuroprotective, anxiolytic, and antipsychotic-like properties. Rich in steroidal lactones called withanolides (e.g., withaferin A and withanone), as well as sitoindosides and various alkaloids, Ashwagandha exerts multidimensional effects on brain physiology, particularly targeting pathways involved in schizophrenia and related psychotic disorders (178).

Several mechanistic studies have elucidated how Ashwagandha modulates key neurotransmitters including dopamine, serotonin, and GABA—all of which play critical roles in the pathology of psychotic conditions. Ashwagandha is shown to enhance GABAergic activity, promote dopaminergic tone in the striatum and prefrontal cortex, and reduce glutamate-induced excitotoxicity—theorized to be hyperactive in schizophrenia. Additionally, it lowers cortisol levels, which aids in regulating the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis that is observed in depression, anxiety, and psychosis (179).

Regarding its anti-inflammatory and antioxidant properties, ashwagandha lowers high levels of pro-inflammatory cytokines such as TNF-α and IL-6, which are frequently elevated in individuals with bipolar disorder and schizophrenia. Additionally, it increases natural antioxidants like glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), which protects neurons and lower oxidative stress, a major cause of psychotic symptoms and cognitive decline (180).

Preclinical studies have strongly supported its role in psychosis management. For instance, Bhattacharya et al. (2000) demonstrated that Ashwagandha extract enhanced memory retention, reduced stress markers, and improved learning in mice. Vohora et al. (2011) showed that Ashwagandha significantly attenuated behavioral abnormalities in a

ketamine-induced psychosis model in rodents, simulating the NMDA receptor hypofunction believed to underlie schizophrenia (181).

Clinically, a noteworthy randomized, placebo-controlled trial by Chengappa et al. (2018) found that patients with schizophrenia who received standardized Ashwagandha extract (containing 5% withanolides) demonstrated a notable improvement in PANSS positive, negative, and overall psychopathology scores when in contrast to a placebo (182). Additionally, Cooley et al. (2009) reported reduced stress, improved cognition, and mood stabilization in patients under psychological duress, suggesting broader psychiatric applicability (183).

Ashwagandha is generally well-tolerated, with mild gastrointestinal disturbances and drowsiness reported in some cases at higher doses. Clinical doses typically range between 300–600 mg/day, while animal studies use 100–200 mg/kg of body weight. Novel innovations such as withanolide-rich nanoformulations, and synergistic formulations with Curcumin, Piperine, Bacopa, and Shankhpushpi, are being investigated to improve therapeutic effect and bioavailability (184).

Despite these promising findings, the current limitations include a relative paucity of large-scale, long-duration randomized trials specifically targeting psychosis and schizophrenia. Most studies focus on anxiety and cognitive stress. In order to manage psychosis, future research must standardize extract compositions, establish the best dosages, and investigate ashwagandha as a complementary therapy, particularly when combined with traditional antipsychotic medications (185). Ashwagandha presents strong scientific potential as a natural antipsychotic adjunct, offering a multifactorial approach that targets inflammation, neurotransmitter imbalance, oxidative stress, and neuroendocrine dysfunction—core elements of psychotic disorders.

Holy Basil (*Ocimum sanctum*)

Compounds with anti-stress and antioxidant qualities, such as rosmarinic acid and eugenol, are found in holy basil, also known as tulsi. These constituents may modulate catecholamines and inhibit MAO, contributing to mood stabilization and potential antipsychotic effects (186).

Ocimum sanctum, widely revered as Holy Basil or Tulsi, has long been a staple in Ayurvedic medicine for its powerful adaptogenic, anxiolytic, and mood-regulating properties. Over the past few decades, modern scientific studies have begun validating its potential as a natural antipsychotic agent, owing to its multi-targeted action on the central nervous system (187).

Holy Basil is rich in phytochemicals like eugenol, ursolic acid, rosmarinic acid, caryophyllene, carvacrol, luteolin, and ocimumosides, which contribute to its neuroprotective and neuromodulatory properties. These substances have demonstrated the capacity to alter serotonergic and dopaminergic neurotransmission, two important pathways involved in the etiology of psychotic and schizophrenia disorders. Tulsi also has a calming effect on the hypothalamic-pituitary-adrenal (HPA) axis, which lowers cortisol levels and aids in mood and behavior stabilization (188).

Preclinical investigations offer robust evidence for its antipsychotic-like actions. The administration of *Ocimum sanctum* extract dramatically decreased anxiety, enhanced memory, and reversed cognitive deficits in rodent models exposed to chronic stress, according to a seminal study by Bhattacharya et al. (2001). Similar results were also reported by Mondal et al. (2011) in a stress-induced behavioral model, emphasizing decreased oxidative damage in brain tissues, increased locomotor activity, and decreased immobility in the forced swim test (189).

One proposed mechanism of action is Tulsi's ability to reduce glutamate-induced excitotoxicity—a major contributor to neuronal death in psychosis. Additionally, it increases natural antioxidants like glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), which fight oxidative stress in vital brain areas like the hippocampus and prefrontal cortex (190).

Though direct studies on schizophrenia are still emerging, clinical trials underscore Tulsi's impact on mood and stress regulation. In a randomized clinical trial by Saxena et al. (2012), healthy volunteers receiving 300 mg/day of Tulsi leaf extract exhibited marked improvements in general anxiety, cognitive clarity, and mood stabilization. These findings suggest its potential as an adjunctive therapy for managing early-phase psychosis or mood disorders with psychotic features (191).

The herb is usually consumed in the form of aqueous/ethanolic extracts or capsules, and is often part of polyherbal formulations with other CNS-active botanicals like Ashwagandha, Bacopa, and Curcumin. It has a high safety margin, with minimal adverse effects reported, making it suitable for long-term use (192).

While *Ocimum sanctum* is not yet a substitute for conventional antipsychotic drugs, its neuroprotective, anti-inflammatory, and neurotransmitter-balancing actions provide a solid foundation for its use as an adjunct in psychiatric care. Future research involving placebo-controlled clinical trials, brain imaging, and bioavailability studies is warranted to establish Tulsi's definitive role in treating schizophrenia and other psychotic disorders (193).

Saffron (*Crocus sativus*)

Saffron's active constituents, crocin and safranal, have demonstrated serotonergic modulation and neuroprotective effects. Clinical trials suggest saffron's potential in improving depressive symptoms and cognitive dysfunctions associated with psychotic disorders (194).

Saffron (*Crocus sativus* L.), one of the most precious medicinal spices in the world, has emerged as a promising natural antipsychotic agent. Traditionally used as a mood elevator in Persian and Unani medicine, saffron's pharmacological profile now includes antidepressant, anxiolytic, neuroprotective, and

antipsychotic effects, validated through contemporary preclinical and clinical investigations (195).

The major active constituents of saffron—crocin, crocetin, safranal, and picrocrocin—exert multimodal neuropharmacological actions. These bioactives modulate the dopaminergic and serotonergic systems, which are critical in psychotic disorders, especially schizophrenia. Furthermore, saffron's antioxidant properties protect against glutamate-induced excitotoxicity, a well-known contributor to neuronal degeneration in psychosis (196).

Preclinical models of psychosis, such as ketamine- and MK-801-induced hyperlocomotion, have demonstrated that both saffron extract and its active compound safranal can significantly reduce psychosis-like behavior and restore behavioral balance.

In a study by Inan et al. (2019), safranal exhibited notable antipsychotic-like effects comparable to haloperidol, showing suppression of stereotypy and agitation in murine models. Moreover, Hosseinzadeh et al. (2005) highlighted crocin's neuroprotective and antidepressant actions through hippocampal upregulation of neurotrophic markers (197).

In the clinical domain, Talaei et al. (2020) reported that saffron, when used as an adjunct to risperidone, improved negative symptoms of schizophrenia such as flat affect and social withdrawal —areas where traditional antipsychotics often fall short. Another double-blind trial by Kashani et al. (2018) confirmed saffron's capacity to improve cognition and overall mental well-being in patients with psychiatric disorders (198).

Saffron works by enhancing monoaminergic tone, inhibiting NMDA receptor-mediated glutamate toxicity, and downregulating pro-inflammatory cytokines (through NF-κB inhibition). Because of this, saffron is particularly useful in treating co-occurring anxiety and depression, as well as both positive and negative symptoms of psychosis (199).

At dosages of 30 to 100 mg per day, saffron is safe and well tolerated, with few adverse effects like mild sedation or gastrointestinal distress. Although higher doses (above 1.5 g/day) are rarely required for therapeutic benefits, they may have negative side effects. With promising results, it has been investigated in a variety of forms, including hydroalcoholic extract, standardized crocin tablets, and even polyherbal combinations (200).

Although its long-term effects in chronic schizophrenia are yet to be fully established, saffron offers great potential as an adjunctive therapy, particularly for treatment-resistant schizophrenia, schizoaffective disorder, and psychotic depression. For crocin and safranal to more successfully cross the blood-brain barrier, future research is necessary in the form of extensive randomized controlled trials, brain imaging studies, and the creation of targeted delivery systems (201).

Cinnamon (*Cinnamomum verum*)

Eugenol and cinnamon aldehyde, which are found in cinnamon, have neuroprotective and anti-inflammatory qualities. These compounds may improve insulin sensitivity and cognitive functions, potentially aiding in the management of psychosis-related metabolic disturbances (202).

Cinnamon (*Cinnamomum verum*), widely cherished as a culinary spice, has attracted growing interest in neuroscience for its neuroprotective, antidepressant, and antipsychotic potential. Rich in bioactive phytoconstituents such as cinnamaldehyde, eugenol, and cinnamic acid, cinnamon exerts multidimensional actions on the central nervous system (CNS) that are relevant in managing psychiatric disorders, including psychosis and schizophrenia (203).

Experimental studies have revealed that cinnamaldehyde, a key volatile constituent of cinnamon, exhibits antipsychotic-like behavior in animal models of psychosis. In a notable study by Jain et al. (2015), administration of cinnamaldehyde ameliorated ketamine-induced hyperlocomotion, a model mimicking the positive symptoms of schizophrenia. This behavioral normalization was associated with modulation of dopaminergic and glutamatergic pathways, both of which are known to be dysregulated in schizophrenia (204).

Furthermore, cinnamon demonstrates potent antioxidant and anti-inflammatory properties, helping mitigate oxidative stress and neuroinflammation—two major pathological hallmarks in the neurobiology of psychosis. Shaikh et al. (2014) confirmed that aqueous cinnamon extract could reduce lipid peroxidation and enhance catalase and superoxide dismutase activities in the brain, thereby offering neuroprotection in chronic stress models. These mechanisms are indirectly tied to improvements in cognition and emotional regulation, often impaired in psychotic disorders (205).

Although clinical trials specifically targeting psychotic populations with cinnamon are still scarce, its nootropic, antidepressant, and anxiolytic effects have been demonstrated in several human studies. Cinnamon improves insulin sensitivity, which also relates to improved cognition and brain plasticity, especially in patients with schizophrenia comorbid with metabolic syndrome. Moreover, cinnamon appears to enhance GABAergic neurotransmission, supporting anxiolytic action and behavioral calming, essential for addressing agitation and restlessness seen in psychosis (206).

The therapeutic dose of cinnamon typically ranges from 500 mg to 2 g/day (in the form of standardized extracts). Importantly, care should be taken regarding the type of cinnamon used—Ceylon cinnamon (*C. verum*) has significantly lower coumarin content than Cassia cinnamon (*C. cassia*), which can be hepatotoxic at higher doses (207).

Cinnamon holds potential as an adjunctive natural agent for managing symptoms of psychosis, particularly by targeting oxidative stress, inflammation, and neurotransmitter imbalances. While preclinical data are robust, there is a clear need for well-designed clinical trials to fully validate its antipsychotic efficacy and establish its long-term safety profile in psychiatric populations (208).

Clove (*Syzygium aromaticum*)

Clove is rich in eugenol, known for its antioxidant and GABAergic modulatory effects. These properties may contribute to its CNS depressant activity, offering calming effects beneficial in psychotic conditions (209).

Clove (*Syzygium aromaticum*), a spice widely used for culinary and medicinal purposes in India, has demonstrated emerging neuropharmacological properties, including antipsychotic, anxiolytic, and neuroprotective actions. This activity is largely attributed to its rich phytochemical profile, especially eugenol, β-caryophyllene, flavonoids, and tannins. Eugenol, the principal constituent of clove essential oil, exhibits significant activity on CNS neurotransmitters and oxidative pathways, making it a compound of interest for psychosis research (210).

Preclinical studies support the antipsychotic potential of clove. In a landmark study by Shyamala et al. (2013), administration of clove extracts in ketamine-induced psychosis models in rodents reversed behavioral abnormalities such as hyperlocomotion, poor social interaction, and stereotypy. These effects were associated with clove's modulation of NMDA receptors and dopaminergic signaling, two major neurotransmitter systems implicated in the pathogenesis of schizophrenia (211).

Additionally, clove's strong antioxidant and anti-inflammatory actions play a supportive role in neuropsychiatric health. Eugenol has been shown to scavenge free radicals and suppress proinflammatory cytokines like IL-6 and TNF-α, as reported by Ali et al. (2014) in murine brain inflammation models. These mechanisms are highly relevant, given the established links between neuroinflammation, oxidative stress, and psychosis in modern neuroscience literature (212).

Although clinical trials in humans focusing specifically on psychosis or schizophrenia are currently limited, clove has been traditionally used in stress and mental fatigue management. Some indirect human studies support its role in improving cognition, mood stability, and stress tolerance, thereby pointing to a potential adjunctive use in mood or psychotic spectrum disorders (213).

Dosage in preclinical studies generally ranges between 10 to 100 mg/kg, with formulations tested including aqueous and ethanolic extracts as well as eugenol-rich fractions. Importantly, while clove is generally regarded as safe at dietary levels, high doses of eugenol may pose hepatotoxic or nephrotoxic risks, thus necessitating proper formulation and standardization for long-term use in therapeutic contexts (214).

Clove represents a promising phytomedicine in the realm of neuropsychiatric disorders, with mechanistic overlap in dopaminergic modulation, NMDA antagonism, GABAergic enhancement, and anti-inflammatory pathways. Future research, especially in the form of randomized clinical trials, pharmacokinetic profiling, and brain imaging studies, is essential to validate and harness its antipsychotic potential in humans (215).

Nutmeg (*Myristica fragrans*)

Nutmeg contains myristicin and elemicin, which exhibit MAO inhibitory and serotonergic activities. At low doses, nutmeg may have calming effects; however, high doses can be psychotoxic, necessitating cautious use (216).

Nutmeg (*Myristica fragrans*), a spice widely used in culinary traditions across India, is increasingly recognized for its neuropsychopharmacological potential. Traditionally employed for its calming, sedative, and euphoric effects, nutmeg contains a rich array of bioactive constituents such as myristicin, elemicin, safrole, and eugenol, which are implicated in central nervous system modulation (217). These compounds, particularly myristicin, exhibit structural similarity to psychoactive agents like mescaline, and they act by modulating monoaminergic neurotransmission, inhibiting monoamine oxidase (MAO), and interfering with NMDA receptor-mediated glutamatergic excitotoxicity—a key mechanism implicated in schizophrenia and psychosis (218,219).

Preclinical studies provide compelling evidence for the antipsychotic-like and mood-enhancing effects of nutmeg. In a landmark study by Dhingra and Sharma (2006), nutmeg extract exhibited antidepressant-like activity in mice, significantly reducing immobility time in the forced swim test (FST) (220). This was supported by a concurrent increase in serotonin and dopamine levels, indicating its role in monoamine regulation. Sheela et al. (2015) further demonstrated nutmeg's neuroprotective and anti-inflammatory properties in models of oxidative stress-induced neuronal damage (221). These results support the expanding theory that neuroinflammation and oxidative stress are linked to psychotic disorders, indicating that nutmeg may be able to reverse these harmful processes (222).

Behavioral studies have also highlighted nutmeg's ability to reduce psychomotor agitation, hyperlocomotion, and anxiety-like conduct during elevated plus maze and open field tests (223). Although direct clinical trials in humans for psychotic disorders remain absent, traditional medicine has long recommended nutmeg for symptoms resembling psychosis, such as agitation, insomnia, hallucinations, and mood instability (224).

However, nutmeg's psychoactivity is a double-edged sword. At low to moderate doses, it may exert beneficial anxiolytic and antidepressant effects. Yet, at higher doses (above 5 grams/day in humans), it can induce hallucinations, confusion, and even delirium, attributed to the anticholinergic and hallucinogenic actions of myristicin and elemicin (225). This necessitates caution in its therapeutic application, emphasizing the need for dose standardization and toxicological profiling (226).

In conclusion, nutmeg offers promising adjunctive antipsychotic benefits through its diverse pharmacological actions on neurotransmitter systems, oxidative stress, and inflammatory pathways. However, its narrow therapeutic index and potential for psychoactive side effects underscore the urgency for rigorous clinical evaluation, controlled dosing

protocols, and the development of non-toxic formulations for mental health interventions (227).

Cardamom (*Elettaria cardamomum*)

Cardamom's active constituents, such as cineole and terpinene, have antioxidant and GABAergic properties. These may confer anxiolytic and mood-enhancing effects, supporting its traditional use in nervous disorders (228).

Cardamom (*Elettaria cardamomum*), known as the "Queen of Spices," is a fragrant herb from the Zingiberaceae family and holds a special place in Indian traditional medicine. While commonly used for digestive and respiratory disorders, its growing relevance in neuropsychopharmacology—particularly as a natural antipsychotic agent—has attracted attention due to the presence of active constituents such as 1,8-cineole, α-terpinyl acetate, limonene, and flavonoids (229).

Cardamom has been shown in scientific studies to have the ability to alter mood and central nervous system (CNS) activity. By raising levels of monoamines like serotonin and dopamine, which are both crucial in the pathophysiology of schizophrenia and bipolar disorder, the hydroalcoholic extract of cardamom demonstrated antidepressant effects in animal models, according to Al-Yahya et al. (2016) (230). Furthermore, cardamom essential oil demonstrated strong anxiolytic effects in the elevated plus maze and open field tests with negligible sedation, according to Savanth et al. (2020), indicating that it modulates the GABAergic and serotonergic systems (231).

Cardamom's antioxidant and anti-inflammatory effects are of particular importance in psychiatric disorders characterized by oxidative damage and neuroinflammation, such as schizophrenia. The spice is known to reduce proinflammatory cytokines like TNF-α and IL-6, which are often elevated in patients with psychosis (232). Furthermore, its ability to stabilize the limbic system and prefrontal cortex activity makes it a promising candidate for modulating cognitive and emotional disturbances seen in psychotic disorders (233).

Although direct clinical evidence for cardamom in psychosis is limited, traditional systems of medicine have long valued its soothing, mood-enhancing, and calming properties (234). Preclinical studies suggest that doses ranging from 100–400 mg/kg in rodents lead to beneficial neurobehavioral outcomes without major toxicity (235). However, for clinical translation, standardized extracts and targeted trials are necessary (236).

Cardamom holds strong promise as a natural, polypharmacological agent with potential adjunctive benefits in psychosis, especially due to its ability to regulate neurotransmitters, reduce inflammation, and combat oxidative stress. Future directions include randomized controlled trials in humans, receptor-target interaction studies, and the development of standardized phytopharmaceutical formulations for mental health applications (237).

Fenugreek (*Trigonella foenum-graecum*)

Fenugreek contains diosgenin, which exhibits antioxidant and neuroprotective effects. Preliminary studies suggest its potential in cognitive enhancement and neuroprotection, which may be relevant in psychotic disorders (238).

Fenugreek (*Trigonella foenum-graecum*), a leguminous spice with deep roots in Indian traditional medicine, has recently garnered attention for its neuroprotective and potential antipsychotic properties. Rich in diverse phytoconstituents such as diosgenin, trigonelline, saponins, flavonoids, and 4-hydroxyisoleucine, fenugreek exerts multiple pharmacological effects relevant to psychiatric health (239). It mostly works through neurotrophic, anti-inflammatory, and antioxidant processes that are linked to the pathophysiology of mood and psychotic disorders (240).

Preclinical evidence has highlighted fenugreek's role in modulating behavior and brain chemistry under stress-induced conditions. In a pivotal study by Puri et al. (2017), ethanolic extract of fenugreek seeds administered to rodents subjected to chronic mild stress (CMS) significantly reversed behavioral despair in the forced swim test and improved anxiety-related metrics in the elevated plus maze (241). These effects were linked to enhanced levels of dopamine and serotonin, along with reduced oxidative markers such as malondialdehyde (MDA) and increased superoxide dismutase (SOD) levels in the brain (242). In another study by Singhal et al. (2020), fenugreek supplementation was shown to suppress proinflammatory cytokines like TNF-α and IL-6, both of which are elevated in psychotic disorders like schizophrenia (243).

By increasing the expression of brain-derived neurotrophic factor (BDNF), particularly in the hippocampus and prefrontal cortex—areas linked to mood disorders and schizophrenia—fenugreek's neurotrophic potential has also been investigated (244). Additionally, trigonelline, a unique alkaloid found in fenugreek, is suggested to possess nootropic and antidepressant properties, enhancing cognitive performance and emotional regulation via cholinergic and serotonergic pathways (245).

While fenugreek has not yet been directly tested in human clinical trials for schizophrenia or psychosis, its extensive use in Ayurveda for mental fatigue, hormonal imbalance, and nervous system rejuvenation adds ethnobotanical value (246). When taken as a dietary supplement, it is usually safe, and at therapeutic dosages, it shows no discernible toxicity. However, especially in diabetic patients, high dosages may result in hypoglycemia or mild gastrointestinal problems (247).

In conclusion, fenugreek represents a promising adjunctive agent for psychotic and mood disorders due to its multi-targeted mechanisms, including oxidative stress reduction, neuroinflammation suppression, and monoamine regulation. Nonetheless, the absence of direct clinical trials and the need to clarify its molecular mechanisms highlight an important research gap. Its

integration into future nutraceutical or polyherbal formulations may provide a holistic approach to managing neuropsychiatric conditions (248).

Coriander (*Coriandrum sativum*)

Coriander is rich in linalool, known for its anxiolytic properties and modulation of GABAergic neurotransmission. These effects may contribute to its traditional use in managing anxiety and related symptoms (249).

Coriandrum sativum, commonly known as coriander (seeds) or cilantro (leaves), is a widely used culinary spice and medicinal herb from the Apiaceae family. Traditionally utilized for its calming, digestive, and detoxifying properties in Ayurveda, coriander is gaining scientific interest for its neuroprotective and psychotropic potential (250). The plant contains a diverse range of bioactive compounds, including linalool, borneol, camphor, apigenin, and quercetin, which contribute to its anxiolytic, antidepressant, and antioxidant effects (251).

Scientific investigations, particularly in preclinical models, have demonstrated coriander's potential to modulate neurotransmitter systems that are crucial in the pathophysiology of psychotic and mood disorders (252). Coriander's linalool-rich essential oil has demonstrated notable anxiolytic-like effects in animal models like the open field test (OFT) and elevated plus maze (EPM). A study by Emamghoreishi et al. (2005) reported that coriander extract reduced anxiety-like behavior in mice through interaction with GABAergic systems, a mechanism shared with benzodiazepines (253).

Additionally, coriander has anti-inflammatory and antioxidant qualities that lower proinflammatory cytokines like TNF-α and IL-6 as well as reactive oxygen species (ROS), both of which are linked to bipolar disorder and schizophrenia (254). This was further supported by the findings of Sreelatha et al. (2009), who demonstrated that Coriandrum sativum seed extract enhanced memory performance and reduced lipid peroxidation in rodent models (255).

Coriander extracts have been shown to shorten immobility times in behavioral tests such as the forced swim test (FST) and tail suspension test (TST), indicating antidepressant-like effects. This is attributed to enhanced serotonin (5-HT) activity and stabilization of the HPA axis, which is often dysregulated in psychiatric illnesses (256).

Despite promising preclinical data, coriander lacks robust clinical studies directly targeting psychosis or schizophrenia. Nonetheless, its safety profile, traditional usage, and synergistic potential with other psychotropic herbs make it an attractive candidate for adjunctive therapy (257). It is often included in polyherbal formulations for cognitive support, stress relief, and mood regulation, especially in traditional Indian and Middle Eastern medicine (258).

Coriandrum sativum exhibits significant antipsychotic-like properties through neurotransmitter modulation, antioxidant protection, and anti-inflammatory mechanisms. However, future research including clinical trials, receptor-targeting studies, and standardized dosage evaluations are essential to validate its efficacy and establish its role in mainstream psychiatric treatment (259).

Fennel (*Foeniculum vulgare*)

Fennel contains anethole, which has antioxidant and GABAergic modulatory effects. These properties may offer calming effects, supporting its traditional use in nervousness and anxiety (260).

Fennel (Foeniculum vulgare), a fragrant and medicinal spice commonly used in Indian cuisine and traditional systems of medicine such as Ayurveda and Unani, possesses promising neuropsychopharmacological properties (261). Its seeds and essential oils contain bioactive constituents such as anethole, estragole, fenchone, limonene, and a variety of flavonoids and phenolic acids that contribute to its diverse pharmacological profile, including neuroprotective, antioxidant, and anxiolytic effects (262).

Fennel's ability to alter neurotransmitter systems and reduce oxidative stress and neuroinflammation—two factors that are closely linked to the pathophysiology of mental illnesses like schizophrenia, bipolar disorder, and major depression—has been shown in experimental models (263). In rats exposed to stress-induced behavioral changes, a study by Kooti et al. (2014) found that foeniculum vulgare seed extract improved mood and cognitive function (264). Similarly, Sayyah et al. (2006) showed that fennel extract exhibits GABA-mimetic activity, producing calming effects similar to conventional anxiolytics (265).

Mechanistically, fennel's antipsychotic-like effects are believed to arise from its ability to boost GABA and serotonin activity, lower corticosterone levels (a key stress hormone), and inhibit inflammatory cytokines such as TNF-α and IL-1β (266). These neurochemical modulations are vital in reversing psychotic-like symptoms, improving affective stability, and enhancing cognitive function.

In animal studies, fennel's anxiolytic and antidepressant-like effects have been demonstrated using behavioral models like the forced swim test (FST) and elevated plus maze (EPM) (267). These results are consistent with the traditional use of fennel to soothe anxiety, relieving mental tension, and improving sleep quality. Moreover, its antioxidant property further supports its neuroprotective action by neutralizing free radicals and preserving neuronal integrity (268).

Although clinical evidence in humans is still lacking, fennel is widely used in herbal CNS formulations, often in combination with other adaptogens and sedatives like valerian root, ashwagandha, and licorice (269). It is considered generally safe at dietary levels, although caution is advised in large doses due to the presence of estragole, a compound under scrutiny for hepatotoxic potential in very high concentrations (270).

In conclusion, Foeniculum vulgare demonstrates promising antipsychotic potential, primarily through neurotransmitter modulation, antioxidant defense, and

anti-inflammatory actions. However, robust clinical trials and mechanistic investigations are necessary to establish its role as a therapeutic or adjunct agent in psychotic disorders (271).

Ajwain (*Trachyspermum ammi*)

Ajwain's active constituent, thymol, exhibits antioxidant and anxiolytic properties. Its traditional use in calming the nervous system may be attributed to these effects, although scientific data is limited (272).

Trachyspermum ammi, commonly known as Ajwain or carom seeds, is a well-known aromatic spice in Indian households and traditional medicine. Belonging to the Apiaceae family, Ajwain is rich in essential oils, predominantly thymol, along with other potent constituents like γ-terpinene, p-cymene, α- and β-pinene, and polyphenolic flavonoids (273). These compounds contribute to a range of pharmacological activities, including antioxidant, anti-inflammatory, anxiolytic, and neuroprotective effects, which are increasingly recognized in the context of mental health and psychosis-related disorders (274).

The antipsychotic-like properties of Ajwain are primarily attributed to thymol, which has been shown in animal models to modulate GABA-A receptors, thus producing anxiolytic and sedative effects similar to benzodiazepines (275). Sharma et al. (2020) showed that Ajwain essential oil, most likely via GABAergic facilitation, dramatically decreased anxiety-like behavior in the elevated plus maze (EPM) and open field test (OFT) (276). Furthermore, methanolic extracts of Ajwain inhibited LPS-induced neuroinflammation, with a significant decrease in proinflammatory cytokines like IL-1β and TNF-α, which are frequently elevated in psychotic disorders like schizophrenia, according to Khan et al. (2022) (277).

Ajwain also modulates dopaminergic and serotonergic pathways, balancing neurotransmitter systems implicated in schizophrenia and mood disorders (278). These neurochemical effects manifest behaviorally as reduced immobility in the forced swim test (FST) and enhanced exploration and social interaction, which are crucial indicators in psychopharmacology (279). While clinical evidence remains sparse, ethnomedicinal accounts support the use of Ajwain for mental calmness, stress relief, and digestive health, which are intricately linked with psychological well-being (280).

Ajwain's essential oil and extracts have shown efficacy in combination therapies, often paired with cumin, black seed, fennel, and turmeric in traditional formulations for enhancing memory and calming the nervous system (281). It has a high safety profile, being generally recognized as safe (GRAS), although excess thymol may cause mucosal irritation in sensitive individuals (282).

Trachyspermum ammi holds promising antipsychotic and anxiolytic potential via neurotransmitter regulation, antioxidant defense, and anti-inflammatory action. However, there is an urgent need for targeted psychosis model studies, clinical trials, and receptor-binding assays to substantiate its role as an adjunct or standalone therapeutic agent in the management of psychiatric conditions such as schizophrenia, anxiety, and stress-induced behavioral disorders (283).

Mustard Seeds (*Brassica juncea*)

Allyl isothiocyanate, which has anti-inflammatory and antioxidant qualities, is found in mustard seeds. Although direct antipsychotic effects are not well established, these effects might promote CNS health (284).

Brassica juncea, commonly referred to as mustard seeds, is a staple in Indian cuisine and Ayurvedic medicine, widely known for its pungent flavor and therapeutic properties. These seeds, especially the brown Indian variety, are packed with potent bioactive compounds including allyl isothiocyanate (AITC), sinigrin, phenolic acids, flavonoids, selenium, and omega-3 fatty acids (285). Emerging scientific evidence suggests that these constituents may exhibit neuroprotective and antipsychotic-like effects, mediated through their antioxidant, anti-inflammatory, and neurotransmitter-regulating actions (286).

In preclinical models, AITC has shown anxiolytic and antidepressant-like activity, which are often relevant in the treatment of negative symptoms of schizophrenia and mood-related psychosis (287). According to Khan et al. (2019), rodents given AITC showed noticeably fewer anxiety-related behaviors in the open field test (OFT) and elevated plus maze (EPM) (288). Additionally, Singh et al. (2021) reported that aqueous mustard seed extract reversed scopolamine-induced memory deficits, highlighting its potential role in managing cognitive dysfunctions commonly seen in psychosis and neurodegenerative diseases (289).

Mechanistically, the antipsychotic-like effects of mustard seeds appear to stem from their ability to modulate neurotransmitters, particularly enhancing GABAergic and serotonergic tone while reducing excitotoxic glutamate and dopaminergic overactivity—key elements in psychotic pathology (290). In particular, in vulnerable brain regions like the hippocampus and prefrontal cortex, the presence of selenium and flavonoids helps to prevent neurodegeneration and promote neuronal integrity by reducing oxidative stress and pro-inflammatory cytokine production (e.g., TNF-α, IL-1β, IL-6) (291).

Although clinical evidence remains limited, traditional use and emerging experimental data position mustard seeds as a promising adjunctive agent for managing psychotic and mood disorders, especially those related to chronic stress, neuroinflammation, and cognitive decline (292). Additionally, formulations containing mustard seed oil and extracts have been used for enhancing cognition, alleviating anxiety, and supporting overall mental wellness in traditional Indian medicine systems (293). Importantly, mustard is generally regarded as safe (GRAS), though high concentrations of AITC may lead to gastric irritation or mucosal toxicity if used without proper dose standardization (294).

https://doi.org/10.47552/ijam.v16iS2.6227 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Brassica juncea offers a novel avenue for integrative psychopharmacology, combining dietary accessibility with neuropsychiatric potential. Future studies should focus on human clinical trials, neuroreceptor-binding investigations, and standardized extract development to further explore and validate its role in the treatment of psychosis, schizophrenia, and related disorders (295).

Standardization Challenges and Ethnopharmacological Validation

Ensuring consistent quality, potency, and repeatability of herbal products is known as standardisation. The following problems make this especially challenging for plant-based CNS drugs and spices:

Changes in the Phytochemical Composition

Active ingredient concentrations, such as allyl isothiocyanate (AITC) (Mustard) or thymol (Ajwain), might differ greatly because of:

- Origin in geography
- Climate and soil type
- Time for harvesting
- Methods of processing and storage

For instance, the amount of thymol in ajwain might vary greatly based on the cultivar and extraction technique, which makes dosing uncertain.

Absence of standardised extraction procedures

Studies frequently employ various extraction methods (cold press, Soxhlet, distillation) and solvents (methanol, ethanol, aqueous), which produces results that are not comparable.

Different ratios of bioactive, inactive, or even hazardous components may be present in extracts.

Contextual and Cultural Bias

It is challenging to distinguish the effects of a single spice in traditional formulations because they are frequently polyherbal (e.g., Ajwain often used with fennel, cumin, or turmeric).

Ritual use, nutrition, and environment are examples of cultural contexts that might impact efficacy and are challenging to reproduce in therapeutic settings.

Discord Between Conventional Use and Research Objectives

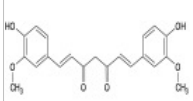
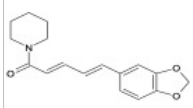
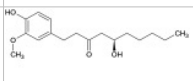
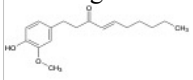
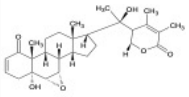
This leap must be made carefully because spices that have historically been used for digestion or overall relaxation are now being researched for complex mental illnesses like schizophrenia.

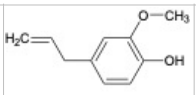
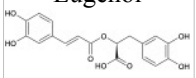
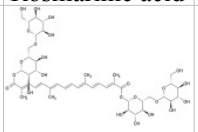
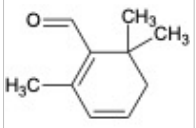
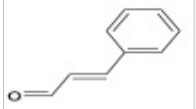
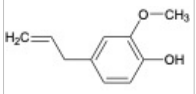
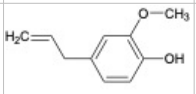
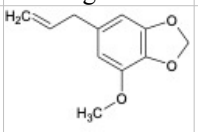
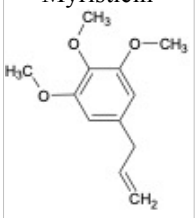
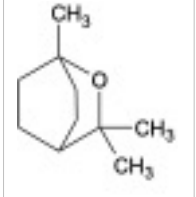
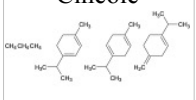
Traditional understanding ignores the need for mechanistic clarity for biological targets like GABA-A regulation or anti-cytokine action.

Issues with Intellectual Property and Ethics

Benefit-sharing and intellectual property rights concerns are frequently brought up by ethnomedical bioprospecting, particularly when the information comes from indigenous groups.

Table 1: Details of spices and their proposed mechanism

S. No.	Indian Spice	Botanical Name	Active Constituents	Proposed Mechanisms	Reported Effects	Evidence Type	References
1	Turmeric	*Curcuma longa*	 Curcumin	Antioxidant, anti-inflammatory, modulates dopamine, serotonin, BDNF	Reduces psychosis symptoms, improves cognition	Preclinical, Clinical	Zhang et al., 2012; Amin et al., 2015
2	Black Pepper	*Piper nigrum*	 Piperine	Enhances curcumin bioavailability, MAO inhibition, dopaminergic modulation	Enhances antipsychotic action with turmeric, anxiolytic effects	Preclinical	Damanhouri et al., 2012
3	Ginger	*Zingiber officinale*	 Gingerol  Shogaol	Anti-inflammatory, GABA modulation	Improves anxiety, memory, and psychotic behaviors	Preclinical	Hasani-Ranjbar et al., 2009
4	Ashwagandha (Indian ginseng)	*Withania somnifera*	 Withanolides	GABAergic, reduces cortisol, dopaminergic stabilization	Reduces schizophrenia symptoms, boosts cognition and sleep	Clinical, Preclinical	Cooley et al., 2019; Chandrasekhar et al., 2012

5	Holy Basil (Tulsi)	*Ocimum sanctum*	 Eugenol  Rosmarinic acid	MAO inhibition, catecholamine modulation, antioxidant	Anti-stress, neuroprotective, mood stabilizing	Traditional, Preclinical	Bhattacharyya et al., 2008
6	Saffron	*Crocus sativus*	 Crocin  Safranal	Serotonergic activity, neuroprotection, anti-inflammatory	Improves depression, cognitive dysfunction in psychosis	Clinical, Preclinical	Akhondzadeh et al., 2004
7	Cinnamon	*Cinnamomum zeylanicum*	 Cinnamaldehyde  Eugenol	Improves insulin sensitivity, antioxidant, anti-inflammatory	Enhances learning and memory; potential adjunct for metabolic side effects of antipsychotics	Preclinical	El-Bassossy et al., 2014
8	Clove	*Syzygium aromaticum*	 Eugenol	GABA receptor modulation, antioxidant	CNS depressant activity, calming effect	Preclinical	Cortés-Rojas et al., 2014
9	Nutmeg	*Myristica fragrans*	 Myristicin  Elemicin	MAO inhibition, serotonergic modulation	Psychotropic and anxiolytic at low doses; hallucinogenic at high doses	Traditional, Preclinical	Suh et al., 2007
10	Cardamom	*Elettaria cardamomum*	 Cineole  Terpinene	Antioxidant, GABAergic modulation	Anxiolytic, mood enhancer	Traditional, Preclinical	Jamshidi et al., 2013

https://doi.org/10.47552/ijam.v16iS2.6227 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

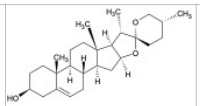
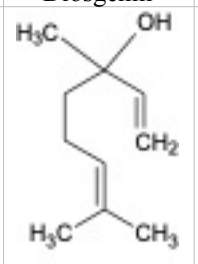
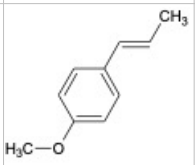
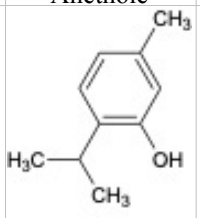
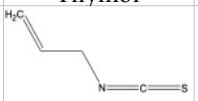
11	Fenugreek	*Trigonella foenum-graecum*	 Diosgenin	Anti-inflammatory, antioxidant, neuroprotective	Memory-enhancing and neuroprotective properties	Preclinical	Raju et al., 2004
12	Coriander	*Coriandrum sativum*	 Linalool	GABA-A modulation, anticonvulsant	Anti-anxiety, calming effect	Preclinical	Emamghoreishi et al., 2005
13	Fennel	*Foeniculum vulgare*	 Anethole	GABAergic and dopaminergic modulation	Traditional use for nervous system disorders	Traditional, Preclinical	Rather et al., 2016
14	Ajwain	*Trachyspermum ammi*	 Thymol	Antioxidant, anxiolytic, GABAergic	Anticonvulsant and calming effects	Preclinical	Pundir et al., 2010
15	Mustard Seeds	*Brassica juncea*	 Allyl isothiocyanate	Anti-inflammatory, neuroprotective	May aid in managing neuroinflammation associated with psychosis		

Table 2: Details of spices along with active phytoconstituent and their observed outcomes

Sr. No.	Spice Name	Active Constituent(s)	Dosage	Model Used	Observed Outcomes
1	Crocus sativus *(Saffron)*	Crocin, Crocetin, Picrocrocin, Safranal	100–200 mg/kg (animal); 30 mg/day (human)	MCAO rats, $AlCl_3$-induced neurotoxicity, Human trials	↓ Glutamate & Aspartate; ↑ Antioxidant enzymes; ↓ Alzheimer's symptoms; ↓ Depression
			40–80 mg/day (extract in clinical trial)	Depression in humans	Combination with fluoxetine improved outcomes over single treatment
2	Nigella sativa	Thymoquinone (TQ), p-Cymene, Carvacrol	500 mg/day (human); varies in animals	Scopolamine-induced memory loss, RA patients, human cognitive test	↑ Memory & learning; ↓ Oxidative stress; ↓ AChE; ↓ Anxiety; ↑ Cognitive function
3	Coriandrum sativum *(Coriander)*	Linalool, Petroselinic acid, Linoleic acid	100–200 mg/kg (mice); 0.5 g/kg (aqueous), 3.5–5 g/kg (ethanolic)	PTZ and electroshock seizure models in mice	↓ Seizure duration; ↑ Anxiolytic behavior; ↑ Social interaction
4	Ferula assafoetida *(Asafoetida)*	E-1-propenyl sec-butyl disulfide, Germacrene B	50–500 mg/kg (animal models)	Peripheral neuropathy, seizure models, passive avoidance tests	↑ Remyelination; ↓ MAO-B & AChE activity; ↑ Memory performance; Anticonvulsant effect

5	Thymus vulgaris	Thymol (40%), Carvacrol (15%), Kaempferol, Linalool	Oral extract; Thymol: unspecified; Carvacrol: EPM-tested	Rodent models (Elevated Plus Maze, amyloid/cognitive impairment models)	Anxiolytic effects, ↑ antioxidant levels, ↓ anxiety, neuroprotective, anticonvulsant, ↑ Ach activity, GABA modulation, antidepressant-like effects
6	Zataria multiflora	Thymol (37.6%), Carvacrol (33.6%), γ-Terpinene, p-Cymene	Intraperitoneal ZEO injection; specific dose not stated	Rat model of Alzheimer's (Aβ-induced)	Antioxidant, anti-inflammatory, antimicrobial, improved learning and memory deficits, reversed cognitive impairments
7	Curcuma longa	Curcumin (0.3–5.4%), Demethoxycurcumin, Bisdemethoxycurcumin	50–200 mg/kg in animals; up to 1500 mg/day in humans	Rodent models (CUMS, 6-OHDA, ischemia, LPS models); human trials	↓ Oxidative stress, ↑ dopamine/5-HT, ↓ cytokines (IL-6, TNF-α), ↓ neuroinflammation, enhanced BDNF, cognitive and behavioral improvements, potential antipsychotic
8	Piper nigrum	Piperine	5–20 mg/day (clinical); synergy with curcumin noted	Rodent models; clinical studies (adjunct in MDD/ psychosis)	↑ Curcumin bioavailability (↑ 2000%), MAO inhibition, ↑ dopamine/ 5-HT, antioxidant, ↑ BDNF, anti-inflammatory, adjunct antipsychotic efficacy
9	Ginger (*Zingiber officinale*)	6-Gingerol, 6-Shogaol, Zingerone	500–1000 mg/day (humans); up to 100 mg/kg (animals)	Ketamine-induced psychosis (mice), clinical trials in depression and cognition	↓ Psychosis-like behavior, ↑ dopamine & serotonin, ↓ TNF-α, IL-6, ↑ BDNF, SOD, catalase
10	Ashwagandha (*Withania somnifera*)	Withanolides (withaferin A, withanone), Sitoindosides	300–600 mg/day (humans); 100–200 mg/kg (animals)	Ketamine-induced psychosis (mice), RCT in schizophrenia	↑ GABA, dopamine; ↓ cortisol, IL-6, TNF-α; ↑ GPx, CAT; Improved PANSS scores, cognition
11	Holy Basil (*Ocimum sanctum*)	Eugenol, Rosmarinic acid, Ocimumosides	~300 mg/day (humans); Variable in animal studies	Chronic stress & behavioral models (mice); clinical anxiety studies	↓ Oxidative stress, anxiety; ↑ cognition, antioxidant enzymes; MAO inhibition; modulates dopamine/ serotonin
12	Saffron (*Crocus sativus*)	Crocin, Safranal, Crocetin, Picrocrocin	30–100 mg/day (humans)	MK-801/ketamine psychosis models; Adjunct trials in schizophrenia	↓ Positive/negative symptoms; ↑ cognition; modulates serotonin/dopamine; ↓ glutamate toxicity
13	Cinnamon (*Cinnamomum verum*)	Cinnamaldehyde, Eugenol, Cinnamic acid	500 mg–2 g/day (Ceylon preferred)	Ketamine psychosis model; Human cognitive/ metabolic studies	↓ Hyperlocomotion; ↑ GABA; ↓ oxidative stress; improves insulin sensitivity and cognition
14	Clove	Eugenol, β-caryophyllene, flavonoids, tannins	10–100 mg/kg	Ketamine-induced psychosis (rodents), inflammation models (mice)	Reversed hyperlocomotion, modulated NMDA/ dopaminergic systems, antioxidant & anti-inflammatory action

15	Nutmeg	Myristicin, elemicin, safrole, eugenol	Not standardized; <5 g/day in humans	FST, oxidative stress, locomotor & behavioral tests (rodents)	Antidepressant, anxiolytic, modulates MAO & NMDA receptors; toxic at high doses
16	Cardamom	1,8-Cineole, α-terpinyl acetate, flavonoids	100–400 mg/kg	FST, EPM, neurotransmitter modulation models (rodents)	Antidepressant, anxiolytic, monoaminergic modulation, reduces IL-6/TNF-α
17	Fenugreek	Diosgenin, trigonelline, flavonoids, saponins	Not standardized	Chronic mild stress, FST, EPM, oxidative stress & cytokine assays (rodents)	Enhances dopamine/ serotonin, increases BDNF, antioxidant/ anti-inflammatory effects
18	Coriander	Linalool, camphor, apigenin, quercetin	Not specified	EPM, OFT, FST, TST, antioxidant & memory tests (rodents)	Anxiolytic, antidepressant, modulates GABA, antioxidant & anti-inflammatory activity
19	Fennel	Anethole, estragole, flavonoids, phenolic acids	Not standardized	FST, EPM, stress-induced behavior models (rodents)	GABAergic modulation, mood improvement, antioxidant & anti-inflammatory effects
20	Ajwain	Thymol, γ-terpinene, p-cymene, flavonoids	Not standardized	EPM, OFT, LPS-induced inflammation, FST (rodents)	Anxiolytic, dopaminergic/ serotonergic modulation, anti-inflammatory, reduces psychosis-like behavior
21	Mustard Seeds (Brassica juncea)	Allyl isothiocyanate (AITC), sinigrin, flavonoids, phenolic acids, selenium, omega-3 fatty acids	AITC: Dose not explicitly stated in rodents; aqueous extract used in scopolamine-induced models	Rodent models: Open Field Test (OFT), Elevated Plus Maze (EPM), scopolamine-induced memory deficits	- ↓ Anxiety-related behaviors (anxiolytic effect) (288) - ↑ Cognitive performance (reversal of memory deficit) (289) - Antioxidant, anti-inflammatory, GABAergic and serotonergic modulation (286–291) - Potential neuroprotection in hippocampus & prefrontal cortex

Table 3. Summary table showing Spices vs. CNS Activity

Sr. No.	Spice / Plant	CNS Activities / Effects	Mechanisms / Bioactive Compounds
1	**Crocus sativus (Saffron)**	- Anti-Alzheimer's - Antidepressant - Anticonvulsant - Neuroprotective	Crocin, crocetin, picrocrocin, safranal Antioxidant, neurotransmitter modulation, opioid system interaction
2	**Nigella sativa**	- Cognitive enhancement - Anti-anxiety - Neuroprotective - Antioxidant	Thymoquinone, p-cymene, carvacrol, thymol Reduces oxidative stress, AChE inhibition
3	**Coriandrum sativum**	- Anxiolytic - Anticonvulsant - Sedative (sleep aid)	Linalool, linoleic acid, monoterpenes GABAergic modulation, anticonvulsant effects

4	**Ferula assafoetida**	- Anticonvulsant - Memory enhancer - Neuroprotective - MAO-B inhibitor	E-1-propyl sec-butyl disulfide, oleo-gum resin AChE inhibition, neuroinflammation reduction
5	**Thymus vulgaris (Thyme)**	- Anxiolytic - Anticonvulsant - Neuroprotective - Antidepressant	Thymol, carvacrol, linalool GABA receptor modulation, antioxidant effects
6	**Zataria multiflora**	- Memory and learning improvement - Antioxidant - Anti-inflammatory	Carvacrol, thymol, beta-caryophyllene, luteolin derivatives Anti-inflammatory, antioxidant
7	**Curcuma longa (Turmeric)**	Antioxidant, anti-inflammatory, neuroprotective, neurotransmitter modulation	- Neuroprotective: antioxidant, anti-inflammatory - Modulates dopamine, serotonin, norepinephrine - Inhibits glutamate neurotoxicity - Upregulates antioxidant enzymes (HO-1, Nrf2) - Suppresses inflammatory cytokines (TNF-α, IL-1β, IL-6) - Inhibits NF-kB and microglial activation - Modulates JAK2/STAT3 signaling - Increases BDNF
8	**Piper nigrum (Black Pepper)**	Monoamine oxidase inhibition, neurotransmitter modulation, bioavailability enhancement	- Enhances bioavailability of curcumin (up to 2000%) - MAO inhibition (raises dopamine, serotonin) - Modulates GABA and glutamate balance - Anti-inflammatory (downregulates TNF-α, IL-6) - Antioxidant - Increases BDNF - Penetrates BBB to exert CNS effects
9	**Zingiber officinale (Ginger)**	Antioxidant, anti-inflammatory, anxiolytic, neurotransmitter modulation	- Anti-inflammatory, antioxidant - Inhibits NF-κB and microglial activation - Lowers pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) - Boosts antioxidant enzymes (SOD, catalase) - Increases dopamine and serotonin levels - Enhances GABAergic transmission, reduces glutamate excitotoxicity - Increases BDNF
10	**Ashwagandha**	Adaptogenic, GABA-mimetic, antioxidant, cortisol reduction	GABA-mimetic activity, dopamine and serotonin modulation, cortisol reduction, anti-inflammatory (↓TNF-α, IL-6), antioxidant (↑GPx, CAT, SOD), neuroprotective, anti-excitotoxic
11	**Holy Basil (Tulsi)**	Adaptogenic, anxiolytic, anti-stress, MAO inhibition, catecholamine modulation, neuroprotection	Modulates catecholamines, inhibits MAO, serotonergic and dopaminergic regulation, HPA axis modulation (↓cortisol), antioxidant (↑GSH, CAT, SOD), neuroprotective
12	**Saffron**	Serotonergic and dopaminergic modulation, NMDA antagonism, neuroprotection, antidepressant, anti-inflammatory	Serotonergic and dopaminergic modulation, NMDA receptor inhibition, antioxidant, anti-inflammatory (NF-κB inhibition), neuroprotective, antidepressant and anxiolytic effects
13	**Clove**	Antipsychotic, Anxiolytic, Neuroprotective	NMDA antagonism, GABAergic modulation, dopamine regulation, antioxidant, anti-inflammatory
14	**Nutmeg**	Antipsychotic-like, Antidepressant, Anxiolytic	MAO inhibition, serotonergic/dopaminergic modulation, NMDA antagonism, anti-inflammatory
15	**Cardamom**	Anxiolytic, Mood-enhancing	GABAergic and serotonergic modulation, monoamine enhancement, antioxidant, anti-inflammatory
16	**Fenugreek**	Neuroprotective, Antidepressant-like	Monoamine modulation, BDNF upregulation, antioxidant, anti-inflammatory, neurotrophic
17	**Coriander**	Anxiolytic, Antidepressant	GABAergic activation, serotonin enhancement, HPA axis regulation, antioxidant, anti-inflammatory
18	**Fennel**	Anxiolytic, Neuroprotective	GABA-mimetic action, serotonin modulation, antioxidant, anti-inflammatory
19	**Ajwain (Trachyspermum ammi)**	Anxiolytic, sedative, antipsychotic-like, neuroprotective	GABA-A receptor modulation, dopaminergic and serotonergic regulation, anti-inflammatory (↓IL-1β, TNF-α), antioxidant
20	**Mustard Seeds (Brassica juncea)**	Anxiolytic, antidepressant-like, cognition-enhancing	Enhances GABAergic/serotonergic tone, ↓ glutamatergic/ dopaminergic overactivity, ↓ oxidative stress and neuroinflammation

Table 4: Summary table showing Preclinical vs. Clinical Evidence of Spices in CNS

Sr. No.	Spice	Preclinical Evidence (Animal/Cell Models)	Clinical Evidence (Human Studies)
1	**Curcuma longa (Turmeric)**	- Increases CNS dopamine, norepinephrine, 5-HT levels - Protects against oxidative brain damage, PD models - Reduces neuroinflammation, ischemia-reperfusion injury - Reverses ketamine-induced psychosis-like behaviors in rats - Reduces proinflammatory cytokines in MPTP-induced animals	- Improved depressive symptoms including psychotic features - Adjunct therapy reducing PANSS scores in schizophrenia - Safe at doses up to 1500 mg/day - Bioavailability improved with piperine co-administration
2	**Piper nigrum (Black Pepper)**	- Cognitive enhancement and anxiolytic effects in rodents - Antidepressant and anti-inflammatory effects - Increases BDNF, modulates neurotransmitters - Inhibits MAO, raises serotonin and dopamine levels - Enhances curcumin bioavailability	- Combined piperine-curcumin treatment improved symptoms in major depressive disorder with psychotic features - Safe at doses 5-20 mg/day in humans - Limited standalone clinical data for psychosis
3	**Zingiber officinale (Ginger)**	- Reduces oxidative stress and behavioral symptoms in ketamine-induced psychosis models - Anxiolytic and mood-enhancing effects in rodents - Increases dopamine, serotonin, and BDNF levels - Antioxidant and anti-inflammatory actions	- Reduced depressive symptoms in type 2 diabetic patients - Improved cognitive function in mild cognitive impairment - Safe at 500-1000 mg/day - Lack of direct clinical trials on schizophrenia or psychosis
4	**Ashwagandha**	Rodent studies: improved memory, reduced stress markers, ketamine-induced psychosis model improvement (181)	RCT: improved schizophrenia symptoms (PANSS scores) (182); improved cognition and mood in psychological stress (183)
5	**Holy Basil (Tulsi)**	Rodent models: decreased anxiety, improved memory, reversed cognitive deficits, oxidative stress reduction (189, 190)	RCT: healthy volunteers showed anxiety reduction, mood stabilization, improved cognition at 300 mg/day (191)
6	**Saffron**	Rodent models: reduced psychosis-like behavior, neuroprotection via crocin and safranal, antidepressant effects (196,197)	Adjunct to risperidone improved negative symptoms of schizophrenia (198); cognition and mood improvements in psychiatric patients (198)
7	**Cinnamon**	Rodent studies: ameliorated ketamine-induced hyperlocomotion, antioxidant and anti-inflammatory effects (204, 205)	Limited clinical trials; evidence mainly from studies on metabolic and cognitive improvements, anxiolytic effects in humans (206)
8	**Clove**	Strong – Ketamine-induced psychosis, neuroinflammation models	Limited – Traditional use, indirect human cognition/stress studies
9	**Nutmeg**	Strong – Antidepressant, anti-inflammatory, behavioral models	Traditional use for agitation, hallucinations; no direct clinical trials
10	**Cardamom**	Moderate – Antidepressant, anxiolytic, oxidative stress models	Traditional calming agent; no psychosis-targeted clinical studies
11	**Fenugreek**	Moderate – CMS models, BDNF upregulation, anti-inflammatory effects	Widely used in Ayurveda; lacks targeted clinical trials
12	**Coriander**	Moderate – Anxiolytic, antioxidant, cognitive enhancement in rodents	Traditional use for anxiety; no psychosis-specific clinical trials
13	**Fennel**	Moderate – GABA-mimetic activity, anti-inflammatory, behavioral assays	Traditional use for anxiety; no direct clinical psychosis trials
14	**Ajwain**	Reduced anxiety in EPM/OFT (Sharma et al., 2020)	

Conclusion

The effects of medicinal plants on the nervous system are the subject of this review, with a particular emphasis on neurotoxicity as measured in several experimental settings (*in vitro* and *in vivo*). The antioxidant actions of the aforementioned medicinal herbs shield neurons from reactive oxygen species (ROS) and enhance superoxide dismutase (SOD) and catalase (CAT) levels, respectively. The 'anti-glutamatergic' or Ca^{2+}, Na^{+}, and K^{+}-lowering activities of these natural substances may contribute to their protective benefits. In the face of illness or injury, neuroprotective agents seek to maintain and safeguard the brain.

The goal of psychotropic drugs is to alter mental processes in order to treat mental illnesses.

Lithium, for example, has both mood-stabilizing and neuroprotective effects, but these are conceptually and functionally different activities. These plants' neuroprotective effects arise from their ability to modulate GABAergic and glutamatergic neurons, reduce inflammatory cytokines while simultaneously increasing anti-inflammatory cytokines, inhibit acetylcholinesterase activity, and lower MDA levels in the brain. Some herbs have been shown to have anti-inflammatory, antioxidant, and immunoregulatory properties, according to data from both basic and clinical studies, which have been used to a number of different conditions. More research is needed in future clinical investigations, however these results support using these herbs and primary ingredient from natural resources in medication development.

Conflict of interest

There is no conflict of interest in this study.

Acknowledgment

None

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Published online in http://ijam.co.in ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Curcumin Reimagined: Harnessing Ionic Liquid Salts for Enhanced Bioavailability and Therapeutic Potential

Review Article

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Abstract

Curcumin, the principal bioactive compound of *Curcuma longa*, has long been celebrated for its potent anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. However, its clinical potential has been severely constrained by poor aqueous solubility, low chemical stability, and rapid systemic elimination, resulting in limited bioavailability. Recent advances in pharmaceutical chemistry have explored novel strategies to overcome these limitations, with ionic liquid salts (ILS) emerging as a promising platform. This review delves into the transformative potential of converting curcumin into ionic liquid salts—either through protonation, salt formation with organic or inorganic cations, or as part of dual-functionalized therapeutic ionic liquids. These modifications significantly enhance curcumin's solubility, permeability, and stability, offering a new paradigm in drug delivery and formulation. The article systematically examines various synthetic approaches, physicochemical characteristics, and in vitro/in vivo studies that demonstrate the superior therapeutic efficacy of curcumin-ILS formulations. Additionally, it explores their potential applications across diverse biomedical domains, including cancer therapy, neuroprotection, antimicrobial coatings, and inflammation regulation. The biocompatibility and tunability of ILS-based systems also make them attractive for targeted and controlled release formulations. Despite promising developments, challenges related to toxicity, scalability, and regulatory approval remain. Future directions include designing task-specific ionic liquids to further tailor curcumin's pharmacokinetics and therapeutic profile.

Keywords: Curcumin, Ionic Liquid Salts, Bioavailability, Drug Delivery, Therapeutic Applications.

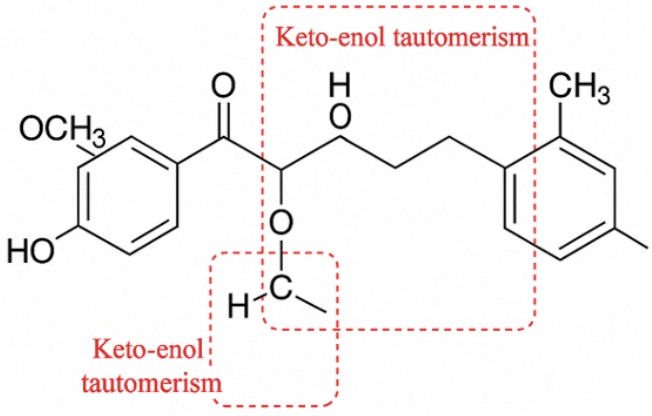
Introduction

The rhizome of Curcuma longa yields curcumin, a hydrophobic polyphenol that is well known for its use in both traditional medicine and cooking. Its distinctive yellow colour and biological activity are attributed to its structure, which consists of two ortho-methoxy phenolic groups joined by a heptadiene-dione linker (1). Curcumin has been used ethnopharmacologically for millennia, but in the contemporary period, its broad range of pharmacological actions and comparatively low toxicity profile have drawn scientific attention. Figure 1 depicts the chemical structure of curcumin, emphasising its hydrophobic constituents and its keto-enol tautomerism. Its limited biological activity and water solubility are caused by these characteristics.

Therapeutic Potential and Limitations

By altering molecular targets such NF-κB, STAT3, COX-2, TNF-α, and different caspases, curcumin has shown anticancer, anti-inflammatory, antioxidant, antibacterial, and neuroprotective qualities (2,3).

Figure 1. Chemical structure of curcumin highlighting its keto-enol tautomerism and hydrophobic moieties



Curcumin's limited water solubility (~11 ng/mL), instability at physiological pH, and significant first-pass metabolism in the liver and gut, however, limit its therapeutic usefulness (4).

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Table 1: Limitations of native curcumin and their pharmacological implications

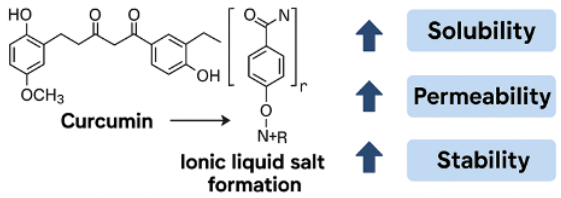
Limitation	Pharmacological Impact
Poor aqueous solubility	Low systemic absorption
Chemical instability	Degradation under light, heat, and pH variation
Rapid metabolism	Short plasma half-life
Low oral bioavailability	Limited therapeutic efficacy

Rationale for Ionic Liquid Salt (ILS) Approach

In pharmaceutical formulations, ionic liquids (ILs), which are often described as organic salts with melting temperatures lower than 100°C, have become adjustable solvents and carriers. Curcumin may show markedly improved solubility, chemical stability, and membrane permeability when it is paired with biocompatible cations or anions to form an ionic liquid salt (5). Furthermore, the ILs themselves may be modified to have biological activity that complements the pharmacodynamics of curcumin. The fictitious schematic of the synthesis of an ionic liquid salt containing curcumin in Figure 2 illustrates how curcumin enhances solubility, membrane permeability, and molecular stability.

Forming curcumin-ILS enhances its delivery and therapeutic results in cancer and inflammatory models, according to recent research (6).

Figure 2: Conceptual schematic of curcumin-based ionic liquid salt formation and its effect on solubility, permeability, and stability



Scope and Structure of the Review

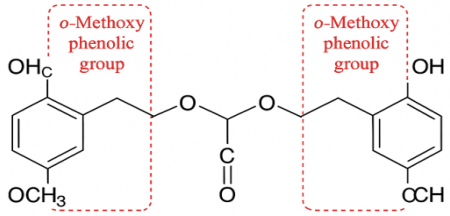
The conversion of curcumin into ionic liquid salts (Cur-ILS) and how this development improves its therapeutic profile are the main topics of this review. After providing a summary of the pharmacokinetic and chemical difficulties associated with curcumin, we go into great detail on the principles of ionic liquids and how they are designed. The synthesis, characterisation, biological effectiveness, and safety aspects of several Cur-ILS systems are then examined. Lastly, we discuss the translational potential and possible future applications of Cur-ILS formulations in personalised treatment and nanomedicine.

Chemical Properties and Pharmacokinetics of Curcumin

Structure and Functional Groups

A symmetrical diarylheptanoid with two aromatic ring systems connected by a seven-carbon α,β-unsaturated β-diketone chain, curcumin ($C_{21}H_{20}O_6$) is the main curcuminoid of turmeric (Curcuma longa) (Figure 1). Its anti-inflammatory and antioxidant qualities are attributed to the presence of hydroxyl (-OH) and methoxy (-OCH_3) groups in each phenyl ring (7). As observed in Figure 3, curcumin's symmetrical diarylheptanoid backbone, two o-methoxy phenolic groups, and a central β-diketone moiety demonstrate its pharmacological actions.

Figure 3. Chemical structure of curcumin showing symmetrical diarylheptanoid backbone with two o-methoxy phenolic groups and a β-diketone moiety



These functional groups play crucial roles in curcumin's bioactivity:

- **Phenolic OH groups**: Responsible for free radical scavenging.
- **Methoxy groups**: Enhance electron-donating ability.
- **β-Diketone moiety**: Enables metal chelation and tautomerization between keto and enol forms (8).

Solubility, Stability, and Bioavailability Challenges

Curcumin's oral bioavailability is severely limited by its near-insoluble nature in water (~11 ng/mL at room temperature) (9). Although its limited water solubility hinders absorption and systemic circulation, it is more soluble in organic solvents such as ethanol, DMSO, and acetone (10).

Additionally, hydrolysis and photodegradation cause curcumin to rapidly degrade at physiological pH, losing its therapeutic effectiveness (11). Even with large oral dosages, its instability and limited absorption lead to low plasma concentration.

Table 2: Key physicochemical challenges of curcumin affecting pharmacokinetics

Property	Observation	Impact on Bioavailability
Water solubility	~11 ng/mL	Poor oral absorption
pH stability	Unstable in neutral/ alkaline pH	Rapid degradation in gut
Metabolism	Extensive first-pass metabolism	Low systemic bioavailability
Photostability	Degrades under light exposure	Reduces shelf-life and efficacy

Numerous approaches, such as complexation with cyclodextrins, nanoformulations, and most recently, the creation of ionic liquid salts, have been investigated to get around these restrictions (12).

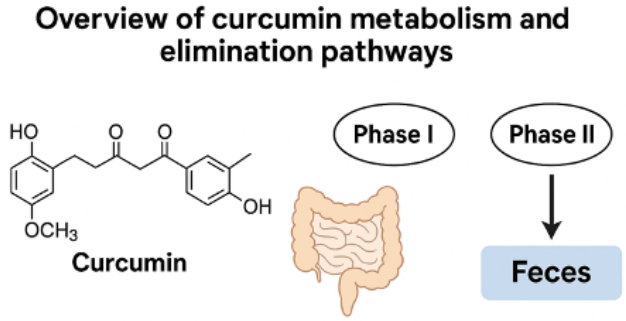
Metabolism and Systemic Elimination

After oral administration, curcumin undergoes extensive first-pass metabolism in the liver and intestinal wall. It is metabolized via:

- **Phase-I reduction** to dihydrocurcumin, tetrahydrocurcumin, and hexahydrocurcumin.
- **Phase II conjugation** to glucuronides and sulfates (13).

Even while these metabolites have some bioactivity, they often lack the potency of pure curcumin. Faecal excretion is the main method of elimination, with renal excretion being negligible. Research indicates that less than 1% of curcumin taken orally enters the bloodstream in its free form (14). Figure 4, which provides a summary of the metabolism and elimination processes of curcumin after absorption, including Phase I reduction and Phase II conjugation events, illustrates that the intestinal route accounts for the majority of its excretion.

Fig. 4. Overview of curcumin metabolism and elimination pathways. After absorption, curcumin is reduced (Phase I) and conjugated (Phase II), with elimination primarily via faeces



Ionic Liquid Salts: Fundamentals and Design Principles

Definition and Classification

Ionic Liquid Salts (ILS) are salts that stay liquid at room temperature or almost there, often below 100°C. These salts are made completely of ions, usually consisting of counter anions and large, asymmetric organic cations. ILS's low melting points, which are far lower than those of conventional salts like sodium chloride, give them their special qualities (15).

ILS can be broadly classified based on:

- **Cation type**: Imidazolium, pyridinium, cholinium, phosphonium, and cations derived from amino acids are examples of common cations (16).
- **Anion type**: Simple halides like chloride and bromide as well as more complicated, functionalised anions like tetrafluoroborate ($[BF_4]^-$) and bis(trifluoromethylsulfonyl)imide ($[NTf_2]^-$) are examples of counterions.
- **Temperature stability**: High-temperature ionic liquids (HTILs) need higher temperatures to stay liquid, while room-temperature ionic liquids (RTILs) stay liquid at room temperature.
- **Protic vs. aprotic**: While aprotic ionic liquids do not include protonated species, prototic ionic liquids (PILs) are made from protonated organic molecules (17).

Table 3: Classification of Ionic Liquid Salts (ILS)

Classification Criteria	Examples	Characteristics
Cation Type	Imidazolium, Pyridinium, Cholinium	Organic cations of varying sizes
Anion Type	$[BF_4]^-$, $[NTf_2]^-$	Simple or functionalized anions
Temperature Stability	RTILs, HTILs	Liquid at room or elevated temperatures
Protic/Aprotic	PILs, APILs	Protonated or non-protonated cations

(RTIL = Room-Temperature Ionic Liquid, HTIL = High-Temperature Ionic Liquid, PIL = Protic Ionic Liquid, APIL = Aprotic Ionic Liquid)

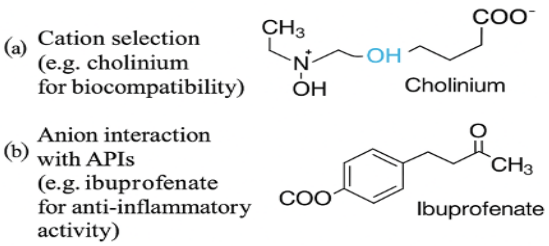
Design of Biocompatible and Therapeutic ILS

Designing ILS with biocompatibility and therapeutic effectiveness in mind is essential for biomedical applications, especially drug delivery. Essential guidelines for the development of biocompatible ILS comprise:

1. **Selection of biocompatible cations and anions**: Because of their non-toxicity and positive interactions with biological systems, cations such cholinium (made from the natural chemical choline), cations based on amino acids, and certain imidazolium derivatives see extensive application (18).
2. **Pharmacological compatibility**: Functional groups may be included into ILS design to enhance solubility, stability, and targeting by interacting favourably with APIs. Hydrophobic medications, such as curcumin, may have their solubility improved using ILS produced from amino acids (19).
3. **Controlled release**: One way to tailor ILS for a particular delivery route (oral, transdermal, injectable) and controlled release profile is to modify its physicochemical characteristics (20).

The design approach for biocompatible and therapeutic ionic liquid salts (ILS) is shown in Fig. 5. It stresses (a) the use of cholinium and other biocompatible cations and (b) the use of ibuprofenate and other functional anion pairings to impart therapeutic activity.

Figure 5: Design strategy for biocompatible and therapeutic ILS: (a) Cation selection (e.g., cholinium for biocompatibility), (b) Anion interaction with APIs (e.g., ibuprofenate for anti-inflammatory activity).



Design strategy for biocompatible and therapeutic ILS

Table 4: Examples of Biocompatible Ionic Liquids for Drug Delivery

Cation Type	Anion Type	Drug/Active Ingredient	Application
Cholinium-based	$[NTf_2]^-$	Curcumin	Solubilization, oral delivery
Imidazolium-based	$[BF_4]^-$	Paclitaxel	Chemotherapy, injectable
Amino acid-based	$[Cl]^-$	Ibuprofen	Anti-inflammatory, transdermal

Physicochemical Properties Relevant to Drug Delivery

ILS have numerous unique physicochemical characteristics that make them perfect for drug delivery uses:

- **Low viscosity**: Many ILS have low to moderate viscosities, which helps to improve the dissolving rate and guarantee consistent medication distribution (21).
- **High solubility for hydrophobic compounds**: By lowering interfacial tension and creating stable solutions, ILS may solvate poorly soluble medications, hence improving bioavailability (22).
- **Tunable properties**: Changing the cation or anion structure can help to customise ILS characteristics such polarity, hydrophilicity, and thermal stability to fit certain drug delivery requirements (23).
- **Thermal stability**: Often thermally stable, ILS may maintain integrity under physiological settings, particularly for sustained-release formulations (24).
- **Biocompatibility and biodegradability**: Some ILS are made with natural or biodegradable materials, hence guaranteeing low toxicity and safe breakdown within the body (25).

Table 5: Physicochemical Properties of ILS Relevant to Drug Delivery

Property	Impact on Drug Delivery
Viscosity	Facilitates easy injection and improves dissolution rates
Solubility	Enhances bioavailability of poorly soluble drugs
Polarity	Customizable to optimize interaction with drug molecules
Thermal Stability	Ensures consistency in drug release over a wide temperature range
Biocompatibility	Reduces toxicity and enhances safety for therapeutic use

Synthesis and Characterization of Curcumin-Based Ionic Liquid Salts

Synthetic Strategies

Curcumin, a naturally occurring polyphenol, is functionalised with suitable ionic liquid cations and anions to create curcumin-based ionic liquid salts (CB-ILS). To maximise curcumin's solubility, stability, and bioavailability, many synthesis pathways have been investigated:

1. **Ion exchange methods**: This calls for using a protic ionic liquid, like choline chloride or imidazolium-based cations, in a solvent to react curcumin and create the curcumin ionic liquid salt. The approach is rather straightforward and allows control of the drug's ionic form (26).
2. **Direct neutralization**: This method starts with curcumin reacting with a suitable acid—e.g., organic or inorganic acids—to create the salt, then couples with a cation like choline or a biocompatible amino acid derivative (27). Often used to improve solubility, this technique guarantees that curcumin is in its bioactive ionic state.
3. **Solvent-free synthesis**: Curcumin may be made using ionic liquids under solvent-free conditions to prevent the use of organic solvents, which might raise toxicity and environmental issues. This sustainable strategy increases curcumin's stability as well as its solubility (28).

Table 6: Synthesis Methods for Curcumin-Based Ionic Liquid Salts

Method	Description	Example
Ion exchange	Reaction with protic ionic liquids	Curcumin with cholinium chloride
Direct neutralization	Neutralization with acid, followed by ion pairing	Curcumin with amino acids (e.g., glycine)
Solvent-free synthesis	Ionic liquids used without solvent	Curcumin with imidazolium cations

Analytical Techniques (NMR, FTIR, DSC, TGA, etc.)

Confirming the chemical structure, purity, stability, and thermal behaviour of curcumin-based ionic liquid salts depends on their characterisation. This goal is served by many methods:

1. **Nuclear Magnetic Resonance (NMR)**: NMR spectroscopy reveals the molecular structure of curcumin-based ionic liquids. Detailed information on the cationic and anionic components is provided by proton (^1H) and carbon (^13C) NMR, hence verifying the effective ionic pairing and the integrity of the curcumin molecule (29).
2. **Fourier Transform Infrared Spectroscopy (FTIR)**: FTIR enables to detect functional groups, hence verifying the existence of curcumin's distinctive phenolic and methoxy groups. Changes in the C=O stretching band in curcumin-based ILs support their creation and structural confirmation (30).
3. **Differential Scanning Calorimetry (DSC)**: DSC evaluates the thermal behaviour and melting temperatures of curcumin-based ionic liquid salts. A drop in the melting point relative to pure curcumin suggests effective ionic liquid creation (31).
4. **Thermogravimetric Analysis (TGA)**: Ionic liquid salts based on curcumin may have their thermal stability assessed with the use of TGA. It guarantees the ionic liquid salts' stability under physiological circumstances by revealing the decomposition temperatures (32).
5. **X-ray Diffraction (XRD)**: XRD identifies the crystalline or amorphous character of curcumin-

ILS. Usually, the amorphous character of ILS suggests more solubility, which helps with medication distribution (33).

Table 7: Common Analytical Techniques for Characterization of Curcumin-Based Ionic Liquid Salts

Technique	Purpose	Insight Gained
NMR	Confirm molecular structure	Verification of cation-anion pairing
FTIR	Identify functional groups	Confirmation of curcumin's functional groups
DSC	Assess thermal properties	Melting point and stability of salts
TGA	Evaluate thermal stability	Decomposition temperatures and stability
XRD	Determine crystallinity	Amorphous or crystalline nature of salts

Stability and Shelf-life Assessments

For curcumin-based ionic liquid salts intended for medicinal use, stability and shelf-life are very important. The chemical and physical interactions between curcumin and the ionic liquid components determine the stability of these ILs. Important techniques for stability testing are:

1. **Chemical stability**: Monitoring deterioration under many conditions—including pH, light exposure, and temperature—helps to determine the stability of curcumin-ILS. Antioxidants and stabilising chemicals help to prolong these ionic liquid salts (34).
2. **Physical stability**: Changes in the appearance, such as crystallisation or phase separation, can suggest physical instability. To guarantee constant solubility and bioavailability, curcumin-ILS should stay in a homogenous, non-crystalline condition (35).
3. **Shelf-life testing**: The goal of doing accelerated shelf-life testing is to mimic the circumstances of actual storage, which include high temperatures and humidity. If the formulation's effectiveness or curcumin concentration decreases over time, then the product has reached the end of its shelf life (36).

Figure 6: Stability Assessment of Curcumin-Based Ionic Liquid Salts: (a) Chemical stability under acidic conditions, (b) Physical stability under varying temperature and humidity

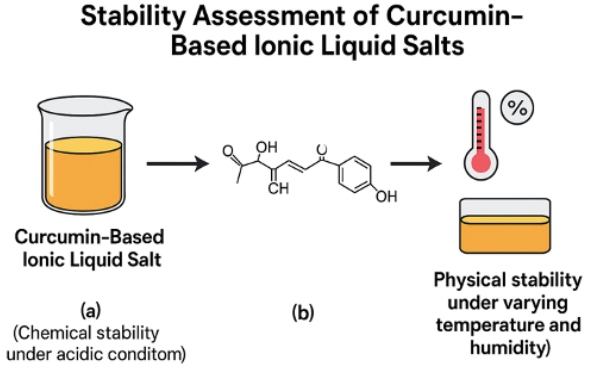


Figure 6 displays the results of the stability assessment of curcumin-derived ionic liquid salts. The results highlight (a) the salts' enhanced chemical stability in acidic conditions and (b) their enhanced physical stability in surroundings with varying degrees of humidity and temperature.

Table 8: Stability and Shelf-life Testing for Curcumin-ILS

Test	Purpose	Methodology
Chemical stability	Assess degradation over time	pH variation, light exposure, temperature tests
Physical stability	Monitor crystallization or phase separation	Visual inspection, centrifugation, solubility tests
Shelf-life testing	Estimate product longevity	Accelerated stability tests, storage at varying conditions

Enhancement of Curcumin's Bioavailability Using ILS

Improved Solubility and Permeability

Curcumin has limited therapeutic effectiveness due to its poor gastrointestinal absorption and low water solubility. By modifying drug partitioning behaviour and boosting mucosal diffusion, ionic liquid systems (ILS), especially those based on imidazolium and choline, have the potential to greatly increase the solubility and membrane permeability of curcumin (37).

Protection Against Degradation

At physiological pH, curcumin degrades quickly. Curcumin may be protected against hydrolysis and photodegradation by creating a stabilising microenvironment with the help of ILS. The chemical stability of curcumin is improved by research showing that ILs may create supramolecular complexes that encase its unstable keto-enol group (38).

Table 9: Summary of Curcumin-ILS Systems and Bioavailability Improvements

ILS Type	Curcumin Solubility Fold Increase	Main Mechanism
Choline chloride IL	~50×	Hydrogen bonding, water structuring (37)
Imidazolium IL	~30–80×	Micelle-like aggregation (38)

Pharmacokinetic Studies and In Vivo Efficacy

Both the systemic bioavailability and the plasma half-life of curcumin-ILS formulations are considerably improved, according to in vivo pharmacokinetic investigations. In inflammatory and cancer models, animal studies show that curcumin accumulates better in target tissues and has better therapeutic effects (39).

Therapeutic Applications of Curcumin-ILS Systems

Anti-inflammatory and Antioxidant Activity

When curcumin is administered by ILS, its anti-inflammatory effects are enhanced because it inhibits NF-κB and COX-2. This, in turn, increases cellular

absorption and redox regulation both in laboratory settings and in living organisms (40).

Anticancer Potential

By increasing apoptotic signalling, ROS production, and mitochondrial membrane rupture, curcumin-ILS systems have shown enhanced cytotoxicity in breast, colon, and prostate cancer cell lines (41).

Neuroprotective Effects

Researchers have shown that curcumin delivered by intravenous ligation has neuroprotective effects in Alzheimer's and Parkinson's disease models. This is achieved by decreasing neuroinflammation and β-amyloid aggregation, as well as enhancing the permeability of the blood-brain barrier (42).

Table 10: Therapeutic Efficacy of Curcumin-ILS Systems in Preclinical Models

Disease Area	Animal/Cell Model	Outcome
Inflammation	LPS-induced mouse model	↓ TNF-α, IL-6, edema (40)
Breast Cancer	MCF-7 cell line	↑ Apoptosis, ↓ proliferation (41)
Alzheimer's Disease	APP/PS1 transgenic mice	↓ Amyloid plaques, ↑ memory scores (42)

Antimicrobial and Antiviral Uses

Because they are more able to penetrate cell membranes and retain cells, mixtures of curcumin and ILS have antiviral action against influenza and SARS-CoV-2 and increased antibacterial activity against E. coli and Staphylococcus aureus (43).

Wound Healing and Skin Applications

For improved transdermal administration and prolonged release, topical formulations using curcumin-ILS gels expedite wound contraction, epithelium regeneration, and collagen deposition (44).

Toxicological and Safety Considerations
Biocompatibility Studies

The majority of research indicates that, when administered at low quantities, ILs formed from naturally existing cations (such as choline or amino acids) are safe and biocompatible. Curcumin may be effectively transported in pharmaceutical systems by these biogenic ILs (45).

Cytotoxicity Assays

The structure of IL determines its cytotoxicity. Modifying the cation/anion mix of ILs may reduce the moderate cytotoxicity seen at higher dosages of some imidazolium-based ILs. In normal cell lines, curcumin-ILS formulations are generally well-tolerated (46).

Regulatory and Approval Perspectives

Despite the lack of widespread approval, regulatory interest in IL-based medication systems is on the rise. The preclinical study of amino acid ILs and GRAS (Generally Recognised as Safe) ILs, such as choline chloride, is being conducted with the purpose of expanding their pharmaceutical application (47).

Challenges and Limitations in Clinical Translation
Scale-Up and Manufacturing Barriers

There is a lack of scalable synthesis techniques and the high cost and restricted availability of pharmaceutical-grade ionic liquids, which hinder industrial-scale manufacturing of curcumin-ILS systems, despite encouraging evidence from lab-scale studies. Another obstacle to clinical preparedness is the safe and effective purification and recycling of ILs (48).

Regulatory Hurdles

Currently, pharmacopeial monographs do not generally acknowledge ionic liquids. Toxicological data, biocompatibility profiles, and long-term environmental safety evaluations are all necessary for regulatory bodies to approve IL-based medication formulations for human use (49).

Long-Term Safety and Environmental Concerns

Biocompatible ILs have shown acceptable short-term safety, however there is a lack of information on their accumulation, degradation products, chronic exposure, and safety. Concerns about ecotoxicity and their environmental destiny also arise when thinking about pharmacological deployment on a wide scale (50). Figure 7 shows the major problems of translating curcumin-ILS systems from the lab to the clinic, including problems with production, toxicity evaluation, scaling, and regulatory approval, among other things.

Figure 7: Translational Barriers of Curcumin-ILS

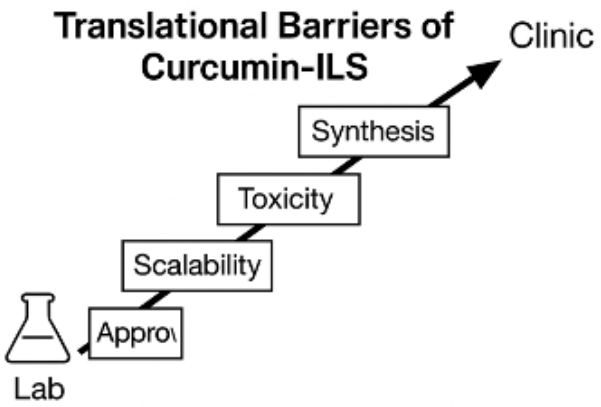


Table 11: Key Clinical Translation Barriers of ILS-Based Formulations

Barrier	Impact on Translation	Potential Mitigation
Lack of scalable production	Limits mass manufacture	Continuous flow synthesis (48)
Unknown long-term toxicity	Hinders regulatory clearance	In vivo chronic toxicity studies
Regulatory classification gaps	Delays clinical trials	ILS classification frameworks

Future Perspectives and Emerging Trends

Task-Specific Ionic Liquids for Personalized Therapy

One promising approach is the development of ionic liquids (ILs) tailored to particular pharmacological requirements, such as improved solubility, stability, or tissue targeting. This innovative idea is called task-specific ionic liquids (TSILs). One potential future direction for precision therapies is hybrids of curcumin and TSIL that target either inflammation or the mucosa (51).

Hybrid Systems and Nanocarriers

ILS has two advantages when used with nanocarriers such as liposomes, micelles, or dendrimers: enhanced loading and regulated release. Compared to non-ionic systems, ILS-loaded curcumin nanoparticles show increased cellular absorption and sustained release (52).

Integration with Smart Drug Delivery Platforms

For site-specific release, ILs may be combined with smart platforms like pH-sensitive carriers or stimuli-responsive hydrogels. For example, curcumin release is only possible in inflammatory or tumoral tissues thanks to ILs with pH-triggered moieties, which raises the therapeutic index (53). Fig. 8, which depicts recent advancements in curcumin-ILS delivery platforms, shows a conceptual progression from task-specific ionic liquids (TSILs) to hybrid IL-nanocarriers and, ultimately, to complex stimuli-responsive drug delivery systems.

Figure 8: Emerging Trends in Curcumin-ILS Delivery

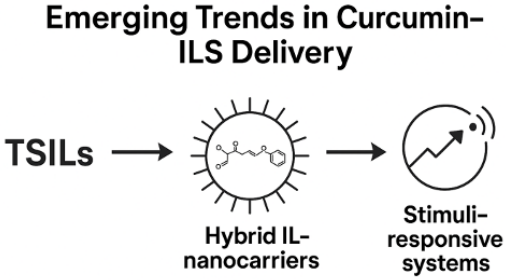


Table 12: Innovations in Curcumin-ILS Drug Delivery

Technology	Advantage	Application
Task-specific ILs	Custom targeting, dual functionality	Anti-cancer, GI targeting (51)
IL-loaded liposomes	Enhanced loading, longer circulation	Neurodegeneration (52)
pH-sensitive IL hydrogels	Triggered release in tumor/inflamed sites	Oncology, arthritis (53)

Conclusion

Curcumin, a polyphenolic molecule with several therapeutic potentials, encounters significant obstacles in clinical use owing to its inadequate solubility, instability, and fast systemic clearance. This study examined the novel use of Ionic Liquid Salts (ILS) to address these challenges, providing a potential framework to improve the bioavailability, stability, and pharmacokinetic efficacy of curcumin. The synthesis and characterisation of curcumin-based ionic liquid systems have exhibited notable enhancements in aqueous solubility and metabolic stability, indicating substantial preclinical efficacy in various applications, such as anti-inflammatory, anticancer, neuroprotective, antimicrobial, and wound healing therapies. Moreover, ILSs provide regulated release profiles and targeted distribution, establishing them as optimal candidates for intelligent and task-specific drug delivery systems. Despite the obvious pharmaceutical benefits of curcumin-ILS systems, issues of toxicity, regulatory ambiguity, and difficulty in scaling must be thoroughly resolved. Long-term evaluations of biocompatibility and environmental effect are crucial for ensuring safe clinical integration. Emerging developments, including task-specific ionic liquids, hybrid nanosystems, and intelligent delivery matrices, signify the next frontier for personalised and responsive therapeutic treatments.

In conclusion, the ILS method provides a revolutionary avenue to reconceptualise curcumin as a therapeutically feasible therapy. Multidisciplinary cooperation among pharmaceutical chemistry, toxicology, regulatory science, and materials engineering will be essential to effectively use and safely transition these systems from laboratory to clinical application.

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https://doi.org/10.47552/ijam.v16iS2.6201 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Green Alchemy: Unveiling the Therapeutic Synergy of Tulsi, Aloe Vera, and Piper betle

Review Article

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Abstract

Traditional medicinal herbs have once again captured the attention of people throughout the world due to the growing need for safer, more environmentally friendly therapeutic options derived from plants. For their wide-ranging pharmacological effects, three of the most venerated plants in traditional medicine—*Ocimum sanctum (Tulsi), Aloe barbadensis miller (Aloe Vera), and Piper betle*—stand out. Reviewing the antibacterial, antifungal, antioxidant, and anti-inflammatory characteristics of these three herbs, this study delves into their synergistic potential. Each herb has strong bioactivity on its own, but by combining them, phytoconstituents like eugenol, aloin, and chavicol may unleash even more effectiveness. This study synthesises data from in vivo, clinical, and in vitro investigations to determine how these herbs influence inflammatory pathways, fight microbial resistance, and scavenge reactive oxygen species (ROS). When applied to wound healing, skin care, dental hygiene, and immunological modulation, the synergy shows great promise. There is scientific evidence that suggests new multi-herb therapies may be possible due to phytochemical interactions' additive or even supra-additive effects. Some of the recent difficulties with standardisation, bioavailability, and formulation, as well as some of the potential benefits of using these plants in herbal medicines of the future. Tulsi, Aloe Vera, and Piper betle form a potent trinity for forthcoming biomedical advancement, which is particularly relevant given the rising popularity of green therapies and evidence-based Ayurveda.

Keywords: *Tulsi, Aloe Vera, Piper Betle*, Phytochemical Synergy, Antimicrobial Activity.

Introduction

Overview of Herbal Medicine Resurgence in Integrative and Modern Therapeutics

Natural, holistic, and side-effect-free therapeutic approaches have been in high demand this century, and herbal medicine has seen a considerable renaissance as a result. There has been a resurgence of interest in phytotherapy as a possible solution to the growing problem of antibiotic resistance and other global health crises, thanks to its ability to combine traditional treatment with scientific data. Complementary and integrative health systems increasingly rely on herbal products, which are backed by growing consumer awareness and solid scientific evidence (1, 2).

Complex, multifactorial illnesses including cancer, diabetes, and neurodegenerative disorders are difficult to treat with conventional treatment, but plant-based therapies have the advantage of multi-targeted activities (3).

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Rationale for Combining *Tulsi* (*Ocimum sanctum*), *Aloe vera*, and *Piper betle*

Tulsi, Aloe vera, and Piper betle are three of the many therapeutic plants that have wide-ranging pharmacological benefits. Ayurveda regards Tulsi (Ocimum sanctum) with great reverence because of its adaptogenic, immunomodulatory, and antibacterial qualities; it is also regarded as the "elixir of life" (4). For a long time, people have turned to aloe vera, often called a "wonder plant," for its anti-inflammatory, wound-healing, and gastrointestinal advantages. These benefits are due to components such acemannan and aloin (5). Bioactive compounds found in the Southeast Asian plant piper betle, such as chavibetol and hydroxychavicol (6), provide it strong antioxidant, antibacterial, and antidiabetic effects.

Because various botanicals work in different ways and may even have synergistic benefits when combined, it makes sense to use them together. Pharmacodynamic and pharmacokinetic synergy, including increased bioavailability, target modulation, and decreased toxicity, may increase the effectiveness of a combination of plants, each of which has its own set of therapeutic advantages (7). Both classical formulations and contemporary systems biology viewpoints are compatible with this polyherbal strategy.

Concept of "Green Alchemy" and Polyherbal Synergy

Green alchemy is a metaphor for the science and art of botanical synergy, which aims to maximise medicinal efficacy while reducing adverse effects. This is in keeping with the old Ayurvedic concepts of Rasayana, which aim to revitalise, balance, and enhance the physiological systems via the use of certain combinations. Phytoconstituent interactions in polyherbal synergy may have additive or multiplicative effects, producing results that are larger than the sum of their parts (8). Researchers can now methodically decipher these intricate relationships with to scientific developments like omics technology and network pharmacology.

Figure 1: Synergistic Pharmacological Targets of Tulsi, Aloe Vera, and Piper Betle

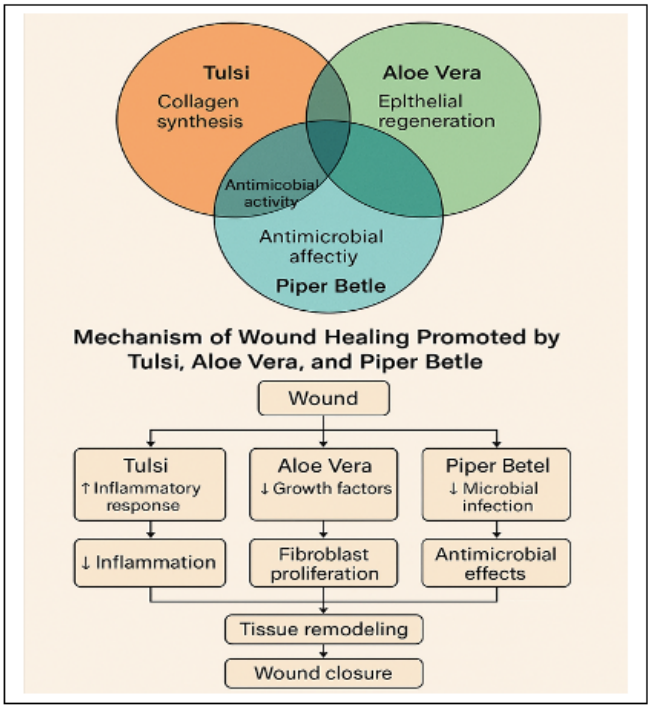


Table 1: Comparative Phytochemical Profile of Tulsi, Aloe Vera, and Piper Betle (9)

Plant	Major Phytochemicals	Therapeutic Effects
Ocimum sanctum	Eugenol, ursolic acid, apigenin	Antioxidant, adaptogen, antimicrobial
Aloe vera	Acemannan, aloin, glucomannan	Wound healing, anti-inflammatory, digestive aid
Piper betle	Chavibetol, hydroxychavicol, eugenol	Antimicrobial, antidiabetic, antioxidant

Ethnobotanical and Historical Perspectives

Traditional Uses across Ayurvedic, Siddha, Unani, and Folk Medicine

It is well-established that Aloe vera, Piper betle, and Ocimum sanctum (Tulsi) are used in traditional medical systems. Tulsi is a rasayana plant in Ayurveda, which means it may help you live longer, adapt better to stress, and strengthen your immune system (10). Aloe vera, also known as Kumari, is used for digestive, menstrual, and skin problems, and it is given to patients with fevers and respiratory problems in Siddha medicine (11). Decoctions or poultices containing Piper betle are often utilised in Unani and folk systems due to its warming, stimulating, and carminative characteristics (12).

Cultural Significance and Ritualistic Roles

These plants are very significant culturally and spiritually, in addition to their medical benefits. As a living goddess representing innocence and safety, Tulsi is revered in Hindu homes as holy (13). A variety of religious and cosmetic traditions have made use of aloe vera, which is referred to as the "plant of immortality" in ancient Egyptian writings (14). The piper betle is a sacred Leaf in several Southeast Asian, Hindu, and Buddhist ceremonies, including marriages and hospitality rites (15).

Historical Co-usage and Empirical Knowledge Base

Traditional polyherbal formulations make use of these plants in an empirical way, with combinations being constructed according to prakriti (body constitution) and guna (qualities). Charaka Samhita and Bhavaprakasha are only two examples of the ancient texts and materia medica that discuss their simultaneous use for vata, pitta, and kapha dosha balance (16). Although these synergies have always made sense intuitively, current research is using mechanical evidence to validate them.

Phytochemical Profiles: Comparative and Synergistic Composition

Key Bioactive Constituents of Tulsi, Aloe Vera, and Piper Betle

- **Tulsi**: eugenol, ursolic acid, apigenin, rosmarinic acid
- **Aloe vera**: acemannan, aloin, barbaloin, aloe-emodin
- **Piper betle**: hydroxychavicol, chavibetol, eugenol, allylpyrocatechol

The anti-inflammatory, antibacterial, antioxidant, and adaptogenic bioactivities are conferred by these components. (16–18)

Flavonoids, Alkaloids, Terpenes, Phenolics, and Glycosides

These plants share key phytochemical classes:

- **Flavonoids** (apigenin, luteolin) in *Tulsi (17)*
- **Phenolics** (hydroxychavicol) in *Piper betle (18)*
- **Terpenoids** (ursolic acid, lupeol) in *Tulsi* and *Aloe vera (19)*
- **Anthraquinone glycosides** (aloin, aloe-emodin) in *Aloe vera* (20)

Synergistic Interactions at the Phytochemical Level

Recent research has brought attention to phytochemical synergy, in which the use of many compounds increases their effectiveness via additive or multiplicative processes. Acemannan improves the

https://doi.org/10.47552/ijam.v16iS2.6201 ISSN No: 0976-5921
Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

mucosal absorption of co-administered phytoconstituents, whereas eugenol and hydroxychavicol suppress bacterial quorum sensing and ROS formation (20, 21).

Analytical Techniques for Compound Identification

Advanced analytical tools are pivotal:

- **HPLC and LC-MS** for flavonoid and glycoside quantification (20)
- **GC-MS** for volatile oils (e.g., eugenol, chavibetol) (21)
- **NMR** for structural elucidation of active compounds (22, 23)

Pharmacological Activities and Molecular Mechanisms

4.1 Antioxidant and Anti-inflammatory Actions

All three botanicals exhibit strong free-radical scavenging properties:

- *Tulsi* has eugenol and rosmarinic acid that block COX-2 pathways and lipid peroxidation. (24).
- *Aloe vera* lowers pro-inflammatory cytokines, including IL-6 and TNF-α. (24).
- In macrophages, *Piper betle's* hydroxychavicol inhibits NF- κB activation and NO generation (25).

Immunomodulatory Effects

Tulsi increases NK cell activity and Th1/Th2 balance, which regulates humoral and cell-mediated immunity (26). Piper betle alkaloids enhance leukocyte proliferation and cytokine balance, whereas aloe vera polysaccharides such acemannan promote macrophage and T-cell proliferation (27).

Antimicrobial and Antiviral Activities

Synergistic antimicrobial effects have been documented:

- Tulsi essential oil inhibits *E. coli*, *S. aureus*, and *Candida albicans* (28)
- Aloe vera gel shows bacteriostatic effects on *P. aeruginosa* and *H. pylori* (28)
- Piper betle extract disrupts biofilms and inhibits multidrug-resistant strains (29)

Mechanistic Insights: Signaling Pathways and Gene Modulation

These botanicals modulate key pathways:

- *Ocimum sanctum* (Tulsi), *Aloe vera*, and *Piper betle* have been shown to influence the JAK/STAT, MAPK, and NF-κB for inflammation
- *Aloe vera* and *Ocimum sanctum* activate PI3K/Akt for the survival of cells
- *Piper betle*—modulate the caspase cascade for apoptosis (30)

Therapeutic Applications in Modern Medicine

Wound Healing and Dermatological Applications

Ocimum sanctum, or tulsi, has shown strong wound-healing qualities because to its antibacterial, anti-inflammatory, and antioxidant qualities. Tulsi topical preparations speed up tissue remodelling and collagen production (31). Mucopolysaccharides and growth factors found in aloe vera are well known for promoting angiogenesis, fibroblast activity, and epithelial regeneration (32). The antibacterial properties of piper betle leaves help to speed up wound healing and combat skin infections (33).

Figure 2: Ethnopharmacological and Cultural Integration of Tulsi, Aloe Vera, and Piper Betle

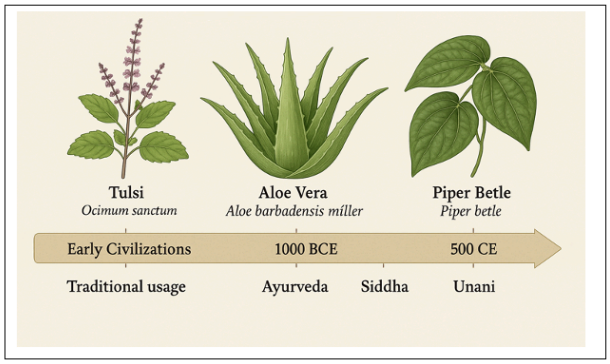
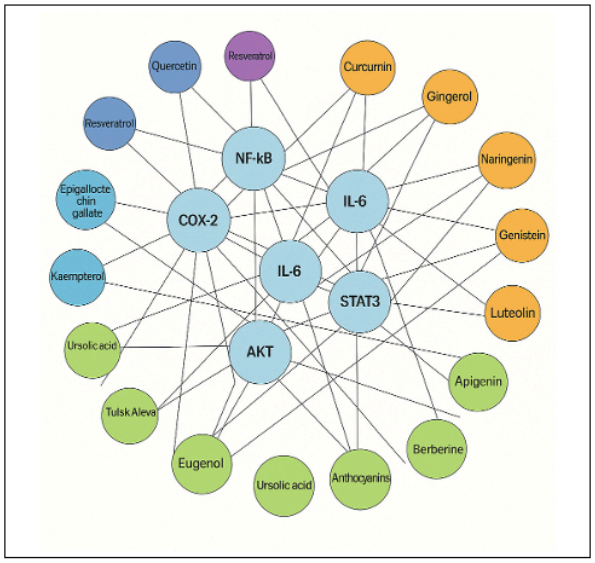


Figure 3: Phytochemical and Mechanistic Overlap Map



Gastrointestinal and Hepatoprotective Benefits

Tulsi has gastroprotective effects by reducing the production of stomach acid and managing oxidative stress (34). Aloe vera's anthraquinones and polysaccharides have laxative and anti-ulcer effects and enhance intestinal flora (35). Piper betle has shown hepatoprotective effects against CCl4-induced liver injury in experimental mice by activating antioxidant enzymes (36).

Table 2: Gastrointestinal and hepatic effects of Tulsi, Aloe vera, and Piper betle. (34, 35, 36)

Plant	GI Benefit	Hepatic Protection	Active Compounds
Tulsi	Anti-ulcer, pro-digestive	Antioxidant-mediated hepatoprotection	Eugenol, ursolic acid
Aloe vera	Laxative, gut healing	Liver enzyme normalization	Aloin, acemannan
Piper betle	Carminative, antimicrobial	Detoxifying, anti-inflammatory	Chavicol, eugenol

Cardiometabolic and Neuroprotective Potential

Tulsi changes blood glucose and lipid profiles by enhancing insulin sensitivity and reducing LDL cholesterol (37). Aloe vera reduces fasting blood sugar and oxidative stress in diabetic animals (38). By inhibiting acetylcholinesterase and reducing amyloid-β aggregation, the phenolic compounds in piper betle have neuroprotective effects (39).

Anti-cancer and Chemopreventive Roles

Each of the three plants have anti-cancer properties, including the ability to induce cell death, halt the cell cycle, and reduce angiogenesis. The anthraquinones found in aloe vera, such emodin, may slow down cancer cell development, flavonoids from tulsi can change the PI3K/Akt and NF-κB pathways (40, 41), and hydroxychavicol from Piper betle can provoke cell death in oral cancer (42).

Clinical Evidence

Clinical investigations have shown that aloe vera gel is useful in treating psoriasis and ulcerative colitis (43). Research on humans has shown that tulsi may help manage stress and metabolic syndrome (44). Additional research on Piper betle is required due to the absence of clinical data, albeit promising preclinical results (45).

Polyherbal Formulations and Synergistic Efficacy

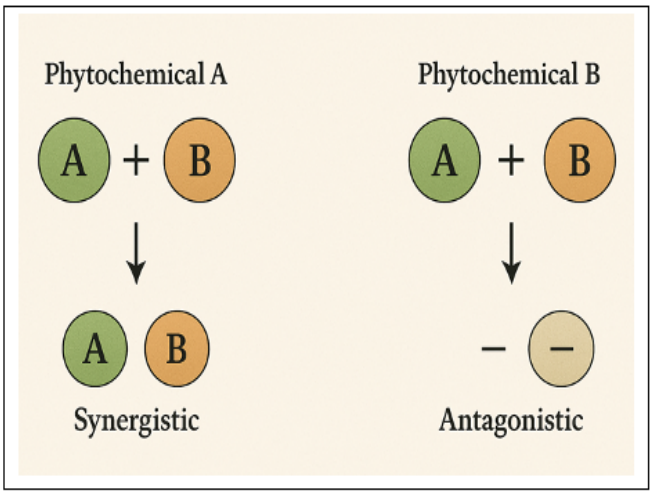
Principles of Polyherbalism in Ayurveda

The Ayurvedic principle of "Yogavahi" provides the theoretical foundation for polyherbal formulations; according to this principle, combining herbs improves therapeutic efficacy, restores dosha balance, and reduces adverse effects (46). All of the parts work together to enhance one another's effects.

Synergy vs. Antagonism in Combined Extracts

Synergy occurs when the combined therapeutic impact is greater than the sum of its separate components, often as a result of interactions between many targets (47). On the other hand, pharmacokinetic problems or phytochemical interactions might cause antagonistic effects.

Figure 4: Diagram showing synergistic vs. antagonistic effects in polyherbal extracts.



Case Studies and Proven Formulations

- **Tulsi + Aloe vera gel** for skin care, there are combinations that are touted as having better anti-inflammatory and healing properties than gels made from just one plant (48).
- **Tulsi + Piper betle extracts** showed combined antibacterial efficacy against Escherichia coli and Staphylococcus aureus (49).
- A **three-herb combination** of Tulsi, Aloe vera, and Piper betle showed better free radical scavenging in vitro than each component alone (50).

Challenges in Standardization and Quality Control

Environmental factors, batch variation, and phytochemical heterogeneity all work against standardisation. Accurate profiling in quality control necessitates the use of modern methods such as HPTLC, HPLC, and DNA barcoding (51). Furthermore, polyherbal regulations are in a constant state of flux.

Table 3: Key challenges and proposed solutions in polyherbal standardization

Challenge	Impact	Proposed Solution
Phytochemical variability	Inconsistent efficacy	Chemoprofiling and marker-based standardization
Herb-drug interaction	Potential toxicity	In vitro and in vivo interaction studies
Regulatory gaps	Limited clinical translation	Harmonized global phytopharmaceutical norms

Toxicological Assessment and Safety Profile

Acute and Chronic Toxicity Studies

When used therapeutically, Tulsi, Aloe vera, and Piper betle have all shown little harm in extensive toxicological studies. Results from acute toxicity tests on rats indicated that Tulsi leaf extracts in water were safe at doses up to 5,000 mg/kg (52). Studies involving acute and sub-chronic oral administration of aloe vera gel did not reveal any notable toxicity (53). Although the LD50 values for Piper betle extracts differ across different types, conventional dosing amounts have not been shown to be toxic (54).

Cytotoxicity and Genotoxicity Evaluation

The anthraquinones included in aloe vera, such as aloin and emodin, may exert dose-dependent cytotoxicity; however, the components of pure gel are often harmless (55). Neither the micronucleus nor the Ames tests showed any genotoxic effects from tulsi extracts (56). Careful dosage monitoring is required because Piper betle, owing to its phenolic components, has modest mutagenesis activity in some in vitro tests (57).

Herb-Drug Interactions and Contraindications

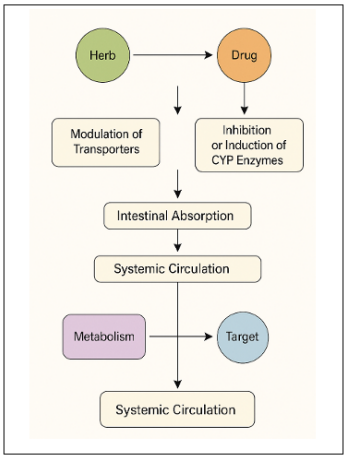
Problems with herb-drug interactions are significant in the field of phytomedicines. It is possible that tulsi, via CYP450 regulation, can increase the efficacy of hypoglycemic or anticoagulant medications (58). Since aloe vera has a laxative effect when

https://doi.org/10.47552/ijam.v16iS2.6201 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

consumed, it may impair the absorption of other medications (59). Use caution while using hepatically metabolised medications while taking piper betle since it may inhibit several CYP isoenzymes (60).

Figure 5: Mechanisms of potential herb-drug interactions



Regulatory Status and GRAS Classification

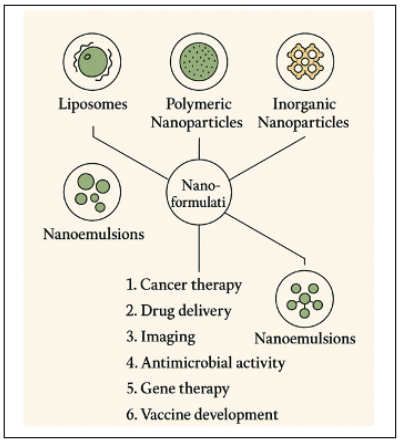
The FDA has granted GRAS (Generally Recognised as Safe) certification to aloe vera, also known as inner leaf gel, for use in food and cosmetics (61). The Ayurvedic Pharmacopoeia includes tulsi, which is well-respected in traditional medicinal systems (62). Despite its long history of usage, Piper betle is not GRAS since there is insufficient evidence of safety in standardised extract forms (63).

Novel Delivery Systems and Biotechnological Enhancements

Nanoformulations (Liposomes, Nanoparticles, Nanoemulsions)

The anti-inflammatory activity of Tulsi oil loaded in liposomes was enhanced (64), wound healing in diabetic rats was improved (65), and the antimicrobial activity of crude extracts of Piper betle was better exhibited by nanoemulsions than by nanoformulations (66). Nanoformulations increase the solubility, bioavailability, and target specificity of phytoconstituents.

Figure 6: Diagram of nanoformulation types and their biomedical applications



Encapsulation for Stability and Bioavailability

The degradation of labile phytochemicals may be prevented via encapsulation. Encapsulating eugenol from Tulsi and aloin from Aloe has improved their shelf life and allowed for regulated release via the use of cyclodextrins and biopolymer matrices (67, 68).

Table 4: Bioenhancement via encapsulation technologies

Compound	Encapsulation Method	Benefits	Reference
Eugenol (Tulsi)	β-Cyclodextrin complex	Improved thermal stability	(16)
Aloin (Aloe vera)	Gelatin nanoparticles	Sustained release, pH stability	(17)
Betel phenolics	Lipid-core nanocapsules	Enhanced antimicrobial efficacy	(15)

Transdermal, Buccal, and Oral Delivery Innovations

For the purpose of wound treatment, transdermal patches containing Aloe vera and Tulsi have been created. These patches provide localised action and prolonged release (69). To treat oral mucosal infections, researchers are looking into Piper betle buccal gels, which may transport active ingredients straight to the affected area (70).

Tissue-targeted Formulations Using Smart Carriers

For distribution to particular tissues, smart carriers such as ligand-conjugated nanoparticles and pH-responsive polymers have been used. Nanoparticles linked with folic acid generated from tulsi have been shown to enhance cytotoxicity while minimising negative effects (71). They exhibit preferential absorption by cancer cells.

Conclusion

The combination of Tulsi (Ocimum sanctum), Aloe vera, and Piper betle exemplifies the latest advancements in medicinal research that combine ancient botanical knowledge with cutting-edge scientific understanding. To provide a full picture of the synergistic and individual potential of these plants, this review has covered their history, phytochemistry, pharmacology, and technology.

Modern analytical techniques have confirmed the presence of potent bioactive constituents—flavonoids, terpenes, alkaloids, and phenolics—that underlie the therapeutic efficacy of these botanicals, while ancient medical systems like Ayurveda, Siddha, and Unani provide credence to the time-honoured use of these plants for a wide range of ailments. These herbs contribute to health-promoting activities ranging from antibacterial and hepatoprotective properties to anticancer and neuroprotective advantages via molecular and cellular pathways including control of oxidative stress, inflammation, immunological responses, and gene expression.

Polyherbal formulations bring the idea of "Green Alchemy" to life by providing comprehensive treatment

options with fewer side effects and amplifying therapeutic benefits via phytochemical synergy. Strict adherence to standards, quality assurance, and risk assessments are necessary, however, for these combinations to reach their maximum therapeutic potential. Although there are still worries about herb-drug interactions and formulation consistency, toxicological studies confirm that all three plants are generally safe when used traditionally.

These botanicals may now be delivered with improved bioavailability, stability, and tailored effectiveness thanks to emerging delivery techniques such encapsulation technologies, nanoparticles, and liposomes. These developments pave the way for these botanicals to be part of evidence-based integrative medicine by connecting traditional knowledge with modern technologies.

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Research Articles

https://doi.org/10.47552/ijam.v16iS2.6165 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Phytochemical Profile, in vitro Antioxidant Potential and HPTLC Fingerprinting of *Acalypha indica* Linn. Leaves

Research Article

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Abstract

The present study aims to standardize and evaluate bioactive profiling of *Acalypha indica* L. leaves through pharmacognostic parameters, phytochemical screening and High-Performance Thin Layer Chromatography (HPTLC) analysis. Comprehensive standardization was carried out using physicochemical constants, including moisture content, total ash, acid-insoluble ash, and extractive values, by pharmacopoeial guidelines. Macroscopic and Microscopic analysis confirmed key diagnostic features of leaves for identification and quality control profiling. Preliminary phytochemical screening of the ethanolic extract revealed the presence of bioactive constituents such as flavonoids, alkaloids, tannins, glycosides, and phenolic compounds. Antioxidant activity was assessed using in vitro assays including DPPH, ABTS, Superoxide, Hydroxyl radical, Nitric Oxide scavenging, and CUPRAC methods. The extract exhibited significant free radical scavenging potential, particularly in the CUPRAC and Superoxide assays. HPTLC analysis showed the best separation of bands at different retention factors (Rf) when using a solvent system of Toluene: Ethyl acetate: Glacial acetic acid in the ratio of (7:2:1 v/v/v). The quantitative estimation revealed that 10 mg of the ethanolic extract of *Acalypha. indica* L. contained 13.90 µg of kaempferol.

Keywords: HPTLC, Standardization, Antioxidant, *Acalypha indica L.*

Introduction

Acalypha indica L., commonly known as *Indian acalypha* (1). belongs to the Acalypha genus species, which is the fourth largest genus in the Euphorbiaceae family. Many plants from this family are used as medicinal herbs in Asian and African regions (2). *Acalypha indica* L. is a prevalent weed medicinal plant that grows across the plains of India (3). *Acalypha indica* L. thrives in wet, tropical regions and temperate countries across Asia (4). This plant is renowned for its notable medicinal properties, which are widely recognised for their human health benefits. This traditional medicinal plant is well-known among older generations in various countries, particularly in Africa and Asia (5). It is commonly found in regions such as a troublesome weed in gardens, along roadsides, and across the plains of India (6). *Acalypha canescens* Wall and *Acalypha ciliata* Wall are invalid synonyms for the plant, and the accepted name to refer to this plant is *Acalypha indica* L. It has been recognized by local communities for its value, widely used in traditional medicine for various therapeutic purposes (7). Extracts from different parts of the *Acalypha indica* L plant, including the leaves, roots, and stems, are widely utilised in the traditional healing practices across several countries (8). The plant has been reported to aid in the management of conditions such as pneumonia, asthma, scabies, and rheumatism. Additionally, it exhibits diuretic, purgative, and anthelmintic properties (3), (8). Furthermore, *Acalypha indica* L. is also recognised for its potential to lower Blood sugar levels (5). Chemical compounds found in *Acalypha indica* L. include acalyphamide, aurantiamide and its acetate, succinimide, calypholactate, 2-methyl anthraquinone, tri-O-methyl ellagic acid, β-sitosterol and its β-D-glucoside, primarily present in the leaves. The plant also contains a cyanogenic glucoside, acalyphine, two alkaloids—acalyphine and triacetonamine. Additionally, it contains kaempferol, quebrachitol, β-sitosterol acetate, tannins (present in the entire plant), and stigmasterol (found in the roots) (9). More recently, four kaempferol glycosides, mauritianin, clitorin, nicotiflorin, and biorobin, have been isolated from the flowers and leaves of the plant (8). This plant is extensively utilized in the treatment of various metabolic and cellular diseases, including diabetes, cancer (10). The antioxidant activity of plant extracts can be measured in vitro using different assay methods. Polyphenolic compounds, flavonoids, play a crucial role in neutralizing free radicals (11). The current study aims to explore the phytochemical constituents, perform qualitative and quantitative analyses, evaluate the antioxidant activity of crude ethanolic extract, and chromatographic profiling by HPTLC.

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Materials & Methods

Plant collection

Acalypha indica L. leaves were collected locally in Chatgaon, located in the Gadchiroli district of Maharashtra. The leaves were authenticated by Prof. Nitin Dongarwar, botanist, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The herbarium specimen was deposited in the Department of Botany, Nagpur, with the Herbarium sheet number 10071.

Chemicals and reagents

The reagents were utilized for studies: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Gallic acid (GA), Cupric chloride ($CuCl_2$), ABTS, trichloroacetic acid which were purchased from SRL chem Laboratories, Ascorbic acid (AA), DMSO, EDTA, Trio barbituric acid (TBA) were purchased from HiMedia, Hydrogen peroxide (H_2O_2) was purchased from Neurochem Laboratories, Trichloro acetic acid (TCA), ferric chloride and sodium nitroprusside were procured from Fisher Scientific, while Sodium phosphate was procured from Rankem laboratory.

Macroscopic and Microscopic Study of *Acalypha indica* Linn.

The macroscopic study was performed on a dry sample. The Macroscopic study involved colour, odour, surface texture, size, and shape of leaves, and microscopic studies of the leaf powder sample were done using a Leica microscope (DM 2000 LED). For a microscopic study, fresh leaves were collected and soaked in acetone to remove excess chlorophyll for better results. The Transverse section (T.S.) was taken using a razor blade. The section was stained with phloroglucinol and HCl and observed under the Leica microscope using a 10x magnification lens (12), (13).

Physicochemical parameters of crude drug

Determination of Moisture Content

A 2 g sample of *Acalypha indica* L. leaves powder and placed in a crucible. The sample was then dried in an oven at 105°C for 3-5 hrs. Initial drying phase, the powder was removed, allowed to cool in a desiccator, and weighed again. The sample was weighed every hour while the drying process continued at 105°C. This procedure was repeated until a constant weight was obtained. The loss in weight was used to calculate the percentage of moisture content representing the loss on drying (12).

Determination of Total Ash

A 2 g sample of the powder was taken into a silica crucible, which had been previously weighed and kept in a muffle furnace until the sample ignited completely. After cooling, the crucible was weighed again. The total ash content was calculated and expressed as a percentage.

% Total ash value = Wt. of ash /Wt. of drug X 100

Determination of acid-insoluble ash

The total ash obtained was boiled with 25 mL of 2 M HCL for 5 minutes, insoluble residue was collected on ashless filter paper, and the residue was ignited for 10-15 minutes at a temperature not exceeding 450°C, then cooled and reweighed. The acid-insoluble ash was then calculated and expressed as a percentage (12).

Determination of water-soluble ash

The total ash obtained was boiled for 5 minutes with 25 mL of water. The insoluble matters were collected on ashless filter paper and washed with hot water. and residue was ignited for 10-15 minutes at a temperature not exceeding 450 °C, then cooled and reweighed, and water-soluble ash was calculated and expressed as a percentage (12), (13).

Determination of the extractive value of *Acalypha indica* L.

Extractive values are helpful to evaluate the nature of constituents present in the crude drug. Water-soluble extractive and alcohol soluble extractive values were determined as per standard procedures. 5 gm of coarsely powder plant material weighed and transferred into a dry 250 mL conical flask then flask was filled with water and alcohol 30 ml separately flasks were corked and kept for 24 hrs at RT with shaking frequently the mixtures were filtered through filter paper the obtained extracts were concentrated to dryness and the extractive value in percentage was calculated (12).

Extraction of *Acalypha indica* Linn.

The leaves of *Acalypha indica* L. were thoroughly cleaned and shade dried. Once dried, the plant material was ground well into coarse powder using a mortar and pestle. A total of 100g of powdered plant material was initially defatted using petroleum ether and extracted with ethanol by maceration. The extraction process was carried out over seven days. The extraction mixture was filtered using a Whatman filter paper no .01. The solvent was recovered by rotary evaporator and drying the extract.

Phytochemical screening

The Phytochemical screening was carried out on ethanol extracts of *Acalypha indica* L. leaves. identify the active phytoconstituents, including alkaloids, glycosides, flavonoids, steroids, saponins, etc., present in the ethanolic extract using the following standard phytochemical tests.

Test for alkaloids

Dragendroff's reagent test: 2 mL of extract was heated with 2% H_2SO_4. A small amount of Dragendroff's reagent was added, and orange-red precipitate was observed.

Mayer's test: 1-2 mL of ethanolic extract was taken into a test tube, then 1-2 drops of Mayer's reagent were added. The result was positive; a creamy white precipitate formed.

Wagner's test: 1-2 mL of extract solution was taken, and 1- 2 drops of Wagner's reagent were added. then, a brown precipitate formed.

Test for glycoside

Borntrager test: 1- 2 mL of extract solution was taken, 2-3 mL of Chloroform was added, then shaken, and the chloroform layer was separated. 10% ammonia solution added. The result was positive, a red coloured solution formed.

Test for flavonoids

Shinoda test: 1- 2 mL of extract was taken, and 5 mL of ethanol added, then added few grains of magnesium, turnings, with a few drops of conc. HCL, the sample was positive for flavonoids, red to pink coloured

Test for Phenolic compounds

Gelatin test: The ethanolic extract was dissolved in 5 mL of distilled water, followed by the addition of 1% gelatin solution and 10 % sodium chloride solution, resulting in the formation of a white precipitate.

Test for Steroids

Salkowski's test: The Alcoholic extract solution was taken, and a few drops of concentrated sulphuric acid were added to the sample was positive for steroids when a red colour formed in the lower layer (12), (15).

Test for tannins

Nitric acid test: 2- 3 mL of the ethanolic extract was taken in a test tube, a few drops of dil. Nitric acid was added, then a reddish yellow colour formed (16).

Test for fixed fats and oils

Spot test/stain test: a small quantity of plant extract is pressed between to filter papers, then an oil stain appears on the paper.

Test for saponin

Foam formation test: 2 mL of aq. the solution was taken into a test tube and shaken vigorously. If foam was formed and did not disappear for 5 min. (15)

Quantitative analysis of the ethanolic leaves extract of *Acalypha indica* L.

Total phenolic content

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of the ethanolic extract of *Acalypha indica* L. leaves (17). Gallic acid was used as the standard. A standard calibration curve was plotted using gallic acid solution at concentrations (10 µg/ml to 50 µg/ml). A stock solution of extract was prepared by dissolving 10 mg of plant extract in 10 mL of methanol. It was diluted even further to get a solution of 100 µg/ml., 1.5 mL of a 20% sodium carbonate (Na_2CO_3) solution was added to 1 ml of this solution, which was then mixed with 0.5 mL of 2 N Folin-Ciocalteau reagent (FCR) for the experiment. To enable the reaction, this solution was left in the dark for two hours. The absorbances were measured with a UV spectrophotometer (Shimadzu UV-2401PC) at 765 nm (18), (19), (20).

Total flavonoid content

The total flavonoid content of the plant extract, determined using the aluminium chloride colorimetric method. A stock solution of plant extract was prepared by dissolving 10 mg sample in 10 mL of methanol. A 10 mL volumetric flask containing 20% methanolic aluminium trichloride was added with an aliquot of 2 mL of the extract and standard solutions of Rutin (0.5 mg/mL). A few drops of acetic acid were added, and the combination was left to stand for 40 minutes. The absorbance of the resulting solution was determined at 415 nm using a spectrophotometer. A standard curve was prepared using varying concentrations (21).

Total tannin content

10 mg of ethanolic extract was dissolved in 10 mL of methanol as a stock solution. The Folin-Ciocalteu Reagent (FCR) was added to 1 mL of this, further treated with 1.5 mL of a 20% sodium carbonate (Na_2CO_3) solution. To enable the reaction, the resulting mixture was left in the dark condition for 15 minutes. Similarly, the standard calibration curve was prepared using the same process as tannic acid as the reference., The absorbance of the resulting solution was determined at 775 nm using a spectrophotometer (22).

Total alkaloid content

A gravimetric analytical method was used to assess the total alkaloid content in the plant material. A 5 g of powdered crude drug was repeatedly extracted with 0.1 N Sulfuric acid (H^2SO^4) in an ultrasonic bath (3x50 ml). The resulting solution was filtered, and the combined acid solution was rinsed four times with 25 mL of chloroform. The chloroform washings were discarded. The acid solution was basified by adding dilute ammonia solution and further extracted with diethyl ether. The combined diethyl ether extracts were washed with distilled water, and the ether was evaporated to dryness in a weighted beaker. The dried extract was then further dried to a constant weight. The total alkaloid content was calculated and expressed as % w/w (23).

Comparative analysis of antioxidant activity using different in vitro assays

DPPH assay

Antioxidant activity is mostly measured using the DPPH radical scavenging test. This technique, which is based on electron transfer, uses an antioxidant to donate electrons to neutralise the stable DPPH radical, which is distinguished by its deep purple. In a 96-well plate, 0.1 mL of a 0.1 mM DPPH solution was mixed with 5 µL of various doses of the test chemical. For every concentration, the reaction was carried out in triplicate, and duplicate blanks were made by mixing 5 µL of the corresponding chemical concentration with 0.2 mL of DMSO/Methanol. For half an hour, the plate was incubated in the dark. Then absorbance was measured at 517 nm against a blank methanol using a UV-Vis

spectrophotometer (Shimadzu UV-2401PC). using a microplate reader (iMark, BioRad). A reaction mixture with 20 µL of deionised water was used as the control. Scavenging activity was expressed as % inhibition relative to the control. The IC_{50} value was calculated using GraphPad Prism 6 software. (24), (25).

%Inhibition = Abs.(control)−Abs. (Sample) /Abs. (control) × 100

Hydroxy Free Radical Scavenging Assay

A reagent mixture was prepared by combining 10 µL of 0.5 M EDTA, 24.14 mg of Deoxyribose, 88 µL of $FeCl_3$ (10 mg/mL), and 28 µL of H_2O_2 (6%), adjusted to a final volume of 33 mL with water. In each well of a 96-well plate, 10 µL of plant extract, 24 µL of phosphate buffer (50 mM, pH 7.4), and 10 µL of ascorbic acid were added to each well, followed by the reagent mixture. The plate was incubated at 37°C for 1 hour. The wells without treatment served as the control, and Gallic Acid was used as the standard. After incubation, 50 µL of 10% TCA and 50 µL of 1% TBA were added to each well. A pink chromogen formed, and absorbance was measured at 540 nm using a microplate reader. IC_{50} was calculated using the Software GraphPad Prism 6. A graph was prepared between X axis (Sample Concentration) Vs. Y axis (% inhibition w.r.t. control (26), (27).

%Inhibition = Abs.(control)−Abs. (Sample) /Abs. (control) × 100

Reactive Nitrogen Oxide Scavenging Assay

A reaction mixture was prepared containing 50 µL of 10 mM sodium nitroprusside, 40 µL of distilled water, and 10 µL of gallic acid. The reaction mixture without treatment was used as the control. The mixture was pre-incubated at room temperature for 15 minutes in the presence of light. After incubation, 100 µL of Griess reagent was added to both the test and control wells. The plate was then incubated for 5-10 minutes at room temperature to allow for chromophore development and stabilization. Absorbance was measured at 540 nm and 660 nm using a microplate reader. The IC_{50} value was calculated using GraphPad Prism 6 software (28).

%Inhibition = Abs. (control)−Abs. (Sample) /Abs. (control) × 100

Super Oxide Anion Radical Scavenging Assay

Different concentrations of the extract (ranging from 1 to 1000 µg/mL) and riboflavin solution (used as the standard, ranging from 1 to 50 µg/mL) were prepared. The reaction mixture was then incubated for 30 minutes in a 96-well plate under light at room temperature. After the incubation, the reaction mixture was added to the previously incubated sample and thoroughly mixed. The wells without treatment were considered the control. Absorbance was then measured using an ELISA plate reader at 560 nm. The IC_{50} value was calculated using GraphPad Prism 6 software (29).

%Inhibition = Abs. (control)−Abs. (Sample) /Abs. (control) × 100

ABTS Radical Scavenging Assay

ABTS radicals were generated by mixing a 2.45 mM APS solution with a 7 mM ABTS solution, which was then diluted 100-fold to prepare the ABTS free radical reagent. To evaluate antioxidant activity, 10 µL of different concentrations of ascorbic acid (standard) and the test samples were added to 200 µL of the ABTS free radical reagent in a 96-well plate. The plate was incubated at room temperature for 10 minutes in the dark. Control wells, containing no treatment, were included for comparison. After incubation, the absorbance of the decolorized solution was measured at 750 nm using a microplate reader. The results were expressed relative to the negative control (30), (31), (32).

%Inhibition = Abs. (control)−Abs. (Sample) /Abs. (control) × 100

CUPRAC Assay

10µl of different concentrations of the sample were added to defined wells of a 96-well plate. Then, 200µl of reagent mixture was added. Reaction mixture in triplicate form and blank in duplicate form were prepared, containing 200µl Methanol and 10µl of compound of different concentrations for sample and standard (Trolox – Ottokemi - Cat no–T7723) and incubated for 30 minutes in the dark. The wells without treatment were considered as controls. At the end of the incubation, absorbance of the decolorization was measured at 490 nm (33).

%Inhibition = Abs.(control)−Abs. (Sample) /Abs. (control) × 100

HPTLC Fingerprint Analysis

A CAMAG HPTLC system equipped with a Linomat 5 applicator fitted with a 100 µL syringe, a CAMAG TLC scanner, and visionCATS software was used.

Sample Preparation and Application

0.1 mg/mL of standard (Kaempferol) solution was prepared in HPTLC grade methanol and 10 mg/mL ethanolic crude extract was prepared in methanol of chromatographic grade and filtered through Whatman filter paper no .01 prepared extract and standard were applied on a TLC aluminium sheet, silica gel 60 F 254 (Merck). Standard reference solution kaempferol (1 to 8 µL) and sample ethanolic extract (2 to 14 µL) were applied sequentially on a 200 ×100 mm plate, each with a band length of 8.0 mm, using a CAMAG Linomat 5 sample applicator fitted with a 100 µL syringe, set at a dosage speed of 150 nL/s.

Sample system development

For the separation of phytoconstituents in the extract, various solvent systems were tried. Optimal resolution and the maximum number of spots were obtained in the solvent system Toluene: Ethyl acetate: Glacial acetic acid in the ratio 7:2:1 (14).

https://doi.org/10.47552/ijam.v16iS2.6165 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Development of Chromatogram

The TLC plate was developed in a twin trough glass chamber of 20 ×10 cm and saturated the mobile phase system for 20 min at RT up to a distance of 70 mm. The standard and sample-loaded plates were developed in an automated development chamber. The plate was then allowed to dry at room temperature for 10 min. the Rf values and colour of the bands were noted.

Scanning and detection of spots

The dried plate was visualised using under UV cabinet at 254nm and 366 nm. All spots are UV visible in the extract, and spectrum scanning of the developed plate was performed on a CAMAG TLC scanner 4 using the deuterium and tungsten lamp at 190-450 nm wavelength and a spectrum speed of 20 nm/s. The separation of per track was used, and Rf values were noted.

Results And Discussion

Macroscopic and Microscopic Study

The leaves of *Acalypha indica* L. are ovate to rhomboid in shape, generally measuring 4 to 5 cm in length and 3-4 cm in width. the upper surface is dark green, while the lower surface is pale green. Leaf margins are serrate to crenate, and the petiole is notably long, from 1 to 8 cm, as observed in macroscopic studies fig.1 and 2. The transverse section (TS) of *Acalypha indica* L. reveals a differentiated structure comprising both upper and lower epidermal layers. Beneath the epidermis, parenchyma cells are present, consisting of loosely arranged, thin-walled cells. Vascular bundles are visible, exhibiting a reddish coloration, and non-glandular types of trichomes are observed in Fig. 3 on the epidermal surface under the Leica microscope (DM 2000 LED). Microscopic evaluation of the powdered material revealed several diagnostic features. Xylem vessels (A) appeared as thick-walled, elongated structures. Parenchyma cells (B) were noted to be thin-walled and polygonal in shape. Volatile oil globules (C) were observed as spherical droplets. Starch grains (D) were observed when iodine solution was applied. Under polarized light, needle-shaped and crystal-shaped calcium oxalate crystals (E) became distinctly visible with 60% H_2SO_4, as shown in Fig. 4.

Table 1: Macroscopic parameters

Macroscopic parameters	Observation
Shape	Ovate to rhomboid
Size	Length – 4 to 5 cm, Width- 3 to 4 cm
Colour	Upper dark green and lower pale green
Margin	Serrate-crenate
Petiole	Very long (1 to 8 cm)

Physicochemical study of crude drug

The percentage of moisture content, total ash, acid-insoluble ash, and water-soluble was carried out and the results are shown in Table 2. The physicochemical standardization parameters results showed that moisture content, total ash, acid insoluble ash, and water-soluble ash values are 6.50±0.06, 14±0.03, 5.6±0.05, and 3.4±0.04 % w/w, respectively. The alcohol soluble and water-soluble extractive values are determined. the alcohol soluble extractive value indicates the presence of polar phytoconstituents like flavonoids, glycosides, phenols, etc., and the water-soluble extractive value indicates the presence of sugar, amino acids. The alcohol and water-soluble extractive values of *Acalypha indica* L. were 12 ±0.02 % and 17.5 ±0.08 %, respectively.

Figure 1: Upper surface

Figure 2: Lower surface

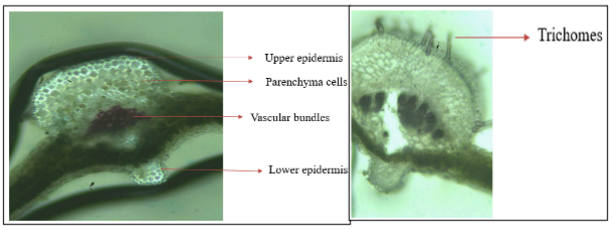

Figure 3: T.S. of *Acalypha indica* L

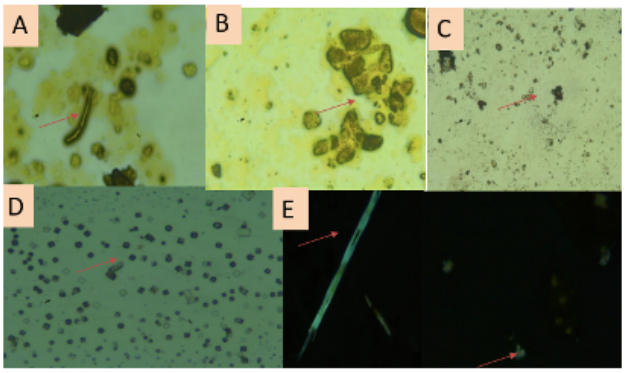

Fig. 4: Powder microscopy of leaves of *Acalypha indica* L

Table 2: Physicochemical Standardization Parameters

Sr. no	Physicochemical parameters	% yield (%w/w)
1	Moisture content	6.50 ± 0.06
2	Total ash value	14.5±0.03
3	Acid insoluble ash	5.6±0.05
4	Water-soluble ash	3.4±0.04
5	Water-soluble Extractive value	17.5 ±0.08
6	Alcohol-soluble extractive value	12 ±0.02

*Values were the means ±SEM

Phytochemical screening

Phytochemical screening (Table 3) revealed that the leaf extract of *Acalypha indica* L. is rich in secondary metabolites, particularly flavonoids, alkaloids, glycosides, tannins, and phenolic constituents present and saponins and fixed fats and oils were absent in the ethanolic extract.

Table 3: Phytochemical screening of leaves extract of *Acalypha indica* L

Sr.no.	Phytochemical tests	Observation	Result
1	Alkaloids Dragendroff's test Mayer's test Wagner's test	A reddish-brown precipitated White precipitated Brown precipitated	+ ve + ve + ve
2	Glycosides Borntrager test	Red colour solution formed	+ ve
3	Tannins Ferric chloride test	Greenish blue colour formed	+ ve
4	Phenolic compound Gelatin test	White precipitated	+ ve
5	Flavonoids Shinoda test	Red to pink precipitated	+ ve
6	Steroids Salkowski's test	Red colour formed at a lower layer	+ ve
7	Saponin Foam test	No foam formed	- ve
8	Fixed Fats and oils Spot test	No oil spot observed	- ve

Key: +ve = Positive test, - ve = Negative

Quantitative estimation

Total phenolic content was expressed as microgram of gallic acid equivalent per mg of crude extract (µg GAE/mg), Total flavonoids content was expressed as microgram of rutin equivalent per mg of extract (µg RT/mg), and total tannin content was expressed as mg of tannic acid equivalent per mg of crude extract. (µg TA/mg). Total phenolic, flavonoids, and tannins content were recorded as 220.9 ± 0.83, 68.50 ± 0.33, and 21.65 ± 0.41, respectively, in Table 4. The alkaloid content was determined to be 0.4 % w/w of the total yield.

Table 4: Quantitative estimation

Sr.no	Quantitative parameters	Result(µg/mg)
1	Total phenolic content (µg GAE/mg)	220.9 ± 0.83
2	Total flavonoid content (µg RT/mg)	68.50 ± 0.33
3	Total tannin content (µg TA/mg)	21.65 ± 0.41

*Values were the means ± SEM

Comparative analysis of antioxidant activity

The in vitro antioxidant activities of ethanolic extracts of *Acalypha indica* L. were evaluated using various antioxidant assays, as shown in Table 5.

The antioxidant activity was evaluated by using different in vitro assays. The DPPH assay showed modest activity with an IC_{50} of 415 ± 0.05 µg/ml. The assays for Superoxide Scavenging and Hydroxyl Radical showed stronger antioxidant activity, with IC_{50} values of 13.07 ± 0.53 µg/ml and 16 ± 0.16 µg/ml, respectively. The CUPRAC assay also showed significant potency (IC_{50} = 9.29 ± 0.9 µg/ml). The IC_{50} values for the Nitric Oxide and ABTS tests were 510.9 ± 0.20 µg/ml and 28.28 ± 0.06 µg/ml, respectively.

Table 5: Comparative analysis of antioxidant activity using different in vitro assays

Sr. no	Antioxidant assay	Name of Standard	IC_{50} value
1	DPPH radical quenching capacity	Ascorbic acid	415 ± 0.05
2	Hydroxy Free Radical Scavenging Assay	Gallic acid	16±0.16
3	Reactive Nitrogen Oxide Scavenging Methods	Gallic acid	510.9 ± 0.20
4	Super Oxide Anion Radical Scavenging Assay	Gallic acid	13.07 ± 0.53
5	ABTS Radical Scavenging Assay	Ascorbic acid	28.28 ± 0.060
6	CUPRAC	Trolox	9.292± 0.09

*Values were the means ± SEM

HPTLC analysis of the ethanolic extract of *Acalypha indica* L.

Fingerprint profiling and separation of bioactive phytoconstituents of the ethanol extract of *Acalypha indica* L. (Leaves) were carried out using HPTLC, with kaempferol used as a standard reference compound. The best separation of the bands was achieved when using the solvent combination of Toluene: Ethyl acetate: Glacial acetic acid in the ratio (7:2:1,v/v/v). The plate was visualised under UV at 254 nm and 366 nm. The plate scanned at 254 nm for the standard reference compound shows that kaempferol has an Rf value of 0.410. The quantitative estimation revealed that 10 mg of the ethanolic extract of *Acalypha. indica* L. contained 13.90 µg of kaempferol.

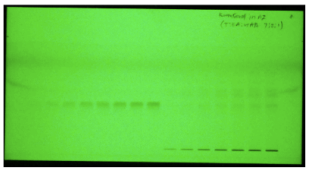

Fig.5 : Scanned image of kaempferol and sample at 254 nm

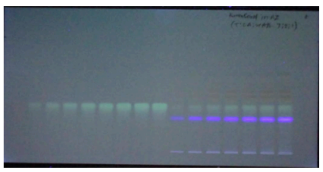

Fig.6: Scanned image of kaempferol and sample at 366 nm

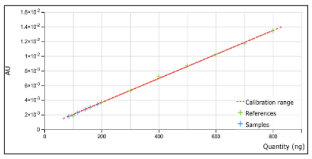

Fig. 7: Area calibration for standard kaempferol

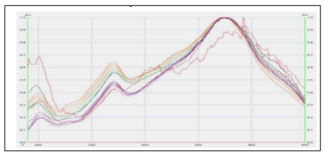

Fig.8: Spectrum scan of 190-450

Discussion

The standardization of *Acalypha indica* L. powdered crude drug by different physicochemical parameters was evaluated, including moisture content, total ash value, acid insoluble value, and extractive values evaluated to assess the purity and stability of the crude drug. The results of the phytochemical screening show that the ethanolic extract contains alkaloids, flavonoids, polyphenolic compounds, tannins, saponins, phenols, steroids, and glycosides. The saponins and fixed fats, and oils were absent. Macroscopic evaluation of the leaves further supports the identification of the plant. The leaves were observed to be ovate to rhomboid in shape with serrate to crenate margins, and

the petiole was generally 1 to 8 cm in length. In microscopic evaluation, Vascular bundles are visible, exhibiting a reddish coloration, and non-glandular types of trichomes are present. Total phenolic, flavonoids, and tannins content were recorded as 220.9 ± 0.83 µg/mg, 68.50 ± 0.33 µg/mg, and 21.65 ± 0.41 µg/mg, respectively. The DPPH assay showed modest activity with an IC_{50} of 415 ± 0.05 µg/ml. The assays for Superoxide Scavenging and Hydroxyl Radical showed stronger antioxidant activity, with IC_{50} values of 13.07 ± 0.53 µg/ml and 16 ± 0.16 µg/ml, respectively. Optimal separation was achieved using a mobile phase solvent system comprising Toluene: Ethyl acetate: Glacial acetic acid (7:2:1, v/v/v) in HPTLC analysis. Quantitative analysis further confirmed the presence of kaempferol in the ethanolic extract, with 13.90 µg in 10 mg of crude extract. These findings support the use of HPTLC as a reliable method for standardization and quality assessment of flavonoid content in *Acalypha indica* L.

Conclusion

In the present study, Phytochemical screening revealed that the leaf extract of *Acalypha indica* L. is rich in secondary metabolites, particularly flavonoids, Alkaloids, glycosides, tannins, and phenolic compounds. The presence of these compounds is consistent with the traditional medicinal uses of *Acalypha indica* and supports its potential antioxidant and therapeutic properties. Comparative analysis of antioxidant activity using different in vitro assays findings imply that the extract has strong antioxidant qualities, especially against cupric, superoxide, and hydroxyl radicals. In the HPTLC profiling analysis, kaempferol was quantified. Rf value of kaempferol found to be 0.410, when the mobile phase solvent system comprising Toluene: Ethyl acetate: Glacial acetic acid (7:2:1, v/v/v), 13.90 µg of kaempferol was found in 10 mg of leaves extract of *Acalypha indica* L.

Abbreviations

- **DPPH:** 2,2-diphenyl-1-picrylhydrazyl
- **ABTS:**2, 2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
- **IC 50**: 50% Inhibitory concentration
- **TPC**: Total polyphenol content
- **TFC**: Total flavonoid content
- **GAE:** Gallic acid equivalent
- **RE:** Rutin equivalent
- **TA**: Tannic acid equivalent
- **HPTLC**: High-performance thin-layer chromatography
- **FCR**: Folin-Ciocalteu Reagent
- **Rf:** Retention factor
- **RT:** Room temperature

Acknowledgment

The author expresses sincere gratitude to the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, for providing the necessary facilities and support throughout the study. Special thanks are extended to my research guide, co-guide, and colleagues for their invaluable guidance and assistance during the research process. The author also gratefully acknowledges the Chhatrapati Shahu Maharaj National Research Fellowship–2022 (CSMNRF–2022) for providing financial support during this research study.

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https://doi.org/10.47552/ijam.v16iS2.6166 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Phytochemical Screening and Thin Layer Chromatography of Successive Solvent Extracts of the Medicinal Plant Maytenus emarginata

Research Article

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Abstract

Medicinal plants have long been recognized for their therapeutic potential, with phytochemical screening serving as a crucial step in understanding their bioactive constituents. *Maytenus emarginata*, a member of the Celastraceae family, has garnered attention for its diverse secondary metabolites. This study aimed to analyze the phytochemical composition of *M. emarginata* leaves through qualitative screening and thin-layer chromatography (TLC). Extraction was performed using chloroform, methanol, and aqueous solvents, followed by the identification of compounds such as flavonoids, alkaloids, tannins, and sterols. TLC profiling provided insights into compound separation, revealing varied retention factor (Rf) values across different extracts. The qualitative analysis of *M. emarginata* leaf extracts identified the presence of secondary metabolites. Alkaloids were detected in chloroform and aqueous extracts, while flavonoids and sterols were present across all extracts. Among the three solvents used, aqueous extraction yielded the highest extractive content (6.03%), followed by methanolic (3.95%) and chloroform (1.44%). TLC confirmed the presence of multiple phytoconstituents across extracts. The chloroform extract exhibited 6 spots under normal and short-wave UV and 7 under long-wave UV, indicating a broad range of compounds with Rf values from 0.62 to 1.0. The methanolic extract showed up to 4 spots under long-wave UV, while the aqueous extract displayed a single UV-active compound (Rf = 0.68). Qualitative phytochemical screening of *M. emarginata* suggests a complex chemical makeup, underscoring the need for quantitative HPLC analysis. Further research should emphasize the isolation and structural characterization (NMR, MS) of individual compounds.

Keywords: Celastraceae, *Maytenus emarginata,* Phytochemical screening, Secondary metabolites, Thin-layer chromatography.

Introduction

Historically, the plant kingdom has served as a substantial reservoir of bioactive molecules. A significant proportion of pharmaceuticals currently available and approved for clinical application are either naturally occurring compounds or their synthetic analogs. Despite the advent of novel synthetic drugs, phytomedicinals continue to be widely employed globally. Numerous secondary metabolites synthesized by medicinal flora function as precursor molecules in the development of novel therapeutic agents (1-3). Ethnobotanical practices, encompassing herbal medicine, represent an ancient and cross-cultural tradition. Medicinal plants have played a crucial role in pharmacognosy and remain integral to contemporary drug discovery pipelines. While traditionally foundational to therapeutics in developing nations, the utilization of herbal remedies has also witnessed increased adoption in developed countries, primarily attributed to their relative affordability, accessibility, and reliance on traditional empirical knowledge (1, 4).

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Recent scientific literature highlights the escalating research interest in the genus *Maytenus*, primarily driven by its extensive variety of bioactive constituents and its well-recognized role in traditional pharmacopoeia (1). *Maytenus emarginata* (Willd.) Ding Hou, a member of the plant family Celastraceae, is an evergreen arboreal species exhibiting tolerance to diverse abiotic stresses prevalent in arid environments. This taxon, vernacularly known as "Kankero" (Hindi) and "Thorny staff tree" (English), demonstrates a broad geographical distribution across various Indian states, including Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, Gujarat, Delhi, Bihar, Tamil Nadu, and Rajasthan (5, 6).

The Celastraceae family, native to tropical and subtropical zones globally, encompasses approximately 88 genera and 1300 plant species. *M. emarginata* , known locally as Bharati, is an erect evergreen shrub within this family, characterized by obovate, glaucous green to reddish-brown leaves with an emarginate apex and serrate margin. Phytochemical analysis of M. emarginata has revealed the presence of sesquiterpene pyridine alkaloids (Emarginate A–E), triterpenes (β-amyrin), sterols (β-sitosterol), fatty acids (palmitic acid in leaves), maytansinoids (maytensine in leaves), and flavonoids (quercetin, myricetin, isorhamnetin in leaves). Traditional medicinal uses include the application of roots for dysentery and root juice for diabetes treatment (7, 8). The study showed that the

chemical profile of *M. emarginata* is characterized by a significant accumulation of triterpene quinone methides (pristimerin, tingenone, iguesterin) and the triterpenoid β-amyrin, as evidenced by their relatively higher extraction yields from different tissues. While sesquiterpene pyridine alkaloids (emarginatines A-G, maytansine, emarginatinine) and other isolated triterpenoids (including novel oxygenated lupanes) occur in trace amounts within stem extracts, flavonoids (myricetin, quercetin, isorhamnetin) and additional triterpenes (friedelan-3-one, 29-norcycloartanol) have been identified in leaf extracts (1, 9).

M. emarginata has been extensively investigated within the scientific community, with a particular focus on elucidating its therapeutic potential. Research findings indicate that the phytoconstituents present in *M. emarginata* demonstrate a diverse spectrum of pharmacological actions, encompassing antioxidant, anti-inflammatory, antimicrobial, and antineoplastic effects. These bioactivities are predominantly correlated with the presence of phenolic compounds, flavonoids, and other secondary metabolites identified within the plant matrix (1, 4, 5, 10).

The present study aims to investigate the phytochemical composition of *M. emarginata* through phytopharmacological screening and thin-layer chromatography to identify bioactive compounds that may contribute to the development of effective plant-based therapeutic agents.

Materials and methodology

Preparation of Plant Material

The leaves of *M. emarginata* (Figure 1) were collected from Nagpur in April. Following collection, the plant material underwent a cleaning process that included the removal of deteriorated components, washing with tap water followed by distilled water, and absorption of excess moisture with blotting paper. The cleaned leaves were then subjected to shade-assisted air drying for preservation.

Figure 1: Procurement of Leaves of *M. emarginata*

Preparation of Plant Extracts

The extraction of phytochemicals, essential for isolating bioactive secondary metabolites from plant matter, was performed on *M. emarginata* leaves. Specifically, 50 g of coarsely ground, shade-dried leaves were subjected to maceration with chloroform, methanol, and water, the maceration process consisted of 48 hours for the first maceration and 24 hours for the second maceration after filtration. (Figure 2) (11). Each solvent-containing filtrate was evaporated at 50°C to yield a concentrated extract. The percentage of extractable material for each solvent system was subsequently quantified based on the dry weight of the recovered extracts.

Figure 2: Extraction of plant leaves by the maceration process

Determination of percentage yield

The percentage yield serves as a quantitative metric for evaluating the overall efficiency of the extraction procedure. This value is determined through the following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening, employing established methodologies, was performed on the extract to identify diverse classes of natural compounds. This screening identified the presence of phenolics, flavonoids, tannins, saponins, alkaloids, and proteins based on characteristic color changes and precipitation reactions observed in freshly prepared extracts (12).

Detection of alkaloids

The presence of alkaloids in plant extracts was investigated through a series of analytical steps. Initially, extracts were dissolved in dilute hydrochloric acid and filtered to remove particulate matter. The resulting filtrates were then subjected to: (a) Hager's test, where the formation of a yellow precipitate upon addition of saturated picric acid confirmed the presence of alkaloids; and (b) Wagner's test, where a reddish brown precipitate observed after the addition of Wagner's reagent served as a positive indicator for alkaloids.

Detection of Glycoside

The different types of glycosides tests are as follows,

Borntrager's test

To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Legal's test

50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Detection of flavonoids

The qualitative chemical characterization of the extract for the estimation of flavonoids involved two distinct color-based assays.

Shinoda test

Crude extract was mixed with a few fragments of magnesium ribbon, and concentrated HCl was added dropwise. Pink scarlet colour appeared after a few minutes, which indicated the presence of flavonoids.

Detection of diterpenes

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Detection of phenols

The qualitative chemical characterization of the extract for phenolic content involved two distinct color-based assays. (a) The ferric chloride test, a rapid method for detecting phenols, relies on the formation of a colored complex (bluish-black) upon interaction with ferric chloride. (b) The Folin-Ciocalteu assay, a more sensitive but less specific test for phenolic antioxidants, was employed, with the appearance of a blue-green color indicating the potential presence of phenolic compounds.

Detection of proteins

The detection of proteins in extracts was performed using the xanthoproteic test, where a yellow color formation upon the addition of concentrated nitric acid served as a positive indicator.

Detection of saponins

The presence of saponins was confirmed by foam test. Following dilution of extracts to 20 mL with distilled water, the solution was mechanically shaken for 15 minutes in a graduated cylinder. The formation and persistence of a foam layer measuring ≥ 1 cm in height served as a positive indicator for the presence of saponins.

Detection of tannins

The gelatin Test serves as a qualitative assay for the presence of tannins. The procedure entails mixing a sample extract with a 1% gelatin solution containing sodium chloride. The formation of a visible white precipitate is interpreted as a positive qualitative indication of tannin presence.

Detection of Sterols

Libermann-Burchard's test

The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols (13).

Separation and Identification

Separation and Identification of phytoconstituents in the extract of *M. emarginata* by thin layer chromatography (TLC). TLC profiling serves as a preliminary step toward the isolation and characterization of these phytoconstituents. Solvent extracts underwent one-dimensional ascending TLC on pre-coated silica gel 60F254 plates (7x6 cm, Merck), manually cut. Sample application (1 µL) was performed using glass capillaries, spotted 1 cm from the base in five tracks. Chromatographic development was conducted in a twin-trough chamber pre-saturated for 20 minutes with a toluene:ethyl acetate:formic acid (5:4:1 v/v/v) mobile phase for flavonoid analysis. Compound migration was quantified by Retention factor (Rf) values. Visualization of developed chromatograms was achieved under visible light, short-wave UV (254 nm), and long-wave UV (365 nm) using a TLC viewing cabinet (Electronic India). Once the chromatogram was developed the R_f Value of the spot was calculated using the formula (14):

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Results

% yield of leaves extract of *M. emarginata*

Table 1 presents the percentage yield (W/W) of different solvent extracts obtained from the leaves of *M. emarginata* . Among the three extracts, the aqueous extract exhibited the highest yield at 6.03%, followed by the methanolic extract with a yield of 3.95%. The chloroform extract showed the lowest yield, amounting to 1.44%.

Table 1: % Yield of leaves extract of *M. emarginata*

Sr. No	Extracts	% Yield (W/W)
1	Chloroform	1.44%
2	Methanolic	3.95%
3	Aqueous	6.03%

Result of phytochemical screening

Phytochemical screening of medicinal plants is crucial for assessing their potential therapeutic applications and identifying the bioactive compounds responsible for their established pharmacological effects. Table 2 presents the results of a qualitative phytochemical analysis conducted on chloroform, methanolic, and aqueous extracts of *M. emarginata* leaves to determine the presence of various secondary metabolites.

Phytochemical screening revealed that alkaloids were present in the chloroform and aqueous extracts, glycosides in methanolic and aqueous extracts, and flavonoids in all extracts. Phenols, proteins, carbohydrates, sterols, and saponins were present in both methanolic and aqueous extracts. Tannins were detected only in the aqueous extract. Diterpenes were absent in all, while saponins were consistently present across all extracts.

Separation and Identification by TLC

The phytochemical constituents present in the extracts of *M. emarginata* were separated and identified using TLC. The analysis was performed using a mobile phase consisting of Toluene: Ethyl acetate: Formic acid in the ratio of 5:4:1. The TLC plates were visualized under normal light, short-wave UV, and long-wave UV to identify the number and nature of phytoconstituents based on their Rf values. As shown in Table 3, the standard flavonoid quercetin exhibited a single spot with an Rf value of 0.64, consistently observed under normal light, short UV, and long UV conditions, which served as a reference for comparison.

Table 2: Result of phytochemical screening of the leaf extract of *M. emarginata*

S. No.	Constituents	Chloroform extract	Methanolic extract	Aqueous extract
1	**Alkaloids** Wagner's Test Hager's Test	 +ve +ve	 -ve -ve	 +ve -ve
2	**Glycosides** Borntrager's Test Legal's Test Keller-kilani Test	 -ve -ve -ve	 +ve +ve +ve	 +ve +ve +ve
3	**Flavonoids** Shinoda Test	 +ve	 +ve	 +ve
4	**Diterpenes** Conc. H_2SO_4 Test	 -ve	 -ve	 -ve
5	**Phenol** Ferric Chloride Test Folin Ciocalteu Test	 -ve -ve	 -ve +ve	 +ve +ve
6	**Proteins** Xanthoproteic Test	 -ve	 +ve	 +ve
7	**Saponins** Froth Test	 +ve	 +ve	 +ve
8	**Tannins** Gelatin test	 -ve	 -ve	 +ve
9	**Sterols** Libermann-Burchard's Test	 -ve	 +ve	 +ve

+Ve = Positive, -Ve= Negative

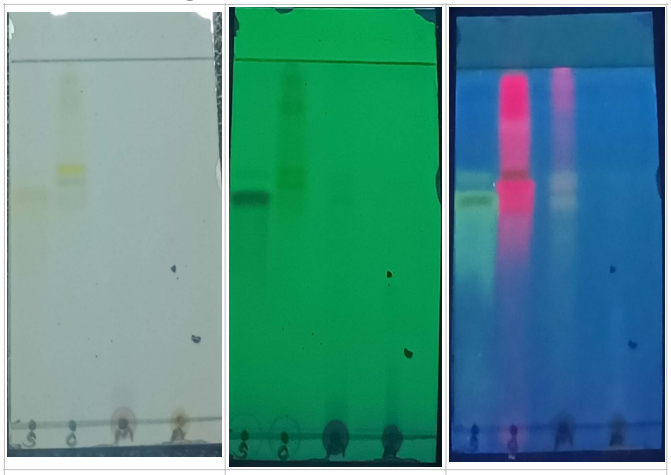
Table 3: TLC of the extract of *M. emarginata*

TLC of *M. emarginata* (Flavonoids)			
S. No.	**Mobile phase** Toluene: Ethyl acetate Formic acid (5:4:1)	**Distance of solute**	***Rf* value**
1	**(Quercetin)** Dis. travel by mobile phase= 5.0 cm No. of spot at normal light = 1 No. of spot at short UV = 1 No. of spot at long UV = 1	 Normal Light- 3.2 Short- 3.2 Long- 3.2	 Normal- 0.64 Short- 0.64 Long- 0.64
2	**(Chloroform extract)** No. of spot at normal light = 6 No. of spot at short UV = 6 No. of spot at long UV = 7	Normal Light- 3.1, 3.3, 3.5, 4.5, 4.8, 5 Short- 3.1, 3.3, 3.5, 4.4, 4.8, 5 Long- 3.1, 3.3, 3.5, 4, 4.4, 4.8	Normal- 0.62, 0.66, 0.7, 0.9, 0.96, 1 Short- 0.62, 0.66, 0.7, 0.88, 0.96, 1 Long- 0.62, 0.66, 0.7, 0.8, 0.88, 0.96
3	**(Methanolic extract)** No. of spot at normal light = 1 No. of spot at short UV = 2 No. of spot at long UV = 4	 Normal Light- 0.4 Short-3, 3.4 Long- 3, 3.5, 4.6, 5	 Normal- 0.08 Short- 0.6, 0.68 Long- 0.6, 0.7, 0.92, 1.0
4	**(Aqueous extract)** No. of spot at normal light = 0 No. of spot at short UV = 1 No. of spot at long UV = 1	 Normal Light- 0 Short- 3.4 Long- 3.4	 Normal- 0 Short- 0.68 Long- 0.68

The chloroform extract of *M. emarginata* showed a total of 6 spots under normal and short UV, and 7 under long UV, indicating the presence of multiple phytoconstituents (Figure 3). The Rf values ranged from 0.62 to 1.0, suggesting the presence of several distinct compounds. The methanolic extract exhibited 1 spot

under normal light, 2 spots under short UV, and 4 spots under long UV, with Rf values ranging from 0.08 to 1.0. This reflects a rich flavonoid and phenolic profile extractable in methanol. The aqueous extract showed no visible spots under normal light but revealed a single spot under both short and long UV light, with an Rf value of 0.68, indicating the presence of at least one UV-active compound.

Figure 3: TLC of Flavonoids



Normal light	Short UV	Long UV

1st spot= Standard Quercetin, 2nd spot= Chloroform extract, 3rd spot= Methanolic extract, 4th spot= Aqueous extract.

Discussion

An analytical study was undertaken to evaluate the phytochemical properties of *M. emarginata*. The plant's leaves were processed through dehydration and pulverization to enhance the efficiency of subsequent extraction. A series of solvents, specifically chloroform, methanol, and water, were utilized to facilitate the comprehensive extraction of the plant's phytochemical constituents. The purpose of the phytochemical analysis was to identify biologically active compounds, determine potential medicinal uses, assess overall quality, contribute to the advancement of pharmaceutical science, and optimize formulation design. Such analytical approaches are crucial for a comprehensive understanding of the therapeutic potential of medicinal flora, guaranteeing their safe and effective application and upholding rigorous quality control protocols. The phytochemical analysis of *M. emarginata* demonstrated the presence of alkaloids, flavonoids, phenolics, tannins, and saponins, compounds that have been extensively documented for their potential antidiabetic activity. TLC confirmed the diversity of these phytoconstituents, reinforcing the plant's pharmacological significance (8, 15).

Several previous investigations have highlighted the antidiabetic properties of medicinal plants rich in bioactive compounds. For instance, a study conducted by Chandak *et al*.(7) evaluated the antidiabetic effects of *M. emarginata* in alloxan-induced diabetic rats, revealing significant reductions in blood glucose levels. The phytoconstituents identified in the present study corroborate these findings, suggesting that the bioactive compounds found in *M. emarginata* may modulate metabolic pathways associated with glucose homeostasis.

Furthermore, prior ethnobotanical surveys by Kifle *et al*., (16) have documented over 1200 medicinal plants with hypoglycemic effects, emphasizing the therapeutic value of natural compounds in diabetes management. The current study aligns with these reports, reinforcing the necessity of further research into the pharmacological mechanisms of M. emarginata in glycemic regulation.

The presence of flavonoids and phenolics has been linked to antioxidant and insulin-sensitizing effects in diabetes, as documented by Alam *et al.,* (17). The study here does not provide mechanistic insights into how these compounds exert their influence. Future research should aim at isolating specific bioactive molecules and conducting detailed pharmacokinetic and clinical studies to determine their efficacy and safety.

Despite the promising findings of this study, several limitations must be acknowledged. The qualitative phytochemical analysis confirms the presence of key bioactive compounds, yet it does not quantify their concentrations, which is essential for determining their pharmacological potency. Without precise quantification, it remains challenging to assess the contribution of individual components to the observed antidiabetic effects. Future research should prioritize the isolation and characterization of bioactive compounds to identify precisely those responsible for the antidiabetic effects. Structural analysis using advanced spectroscopic techniques such as NMR and LC-MS would aid in determining the molecular identity and potential pharmacological targets of these compounds. Investigations into the mechanisms of action through in vitro enzyme inhibition assays and molecular docking studies could provide valuable insights into how *M. emarginata* influences glucose metabolism. Additionally, in vivo studies in diabetic animal models are necessary to validate its efficacy, alongside pharmacokinetic studies that assess absorption, distribution, metabolism, and excretion. Clinical trials must be undertaken to establish their safety, efficacy, and dosage parameters for human use. Furthermore, comparative studies evaluating *M. emarginata* alongside conventional treatments like Metformin could provide insight into its relative therapeutic potential. By addressing these gaps, future research can better elucidate the role of *M. emarginata* in diabetes management, paving the way for its potential integration into modern medicinal applications.

Conclusion

The present study highlights the rich phytochemical composition of *M. emarginata* and its potential as a natural antidiabetic agent. The detection of key bioactive constituents such as alkaloids, glycosides, and flavonoids, supported by TLC profiling, reinforces the therapeutic relevance of this plant in traditional medicine. These findings provide a scientific

basis for its use in diabetes management and warrant further investigation into the isolation, characterization, and pharmacological validation of its active compounds. Future studies involving in vivo models and clinical trials are essential to establish the efficacy, safety, and mechanism of action of *M. emarginata-derived* compounds in the treatment of diabetes mellitus.

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https://doi.org/10.47552/ijam.v16iS2.6167 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

High-performance Thin Layer Chromatographic quantification of hesperidin and naringenin from *Citrus sinensis* peel extract

Research Article

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Abstract

Orange peel is a member of *Citrus sinensis*, the Rutaceae family, and is widely used for its nutritional properties. Orange peels are the waste part of the fruit and contain various therapeutic molecules. Hesperidin and naringenin are two flavonoids present in orange peel. The present study depicted the quantification of high-performance thin layer chromatography of orange peel extract using hesperidin and naringenin. The HPTLC densitometric analysis of the ethanolic extract of orange peel was carried out using a CAMAG HPTLC system, Linomat 5 applicator, and Vision CAT software. The results were obtained in the form of chromatograms scanned at 254 & 366 nm. The Rf values of hesperidin were 0.5 with mobile phase Ethyl acetate: Methanol: Water (15:04:01) and 25.93 nanograms in 1 mg of orange peel extract. Naringenin was quantified at 254 & 366 nm with Rf value 0.6 & mobile phase used was Toluene: Ethyl acetate: Formic acid (12:8:1.6v/v/v), and 14.92 nanograms of naringenin were found in 1 mg of orange peel extract.

Keywords: Orange peel extract, HPTLC, Hesperidin, Naringenin, Densitometry.

Introduction

Oranges are currently the most produced fruit in the world. Tropical and subtropical regions are home to the sweet orange, *Citrus sinensis*, a citrus fruit that belongs to the Rutaceae family. Today, the world produces around 110 million tons of citrus fruit per year (1). Orange juice will be made from about 8.3% (2 million tons) of the 24 million tons of oranges that are anticipated to be produced globally in 2016–17 (2). However, as orange peels comprise around 44% of the fruit body, they will produce a large number of by-products. Since these orange peels are typically thrown away as waste, there may be major disposal issues (3). There are numerous techniques to prepare orange peels for use in cosmetics, medications, and food (4). Orange peel extracts are high in physiologically active substances, including phenolic acids and flavonoids, which have antioxidant, anti-inflammatory, anti-atherosclerosis and anti-carcinogenic potential, and they are also a major source of dietary fiber (5). Orange peel is a common waste product that contains both soluble fibers like pectin and insoluble fibers, including lignin, cellulose, hemicellulose (6). Orange peels also contain bioactive chemicals and essential oils with antioxidant and antibacterial potentials, such as α- α-pinene, β-pinene, farnesene, limonene, γ-terpinene, myrcene and α-terpinolene. Orange peels contains compounds like essential oils and pectin that have antibacterial activity and can be used to prolong the shelf life of some food products. Numerous extraction methods are employed to extract natural chemicals from orange peels (7). Flavonoids and phenolic acids are among the many phenolic chemicals found in citrus fruit peels and seeds, out of both flavonoids are more prevalent in the peel than in the seeds (8). Flavonoids are naturally occurring compounds with different phenolic structures that are found in plants. They are oxidized by radicals to form a less reactive and more stable radical. They include flavonols (such as kaempferol, quercetin, fisetin and myricetin), flavonones (such as flavanone, hesperidin, and naringenin), flavones (such as flavone, luteolin and apigenin), and additional classes (9). Certain flavonoids can directly scavenge superoxides, but other flavonoids can scavenge peroxynitrite, a highly reactive radical generated from oxygen. Epicatechin and rutin are also powerful radical scavengers. Owing to its inhibition of the xanthine oxidase enzyme, rutin may possess scavenging capabilities (10). Numerous flavonoids, such as apigenin, catechin, quercetin, naringenin, rutin, venoruton, and, have been demonstrated to possess hepatoprotective qualities (11). Hesperidin is a flavanone glycoside found in several citrus fruits. Among its many pharmacological activities are antioxidant, anti-inflammatory, neuroprotective, anticancer, and antiallergy (12). According to Wei et al., treating asthmatic mice decreased blood levels of IgE as well as BALF levels of eosinophils, neutrophils, macrophages, IL-4, IL-5, and IL-13. This data suggests that hesperidin helps to cure allergic asthma by suppressing the Th2 response and reducing

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inflammation (13). Citrus fruits, such as oranges and grapefruit, and tomatoes are the main sources of naringenin (4′,5,7-trihydroxyflavonone). It comes in a variety of conjugated forms, mostly as aglycone glycosylated, neohesperidoside, which is derived from phenylalanine, the aromatic amino acid (14). Each of these forms has altered pharmacokinetic properties, including absorption, distribution, metabolism, and elimination. Grapefruits are the most common source of naringenin, where it is present as "naringin," an inactive glycone form. Naringin, a 4′,5,7-trihydroxyflavonone 7-rhamnoglucoside, (15). The most active aglycone form. Naringenin is reported to enter the bloodstream fast due to its easy absorption by the gastrointestinal tract. Ever since its rapid absorption, Naringenin is the most pharmacologically effective form of naringin (16). Two intermediates, naringenin and rhamnose, are produced shortly from naringin after consumption by the intestinal bacterial naringinase enzyme (17).

Materials and Methods

Collection and authentication

The orange peels were collected from the local market and authenticated by Dr. Nitin Dongarwar, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur

Extraction

The orange peels were collected and shade dried for 5 days. The dried peels were powdered using a mechanical grinder into coarse powder (18). As per the literature and our study revealed that the maceration is the best process for extraction of phytochemicals. The 500 g of coarse powdered material was placed in a glass jar and macerated for 3-5 days using ethanol (3 volumes). The extract was filtered and concentrated using a rotary evaporator with 50-60˚C temperature (19)

Phytochemical Study

The phytochemical screening of the orange peel extract was performed and shown the presence of flavonoids, phenolics, tannins, and alkaloids (20)

Test for flavonoids

Shinoda test: A little piece of magnesium, 1.5 mL of 50% methanol solution, and 4 mL of extract solution were heated. When 5–6 drops of concentrated HCl were added, flavonoids showed a crimson hue (21).

Dil. NH_3 test: 5 mL of diluted NH3 solution in extract was taken, and concentrated H_2SO_4 was added. Flavonoids were suggested when a yellow-colored precipitation appeared (22).

Test for polyphenol

Ferric Chloride test: When 3 to 4 drops of 10% $FeCl_3$ were added to the diluted extract, gallo tannins became blue while catechol tannins caused the solution to become green (23).

Test for Tannins

Lead acetate Test: 10 mg of plant extract was taken and 0.5 ml of 1 % lead acetate solution was added and the formation of precipitate indicates the presence of tannins (23).

Test for alkaloids

Meyer's test: 1ml of Meyer's reagent was added to 2 ml of extract. Presence of pale yellow precipitate confirms the presence of alkaloids in the OPEE (24).

Dragendorff's reagent test: 2ml of extract were heated using 2% H_2SO_4. A little amount of Dragendorff's reagent was added. The presence of alkaloids was revealed by an orange-red precipitate (25).

HPTLC Instrument Specifications

Instrumentation

A CAMAG HPTLC system equipped with LINOMAT 5 applicator fitted with a 100 µl syringe with CAMAG TLC scanner and vision CAT software was used (26).

Solvents and Chemicals

Standard hesperidin & naringenin was purchased from Sigma-Aldrich, St. Louis, MO, USA. All the HPLC grade solvents were used for HPTLC analysis, and all the chemicals of analytical grade were used for the above study.

Sample preparation

Dried orange peel extract 10 mg was dissolved in 10 mL of methanol and then filtered.

Standard preparation

1 mg hesperidin dissolved in 10 ml of methanol.1 mg of naringenin dissolved in 10 ml of methanol. Both the standard solutions were sonicated using a sonicator, used for an HPTLC quantification study.

Chromatographic Conditions

A 10 x 20.0 cm pre-coated silica gel 254 HPTLC plate (E. MERCK) was used for the HPTLC densitometric analysis. No plate modification or pre-washing. A CAMAG Linomat applicator with a 100 µl syringe was used to apply the sample solution on the plate in the form of a band. Samples were applied to the TLC plates as 8 mm bands using a Camag Automatic TLC Sampler 4 (ATS4) sample applicator (Switzerland) fitted with a Camag microlitre syringe. A constant application rate of 150 nL/s was used. Linear ascending development of the plates to a distance of 70 mm was performed with ethyl acetate-methanol-water 15:4:1 (%, v/v) previously saturated for 20 min and the hesperidin standard and sample-loaded plates were developed in an automated development chamber. The procedure repeated for naringenin using Toluene: Ethyl acetate: Formic acid (12:8:1.6 v/v/v) mobile phase previously saturated for 20 min and the naringenin standard and sample-loaded plates were developed in an automated development chamber with distance of 70 mm and the application rate was 150 nL/s.

https://doi.org/10.47552/ijam.v16iS2.6167 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Results and Discussion

The *Citrus sinensis* L. peels were subjected to ethanolic extraction by using maceration and the extract was evaporated to dryness by rotary evaporator, and the extract were concentrated and tested for different phytoconstituents, by phytochemical study as shown in Table 1.

Table 1: Phytochemical study of Orange peel ethanolic extract

Sr. No	Phytochemical Test	Observation
1	Flavonoids (Shinoda test)	Positive
2	Tannins (Lead acetate Test)	Positive
3	Phenolics (Ferric Chloride test)	Positive
4	Alkaloids (Dragendorff test)	Positive

The polyphenolic compounds were found in the extract based on the above qualitative phytochemical study. The extract was assessed using HPTLC and fingerprinting using naringenin and hesperidin as standards. The outcomes are shown in Tables 2 and 3.The chromatograms were obtained upon scanning at UV at 254 & 366 nm. Figures 1, 2 & 3 depicted for hesperidin and Figures 4, 5 & 6 depicted for naringenin, respectively. The Rf values, area percentage, and peak height of both hesperidin and naringenin were calculated.

Table 2: Tracks of hesperidin as a standard & Orange-peel ethanolic extract

Track no	Ref/Sample ID	X (mm)	Y (mm).	Appl. Volume	Rf	Conc.
1	Hesp	20.0	40.7	1.0 µl	0.527	0.1 mg/ml
2	Hesp	31.4	40.8	2.0 µl	0.529	0.1 mg/ml
3	Hesp	42.8	40.1	3.0 µl	0.518	0.1 mg/ml
4	Hesp	54.2	40.4	4.0 µl	0.523	0.1 mg/ml
5	Hesp	65.6	40.3	5.0 µl	0.521	0.1 mg/ml
6	Hesp	77.0	40.1	6.0 µl	0.518	0.1 mg/ml
7	Hesp	88.4	40.1	7.0 µl	0.518	0.1 mg/ml
8	Hesp	99.8	40.2	8.0 µl	0.519	0.1 mg/ml
9	OPE	111.2	40.3	2.0 µl	0.521	1mg/ml
10	OPE	122.6	39.8	4.0 µl	0.513	1mg/ml
11	OPE	134.0	40.1	6.0 µl	0.518	1mg/ml
12	OPE	145.4	40.4	8.0 µl	0.523	1mg/ml
13	OPE	156.8	40.3	10.0 µl	0.521	1mg/ml
14	OPE	168.2	40.0	12.0 µl	0.516	1mg/ml
15	OPE	179.6	39.7	14.0 µl	0.511	1mg/ml

Hesp - Hesperidin, OPEE - Orange peel ethanolic extract

Figure 1: Calibration curve of Hesperidin standard

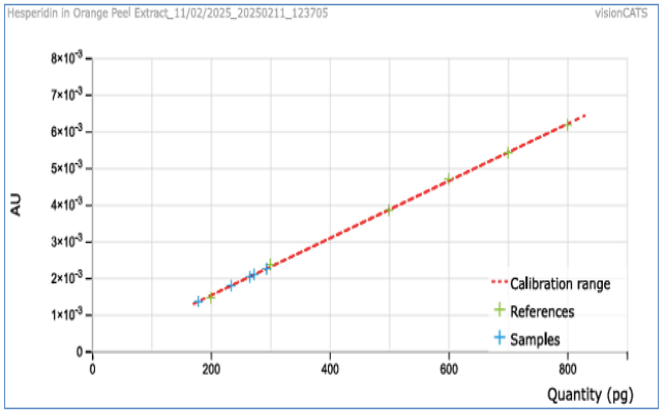
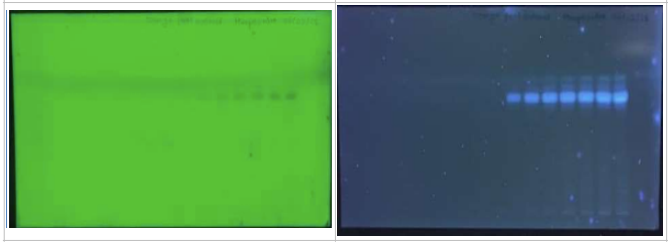


Figure 2: Scanned image of Hesperidin and OPEE at 254 nm

Figure 3: Scanned image of Hesperidin and OPEE 366 nm



After performing the phytochemical study revealed that the polyphenolic compounds are abundantly present, and the same extract was studied using High-performance thin layer chromatography (HPTLC), and the extract was fingerprinted with the use of naringenin and hesperidin as standards, and the results are displayed in Table 2 & 3 respectively. The chromatograms were obtained upon scanning at UV at 254 & 366 nm. Figure 7 shows the chromatographic overlay of hesperidin standard & OPEE while figure 8 shows the UV overlay spectra of OPEE extract at 254 nm. Figure 9 displays the chromatographic overlay of naringenin & OPEE while figure 10 shows the UV overlay spectra of naringenin and OPEE at 254 nm.

Table 3: Tracks of naringenin & orange peel ethanolic extract (OPEE)

Track no	Ref/Samp.ID	X (mm)	Y (mm)	Appl. Volume	Rf	Conc.
1	Naring	20.0	45.9	1.0 µl	0.611	0.1 mg/ml
2	Naring	31.4	44.9	2.0 µl	0.595	0.1 mg/ml
3	Naring	42.8	45.4	3.0 µl	0.603	0.1 mg/ml
4	Naring	54.2	45.4	4.0 µl	0.603	0.1 mg/ml
5	Naring	65.6	45.0	5.0 µl	0.597	0.1 mg/ml
6	Naring	77.0	44.8	6.0 µl	0.594	0.1 mg/ml
7	Naring	88.4	44.7	7.0 µl	0.592	0.1 mg/ml
8	Naring	99.8	44.7	8.0 µl	0.592	0.1 mg/ml
9	OPEE	111.2	45.2	2.0 µl	0.600	1mg/ml
10	OPEE	122.6	45.9	4.0 µl	0.611	1mg/ml
11	OPEE	134.0	45.2	6.0 µl	0.600	1mg/ml
12	OPEE	145.4	45.3	8.0 µl	0.602	1mg/ml
13	OPEE	156.8	42.8	10.0 µl	0.561	1mg/ml
14	OPEE	168.2	44.6	12.0 µl	0.590	1mg/ml
15	OPEE	179.6	44.6	14.0 µl	0.590	1mg/ml

Naring – Naringenin, OPEE – Orange peel ethanolic extract

Figure 4: Calibration curve for Naringenin Standard

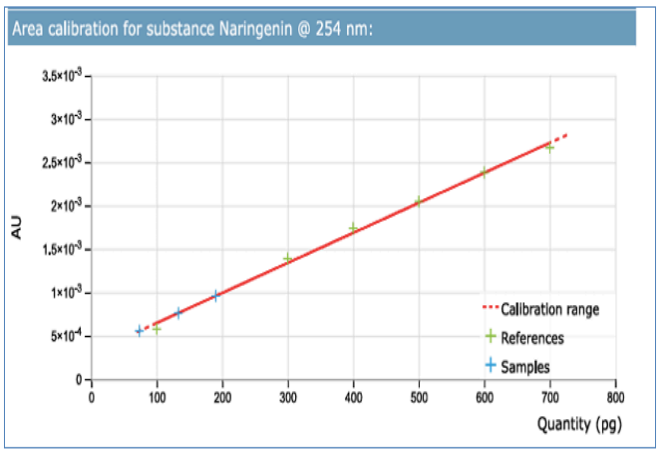


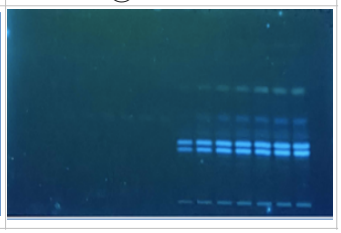
Figure 5: Scanned image of naringenin and OPEE @ 254 nm	Figure 6: Scanned image of naringenin and OPEE @ 366 nm
	

Table 4: Calibration curve parameters of Hesperidin Standard

Parameters	Values
Regression mode	Linear-2
Range deviation	5.00%
Related substances	None
Assignment mode	Single
Number of references	6
Calibration function	$y=3.463\times10^{-6}x+3.002\times10^{-4}$
Coefficient of variation	2.61%
Correlation coefficient	R=0.997633

Table 5: Calibration curve parameters of Naringenin Standard

Parameters	Values
Regression mode	Linear-2
Range deviation	5.00%
Related substances	None
Assignment mode	Single
Number of references	6
Calibration function	$y=7.803\times10^{-6}x$
Coefficient of variation	1.17%
Correlation coefficient	R=0.999601

Figure 7: Overlay Chromatograms of hesperidin in OPEE	Figure 8: UV Overlay Spectra of hesperidin in OPEE at 254 nm

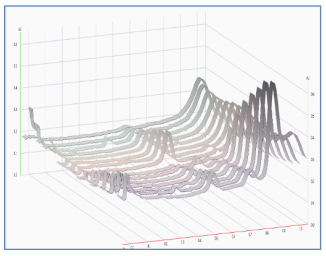
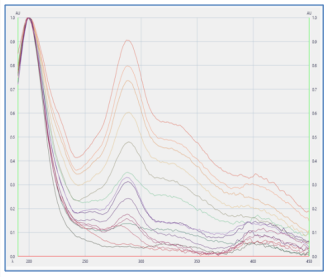
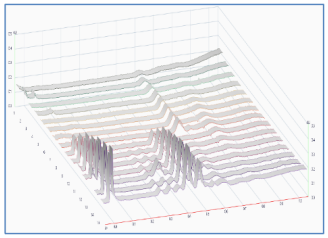
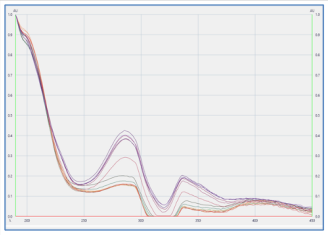

	

Figure 9: Overlay chromatograms of Naringinin in OPEE.	**Figure 10: UV Overlay Spectra of Naringinin in OPEE at 254 nm.**
	

Conclusion

Orange fruit is a waste part of the orange fruit contains different chemical constituents, which are responsible for various therapeutic potential. Hesperidin is a major flavonoid present in orange peel. In the present study, the HPTLC analysis of orange peel extract was performed and the densitometric analysis of the ethanolic extract of orange peel was carried out using a CAMAG HPTLC system and Vision CAT software. The results were observed in the form of chromatograms scanned at 254 & 366 nm. The Rf values of hesperidin was found to be 0.5 with mobile phase Ethyl acetate: Methanol: Water (15:04:01). 25.93 nanograms of hesperidin was found in 1 mg of orange peel extract. Naringenin was quantified at 254 & 366 nm with Rf value 0.6 & mobile phase used was Toluene: Ethyl acetate: Formic acid (12:8:1.6v/v/v). The hesperidin and naringenin was done 14.92 nanograms of naringenin was found in 1 mg of orange peel extract.

Acknowledgement

Authors are thankful to the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, for providing technical facilities.

The authors are thankful to the Mahatma Jyotiba Phuley Research Fellowship (MJPRF) for providing financial assistance during research work.

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https://doi.org/10.47552/ijam.v16iS2.6168 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

A Research on Comparative Methods of Isolation, Evaluation and Identification of *Clitoria ternatea* Plant

Research Article

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Abstract

Clitoria ternatea (Butterfly pea) is a traditional medicinal plant known for its therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities. This study aimed to evaluate its phytochemical composition, physicochemical characteristics, and extraction efficiency using different techniques to support its pharmacological potential. Ethanolic extracts of *Clitoria ternatea* flowers were prepared using maceration, Soxhlet extraction, and ultrasonic-assisted extraction. Qualitative phytochemical screening was conducted to identify secondary metabolites, while physicochemical parameters such as total ash, water-soluble ash, loss on drying, and alcohol- and water-soluble extractives were assessed. The Soxhlet method yielded the highest extractive value (89%), followed by maceration (62%) and ultrasonic extraction (57%). Phytochemical screening confirmed the presence of key bioactive compounds, including flavonoids, alkaloids, saponins, tannins, steroids, and cardiac glycosides. Physicochemical evaluations were within acceptable limits, supporting extract quality and reproducibility. This study validates the traditional use of *Clitoria ternatea* and demonstrates its potential for further development as a phytopharmaceutical agent. Soxhlet extraction is recommended for optimal recovery of bioactive constituents. Further research is warranted to isolate specific compounds and explore their therapeutic applications through pharmacodynamic and clinical studies.

Keywords: *Clitoria ternatea,* Phytochemical screening, Soxhlet extraction, Physicochemical analysis, Herbal medicine, Bioactive compounds.

Introduction

Since ancient times, herbal or plant-based remedies have been utilized for the prevention and treatment of illnesses, and there are still many more components from these natural sources that remain to be investigated. This realization has inspired researchers to discover new compounds from herbal sources to combat various infectious diseases. Studies indicate that a majority of medicinal plants exhibit antimicrobial, antioxidant, and anti-inflammatory properties, which have contributed to the prevention of numerous infectious diseases and also provide potential advantages for society. The current landscape of infectious diseases indicates a concerning rise in the occurrence of both new and re-emerging infectious diseases. Another critical issue is the emergence of resistance to antibiotics currently used in clinical settings. Therefore, there is an urgent requirement to develop a natural formulation that can effectively target the microorganisms responsible for skin diseases (1).

An increasing number of individuals worldwide are utilizing medicinal plants and herbs for health-related purposes. As a result, it will be beneficial to scientifically examine their therapeutic capabilities, biological properties, and safety to make informed choices regarding their use. Numerous important drugs and biologically active compounds have been derived from traditional medicinal plants. These plants demonstrate a wide variety of pharmacological activities, including antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous system, respiratory, immunological, anti-inflammatory, analgesic, antipyretic, and many other pharmacological effects. Preliminary phytochemical analysis revealed that *Clitoria ternatea* contains tannins, phlobatannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, Stigmast-4-ene-3,6-dione, volatile oils, and steroids (2).

Clitoria ternatea, commonly referred to as Butterfly pea, is a perennial leguminous vine that belongs to the Fabaceae family and the Papilionaceae subfamily. The Clitoria genus includes 60 species, primarily found in tropical regions, with a few species occurring in temperate zones. The species most often noted is *Clitoria ternatea*. This plant is primarily utilized as animal fodder due to its high palatability for livestock and its adaptability to different climatic conditions (3). Indigenous to Ternate Island in the

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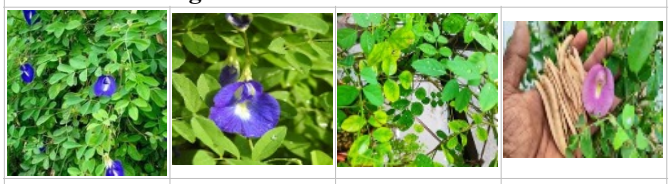
Moluccas, this species is now commonly cultivated as an ornamental, fodder, or medicinal plant (4). Native to Ternate Island in the Moluccas, this species is now frequently grown as an ornamental, feed, or medicinal plant. It has become naturalized in the East and West Indies, China, and India (5).

Since ancient times, "Shankhpushpi" has been recognized as a well-regarded herbal remedy in Ayurveda, known for its qualities as a brain and nervine tonic, as well as a laxative. In Ayurvedic literature, it is referred to as a "Medhya-Rasayana." This herbal preparation includes the whole plant, featuring the following botanicals: *Convolvulus pluricaulis* (from the Convolvulaceae family), *Evolvulus alsinoides* (also from the Convolvulaceae family), *Clitoria ternatea*(from the Papilionaceae family), and *Conscora decusata* (from the Gentianaceae family). These Ayurvedic formulation of above mentioned plants having antioxidant potential are utilized for its effects on the central nervous system (CNS), particularly in enhancing memory and intellect (6). The blossoms of the Clitoria plant are utilized for treating snake bites and scorpion stings in India (7).

Fig 1: *Clitoria ternatea* twiner



Twiner	Flower	Leaves	Pods

Plant profile

Synonym: Blue-pea, butterfly-pea, cordofan-pea, Darwin-pea

Taxonomical classification

- Kingdom- Plantae
- Order- Fabales
- Family- *Fabaceae*
- Tribe- Phaseoleae
- Subtribe- clitoriinae
- Genus-clitoria

Plant Description

This is a perennial herb that can climb or trail, originating from a woody root system. The leaves are imparipinnate, consisting of 2-4 pairs of leaflets along with a terminal leaflet. The leaflets are either ovate or elliptic-oblong, measuring up to 6.5 × 4 cm, with a mostly hairless upper surface and a pubescent underside. The flowers are found in the axils, either solitary or in pairs, and are large and vibrant, displaying a bright blue colour. The pods are linear and oblong, ranging from 6 to 13 cm in length, flattened, and have a pointed tip, being either hairless or covered in fine hairs (8).

Flowers

- The flower is the appealing part of the plant. They can be found in either single or paired forms and come in a variety of colours such as mauve, white, and both dark and light blue.
- The pedicels and bracts can reach lengths of 4-9 mm and 12 m, respectively, while the corolla is made up of one standard petal, two keels, and two wing petals. The standard petal is the most prominent among all the petals.
- The flower has a bilateral symmetry, although there are also naturally occurring petal mutants that exhibit a radial appearance. The variation in flower colour is attributed to the presence of flavonoids (9).

Leaves

- The leaves exhibit pinnate venation with seven leaflets. The terminal leaflets are larger, while those at the base are smaller. A dorsiventral structure can be observed when a transverse section of the leaves is made (10).
- Prismatic calcium oxalate crystals are found along the veins. The palisade ratio measures 6.0, and the vein-islet number is 7.5.
- The leaf blade has linear trichomes present on both surfaces. The shape of the lamina is ovate, and its surface is smooth but has a hairy texture (11).

Pods

- The pods are elongated and flat, featuring colours such as olive, brown, and black, typically measuring between 5 to 7 cm in length, with each pod containing 6 to 10 seeds (12).

Seeds

- The seeds can be soaked in water for a night to initiate germination. Following that, germination occurs within 1 to 2 weeks, and blooming takes place after 4 weeks. For optimal growth, the plant requires full sunlight or a partially shaded environment (13).

Stems

- The stems can reach lengths of 3 to 5 meters and may be hairy, smooth, or sometimes upright. They are characterized by their long, slender, and flexible form. The colour ranges from light green to brownish (11).

Roots

- The root structure of this plant consists of a robust taproot system (13). The nodules present on the roots exhibit a symbiotic relationship with nitrogen-fixing bacteria, enabling them to capture atmospheric nitrogen. It has a brown hue, a bitter flavour, and a distinct smell (12).

Traditional Uses

The plant is highly regarded for its diverse healing properties in various traditional practices and folk remedies. The medicinal advantages of the different components of C. ternatea are presented in Table No 1.

https://doi.org/10.47552/ijam.v16iS2.6168 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Table 1: *C. ternatea* traditional uses

Plant Part	Uses	References
Flower	The flower paste is employed in treating eye infections and headaches. Flowers can also serve as an antidote for snake bites.	Alok *et al.* (14)
Leaves	When a headache or swelling of a nearby gland happens, juice extracted from the leaves is mixed with salt and applied around the ears to alleviate discomfort. The juice of the leaves serves as an antidote for snakebites. It is also used to treat swollen joints and is applied as poultices.	Alok *et al.* (14)
Seeds	Applied for alleviating colic, dropsy, joint swelling, and the enlargement of abdominal organs. It also has properties as a laxative, a mild emetic, and is effective against worms. Utilized as green manure and as a remedy for toxins.	Ashraf, *et al.*, (15)
Stem	Serves as an antidote for snake and scorpion bites. Due to certain phytochemicals, it functions as a tonic for the brain and is also beneficial for urinary issues, as well as problems related to the throat and eyes.	Sarma *et al.*, (16)
Roots	Ascetics, epilepsy, enlarged abdominal organs, skin disorders, and throat irritation. Utilized as a diuretic, laxative, mental tonic, and purgative. It is employed to address various illnesses like constipation, dyspepsia, eye conditions, enlarged abdominal organs, and fever.	(14, 15)

Selection and Pre-treatment of Raw Material

The plant was collected from nearby locality of Wardha district, Maharashtra, during the month of December. The plant was authenticated by Dr. Swati Kalode, Department of botany, Bajaj college of Science, Wardha for Voucher specimen number 12/Botany/ 2024-2025.

Then leaves were separated, dried, coarsely powdered, passed through sieve No. 40, and stored in a closed container for further use.

Figure 2: Authentication of the *clitoria ternatea* plant

Plant parts used: Flower, leaves, roots
Cleaning: wash fresh plant material with distilled water to remove dust and contaminants.

Drying:

Shade drying: Flowers were air-dried in a well-ventilated, shaded area for 5-7 days to reduce moisture content to <10%.

Size Reduction: Grind the dried material into course powder (40 -60 mesh size).

Extraction methodology

The method of extraction is:

1) maceration extraction
2) Soxhlet extraction
3) Ultrasonic extraction

Maceration Extraction

A total of 100 g of dried *Clitoria ternatea* flower powder was accurately weighed and placed in a clean, amber-coloured glass container. To this, 1000 mL of 70% ethanol (v/v) was added as the extraction solvent. The mixture was sealed and kept at room temperature (25 ± 2°C) for 72 hours with intermittent shaking (twice daily) to facilitate maximum extraction of phytoconstituents.

After the maceration period, the mixture was filtered through Whatman No. 1 filter paper to separate the extract from the plant residue. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C until a semi-solid mass was obtained. The concentrated extract was then transferred to a pre-weighed glass vial and stored at 4°C for further phytochemical and pharmacological evaluations (18).

Soxhlet Extraction

A total of 50 g of the dried *Clitoria ternatea* flower powder was packed into a cellulose thimble and placed in the Soxhlet extractor. The extraction was carried out using 500 mL of 70% ethanol as the solvent. The apparatus was maintained at the solvent's boiling point (~78°C) and run continuously for 6 hours or until the solvent in the siphon tube became colourless, indicating exhaustive extraction.

After extraction, the solvent was recovered using a rotary evaporator under reduced pressure at 40°C. The concentrated extract was then dried to a constant weight in a vacuum desiccator. The resulting crude extract was stored in a refrigerator at 4°C for further analysis (18).

Ultrasonic extraction

About 10 g of powdered *Clitoria ternatea* flowers was mixed with 100 mL of 70% ethanol in a 250 mL conical flask. The flask was placed in a water bath maintained at 30 ± 2°C. Ultrasonication was carried out at a frequency of 20 kHz and power of 200 W for 30 minutes.

The pulse was set to 5 seconds on and 2 seconds off to avoid overheating. During the process, the

mixture was stirred intermittently to ensure uniform cavitation and solvent penetration.

After sonication, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. The concentrated extract was then dried in a vacuum desiccator and stored at 4°C for subsequent analysis (17).

Phytochemical Screening

- **Alkaloid:** Few ml of extract and few drops of Mayer's reagent added by side of test tube. A white and creamy precipitate indicates presence of alkaloids.
- **Amino Acid:** 1 ml of extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of amino acid.
- **Anthraquinone Glycoside:** To 2.0 mL of each extract of the plant, concentrated ammonia (1.0 mL) was added. The formation of a red-rose colour indicates a negative result, suggesting the absence of anthraquinone glycosides.
- **Cardiac Glycosides:** To the solution of extract, add glacial acetic acid, few drops $Fecl^3$ and conc. Sulphuric acid. Then observe reddish brown colour at the junction of two-layer shows presence of CO^2.
- **Flavonoids:** On 1.0 mL of each acidic extract, sodium hydroxide (4.0 M) solution was added until the pH reached 10. The formation of yellow colour indicates a positive result, suggesting the presence of flavonoids (20).
- **Saponin:** 5 ml of extract boiled in 10 ml of D.W. in test tube, shake vigorously for 30 sec. and allowed to stand for half an hour formation of forth indicates the absence of saponins.
- **Steroids:** 2 ml of chloroform was added to extract and few drops of concentrated H^2SO^4. The presence of steroids was indicated by the appearance of red colour in the upper layer while yellow with greenish fluorescence appears in the H2SO4 layer.
- **Tannins:** About 2 ml of extract was boiled with 1 ml of 1% aqueous HCl acid was taken and observed for the red precipitation which showed that presence of tannins (21).

Physicochemical study of crude drugs

Loss on Drying

(LOD) refers to the mass loss expressed as a percentage weight per weight and can be assessed by the following method.

Methodology: A glass stopper and a shallow weighing bottle, previously dried for 30 minutes, were weighed. A sample of powdered twigs was placed in the bottle and weighed precisely. The sample was evenly spread by gently shaking it from side to side. The bottle was then placed in the oven. The sample was dried until a constant weight was achieved. Once drying was complete, the bottle was allowed to cool to room temperature in a desiccator before being weighed. The difference between the initial and final weights provided the LOD.

Ash content

The non-volatile inorganic compounds found in any organic material make up its ash.

Total ash

Procedure: Accurately weigh about 2-4 grams of ground, air-dried material and place it in a °C until it becomes white, indicating that carbon is no longer present. Allow it to cool in a desiccator, then moisten the residue with approximately 2 ml of water or a saturated solution of ammonium nitrate. Dry it on a water bath and then on a hot plate, followed by igniting it to achieve a constant weight. Let the residue cool in a suitable desiccator for 30 minutes, and then weigh it promptly. Compute the total ash content in mg/g of the dried material. A sample of 1 gram was weighed and air-dried in a pre-weighed silica dish. It was incinerated at a temperature no greater than 450 °C until all carbon was removed, then cooled, and the ash was weighed, from which the percentage of ash was calculated.

Water-soluble ash

Method: The ash was produced following the procedure outlined earlier for total ash. The collected ash was boiled for 5 minutes with 25 ml of water. It was filtered, and the insoluble residue was gathered in a Grouch crucible, rinsed with hot water, and then ignited for 15 minutes at a temperature not exceeding 450° C. The weight of the insoluble residue was deducted from the weight of the ash. The resulting weight difference indicated the amount of water-soluble ash. The percentage of water-soluble ash was calculated based on the air-dried drug.

Acid-insoluble ash

Method: The ash was prepared following the procedure outlined earlier for total ash. The resulting ash was treated with 25 ml of 2M hydrochloric acid and boiled for 5 minutes. It was then filtered, and the insoluble residue was gathered in a Gooch crucible, rinsed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid-insoluble ash was computed based on the air-dried sample.

Extractive value determination

This method determines the number of active constituents in given amount of medicinal plant material when extract with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these Phytoconstituents in that particular solvent yields a solution containing different phytoconstituents. The composition of these Phytoconstituents in that particular solvent depends upon the nature of the nature of the drug and solvent used.

Alcohol soluble extractive value

A precisely weighed 5 grams of air-dried crude drug was placed in a sealed flask and macerated with 100 ml of 95% ethanol for 24 hours. During the first 6 hours, it was shaken frequently and then allowed to sit for an additional 18 hours. The mixture was then

filtered quickly, taking care to prevent any loss of ethanol. A volume of 25 ml from the filtrate was taken and evaporated to dryness in a tared shallow dish at 105°C and subsequently weighed. The percentage of the ethanol soluble extractive value was determined in reference to the air-dried drug.

Water soluble extractive value

For the determination of water-soluble extractive value, 5 grams of air-dried crude drug was accurately weighed and placed in a closed flask, where it was macerated with 100 millilitres of distilled water for 24 hours. During the initial 6 hours, the mixture was shaken frequently and then allowed to rest for 18 hours before filtering rapidly while taking care to prevent the loss of distilled water. A volume of 25 millilitres of the filtrate was taken and evaporated to dryness in a tared shallow dish at 105 °C, after which it was weighed. The percentage of water-soluble extractive value was then calculated in relation to the air-dried drug (22).

Results and Discussion

Preliminary Phytochemical screening

The phytochemical analysis of the methanolic extract was performed and the results have been given in (Table 3).

The preliminary phytochemical screening of extracts revealed the presence of phytoconstituents like alkaloid, tannins, steroids, flavonoids, cardiac glycosides, anthraquinone glycosides, etc.

Table 2: Observation for Phytochemical screening

Sr no.	Extract	Observation
1)	Alkaloids	+
2)	Amino Acid	+
3)	Anthraquinone Glycoside	-
4)	Cardiac Glycosides	+
5)	Flavonoids	+
6)	Saponin	-
7)	Steroids	+
8)	Tannins	+

Physicochemical study of crude drugs

The physicochemical properties such as ash values, extractive values, loss on drying, etc were determined and given in (Table 3)

Table 3: Physicochemical study of crude drugs

Sr. No.	Parameter	Value
1)	Total ash	16%
2)	Acid in soluble ash	1.5%
3)	Water soluble ash	5%
4)	Loss on drying	9%
5)	Alcohol Soluble extractive value	8.9%
6)	Water soluble extractive value	25.6%

Extraction Method

The percentage yield of the extraction method was performed and result have been given in (Table 4).

The extraction yield and phytochemical content of *clitoria ternatea* varied significantly across the three extraction methods: Maceration extraction, Soxhlet extraction and Ultrasonic Extraction.

The Soxhlet extract showed the highest extraction yield (89%), followed by maceration extraction (62%), and ultrasonic maceration (57%).

Table 4: Percentage yield of extraction method

Sr no.	Extraction method	%Percentage
1)	Maceration Extraction	62%
2)	Soxhlet Extraction	89%
3)	Ultrasonic Extraction	57%

These results suggest that the Soxhlet method is more effective in extracting bioactive compounds from *Clitoria ternatea* compared to the other two methods.

Conclusion

The findings of this study establish *Clitoria ternatea* as a promising source of bioactive phytoconstituents, notably flavonoids, alkaloids, tannins, saponins, and steroids. Comparative extraction analysis revealed Soxhlet extraction as the most efficient method, yielding the highest concentration of phytochemicals. Physicochemical characterization supported the consistency and quality of the extracts, suggesting their suitability for formulation development. These results scientifically validate the traditional medicinal use *of Clitoria ternatea* and underscore its potential for further development into standardized phytopharmaceuticals. Future investigations should focus on the isolation of specific active compounds, their mechanistic pathways, and in vivo efficacy through preclinical and clinical studies.

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https://doi.org/10.47552/ijam.v16iS2.6169 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Comparative evaluation of Punarnavadi guggul tablet formulation for anti-inflammatory activity

Research Article

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Abstract

Punarnavadi guggul represents a traditional polyherbal formulation utilized in the treatment of dermatological conditions, jaundice, dropsy, edema, hyperuricemia, and rheumatism. The present investigation sought a comparative analysis of the three marketed formulations of *Punarnavadi Guggul* tablet for its in vivo anti-inflammatory properties. The anti-inflammatory effects were assessed using the carrageenan-induced rat paw edema model and the cotton pellet granuloma method. The animal groups treated with laboratory-prepared formulation PF (150 mg/kg) showed significant antiinflammatory activity comparable to the standard drug. Existing literature indicates that guggulsterone E, Z, and deodarone are implicated in the anti-inflammatory effects, whereas gallic acid, ascorbic acid, polyphenols, and flavonoids contribute to its antioxidant properties. The formulated tablet exhibited pre-eminent anti-inflammatory properties when compared to commercial formulations, but all formulations showed noteworthy bioactivity.

Keywords: Anti-inflammatory activity, Carrageenan, Cotton pellet, Polyherbal, *Punarnavadi guggul*.

Introduction

Inflammation is a dynamic sequence as a protective response against various etiological agents, either infectious or noninfectious, by the body (1). The inflammatory process constitutes a pathological reaction of living tissues to injuries, culminating in the localized aggregation of plasma fluid and blood cells. This physiological response serves to eradicate or mitigate the propagation of harmful agents while simultaneously facilitating the removal of necrotic cells and tissues that arise as a consequence. The currently available anti-inflammatory pharmacological agents impose significant challenges within the realm of medical science, exhibiting restricted utility due to their associated adverse effects (2). Punarnavadi Guggul is an Ayurvedic preparation acclaimed for its therapeutic efficacy, particularly in the management of diverse health disorders. This formulation amalgamates the advantageous properties of Guggul (*Commiphora* species) with a selection of other herbal constituents, rendering it effective in addressing conditions such as obesity, inflammation, hypothyroidism, and chronic renal failure. The formulation primarily comprises the desiccated roots of *Boerhaavia diffusa* Linn. (Nyctaginaceae) and the heartwood of Cedrus deodara (Roxb.). Loud. (Pinaceae), The stems of *Tinospora cordifolia* Miers. (Menispermaceae), The fruits of *Terminalia chebula* Retz. (Combretaceae), In conjunction with *Commiphora mukul* Engl. (Burseraceae) to create the Punarnavadi guggul tablet formulation (3,4). In the present investigation, the Laboratory prepared formulations (PF) and commercially available formulations of Punarnavadi guggul of various manufacturing companies, designated as Marketed formulation 1 (PN), Marketed formulation 2 (PM), and Marketed formulation 3 (PP), were subjected to comparative analysis regarding their anti-inflammatory efficacy. A review of the literature has indicated that guggulsterone E and Z, derived from Commiphora mukul (5), along with deodarone from *Cedrus deodara* (6) polyphenols and flavonoids, bioactives of Punarnava, are implicated in the mediation of anti-inflammatory activity (3,7). Consequently, Formulation and comparative evaluation of the Punarnavadi guggul formulation concerning its anti-inflammatory properties has been undertaken. The anti-inflammatory effects of the formulations were comparatively assessed utilizing the carrageenan-induced rat paw edema method and the cotton pellet granuloma method at dosages of 100 and 150 mg.

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Methodology

Physicochemical investigation

The assessment of the physicochemical parameters associated with the pharmaceutical substances constitutes an essential procedure for

identifying adulteration or improper handling of drugs. This evaluation encompasses various foreign organic matter, extractive values (alcohol and water soluble), ash values (Total ash value and acid insoluble ash value), Loss on drying (Table 2).

Ash content: The powdered botanical drugs were analyzed for total ash value and acid-insoluble ash value in accordance with the standardized protocols outlined by the quality control regulations for herbal products as established by the World Health Organization (8).

Determination of extractive values: The extractive values were ascertained utilizing an array of solvents, namely alcoholic and hydroethanolic solvents. Approximately 5g of dried, coarsely ground plant material was introduced into a sealed glass conical vessel. Subsequently, 100 mL of the aforementioned solvents was added. The sealed conical vessel was permitted to remain undisturbed for a period of 24 hours while subjected to continuous agitation. Following this, the mixture was filtered, and 25 mL of the resultant filtrate was transferred to a pre-weighed petri dish. The liquid was then subjected to evaporation until a dry state was achieved in a hot air oven maintained at 105°C, followed by a cooling phase of 30 minutes in a desiccator prior to weighing (8).

Moisture content: A precisely measured 1 g sample was placed into a pre-dried crucible. The samples underwent desiccation in an oven maintained at a temperature range of 100-110°C for a duration of 2 hours, subsequently removed, allowed to cool within a desiccator, and then reweighed. This methodology was persistently applied until equilibrium in weights was attained. The moisture content within the samples was computed utilizing the following equation: % moisture = (ab-ac) * 100/ab, where ab represents the combined weight of the dish and the sample prior to drying (g), and ac denotes the combined weight of the dish and the sample subsequent to drying (g) (8).

Foreign organic matter: A quantity of 100 g of the drug was accurately measured and uniformly distributed across a white tile, ensuring that there was no overlap. The sample was then examined visually or with the aid of a lens with a magnification of 10x or greater. Any foreign organic materials were subsequently isolated. Upon completion of the separation process, the weight of the foreign organic matter was recorded, and the w/w percentage present within the sample was calculated.

Phytochemical Screening

The bioactivity of herbal constituents was determined by the phytoconstituent present in it. Therefore hydroalcoholic extract of plant parts was screened to ascertain the presence of phytoconstituents by using different chemical tests as per standard procedures (8) (Table 3).

Plant Material and Formulation Preparation

The validated raw materials were acquired from Natural Remedies Pvt. Ltd., Bangalore, and utilized for the formulation development. The raw materials underwent comprehensive pharmacognostic, physical, and chemical assessments as per standard protocols. Three batches of the formulation were formulated from these evaluated raw materials, *viz*. PF1, PF2, and PF3, following the methodology delineated in Baishajya Ratnawali (9). The tablets formulated successfully met the conventional physical and chemical standards, as well as the evaluative criteria specified for tablet formulation.

Preparation of Punarnavadi Guggul tablet formulation

The Punarnavadi guggul tablets were prepared by the dry granulation method. Dried raw materials Haritaki, deodar, gudvel, and punarnava were procured from Natural Remedies, Bangaluru. Materials were grounded by a mechanical grinder and sifted through a mesh number 20 to create 300 mg pills. For a 300 mg tablet of the Punarnavadi guggul formulation mentioned in Table 1 (10).

Table 1: Ingredients of Punarnavadi guggul formulation

Ingredients	Quantity
Boerhaavia diffusa	1 part
Cedrus deodara	1 part
Terminalia chebula	1 part
Tinospora cordifolia	1 part
Commiphora mukul	4 parts
Pulverised sugar	1 part
Talc	0.25 part
Crospovidone	0.75 part

Evaluation parameters of granules

Angle of repose

The fixed height funnel method was employed to determine the angle of repose of tablet blends. The funnel (10 mm inner diameter) was designed to allow the mixtures to flow easily onto the platform. The angle of repose was determined by measuring the diameter of the powder cone using the formula: Tan θ = h/r. Where 'h' represents the height and 'r' denotes the radius of the powder cone, respectively (11,12).

Bulk density

To assess the apparent bulk density, a specific amount of tablet mixtures was added to a graduated cylinder, and both the volume and weight of the cylinder were recorded (12).

Bulk Density = Weight of powder / Total Volume of the powder

Tapped density

A graduated cylinder containing a measured tablet blend was allowed to fall from a height of 10cm onto a hard surface every 2 seconds, falling solely under its weight. The tapping continued until there was no noticeable difference in the volume (11).

Tapped density = Powder weight / Powder tapped volume

Carr's index

The compressibility index created by Carr is calculated as (12):

$$CI = (\rho t - \rho a) / \rho t = (Va - Vt) / Vt$$

Where ρt and ρa denote tapped and poured bulk density, and Vt and Va represent tapped and poured bulk volume, respectively.

Hausner's ratio:

Hausner's ratio was calculated using the formula, and the results were expressed as a percentage (11).

$$H = Dt / Db$$

While Dt indicated the density of the powder after it was tapped, Db represented the density of the powder when assessed in bulk.

Assessment of tablets:

The Thickness of the 20 formulated tablets of each batch was calculated by Vernier caliper.

Uniformity in weight

Weight variation was determined using 20 tablets. The tablets were weighed separately, and computed the average weight of the 20 tablets was computed. Then determine the upper and lower limits (12).

Hardness and brittleness

The Electro lab friabilator apparatus and the Pfizer hardness tester were utilized to assess the friability levels and hardness of 20 tablets (11).

Time for disintegration

The disintegration test was conducted using a disintegration apparatus with water as the medium. The container was filled with 900 mL of disintegration medium and maintained at a temperature of 37 ± 0.2°C. Six tablets were placed in each of six tubes, and a plastic disc was positioned over the tablets. The test tubes were allowed to oscillate up and down at a frequency of 29-32 cycles per minute. The time taken for the tablet to disintegrate was determined, and the duration required for all tablets to pass through mesh 8-10 (11,13).

In vitro release investigation

The dissolution studies of polyherbal tablets were evaluated employing the USP dissolution apparatus II with 900 mL of 0.1 M phosphate buffer maintained at 37±0.5 °C and a stirring rate of 100 revolutions per minute. The absorbance at 254 nm was measured with a UV spectrophotometer after various 5 ml samples were taken and replaced with an equal volume of simulated fluid at 1, 2, 4, and 8 hours, respectively. The samples were then filtered using Whatman filter paper before measuring the absorbance (11,13).

Design of an Anti-Inflammatory Study

The Anti-inflammatory study was done by using two animal models: Carrageenan induced rat paw oedema method and cotton pellet granuloma method at 100 and 150 mg doses.

Animals used

Sprague-Dawley rats of either sex (150-180 g) were used for the present investigation. They were maintained under standard environmental conditions and were fed with a standard pellet diet and water *ad libitum*. Twelve hours before the start of the experiment, rats were deprived of food but given free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee (Registration No.92/1999/CPCSEA).

Acute toxicity study

Sprague Dawley rats of either sex (n=3) were randomly selected and kept in their cages for at least 5 days prior to dosing for acclimatization to the laboratory conditions. The animals were fasted overnight with free access to water. The hydroalcoholic extracts of Punarnavadi guggulu tablet were administered orally with an initial dose of 2000 mg/kg body weight. The general behaviour of the animals was observed continuously for the initial 4h and intermittently for the next 6h and then again at 24h and 48h following drug administration. The following parameters were used for observation: stimulation (hyperactivity, irritability, tremor, convulsion, piloerection), depression (sedation, anaesthesia, loss of reflex, analgesia), respiration, diarrhoea, salivation, and motor activity. The mortality was observed for seven days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered a toxic dose. However, if the mortality was observed only in one rat out of three animals, then the same dose was repeated to confirm the toxic effect (OECD, Guideline 425 200) (14).

Carrageenan-induced Rat paw oedema

The rats were divided into ten groups (n=6). An acute inflammatory response was elicited through the administration of 0.1 ml of 1% carrageenan (Himedia RM 1576) in normal saline, to the sub-plantar area of the right hind paw of rats, previously demarcated with ink at the level of the lateral malleolus, and subsequently immersed in a perspex cell up to this designated mark. The volumetric measurement of the paw was conducted at time intervals of 0, 1st, 3rd, and 5th hours post-carrageenan injection, utilizing a plethysmometer (Ugo Bastile). Group I was administered normal saline (3 ml/kg) orally, Group II received diclofenac (10 mg/kg) orally, and Groups III through X were subjected to treatment with formulations PF, PN, PM, and PP at dosages of 100 and 150 mg, respectively. The animals underwent pretreatment with the respective drug one hour prior to the administration of carrageenan (15–17).

Cotton pellet-induced granuloma

Following the removal of their pelage, the rodents were subjected to light ether anesthesia, after which 20 mg of sterile cotton pellets were implanted, one within each rodent's axillary region. A control vehicle, indomethacin (10 mg/kg), along with formulations PF, PN, PM, and PP at dosages of 100 and 150 mg, were administered via the oral route for seven consecutive days, commencing from the date of cotton pellet implantation. On the eighth day, the subjects were sedated, and the cotton was surgically excised and

meticulously cleared of any extraneous tissue. The pellets were subsequently desiccated to a constant weight at a temperature of 60°C. The increase in the desiccated weight of the pellet was utilized as an indicator of granuloma formation (15,16,18).

Statistical analysis

The results were expressed as mean ± S.E.M. The significance statistical analysis was performed by two-way ANOVA, $p \leq 0.001$ implied significance.

Results and Discussion

Table 2: Physicochemical analysis of plant parts

Sample	Foreign organic matter (% w/w)	Total ash (% w/w)	Acid insoluble ash (% w/w)	Alcohol soluble extractive value (% w/w)	Water soluble extractive value (% w/w)	Loss on drying (% w/w)
Guggul	3.78	4.2	1.0	33.0	59.3	5.70
Hirda	0.35	4.5	4.51	46.38	69.4	6.37
Punarnava	1.6	1.0	0.8	4.38	11.2	8.36
Gudvel	1.18	9.2	1.74	3.14	11.2	7.41
Deodara	0.5	2.0	0.94	19.65	4.20	8.33

(% w/w = Percent weight by weight)

Table 3: Phytochemical Screening

Plant Constituent	Test Reagent	Guggul	Hirda	Gudvel	Punarnava	Deodara
Steroids	Salkowaski reaction	+	+	+	-	+
Triterpenoids	Liebermann-Burchard test	-	-	-	+	+
Alkaloids	Dragendorff's reagent	-	-	+	+	-
	Mayer's reagent	-	-	+	+	-
	Hager's reagent	-	-	+	+	-
	Wagner's Reagent	-	-	+	+	-
Tannins	Ferric chlorideTest	-	+	+	-	-
	Lead acetate test	-	+	+	-	-
	Potassium dichromate test	-	+	+	-	-
Flavonoids	Shinoda test	-	-	-	-	+
Carbohydrates	Molish's test	+	+	+	+	-
	Fehling's test	+	+	+	+	-
Proteins	Biuret test	-	+	-	-	-
	Xanthoproteic test	-	+	-	-	-
Coumarins	Fluorescence test	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-

Evaluation Parameters of Tablet Blend

Based on the findings from the angle of repose, Carr's Index, and Hausner ratio, the powder mixtures of tablet blend exhibit favorable flow characteristics and effective packing capability (Table 4).

Table 4: Evaluation parameters of tablet blend for prepared formulations

Parameter	PF1	PF2	PF3
Bulk density (g/cm³)	0.88 ± 0.17	0.89 ± 0.21	0.91 ± 0.14
Tapped density (g/cm³)	0.97± 0.23	1.03 ± 0.17	1.01± 0.31
Angle of repose	$30.76^0 \pm 0.08$	$31.25^0 \pm 0.18$	$31.76^0 \pm 0.14$
Compressibility Index (%)	18.36	16.41	13.89
Hausner's Ratio	1.25 ± 0.09	1.18 ± 0.05	1.27 ± 0.04

The value shown in the tables is the mean of three determinations.

Table 5: Evaluation parameters of polyherbal tablets

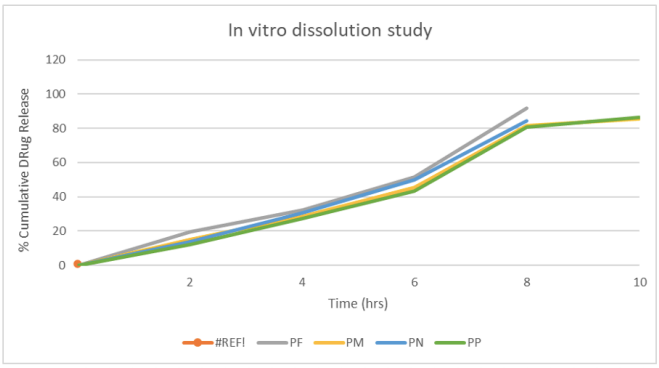
Parameters	PF	PM	PN	PP
Uniformity of weight (mg)	306.67 ± 2.08	307.05 ± 5.29	308 ± 3.65	310.25 ± 32.86
Thickness (mm)	4.32 ± 0.181	5.12 ± 0.250	4.99 ± 0.314	4.16 ± 0.147
Friability (%)	0.169 ± 0.046	0.324 ± 0.51	0.141 ± 0.35	0.172 ± 0.024
Tablet Hardness (Kp)	3.33 ± 0.29	2.67 ± 0.76	3.17 ± 0.29	2.75 ± 0.69
Disintegration time (min)	11.23 ± 0.65	12.64 ± 0.41	13.65 ± 0.21	14.62 ± 0.82

https://doi.org/10.47552/ijam.v16iS2.6169 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

The evaluation parameters for each batch of tablets were within acceptable limits. The herbal tablets exhibit low friability, indicating that they are dense and hard to break. It was found that all the formulations showed a disintegration time ranging from 11.23 to 14.62 minutes, which may be due to the binding property of guggul (Table 5). Based on the results of the drug release profile, the formulation PF reaches its peak release of 91.6% after eight hours.

Figure 1: Dissolution study



Anti-inflammatory activity assessment by Carrageenan-induced rat Paw oedema method

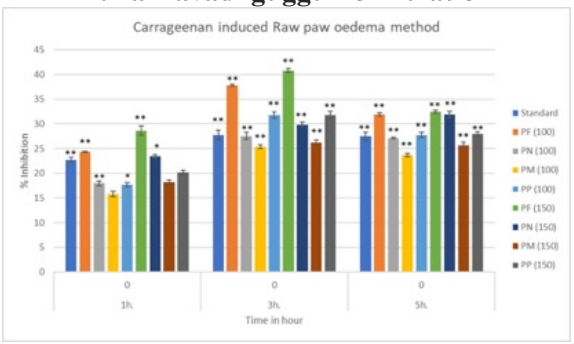
The formulations that were prepared and subsequently marketed exhibit a remarkable ($p<0.001$) inhibition of carrageenan-induced paw oedema in rats, with the results delineated in Table 6. Administration of doses of (100 and 150 mg) of these formulations results in a considerable ($p<0.001$) and dose-dependent attenuation of the swelling provoked by carrageenan at the 1st, 3rd, and 5th hours. The formulations PF, PN, PM, and PP demonstrated a maximum inhibition of paw oedema volume at 31.93 %, 27.13 %, 23.90 %, and 27.74 %, respectively, at a dosage of 100 mg/kg, whereas they exhibited a maximum inhibition of 32.46 %, 31.93 %, 25.66 %, and 28.00 %, respectively, at a dosage of 150 mg/kg. The formulations PF, PN, and PP maintained statistical significance at the 1st, 3rd, and 5th hours, whereas the formulation PM exhibited significance only at the 3rd and 5th hours, across both 100 and 150 mg/kg dosages, while the standard drug diclofenac achieved a maximum inhibition of paw oedema volume at 27.76 % (Figure 2).

Table 6: Anti-inflammatory activity of Punarnavadi guggul formulation on carrageenan-nduced rat paw oedema

Treatment	Dose (mg/kg)	Carrageenan induced paw oedema (mL)			% inhibition		
		1st h.	3rd h.	5th h.	1st h.	3rd h.	5th h.
Control	-	1.402±0.045	1.825±0.039	1.910±0.069	-	-	-
Standard	10	1.083±.0073**	1.330±0.085**	1.385±0.088**	22.71	27.76	27.48
PF	100	1.06±0.026**	1.13±0.029**	1.3±0.01**	24.37	37.8	31.93
PN	100	1.07±0.044**	1.28±0.065**	1.30±0.070**	17.95	27.57	27.13
PM	100	1.180±0.08	1.36±0.111**	1.45±0.115**	15.81	25.38	23.90
PP	100	1.15±0.025*	1.245±0.029**	1.38±0.0559**	17.71	31.78	27.74
PF	150	1.00±0.037**	1.08±0.027**	1.29±0.011**	28.65	40.82	32.46
PN	150	1.15±0.054*	1.32±0.026**	1.39±0.071**	23.42	29.86	31.93
PM	150	1.146±0.035	1.340±0.05**	1.42±0.046**	18.19	26.21	25.66
PP	150	1.118±0.025	1.245±0.030**	1.41±0.044**	20.21	31.78	28.00

*Significant at $p<0.01$, **significance at $p<0.001$, p-value was calculated by comparing with control by ANOVA, values are expressed as ± SEM

Figure 2: Graph showing percentage inhibition of Punarnavadi guggul formulation



Anti-inflammatory activity assessment by Cotton pellet induced granuloma

The formulations at tested doses of 100 and 150 mg/kg produced a significant ($p<0.01$) reduction in granuloma weight when compared to the control group. The formulations PF, PN, PM, PP at 100 mg of dose shows % inhibition of 36.35 %, 32.01 %, 29.37 %, 34.09 % respectively whereas at dose 150 mg it shows % inhibition of 45.01 %, 43.12 %, 33.15 %, 44.25 % respectively and the effects were comparable to that of the standard drug indomethacin 10 mg/kg which shows the % inhibition of 43.69 % (Table 7, Figure 3).

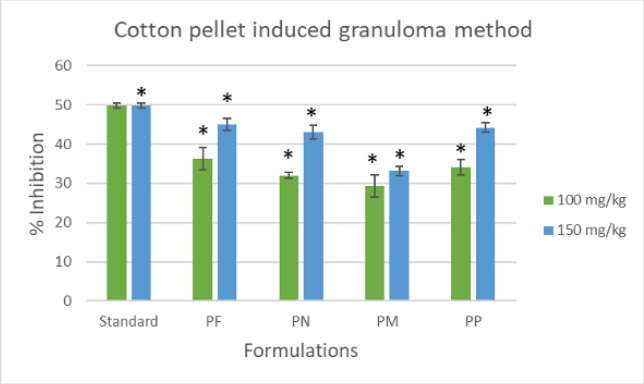
Table 7: Anti-inflammatory activity of Punarnavadi guggul on cotton pellet induced granuloma method.

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	% inhibition
Control	-	88.5±1.258	-
Standard	10	49.83±0.654*	49.83
PF	100	56.33±2.883*	36.35
PM	100	60.16±0.8062*	29.37
PN	100	62.5±2.798*	32.01

PP	100	58.33±1.961*	34.09
PF	150	48.66±1.585*	45.01
PM	150	50.33±1.778*	33.15
PN	150	58.16±1.229*	43.12
PP	150	49.33±1.174*	44.25

* Significant at p<0.01, p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM

Figure 3: Cotton pellet granuloma method for Punarnavadi guggul formulation.



Discussion

In the current study, three distinct batches of the punarnavadi guggul tablet were formulated in a laboratory and subsequently compared with three separate batches of commercially available formulations from different manufacturers. The tablets were formulated utilizing the direct compression technique. Preliminary formulation investigations were conducted on the tablet blends. The findings pertaining to bulk and tap density, angle of repose, Carr's Index, and Hausner ratio indicated that the powder mixtures exhibited favourable flow characteristics and excellent packing capabilities. Moreover, the formulated tablets underwent evaluation based on weight variation, hardness, drug content, and friability, with the results being compared against those of other polyherbal tablets for consistency. All tablet weights across the formulations were determined to be within the parameters established by the USP, which specified a range from 295 to 310 mg. The hardness and friability levels of the tablets produced from each batch of herbal tablets conformed to acceptable standards. The herbal tablets demonstrated a relatively low friability, implying a dense compaction. It was observed that all formulations exhibited disintegration times ranging from 11.23 to 14.62 minutes (Table 5). According to the analysis of the drug release profile, formulation PF attained its peak release of 91.6% after a duration of eight hours (Figure 1). In addition, the formulations were assessed for their anti-inflammatory efficacy. The carrageenan-induced paw oedema and cotton pellet-induced granuloma methodologies were employed as experimental models for the assessment of anti-inflammatory activity. All formulations demonstrated significant anti-inflammatory effects. The formulation-maintained significance at the 1st, 3rd, and 5th hours, exhibiting dose-dependent activity; however, when comparing the prepared formulations with various marketed formulations for anti-inflammatory activity, the highest percentage of inhibition was observed in PF, followed by PP, whereas formulation PM exhibited a lower percentage of inhibition for both acute and chronic activities at dosages of 100 and 150 mg/kg. The discrepancies in the percentage of inhibition exhibited by the different formulations can be attributed to variations in the concentrations of active constituents such as guggulsterone E and Z, punarnavin, deodarone, polyphenols, and flavonoids present, which are integral to the anti-inflammatory activity.

Conclusion

In the current research endeavor, efforts were directed towards the comparative analysis of the Punarnavadi guggul tablet formulation concerning its anti-inflammatory efficacy. Upon conducting the comparison, it was discerned that the formulation designated as PF exhibited the highest percentage of inhibition, followed by formulation PP; formulation PM demonstrated a comparatively lower percentage of inhibition during both acute and chronic phases. The observed discrepancies in the percentage of inhibition among the various formulations may be attributed to the differences in the concentrations of active constituents present, such as guggulsterone E and Z, punarnavin, and deodarone, which are integral to the manifestation of anti-inflammatory effects. The results clearly spell out the importance of using standard raw materials to ensure proper activity.

Acknowledgement

The authors would like to thank the head of Natural Remedies Bangalore for providing authenticated raw material.

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https://doi.org/10.47552/ijam.v16iS2.6170 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Gastro Retentive Drug Delivery System of Perindopril using Natural Polymer - Development and Optimization

Research Article

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Abstract

Gastro retentive floating matrix tablet provides drug delivery at the controlled rate, improve bioavailability and prolong the retention of dosage forms in gastrointestinal tract. Okra gum, a mucilage-rich extract derived from the okra plant (*Abelmoschus esculentus*), holds significant value in both Ayurvedic medicine and modern pharmaceutical sciences. Knowing the importance of Okra gum, aim of present investigation is to develop gastro retentive floating matrix tablet of Perindopril using Avicel pH 102 as a directly compressible material; Citric acid for production of acidic microenvironment and sodium-bi-Carbonate as gas generating agent. Pre-compression parameters of powdered blend as well as prepared batches were studied and found within the range. FTIR of physical mixture (Perindopril, Okra gum and HPMCK4M) suggesting no incompatibility. Formulation batch F8 floated, and remained buoyant without disintegration with swelling index value 42.32%, released Perindopril 92.92% about 12 hours might be due to combine use of HPMCK4M & Okra gum; showed higher correlation coefficient (r^2-value) followed Korsmeyer Peppas release kinetics. DSC thermogram of F8 confirms uniform dispersion of drug in an amorphous form as endothermic peak was below 126.0^0. No significant changes in physiochemical properties, drug release profile as well as drug content of optimized F8 batch when subjected to stability at 40± 2^0 temperature with relative humidity 75±5% for three months, indicating there was no degradation and change in the matrix system.

Keywords: Gastroretentive Drug Delivery System, Perindopril, Hypertension, FTIR, DSC.

Introduction

Oral sustained drug delivery system is complicated by limited gastric residence time. Rapid gastrointestinal transit can prevent complete drug release in the absorption zone and reduce the efficacy of administered dose, since the majority of drugs are absorbed in stomach or the upper part of small intestine Floating drug delivery offers several applications for drugs having poor bioavailability because of the narrow absorption window in the upper part of the gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability (1).

Okra gum, a mucilage-rich extract derived from the okra plant (Abelmoschus esculentus), holds significant value in both Ayurvedic medicine and modern pharmaceutical sciences. In Ayurveda, its snigdha (unctuous) and sheetala (cooling) qualities make it beneficial for soothing the gastrointestinal tract, helping to pacify aggravated Pitta and Vata doshas, and managing conditions such as gastritis and acid reflux. As a functional food, it supports digestive health and overall wellness due to its demulcent and nourishing nature.From a pharmaceutical perspective, okra gum is gaining attention as a natural excipient, particularly for its role as a binder in tablet formulations. Its biocompatibility, non-toxicity, and gel-forming ability make it a promising candidate in drug delivery systems, offering a plant-based alternative to synthetic binders while aligning with the principles of green pharmacy and Ayurvedic herbal formulation.

In present investigation attempt has been made to develop and evaluate gastro retentive floating tablets of Perindopril, (as have short elimination time 1.2 hr and can sustain the release), by direct compression method (as Avicel pH 102 as a directly compressible material) using Okra gum and HPMCK4M polymer ratio in single or in combination to achieve controlled drug release with reduced frequency of drug administration, reduced side effects, patient compliance as well as to prolong the drug release in GIT and consequently into the plasma. Perindopril, used for the treatment of hypertensionand heart failure and is suitable candidate for controlled release administration.

Materials And Methods

Perindopril was gift sample procured from Hetero Drugs Ltd., Hyderabad. Okra gum procured from Gold king Biogene Private Ltd., Ahmedabad. HPMCK4M procured from Mahalaxmi Chemicals, Hyderabad, whereas Sodium bicarbonate, Citric Acid, Micro-crystalline Cellulose, Magnesium stearate and Talc are procured from Samar Chemicals, Nagpur.

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Organoleptic Properties

Organoleptic properties such as colour, taste, odour and melting point has been determined.

Preparation of Stock solution

20 mg of Perindopril was accurately weighed in a 100 ml volumetric flask then the volume was made up to 100 ml with 0.1N HCl.

Determination of wavelength maxima (λ_{max}) and Standard Calibration Curve of Perindopril

The solution of 10µg/ml in 0.1N HCl was prepared and scanned in the range of 200-400 nm and wavelength maxima was determined. For standard calibration curve, from the stock solution, 10 ml was pipetted out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with 0.1N HCl containing concentration of 20 µg/ml. From this solution, aliquots 1, 2, 3, 4, …….10ml were pipetted out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with 0.1 N HCl so as to make final concentration equivalent to 2-20 µg. The absorbance of these solutions was measured against 0.1 N HCl as blank at 215 nm using Shimadzu UV-Visible double beam spectrophotometer (2).

Solubility study

The solubility of Perindopril was determined in solvents of different polarities. The solubility of Perindopril is usually determined by the equilibrium solubility method (3), which employs a saturated solution of Perindopril, obtained by adding an excess amount of Perindopril in the solvent to promote drug precipitation, and then stirring for two hours until equilibrium was reached. The mixture was filtered and amount of Perindopril was determined by using UV Spectrophotometer at 215 nm.

Drug Excipient Compatibility Study using Fourier Transform Infrared Spectroscopy

The samples were crushed with KBr to make pellets under hydraulic pressure of 10 tons, and then the FTIR spectra were recorded between 400 and 4000 cm^{-1}. It was used to study the interactions between the drug and polymer. The drug and polymer must be compatible with one another to produce a stable product. Drug and polymer interactions were studied by using FTIR (4,5).

Evaluation of powder parameters

Parameters including bulk density, tapped density, Carr's index, Hausner ratio and angle of repose of powder were evaluated according to the procedure given in Indian Pharmacopoeia, 2014 (3).

Development and optimization of Perindopril floating matrix tablets

Perindopril, selected polymers, sodium bicarbonate, citric acid and Avicel pH 102 were taken in required quantities and passed through 60 meshes separately. In dry state, the drug with other ingredients (for each batch containing blend of 50 tablets) along with Avicel pH 102 as a directly compressible material was mixed for the period of 10 min in mortar to get uniform mixture powder. The mixture was blended with Magnesium stearate and talc for 2-3 min to improve flow property. The powder materials were compressed using 8 mm diameter, round, biconcave punches on a Fluid pack multi station rotary tablet machine. The tablet weight was kept 120 mg and hardness between 5-7 kg/cm^2. The weights of the tablets were kept constant for all formulations as shown in Table 1.

Table 1: Composition of floating tablets of Perindopril

Sr. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Perindopril	8	8	8	8	8	8	8	8	8
2	Okra Gum	4	8	12	--	--	--	4	8	12
3	$HPMCK_4M$	--	--	--	4	8	12	4	8	12
5	Sodium Bi-Carbonate	8	8	8	8	8	8	8	8	8
6	Citric Acid	4	4	4	4	4	4	4	4	4
7	Avicel pH 102	93	89	85	93	89	85	89	81	73
8	Magnesium-stearate	2	2	2	2	2	2	2	2	2
9	Talc	1	1	1	1	1	1	1	1	1
10	Total (mg)	120	120	120	120	120	120	120	120	120

Evaluation of Post Compression Parameter of Floating Tablets

Floating tablets parameters like taste, color, size, thickness, shape, hardness, friability, weight variation and drug content were determined as per the procedures given in Indian Pharmacopoeia (3).

Tablet Density

Tablet density was an important parameter for floating tablets. The tablet would float only when its density was less than that of gastric fluid (1.004). The density was determined using following relationship.

$$V = pr^2h \qquad d = m/v$$

Where, v = volume of tablet (cc), r = radius of tablet (cm), h = crown thickness of tablet (g/cc), m = mass of tablet

Floating lag time

The lag time was carried out in beaker containing 100ml of 0.1 N HCl as a testing medium maintained at

37 ± 0.5^0. The time required for the tablet to rise to the surface and float was determined as floating lag time.

Floating time/Buoyancy Study

The tablets were placed in a 100 ml beaker containing 0.1N HCl. The time required for the tablet to rise to the surface and float was taken floating lag time.

Swelling Characteristics

The swelling properties of matrix tablet containing drug were determined by placing the tablet matrices in the glass beaker containing 200ml of 0.1N HCl and incubated at 37±1^0. At regular 1hr time interval until 10hrs, the tablet was removed from beaker and the excess surface liquid was removed carefully using the filter paper. The swollen tablet was then re-weighted. Swelling characteristics (5,6) were expressed in term of percentage water uptake (WU %) according to the equation given below:-

$$\text{Swelling Index} = \frac{\text{Wt. of swollen tablet- Initial wt. of tablet}}{\text{Initial wt. of tablet}} \times 100$$

In-Vitro drug release study

The dissolution rate of Perindopril from floating matrix tablets was determined using 0.1 N HCl as dissolution medium to determine the drug release by using USP type II (Paddle apparatus) (Electro lab India) containing 900 ml. The tablet was placed in dissolution flask containing dissolution medium, maintained at 37±0.5^{0}C and the agitation speed was 50 rpm. A solution of (1.0 ml) of the dissolution medium was withdrawn at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hour time intervals and the same amount was replaced with the fresh medium. Absorbance of these solutions was measured by UV spectrophotometer at the $\lambda_{max}$of 215 nm. The cumulative percentage drug release was expressed as each value is determined and results were summarized.

Dissolution Kinetic Model

Model dependent methods (Table 2) are based on different mathematical functions, which describe the dissolution profile. Once a suitable function has been selected the dissolution profiles are evaluated depending on the derived model parameters (5,7).

Table 2: Mathematical models for drug dissolution curves

Sr. No.	Models	Equation
1	Zero Order release Equation	$Q_t = Q_0 + K_0 t$
2	First Order release Equation	$\ln Q_t = \ln Q_0 + K_1 t$
3	Higuchi Plot Equation	$Q_t = K_H t_{1/2}$
4	Hixson – Crowell Equation	$Q_{01/3} - Q_{t1/3} = K_s t$
5	Korsmeyer-Peppas Equation	Log (M t / M f) = Log k + n Log t

N	Mechanism
0.5	Fickian diffusion (Higuchi Matrix)
0.5 < n < 1	Non-Fickian diffusion
1	Case II transport

Differential Scanning Calorimetry (DSC)

Thermal properties of pure Perindopril and the optimized formulation were analyzed using DSC (8). The samples were heated in a hermetically sealed aluminum pans. Heat runs for each sample were set from 30^0 to 35^0 at a heating rate of 10^{0}min, using nitrogen as blanket gas.

Stability Studies

The optimized tablet batch was selected and wrapped in aluminum foil of thickness 0.04 mm and stored at temperature 40± 2^{0}with relative humidity of 75±5%. The sampling was done after every one month and evaluated for appearance, thickness, hardness, friability, drug content and cumulative % drug release (4,5,9).

Results and Discussion

Perindopril evaluated for parameters like color, odor, taste and melting point was found to be complying the specifications given in the Indian Pharmacopoeia (10). Perindopril, a BCS Class III drug, is highly soluble but suffers from low permeability and a short half-life. It is primarily absorbed in the upper GI tract, was observed to be white colored, odorless and tasteless powder with melting point of 126-128^0.The solution of 10µg/ml in 0.1N HCl was prepared and scanned in the range of 200-400 nm and wavelength maxima (fig.1) was found to be 215 nm.In order to prepare standard calibration curve of Perindopril (fig. 2), absorbance values of different concentrations of Perindopril were determined (Table 3). Solubility of Perindopril in water, 0.1N HCl and ethanol was found to be 28.82mg/ml, 14.70 mg/ml and 20.77 mg/ml respectively.

Table 3: Absorbance values of perindopril in 0.1 NHCl

Concentration (µg\ml)	Absorbance
0	0
2.0	0.074±0.04
4.0	0.151±0.07
6.0	0.234±0.02
8.0	0.299±0.05
10.0	0.378±0.07
12.0	0.456±0.09
14.0	0.528±0.04
16.0	0.603±0.05
18.0	0.677±0.07
20.0	0.755±0.04

(n=03)

Fig. 1: Determination of wavelength maxima (λ^{max}) of Perindopril in 0.1N HCl (Abs= Absorbance)

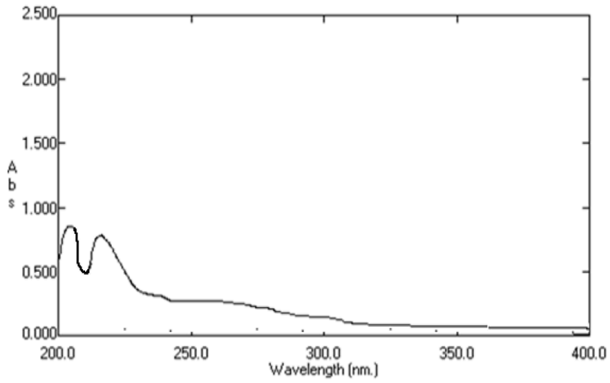
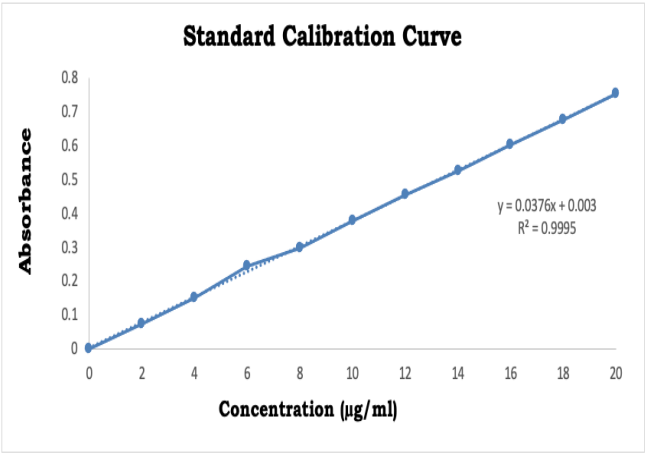


Fig. 2: Standard Calibration curve of Perindopril in 0.1 N HCl



The interaction studies of drug with polymers suggests no incompatibility.Perindopril shows retention of basic characteristics asN-H stretch at 2927.91cm^{-1}, N-H bending at 1642.01 cm$_{-1}$, C=C bonding at 1730.51, 1745.03cm^{-1}, -C-H stretch at 2747.24cm^{-1}, O-H bendingat 1390.61cm^{-1}, C=O Stretch at 1290.44cm^{-1}, C-N stretch at 1317.30cm^{-1} as shown in FTIR of drug and excipients. The typical FTIR curves shown in fig.3 [A], [B], [C] and [D] and wave number values for major peaks present of Perindopril are shown in Table 4.

Fig. 3: FTIR of [A] Perindopril, [B] Okra gum, [C] HPMCK4M, [D] Physical mixture of Perindopril with HPMCK4M and Okra gum

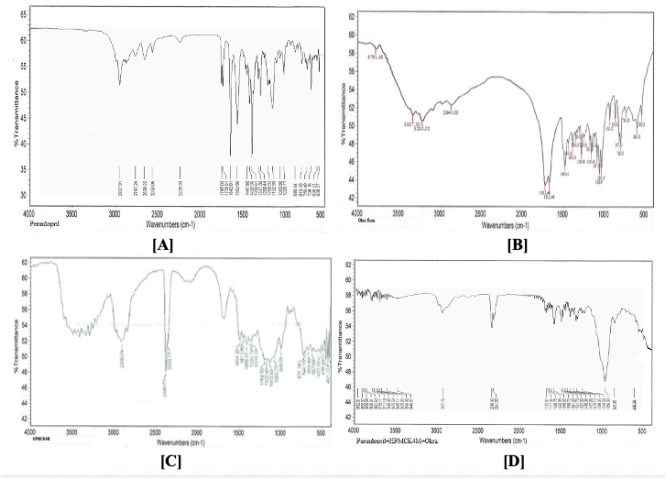


Table 4: Majorpeaks present in ir spectra of perindopril

Peak at wave number	Peak report	Peak observed
N-H stretch	2900-3600	2927.91
N-H bending	1500-1700	1642.01
C=C	1700-2000	1730.51, 1745.03
C-H stretch	2700-3300	2747.24
O-H bending	1200-1400	1390.61
C=O Stretch	1050-1300	1290.44
C-N stretch	1180-1360	1317.30

Powder characteristics were evaluated and found to be passing the tests for various batches according to the procedure given in Indian Pharmacopoeia (Table 5).Evaluation of tablets of batches F1 to F9 were carried out and thickness was found in range of 2.81±0.02 to 2.93±0.06mm; Hardness 5.25±0.17 to 5.50±0.13kg/cm^2; friability around 0.23±0.09; weight variation about 121±2.02 mg and drug content around 98.98±0.12 (Table 6). To provide good floating behavior in the stomach, the density of the device found to be less than that of the gastric contents (1.004g/cm^3). All the batches showed density below than that of gastric fluid (1.004). The values are shown in Table 7.

Table 5: Preformulation studies of various batches

Batches	Angle of repose (θ) ±SD	Bulk density (g/ml) ±SD	Tapped density (g/ml) ±SD	Compressibility Index (%)±SD	Hausner's ratio±SD
F1	25.22±0.12	0.191±0.05	0.235±0.31	14.76±0.32	1.18±0.04
F2	27.30±0.28	0.185±0.25	0.266±0.05	13.89±0.16	1.12±0.07
F3	27.75±0.33	0.225±0.07	0.224±0.09	16.41±0.12	1.13±0.09
F4	26.88±0.16	0.205±0.21	0.218±0.34	14.42±0.06	1.15±0.06
F5	25.55±0.12	0.171±0.08	0.202±0.24	10.75±0.22	1.16±0.05
F6	29.11±0.09	0.205±0.05	0.199±0.16	11.63±0.17	1.19±0.04
F7	25.44±0.15	0.219±0.21	0.191±0.40	12.60±0.18	1.11±0.01
F8	26.67±0.55	0.173±0.01	0.189±0.42	12.30±0.19	1.17±0.11
F9	24.22±0.12	0.186±0.21	0.236±0.14	12.36±0.21	1.14±0.07

https://doi.org/10.47552/ijam.v16iS2.6170 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Table 6: Physical evaluation of formulated tablet

Batches	Thickness (mm) ±SD	Hardness (kg/ cm²)±SD	Friability (%) ±SD	Weight variation (mg)	Drug content uniformity (%) ±SD
F1	2.88±0.07	5.25±0.09	0.16±0.09	120±1.05	98.25±0.21
F2	2.84±0.03	5.35±0.11	0.21±0.05	121±2.02	98.38±0.30
F3	2.81±0.02	5.50±0.08	0.19±0.06	120 ±1.23	98.30±0.24
F4	2.93±0.06	5.50±0.13	0.11±0.02	120±1.12	98.49±0.19
F5	2.91±0.05	5.45±0.12	0.19±0.04	121±0.57	98.29±0.31
F6	2.83±0.01	5.30±0.03	0.17±0.02	119 ±1.37	98.70±0.11
F7	2.88±0.04	5.25±0.17	0.14±0.08	121 ±0.17	98.98±0.12
F8	2.86±0.07	5.40±0.25	0.14±0.09	119±0.09	98.44±0.32
F9	2.83±0.04	5.35±0.35	0.23±0.06	121±1.14	98.81±0.15

Table 7: Tablet densities, buoyancy lag time and total floating time

Batches	Tablet density (g/cc)	Buoyancy lag time (Sec)	Total floating time (Hr)
F1	0.91±0.02	96±0.03	>12
F2	0.88±0.01	97±0.02	>12
F3	0.86±0.02	97±0.01	>12
F4	0.89±0.01	95±0.02	>12
F5	0.87±0.03	96±0.03	>12
F6	0.82±0.01	96±0.01	>12
F7	0.85±0.03	93±0.02	>12
F8	0.84±0.05	92±0.02	>12
F9	0.83±0.02	91±0.01	>12

(n=03)

On immersion in 0.1N HCl solution pH (1.2) at 37⁰, the optimized (F8) tablets floated, and remained buoyant without disintegration. Table 7 shows the results of Buoyancy study showing buoyancy character of prepared tablet. The formulation's rapid buoyancy and prolonged floating behavior ensured retention in the stomach, a prerequisite for improved absorption. The combination of polymers facilitated swelling and gel formation, which controlled the drug release rate. The sustained release not only aligns with the absorption window of Perindopril but also potentially reduces the frequency of administration and enhances patient compliance. Thus, a gastroretentive drug delivery system can improve its bioavailability by extending the gastric residence time.

Swelling is used to describe the process that a polymer system undergoes addition to solvent; this is a composite, and not simple, term that encompasses all of the processes viz. hydration, gelling, swelling and erosion of polymer. After 10 hours, swelling index for prepared batches was found to be 26.47% to 42.32% which was maximum for F8 batch summarized in Table 8 and figure 4.

In-vitro dissolution study of prepared tablets namely F1-F9 (Table 9, fig. 5) were carried out. Batches F1 to F6 releases Perindopril early i.e. upto ten hours in range of 99.69±0.15, might be due to use of one polymer in formulation whereas use of combination of polymers (Okra gum and HPMCK4M as a matrix forming polymer which control the release) in batches F7 to F9, releases Perindopril upto twelve hours. F8 promisingly releasing 92.92±0.12% of drug considered as optimized batch. The kinetic treatment data of dissolution profiles of formulations F1-F9 has been summarized in Table10. The *in-vitro* drug release pattern of F8 showed the highest regression value (r_2 = 0.9966) for Korsmeyer- Peppas model. The 'n' value was found to below 0.5 (i.e 0.3133) suggesting that release of drug follows Fickian diffusion (Higuchi Matrix) mechanism. Release kinetics may be following diffusion mechanism from the formulation.

Table 8: Swelling index of formulations

Time (Hours)	Swelling index (%) or % Hydration								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	19.21	18.60	20.12	21.33	29.86	25.19	22.13	23.09	27.03
2	42.52	37.36	29.64	44.41	31.87	28.11	37.26	27.26	45.14
3	41.66	42.94	37.60	49.11	37.37	42.03	50.05	41.40	46.37
4	39.22	54.32	50.51	60.02	47.50	47.12	54.58	47.61	49.12
5	32.23	51.73	53.14	58.19	46.70	45.22	50.02	52.51	37.22
6	28.21	37.38	40.19	50.09	44.06	43.91	43.10	47.51	35.32
7	23.12	31.14	38.66	42.32	41.16	37.11	40.04	42.32	34.23
8	25.11	26.86	26.47	32.65	45.22	34.10	40.04	42.32	31.52
9	28.21	26.86	26.47	32.65	48.87	34.10	40.04	42.32	31.52
10	28.21	26.86	26.47	32.65	48.87	34.10	40.04	42.32	31.52

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Figure 4: Relationship between Swelling Index & Time of batches F1- F9 (Hrs= Hours)

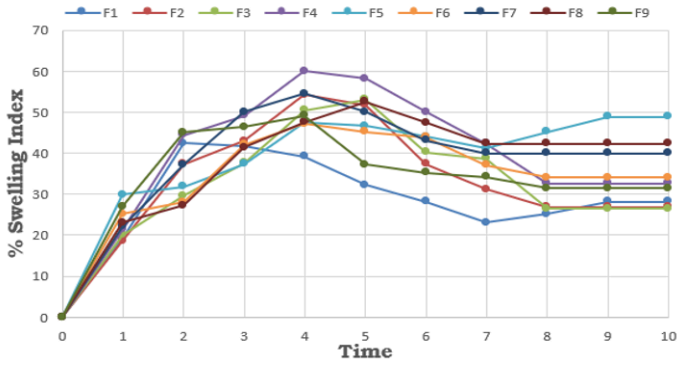


Figure 5: % cumulative Perindopril release (F1 – F9)

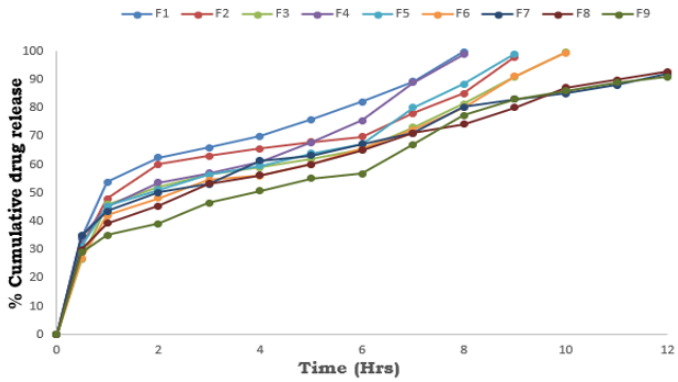


Table 9: In-vitro drug release profile of formulations

Time (Hours)	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	33.92±0.33	31.22±0.29	26.52±0.23	34.56±0.14	31.07±0.41	26.78±0.22	35.00±0.11	30.03±0.19	29.02±0.41
1	53.87±0.89	48.06±0.35	46.01±0.29	45.00±0.21	45.36±0.34	42.15±0.48	43.48±0.18	39.40±0.23	35.11±0.12
2	62.45±0.13	60.06±0.19	52.01±0.11	53.63±0.12	51.10±0.38	48.10±0.11	50.16±0.25	45.48±0.14	39.19±0.32
3	65.96±0.11	62.99±0.12	56.65±0.28	57.00±0.06	56.45±0.43	54.55±0.15	53.04±0.43	53.24±0.43	46.56±0.45
4	69.95±0.29	65.57±0.16	59.07±0.19	60.94±0.06	59.57±0.31	56.15±0.23	61.36±0.21	56.18±0.19	50.68±0.49
5	75.91±0.22	67.99±0.02	62.01±0.21	67.86±0.09	64.00±0.40	60.08±0.22	63.19±0.23	60.12±0.57	55.15±0.19
6	82.21±0.22	69.92±0.24	65.22±0.40	75.47±0.05	67.01±0.16	65.89±0.31	67.33±0.09	65.10±0.42	56.78±0.28
7	89.30±0.66	78.07±0.17	73.11±0.41	89.02±0.06	80.11±0.41	72.17±0.38	71.11±0.21	71.07±0.16	67.19±0.77
8	99.89±0.31	85.15±0.27	81.41±0.24	98.99±0.08	88.49±0.13	80.21±0.31	80.34±0.51	74.19±0.53	77.31±0.28
9	-------	97.97±0.45	91.04±0.13	-------	99.01±0.10	91.19±0.19	83.17±0.17	80.23±0.24	83.12±0.32
10	-------	-------	99.69±0.15	-------	-------	99.42±0.22	85.04±0.48	87.21±0.51	85.98±0.11
11	-------	-------	-------	-------	-------	-------	88.04±0.12	89.89±0.22	89.01±0.16
12	-------	-------	-------	-------	-------	-------	92.03±0.23	92.92±0.12	91.04±0.32

Table 10: Kinetic treatment profiles

Batch	Variables	Zero order	First order	Hixson crowell	Korsmeyer	Higuchi plot
F1	r^2	0.8994	0.6274	0.7299	0.9907	0.9740
	n	0.1545	0.0030	0.0165	0.3133	0.2388
	K	29.515	1.0685	4.2554	0.2135	-1.9481
F2	r^2	0.8931	0.6192	0.7083	0.9894	0.9591
	n	0.1279	0.0029	0.0152	0.3018	0.2719
	K	28.880	1.0520	4.1547	0.1998	-2.2990
F3	r^2	0.9296	0.6294	0.7186	0.9890	0.9658
	n	0.1237	0.0029	0.0148	0.3194	0.2895
	K	24.831	1.0157	3.9010	0.1172	-1.9336
F4	r^2	0.9335	0.6288	0.7345	0.9928	0.9764
	n	0.1561	0.0030	0.0155	0.2859	0.2458
	K	24.049	1.0394	4.0178	0.2228	-1.1375
F5	r^2	0.9323	0.6222	0.7167	0.9921	0.9728
	n	0.1367	0.0029	0.0147	0.2840	0.2743
	K	24.437	1.0308	3.9808	0.2047	-1.7217
F6	r^2	0.9452	0.6387	0.7366	0.9943	0.9776
	n	0.1264	0.0029	0.0148	0.3231	0.2968
	K	22.970	0.9991	3.7768	0.0927	-1.8440
F7	r^2	0.9185	0.6161	0.7121	0.9899	0.9682
	n	0.0953	0.0028	0.0145	0.2538	0.3012
	K	30.314	1.0377	4.0145	0.2708	-2.6366
F8	r^2	0.9423	0.6355	0.7373	0.9966	0.9802
	n	0.1008	0.0029	0.0147	0.2973	0.3133

	K	26.422	1.0017	3.7658	0.1454	-2.3243
F9	r^2	0.9604	0.6327	0.7299	0.9957	0.9768
	n	0.1059	0.0028	0.0137	0.2738	0.3201
	K	21.952	0.9765	3.5995	0.1485	-1.4521

The DSC thermogram of Perindopril (fig.6A) records two endothermic peaks corresponding to the melting point of drug (126.0^0) whereas Perindopril Loaded optimized formulation, F8 (fig.6B), showed lesser melting point (122.4^0), suggesting the possibility of interaction. Optimized F8 formulation was studied for stability at 40 ± 2^0 and 75 ± 5% RH for about 3 months according to the ICH guidelines (Table 11). After every one month sampling, no significant changes in appearance, thickness, hardness, friability, drug content and cumulative % drug release were observed and summarized in Table 12 & Fig. 7.

Fig. 6: DSC thermogram of [A] Perindopril [B] Drug Loaded optimized formulation

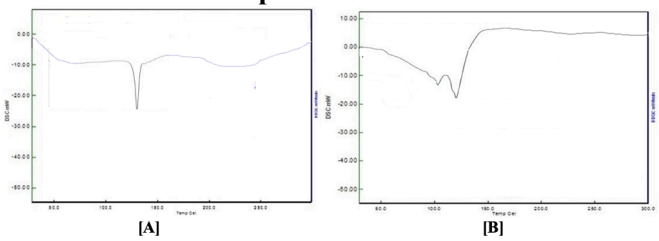


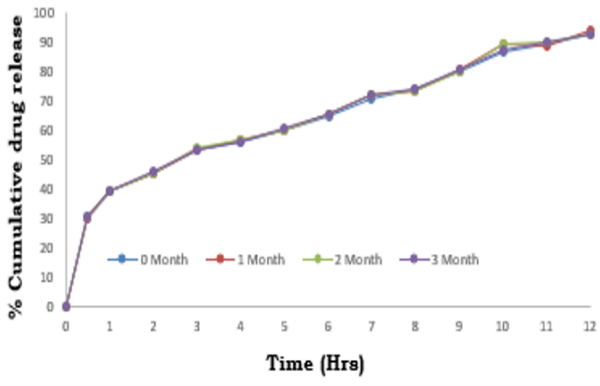
Table 11: Stability studies of formulation f8 at 40^{0}c /75% rh

Parameters	0 month	1 month	2 months	3 months
Appearance/Colour	White	White	White	White
Thickness (mm)	2.86	2.86	2.86	2.85
Hardness (Kg/cm^2)	5.40	5.30	5.30	5.30
Friability (%)	0.14	0.13	0.15	0.14
Drug content (%)	98.44	98.38	98.39	98.38

Table 12: In-vitro drug release study of formulation f8

Time (Hours)	Cumulative % Perindopril release			
	0 month	1 month	2 months	3 months
0	0	0	0	0
0.5	30.03	29.85	30.75	30.67
1	39.4	39.19	39.62	39.62
2	45.48	45.43	45.38	45.99
3	53.24	53.69	53.77	53.71
4	56.18	56.77	56.73	56.22
5	60.12	60.39	60.19	60.71
6	65.1	65.35	65.32	65.42
7	71.07	71.82	71.82	71.82
8	74.19	73.74	73.74	74.48
9	80.23	80.73	80.37	80.57
10	87.21	89.36	89.86	87.36
11	89.89	89.05	89.95	89.99
12	92.92	94.25	92.96	92.93

Fig. 7: In-vitro release profiles of formulation F8 kept for stability at 40^0 ± 2^{0}C and 75 ± 5% RH for 3 months



Conclusion

A gastro-retentive tablet formulation of Perindopril was successfully developed offering prolonged gastric retention and sustained drug release. Okra gum used in formulation is gaining attention as a natural excipient, its biocompatibility, non-toxicity, and gel-forming ability make it a promising candidate in drug delivery systems, offering a plant-based alternative to synthetic binders while aligning with the principles of Ayurvedic herbal formulation.The resulting formulation strategy can enhance the oral bioavailability of Perindopril, a BCS Class III drug, by synchronizing drug release with its narrow absorption window in the upper GI tract and demonstrated desirable characteristics, including high drug content,

optimal tablet hardness, effective floatability, a favorable swelling index, and controlled drug release behavior.

The high floating capacity of the formulation is expected to prolong its gastric residence time, potentially enhancing bioavailability, reducing dosing frequency, and minimizing both the required dose and associated side effects. Overall, this polymer-based approach for Perindopril shows promise for developing gastro-retentive drug delivery systems and has the potential to improve therapeutic outcomes and patient adherence in the management of hypertension, warranting further detailed investigation.

Acknowledgements

Authors would like to acknowledge the entire staff members of Agnihotri College of Pharmacy, Wardha – 442001 for their support during this research work.

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https://doi.org/10.47552/ijam.v16iS2.6171 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Acne Treatment Practices and Patient Preferences for Herbal Products: A Conjoint Analysis

Research Article

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Abstract

This study aims to understand the acne treatment practices and patient preferences, focusing on herbal products and identifying the most and least preferred attributes using conjoint analysis. Five key attributes were evaluated: product form (cream, lotion, gel), expected treatment time (1 week, 2 weeks, 4 weeks), packaging (tube, jar), type of medication (over the counter or prescription), and price (economy, mid-priced, premium). A survey was conducted with 408 respondents using purposive, snowball, and availability sampling methods. Data were analysed using SPSS software through conjoint analysis to determine the cardinal utility of each attribute. Results showed that 50% of respondents used medication for acne, while others relied on home remedies or lifestyle changes. Only 20% reported using herbal products. Among those using acne medication, 46.6% observed noticeable results within one week, and most reported no side effects. While 40% of participants were satisfied with their current treatments, 34.3% expressed dissatisfaction. About 34% of patients preferred herbal acne treatments, showing a liking for products that are applied once daily, white in colour, fragrant, and dispensed in fingertip quantity. The most significant attribute influencing patient preference was treatment time, followed by product form, price, packaging, and medication type. Patients preferred herbal gels that are over the counter, low cost, packaged in jars, and deliver results within a week. These findings provide valuable insights into consumer expectations and can aid in the development of effective, patient friendly herbal acne treatments that align with real world preferences.

Keywords: Acne, Herbal Products, Conjoint Analysis, Patient Preferences, Acne Treatment.

Introduction

Acne is a widespread dermatological skin condition that affects individuals globally, irrespective of age, gender, or ethnicity, and its impact extends beyond mere physical manifestations (1,2,3). It encompasses various inflammatory and non-inflammatory lesions such as comedones, papules, pustules, nodules, and cysts, significantly influencing an individual's psychological well-being and social interactions (4,5). Despite the availability of different treatments such as topical creams, oral antibiotics, and retinoids, managing acne effectively remains challenging for both patients and healthcare providers (6,7). This challenge has led to a growing interest in alternative or complementary approaches, particularly in recent years, where there has been a noticeable surge in the popularity of herbal treatment products (8,9).

As a result, many patients are turning to herbal remedies as they perceive them to be safe and effective alternatives to conventional medications (10,11). Herbal treatments, which have been used for centuries in different cultures, are gaining popularity due to their supposed efficacy and fewer side effects compared to traditional medicines (12). However, despite their widespread use, there's a lack of research on acne patients' preferences and practices regarding herbal products (13,14). Understanding these preferences is crucial for tailoring interventions that meet patients' needs and improve treatment adherence and satisfaction (15).

In the world of acne treatments, it's really important to know what patients like and what they want. This helps doctors make sure patients stick to their treatment and are happy with how it works. Additionally, the appearance of antibiotic-resistant strains of Propionibacterium acnes highlights the urgent need for alternative treatment methods (16-19). In this context, herbal treatment products have emerged as potential alternatives due to their perceived efficacy and minimal contribution to antibiotic resistance (20,21, 22).

Conjoint analysis, a powerful research methodology, offers a systematic approach to unraveling patients' preferences for various attributes of healthcare products (23). By dissecting the relative importance of different product attributes such as

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efficacy, safety, cost, and formulation characteristics, conjoint analysis provides valuable insights into patient decision-making processes (24). This study aims to delve into the treatment practices and preferences of acne patients regarding herbal treatment products through the lens of conjoint analysis. By elucidating the most and least preferred attributes of herbal products, this study aims to provide actionable insights for healthcare practitioners and researchers (25,26).

The findings of this research will contribute to a comprehensive understanding of patients' perspectives on herbal acne treatment, informing healthcare providers and policymakers about the factors driving patient preferences in this domain (27,28). This study will also contribute to the existing literature on acne treatment. Ultimately, this knowledge can facilitate the development of patient-centered approaches to acne management, thereby enhancing treatment outcomes for individuals seeking alternative therapeutic options (29).

About the Present Study

The main objective of this study is to understand the acne treatment practices & experiences of patients with acne and their preferences for herbal products used to treat acne. This study makes an effort to determine the most preferred and least preferred attributes and various choices within each attribute of herbal products used by patients to treat acne by computing the cardinal utility of each attribute. This study endeavours to investigate empirically the preferences of the patients of acne towards five attributes of herbal products used to treat acne viz; product form (cream, lotion, and gel), expected treatment time to get noticeable results (1 week, 2 weeks, and 4 weeks), packaging (tube and jar), type of medication (over the counter i.e. without prescription and prescription required i.e. Rx medication) and price preference there are three levels (economy, mid-priced, and premium) by performing conjoint analysis. This study also aims to find out the relative importance of these attributes among the respondents. It endeavours to address the following research questions:

- RQ 1: What are the clinical characteristics of acne experienced by the patients?
- RQ 2: What are the acne treatment practices of acne patients?
- RQ 3: What is their experience of following an acne medication?
- RQ 4: What are their preferences for herbal products used to treat acne?

Materials and Methods

Sampling

This research study is based on descriptive and analytical research design. A sample survey of acne patients in Maharashtra State, India was conducted to gather primary data. The sample size was determined by using Cochran's (Cochran, 1977) formula for an infinite population with 95% confidence level and 5% sampling error (30). The minimum sample size computed by using Cochran's formula was 384. The researchers surveyed 408 respondents which is much more than the minimum sample size required. The data for the study were gathered from those patients who had been experiencing acne at the time of conducting a survey and those patients who had experienced acne at some point in the past. Non-probability sampling techniques viz; availability, snowball, and purposive sampling techniques were adopted for the final selection of the respondents. Non-probability sampling techniques are typically used when the target population is infinite. The sample characteristics are presented in (Table 2).

Measure

This research study is based predominantly on the primary data. Primary data was gathered by administering a well-structured questionnaire. The survey instrument constitutes four sections viz; socio-demographic characteristics, clinical characteristics of acne, acne treatment practice & experience, and preferences of acne patients for herbal treatment products. A multiple-choice scale was used to measure socio-demographic characteristics viz; gender, age, education, monthly household income, and weight in kg. For assessing the clinical characteristics of acne viz; skin type of patients, age when they experienced first acne, appearance & feel of acne, concerns about acne, and places of acne spots on face on binary and multiple response scales. Two questions viz; concerns about acne, and places of acne spots on the face were adopted from Rendon et al., 2015. Acne treatment practice is measured by using three questions viz; whether any acne treatment, type of treatment, a form of medication and were adopted from Rendon et al., 2015. The question of whether to use any acne treatment was measured on a binary scale of 'yes' and 'no' type; and 'type of treatment' and 'form of medication' were measured by using checklist questions. The acne treatment experience of patients was measured by using four questions viz; 'time taken to get noticeable results in healing acne', 'time taken to heal acne completely', 'side effects experienced while using acne medication', and 'extent of satisfaction with acne treatment'. These questions were adopted from Rendon et al., 2015 (31). The first three questions are measured using multiple choice questions and for measuring the extent of satisfaction five-point Likert scale from 'highly dissatisfied' to 'highly satisfied' was used. Expectations of patients from herbal treatment products were measured by using five multiple-choice questions (Annexure 1). For determining the preferences of acne patients for herbal treatment products conjoint profiles are created. The procedure followed to create conjoint profiles is explained in the next section.

Conjoint Analysis: Attributes and Levels

In this study, conjoint analysis is performed to determine the relative importance of the attributes of herbal products used to treat acne. Conjoint analysis is a statistical method used to determine the value of the attributes of a product for its consumers. It is a method for analysing the preferences of customers. It is also a useful tool for predicting and determining the responses of customers to new product features and totally new

https://doi.org/10.47552/ijam.v16iS2.6171 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

products (32). In this research study, choice-based conjoint analysis is used to evaluate the preferences of patients with acne for herbal products used to treat acne.

Based on extensive literature review and studying the present herbal products available in the market to treat acne, the researchers have selected five attributes of herbal products viz; product form, expected treatment time to get noticeable results, packaging, type of medication, and price preference. All the attributes consist of different levels. For 'product form' there are three levels (cream, lotion, and gel), for 'expected treatment time to get noticeable results' there are three levels (1 week, 2 weeks, and 4 weeks), for 'packaging' there are two levels (tube and jar), for 'type of medication' there are two levels (over the counter i.e. without prescription and prescription required i.e. Rx medication) and for 'price preference' there are three levels economy, mid-priced, and premium). There are 3 × 3 × 2 × 2 × 3 = 108 profiles after combining all the attributes and levels. In this research, 20 conjoint cards (profiles) have been created by using an orthogonal design and distributed to the participants. The various choices under each attribute are depicted in the conjoint lay-out (Table 1) prepared by the researchers. 20 profiles were generated by using SPSS software (Annexure 2). The respondents were asked to rate each profile on a 1 to 10 scale. '1' for the least preferred profile and '10' for the most preferred profile.

Table 1: Conjoint Lay-out

Attributes	Product Form	Expected treatment time to get	Packaging	Type of Medication	Price Preference
Choices	Cream	1 week	Tube	Without Prescription	Economy
	Lotion	2 weeks	Jar	Prescription Required (Rx	Mid-Priced
	Gel	4 weeks			Premium

Data Collection

The data was gathered by administering a well-structured questionnaire on acne patients in Maharashtra, India from December 2023 to February 2024. The data was gathered by two methods viz; field survey and online survey. The researchers visited various colleges and institutions in Maharashtra. After seeking permission from the respective college authorities, the researchers visited the available students and faculties in the classrooms and college campus area. They have explained the nature of the research study. Survey forms were distributed to them. Subsequently, required instructions were rendered to them to fill up the form correctly. The questionnaire was also transformed into an electronic Google survey form Linge et al. 2023 (33). The link of the Google survey form was shared with the potential respondents in various WhatsApp groups and individual WhatsApp windows along with a cover letter requesting to participate in the survey. Additionally, telephone numbers of the potential respondents were gathered by using numerous known respondents in various cities and towns. A regular follow-up was taken by making telephone calls to the potential respondents. After surveying the acne patients, they were asked to give the references of potential respondents in their contact. The respondents were also requested to forward the link to their known potential contacts. The potential respondents were motivated to participate in the survey throughout the data collection period.

Results

Sample Characteristics

This study is conducted to understand the acne treatment practices & experiences of acne patients and their preferences for herbal products used to treat acne. The socio-demographic characteristics of the acne patients are presented below:

Table 2: Sample Characteristics

Characteristic	Choices	No. of Respondents	%
Gender	Male	134	32.8
	Female	274	67.2
Age	15 to 20	150	36.76
	21 to 30	214	52.45
	Above 30	44	10.78
Education	Up to HSSC	39	9.6
	Above HSSC	369	90.4
Monthly Household Income	Below Rs 25000	152	37.3
	Rs 25,000 to Rs 50000	146	35.8
	Above Rs 50000	110	27.0
Weight (in Kg)	25 to 45	94	23.04
	46 to 65	227	55.64
	Above 65	87	21.32

N = 408

A survey of 408 acne patients was conducted. The sample constitutes 134 (32.8%) male and 274 (67.2%) female patients. There were 150 (36.76%) patients in the age group of 15 to 20 years, 214 (52.45%) in the age group of 21 to 30 years and 44 (10.78%) were above the age of 30 years. 39 (9.6%) respondents studied up to HSSC and the majority of them i.e. 369 (90.4%) were above HSSC educational qualification. The monthly household income of 152 (37.3%) respondents was below Rs 25000, 16 (35.8%) respondents' monthly household income was between Rs 25000 to Rs 50000, and 110 (27%) respondents' monthly household income was above Rs 50000. As far as BMI is concerned, 94 (23.04%) respondents weigh 25 to 45 kg, 227 (55.64%) have between 46 to 65 kg, and 87 (21.32%) weigh 65kg.

Clinical Characteristics of Acne

The clinical characteristics of facial acne are presented in Table 3.

Table 3: Clinical Characteristics of Acne (N= 408)

Characteristic	Choices	No. of Respondents	%
Acne Experience	Presently Experiencing	295	72.3
	Experienced in the past	113	27.7
Skin Type	Dry	54	13.2
	Oily	124	30.4
	Sensitive	44	10.8
	Normal	79	19.4
	Combination	107	26.2
Age When Experienced First Acne	13 to 15 years	96	23.5
	16 to 18 years	173	42.4
	19 to 20 years	89	21.8
	Above 20 years	50	12.3
Appearance of Acne	Small Red Spots	276	67.6
	Yellow Spots	71	17.4
	Large painful Red Spots	61	15.0
Feeling of Acne	Itchy	130	31.9
	Painful	100	24.5
	Stingy	28	6.9
	Inflammation	59	14.5
	No Feeling	91	22.3
Concerns about Acne	Actual Pimples	91	22.3
	Pimples with Inflammation	58	14.2
	Dark marks (discoloured skin) left by pimples	107	26.2
	Both pimples and dark marks (discoloured skin)	152	37.3
Places of Acne Spots on Face	Chin	23	5.6
	Chik	241	59.1
	Forehead	100	24.5
	Hairline	3	0.7
	Jawline	6	1.5
	Nose	35	8.6

There were 295 (72.3%) patients experiencing acne at the time of conducting a survey and 113 (27.7%) experienced acne at some point in their lifetime. 54 (13.2%) patients had dry skin, 124 (30.4%) had oily skin, 44 (10.85%) had sensitive skin, 79 (19.4%) had normal skin and 107 (26.2%) had a combination skin type. 96 (23.5%) acne patients experienced acne for the first time between the age of 13 to 15 years, 173 (42.4%) experienced it at the age of 16 to 18 years, 89 (21.8%) experienced it at the age of 19 to 20 years and only 50 (12.3%) experienced acne for the first time when they were above the age of 20 years. As far as the appearance of acne is concerned, a maximum i.e. 276 (67.6%) patients reported that their acne looked like small red spots, 71 (17.4%) said it looked like yellow spots, and 61 (15%) said that their acne was large painful red spots. In terms of the feeling of acne, 130 (31.9%) said that their acne was itchy, 100 (24.5%) said it was painful, 28 (6.9%) felt it painful, 59 (14.5%) experienced inflammation and 91 (22.3%) patients said there was no noticeable feeling of acne. 91 (22.3%) patients were concerned about their actual pimples, 58 (14.2%) were concerned about pimples with inflammation, 107 (26.2%) were concerned about dark marks left by pimples and 152 (37.3%) were concerned about both pimples and dark marks. Moreover, 23 (5.6%) patients experienced acne on the chin, 241 (59.1%) on the chik, 100 (24.5%) on the forehead, 3 (0.7%) on the hairline, 6 (1.5%) on the jawline, and 35 (8.6%) patients experienced acne on nose.

Acne Treatment Practice

The researchers also investigated the acne treatment practices of patients viz; whether they use any acne treatment medication, if yes, then, the type of treatment used by them to heal acne, and the form of medication they used. The responses of acne patients show that 203 (49.8%) patients use some sort of medication to heal acne and 205 (50.2%) acne patients don't use any typical medication to heal acne. They rely more on home remedies and lifestyle changes to heal their acne naturally. It has been observed that acne patients use more than one type of treatment to heal acne. The related data gathered using checklist questions show that maximum patients i.e. 189 (35.93%) had been using home remedies, 107 (20.34%) using herbal products, 100 (19.01%) preferred to make changes in their lifestyles, 76 (14.45%) use prescription-based medication and only 54 (10.27%) patients are using over the counter medications. Similarly, it is observed that acne patients use more than one form of medication for acne treatment. The related data gathered using checklist questions show that maximum acne patients i.e. 243 (30.92%) use facewash, 154 (19.59%) use cream, 148 (18.83%) use gel, 99 (12.6%) use soap, 67 (8.52%) take tablets, and only 59 (7.51%) acne patients use an ointment to heal acne. The data is exhibited in Table 4.

Table 4: Acne Treatment Practices of Patients

Characteristic	Choices	No. of Respondents	%
Whether used any acne treatment	Yes	203	49.8
	No	205	50.2
Type of Treatment*	Home Remedies	189	35.93
	Herbal Products	107	20.34
	Lifestyle Changes	100	19.01
	Prescription based (Rx medication)	76	14.45
	Over the Counter (OTC)	54	10.27
Form of Medication*	Facewash	243	30.92
	Cream	154	19.59
	Gel	148	18.83
	Soap	99	12.60
	Tablet	67	8.52
	Ointment	59	7.51
	Patches	16	2.04

*Note: * indicates checklist questions*

https://doi.org/10.47552/ijam.v16iS2.6171 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Acne Treatment Experience

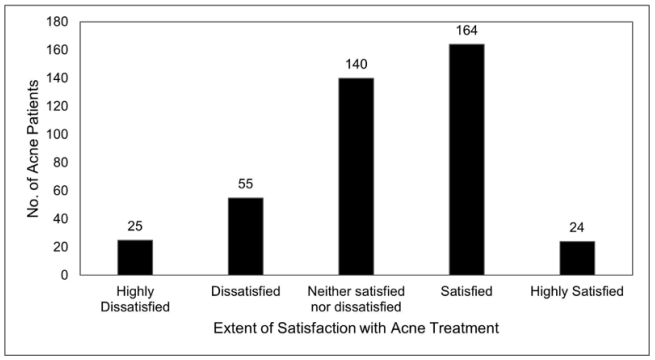
One of the objectives of this research study is to know the acne treatment experiences viz; time taken to get noticeable results while using a particular type of treatment, time taken to heal acne completely, side effects experienced while using acne medication, and the extent of satisfaction of the patients with acne treatment. The relevant data is presented in Table 5.

Table 5: Acne Treatment Experience

	Choices	No. of Respondents	%
Time Taken to Get Noticeable Results in Healing Acne	1 Day	31	7.6
	1 Week	190	46.6
	2 Weeks	103	25.2
	More than 2 Weeks	84	20.6
Time Taken to Heal Acne 100%	1 Week	109	26.7
	2 Weeks	102	25.0
	3 Weeks	76	18.6
	More than a Month	121	29.7
Side Effects Experienced while using Acne Medication	No Side Effects	235	57.6
	Dry Skin	85	20.8
	Allergic Reaction	19	4.7
	Skin Flakiness	20	4.9
	Other	49	12.0
Extent of Satisfaction with Acne Treatment	Highly Dissatisfied	25	6.1
	Dissatisfied	55	13.5
	Neither satisfied nor dissatisfied	140	34.3
	Satisfied	164	40.2
	Highly Satisfied	24	5.9

As shown in Table 5, only 31 (7.6%) patients got noticeable results in just one day of using acne treatment. 190 (46.6%) patients got noticeable results in one week, 103 (25.2%) in 2 weeks and 84 (20.6%) patients got noticeable results in more than two weeks. Moreover, 109 (26.7%) patients experienced that it takes only a week to heal acne completely. 102 (25%), 76 (18.6%), and 121 (29.7%) patients experienced two weeks, three weeks, and more than a month time to completely heal acne. Maximum acne patients i.e. 235 (57.6%) had no side effects of acne treatment, 85 (20.8%) experienced dry skin, 19 (4.7%) experienced an allergic reaction, 20 (4.9%) experienced skin flakiness and 49 (12%) acne patients experienced other side effects of acne treatment. As far as the extent of satisfaction of acne patients with their acne treatment is concerned, 25 (6.1%) patients were found to be highly dissatisfied, 55 (13.5%) were dissatisfied, 140 (34.3%) were neither dissatisfied nor satisfied, 164 (40.2%) satisfied and 24 (5.9%) found to be highly satisfied. The results are presented in Figure 1.

Figure 1: Extent of Satisfaction with acne treatment



Expectations of Acne Patients from Herbal Treatment Products

Table 6: Expectations of Acne Patients from Herbal Treatment Products

Questions	Choices	No. of Respondents	%
Whether patients prefer to use herbal acne treatment product?	Do not Prefer to use it	73	17.89
	May consider using it	196	48.04
	Would certainly prefer it	139	34.07
Preference for frequency of application of herbal product	Twice a week	118	28.92
	Once in a Day	195	47.79
	Twice a Day	95	23.28
Preference for the Colour of Herbal Acne Treatment Product	White	326	79.90
	Brown	82	20.10
Preference for the fragrance of herbal acne treatment product	With Fragrance	237	58.09
	Without Fragrance	171	41.91
Preference for herbal acne treatment product application method	Finger Tip	300	73.53
	Roller Ball	108	26.47

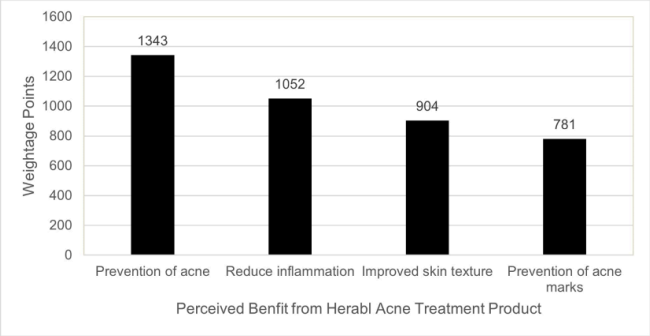
As mentioned earlier, the main aim of this study is to understand acne patients' expectations from herbal products used to treat acne. As shown in Table 6, 17.89% of acne patients do not prefer to use herbal products to heal their acne, 48.04% of patients may consider using it and 34.07% of patients would certainly prefer it. The majority i.e. 47.79% of patients prefer to apply the herbal product once a day, 28.92% twice a week, and only 23.28% of patients prefer to apply it twice a day. As far as the colour of the herbal treatment product is concerned, the maximum i.e. 79.9% of patients prefer to have it in white colour and only 20.1% of patients wish to have it in brown colour. Moreover, 58.9% of customers prefer to have herbal acne treatment products with fragrance and 41.91% want it without fragrance. In terms of application of the herbal product, the majority i.e. 73.53% of patients prefer it in

'fingertip' form and only 26.47% of customers prefer it in 'rollerball' form.

Table 7: Benefits perceived by acne patients from an Herbal Acne Treatment Product

Benefits perceived	Weightage	Rank
Prevention of acne	1343	1
Reduce inflammation	1052	2
Improved skin texture	904	3
Prevention of acne marks	781	4

Figure 2: Benefits perceived by acne patients from an Herbal Acne Treatment Product



As shown in Table 7 and Figure 2, acne prevention is the most preferred benefit expected by acne patients from a herbal treatment product followed by reduced inflammation, improved skin texture, and prevention of acne marks.

Preferences for Herbal Acne Treatment Products: A Conjoint Analysis

As mentioned earlier, conjoint analysis is performed to investigate the acne patients' preferences for attributes and various choices within each attribute of herbal products for acne treatment. The hypothesis is that all the attributes of herbal acne treatment products are not significantly different. This hypothesis is tested by performing conjoint analysis at a .05 significance level using the SPSS computer program. The results show that the attributes of herbal acne treatment products are significantly different from each other ($p<.05$, $F=20.352$) (Table 8).

Table 8: Test of Significance - Conjoint Analysis

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6.267	8	0.783	20.352	0.000
	Residual	0.423	11	0.038		
	Total	6.690	19			

The R^2 for the model shows that the preferences for the attributes of herbal acne treatment products independently account for 93.7% of the variance in different choices (Table 9). It indicates that the patients with acne are very clear about the attributes of each herbal acne treatment product.

Table 9: Model Summary

Model	R	R Square	Adjusted R Square	SE of the Estimate
1	.968[a]	0.937	0.891	0.196

The partworth utility table (Table 10) is useful for comparing the partworth utility of the choices in each attribute. After comparing the utilities of 'product form', it is found that gel form is the most preferred herbal acne treatment product followed by lotion form. However, the herbal treatment product in cream form is found to be the least preferred form. As far as the expected time of herbal acne treatment products to get noticeable results is concerned, the patients associated the maximum utility with '1 week' followed by '2 weeks' and '4 weeks.' This means, the patients prefer to purchase those herbal acne treatment products which could give noticeable results in just one week. The herbal acne treatment products packaged in jars are preferred by the patients than tube packaging. The part-worth utility of 'over-the-counter (OTC)' herbal acne treatment products is found to be more than prescription-based medication. In terms of price preferences, acne patients are found to prefer economy i.e. low-priced herbal acne treatment products over mid-priced and premium products.

A conjoint calculator was used to calculate the part-worth utilities of different attributes. Table 11 shows the relative importance of each attribute of herbal acne treatment products specified in this research. The results show that 'expected treatment time' has the maximum relative importance. 'Product form' is the second most preferred attribute of herbal acne treatment products followed by 'price' and 'packaging'. 'Type of medication' is found to be the least considered attribute while purchasing herbal acne treatment products. The relative importance of 'product form' 'expected treatment time to get noticeable results', 'packaging', 'type of medication', and 'price' are 22.61%, 41.47%, 17.87%, 0.15%, and 17.9% respectively. The higher percentage of relative importance indicates a higher contribution of the attribute in deciding to purchase herbal acne treatment products.

Table 10: Part-worth Utility Table

Attribute	Choices	Utility	Preferences
Product Form	Cream	-0.200	Gel > Lotion > Cream
	Lotion	-0.184	
	Gel	0.384	
Expected treatment time to get noticeable results	1 week	0.474	1 Week > 2 Weeks > 4 Weeks
	2 weeks	0.123	
	4 weeks	-0.597	
Packaging	Tube	-0.231	Jar > Tube
	Jar	0.231	
Type of Medication	OTC	0.002	OTC > Rx
	Rx	-0.002	
Price Preference	Economy	0.218	Economy > Mid-Priced > Premium
	Mid-Priced	0.026	
	Premium	-0.244	

https://doi.org/10.47552/ijam.v16iS2.6171 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Table 11: Relative importance of different attributes

Attributes	Relative Importance	Preference
Product Form	22.61%	Expected treatment time > Form > Price > Packaging > Type of Medication
Expected treatment time to get noticeable results	41.47%	
Packaging	17.87%	
Type of Medication	0.15%	
Price Preference	17.90%	

Figure 3: Part-worth Utilities of all the Choices with each attribute

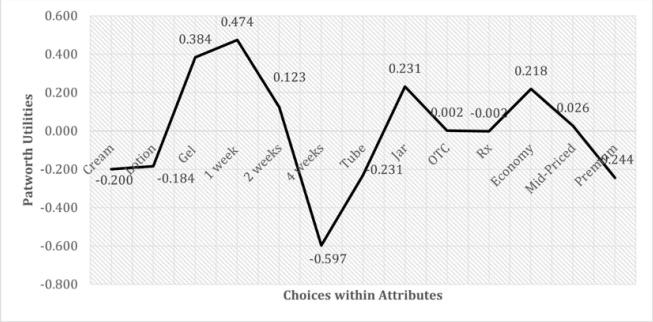
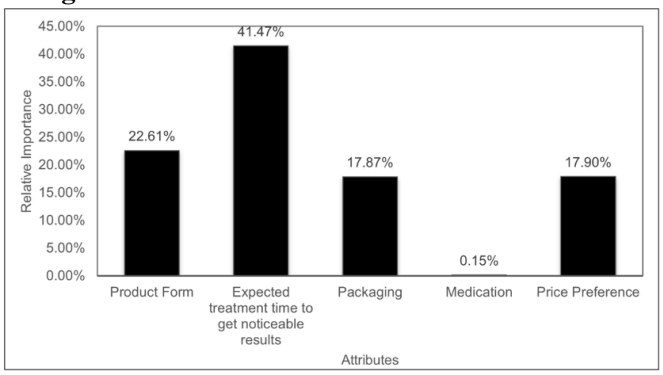


Figure 4: Part-worth Utilities of Each Attribute



Discussion

Implications of the Study

The findings of this research study hold significant implications for various stakeholders within the acne treatment industry. Primarily, manufacturers producing herbal-based products for acne treatment can utilize the understanding obtained from this study to refine existing products or develop new ones that align more closely with the preferences of acne patients (34,35).

By identifying the most desired attributes and features of herbal acne treatment products, manufacturers can better meet the needs and expectations of their target demographic (36,37).

Furthermore, the study sheds light on the clinical characteristics of acne, providing valuable insights into the appearance and sensory experiences of acne as reported by patients. This information can aid industry professionals in developing treatments that more precisely target the symptoms and concerns of acne patients.

Additionally, understanding the current acne treatment practices adopted by patients can inform managerial decisions within the industry, allowing for more strategic product positioning and marketing strategies (38).

From a theoretical perspective, this study contributes to the existing literature on acne treatment by providing empirical evidence regarding patient preferences and treatment practices. It offers valuable insights for research scholars and academia, serving as a basis for further investigation into the efficacy and acceptability of herbal acne treatment products (39).

Understanding Patient Preferences for Herbal Acne Treatment

This study offers valuable insights into the treatment practices and preferences of patients suffering from acne, particularly regarding their preferences for herbal products. Utilizing conjoint analysis, the research delves into the subtle differences in preferences among acne patients regarding the attributes of herbal acne treatment products (40).

Significance of the Study

Acne remains a prevalent dermatological concern affecting millions worldwide, and patient preferences play a crucial role in determining treatment adherence and efficacy. Herbal products have emerged as popular alternatives for individuals seeking complementary treatments for acne (41). Understanding patient preferences for these products is paramount for healthcare providers and policymakers in delivering patient-centered care and improving treatment outcomes (42).

Conclusion

The study was to understand the acne treatment practices & experiences of patients with acne and their preferences for herbal products used to treat acne. The results of the survey conducted of 408 acne patients show that about 50% of patients use medication for acne treatment and the rest rely on home remedies and lifestyle changes. It is also found that only 20% of patients use herbal products to heal acne. The results also show that a maximum i.e. 46.6% of patients got noticeable results in one week time of treatment. Maximum patients reported that they didn't have any side effects of using acne treatment medication. About 40% of patients are satisfied and 34.3% of patients are dissatisfied with their acne treatment.

The results of expectations of patients from herbal treatment products indicate that 34% of patients certainly prefer to use herbal acne treatment products. Majority of the acne patients prefer to apply it once in a day. They prefer to have it in fingertip form, available in white colour that too with some fragrance. Conjoint analysis was performed to understand the preferences of acne patients for herbal products. This research study has used five attributes of herbal acne treatment products viz; 'product form', 'expected treatment time to get noticeable results', 'packaging', 'type of medication', and 'price preference' to understand which

of these attributes are the most and least preferred by the patients with ache while purchasing herbal acne treatment products. The results of conjoint analysis show that 'expected treatment time' has the maximum relative importance. 'Product form' is the second most preferred attribute of herbal acne treatment products followed by 'price' and 'packaging'. 'Type of medication' is found to be the least considered attribute while purchasing herbal acne treatment products. Further, the part-worth utilities of the choices within each of these five attributes were computed. It was also found that the acne patients are very clear about the choices within various attributes. The results show that patients with acne prefer to purchase herbal acne treatment products that are available over the counter in gel form, packaged in a low-priced jar, and which could give noticeable results in just one week.

Limitations & Future Research

The main focus of this study is to understand the practice & experience of acne treatment and to determine the preferences of ache patients for herbal products used to heal acne. Future studies may consider other medications in various forms. The present study focuses on Maharashtra, India, which may limit global applicability. The future studies could be conducted in other geographic locations covering multi-regional data so as to gain more insights on the topic. This study is confined to the evaluation of preferences of only five attributes of herbal products used to heal acne. Future studies could involve some other attributes and other specific herbal ingredients so that further information on this topic can be explored in depth. This study is conducted by applying conjoint analysis technique. Future studies may also involve a deeper comparison between herbal and conventional acne treatments. One of the limitations of this study is that it has used non-sampling techniques for selecting the samples. The study also does not focus on post-treatment analysis. The future studies may involve post-treatment analysis. Future studies may also be conducted on several other target populations.

Conflicts of Interest

The authors declare no conflict of interest.

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Annexures

Annexure 1: Questionnaire on Acne Treatment Preferences

Section 1: Socio-Demographic Characteristics of Acne Patients

Name
Gender
a) Male
b) Female
Age:
Education
a) SSC
b) HSSC
c) Above HSSC
Total Monthly Household Income
a) Below Rs 25,000
b) Rs 26,000 to Rs 50,000
c) Above Rs 50,000
Weight (in Kg):

Section 2: Clinical Characteristics of Acne

Q: 1. Are you suffering from acne?
a) Yes. Presently Suffering
b) Experienced acne in the past
Q: 2. How would you describe your skin type?
a) Dry
b) Oily
c) Sensitive
d) Normal
Q: 3. How old were you when you get first acne?
a) 13-15
b) 16-18
c) 19-20
Q: 4. How does your acne look like?
a) Small red spot
b) Yellow spot
c) Large painful red spot
Q: 5. How does your acne feel?
a) Itchy
b) Painful
c) Stingy
d) Inflammation
Q: 6. What bothers or concerns you most about your acne?
a) Actual pimples
b) Dark marks (discoloured skin) left by pimples
c) Both pimples and dark marks (discoloured skin)
Q: 7. Where on your face do you most often experience acne?
a) Chin
b) Cheeks
c) Forehead
d) Hairline
e) Jawline
f) Nose

Section 3: Acne Treatment Practice & Experience

Q: 8. Have you used some type of acne treatment or procedure?
a) Yes
b) No
Q: 9. What treatment you have been using for last 4 weeks? (You may tick mark more than one choice)
a) Over-the-counter (OTC)
b) Prescription based (Rx medication)
c) Home-remedies
d) Lifestyle changes
Q: 10. What form of medication you have been taking? (You may tick mark more than one choice)
a) Gel
b) Ointment
c) Tablet
d) Patches
e) Cream
f) Soap
g) Facewash
Q: 11. According to your experience, how long does it take to get noticeable results in healing acne with the medication you are using?
a) 1 day
b) 1 week
c) 2 weeks
d) More than 2 weeks
Q: 12. According to your experience, how long does it take to heal your acne 100% with the medication you are using?
a) 1 Week
b) 2 weeks
c) 3 weeks
d) More than a month
Q: 13. Did you experience any side effects while using acne medication?

https://doi.org/10.47552/ijam.v16iS2.6171 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

a) No side effects
b) Dry skin
c) Allergic reaction
d) Skin flakiness
e) Other (please specify)

Q: 14. What is your extent of satisfaction with your present acne treatment?
a) Highly dissatisfied
b) Dissatisfied
c) Neither satisfied nor dissatisfied
d) Satisfied
e) Highly satisfied

Q: 15. What is the extent of acne clearing achieved by you with your present treatment?
a) No clearing
b) Minimal clearing
c) Moderate clearing
d) Good clearing
e) Full clearing

Q:16 What is the composition of your medication?
a) Aloe vera
b) Salicylic Acid
c) Other Herbal Products
d) Niacinamide
e) Tea Tree Oil
f) Benzoyl Peroxide
g) Clindamycin & Benzoyl Peroxide
h) Alpha Hydroxy Acids (AHAs)
i) Sulphur
j) Licorice Extract
k) Topical Retinoids

Q: 17 What was the composition of Rx medications?
a) Oral antibiotics
b) Topical antibiotics
c) Both a and b
d) Topical retinoids
e) Do not know

Section 4: Herbal Treatment Expectations

Q: 18 Have you ever used herbal product for your acne treatment?
a) Yes
b) No

Q: 19 Would you prefer to use herbal acne treatment?
a) No, not at all
b) I may consider
c) Yes, I would certainly prefer

Q: 20 How much of your lifetime would you be willing to give to get your face completely (100%) clear after using herbal product?
a) Overnight
b) 1 week
c) 1 Month
d) 6 Months
e) More than six months

Q: 21 What would be your preference for frequency of application of herbal acne treatment product?
a) Twice a week
b) Once in a day
c) Twice a day

Q: 22 What is your preference for acne treatment product colour?
a) White
b) Brown

Q: 23 What is your preference for acne treatment product fragrance?
a) With fragrance
b) Fragrance-free

Q: 24 What is your preference for acne treatment product application method?
a) Finger tip
b) Roller ball

Q: 25 What benefit in addition to acne clearing you are expecting from a herbal product?

Kindly rank the following benefits from 1 = most preferred benefit to 4 = least preferred benefit

Benefit in addition to acne clearing	Rank
· Prevention of Acne Scarring	
· Reduction in Break out frequency	
· Improved skin texture	
· Prevention of acne marks	

Annexure 2: Conjoint Profiles

Q: Kindly rate the following profiles in the scale of '1=Least Preferred' to '10=Most Preferred'

	Product Form	Expected Time to Get Noticeable	Packaging	Type of Medication	Price Preference	Rating
1	Cream	Two Weeks	Jar	Over the Counter	Economy	
2	Gel	Four Weeks	Tube	Over the Counter	Premium	
3	Lotion	Two Weeks	Tube	Over the Counter	Economy	
4	Gel	One Week	Jar	Over the Counter	Economy	
5	Cream	Two Weeks	Jar	Over the Counter	Mid-Priced	
6	Gel	Two Weeks	Tube	Prescription Based	Premium	
7	Lotion	One Week	Tube	Over the Counter	Economy	
8	Lotion	Two Weeks	Tube	Prescription Based	Premium	
9	Gel	One Week	Jar	Prescription Based	Mid-Priced	
10	Gel	Two Weeks	Tube	Over the Counter	Economy	
11	Lotion	One Week	Tube	Over the Counter	Premium	
12	Gel	One Week	Jar	Over the Counter	Premium	
13	Lotion	Two Weeks	Tube	Over the Counter	Mid-Priced	
14	Gel	Two Weeks	Tube	Over the Counter	Mid-Priced	

15	Gel	Four Weeks	Tube	Over the Counter	Mid-Priced	
16	Cream	Four Weeks	Tube	Over the Counter	Mid-Priced	
17	Cream	Two Weeks	Jar	Prescription Based	Premium	
18	Gel	Four Weeks	Tube	Prescription Based	Economy	
19	Cream	One Week	Tube	Over the Counter	Economy	
20	Lotion	One Week	Tube	Prescription Based	Mid-Priced	

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https://doi.org/10.47552/ijam.v16iS2.6172 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Comparative Anthelmintic activity of three Ayurvedic medicinal plants

Research Article

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Abstract

Helminthiasis, which is the infection by helminths or worms, is a major public health issue, especially in developing nations where it leads to malnutrition, anaemia, and death. The traditional treatment with synthetic anthelmintic drugs like albendazole and mebendazole is usually accompanied by adverse effects like gastrointestinal upsets and neurological manifestations. Additionally, the growing problem of drug resistance has created a need for safer and more effective natural alternatives. The present study aims to explore, evaluate and compare the anthelmintic potential of three traditionally used Ayurvedic medicinal plants *Annona squamosa, Murraya koenigii,* and *Cassia tora* as herbal alternatives to synthetic deworming agents. Method: Plant materials were obtained from parts of Maharashtra state and identified. Petroleum ether and hydroalcoholic extracts of each plant were obtained through Soxhlet and reflux processes. Phytochemical screening was carried out using standard procedures. Anthelmintic activity was analysed on *Pheretima posthuma* (Earthworm) at concentrations of 50, 100, and 150 mg/ mL, with Albendazole as reference standard. The activity was found to be dose dependent on concentration with maximum in *Murraya koenigii.* Results: All three plants exhibited dose-dependent anthelmintic activity, with hydroalcoholic extracts generally showing greater efficacy than petroleum ether extracts. Among them, the hydroalcoholic extract of *Murraya koenigii* exhibited the highest efficacy, compared to the standard drug. Conclusion: The findings support the traditional use of these plants in managing helminth infections. Given their promising efficacy and presumed safety, these herbal candidates may serve as potential alternatives or complementary therapies to conventional anthelmintic drugs. Further studies involving phytochemical analysis and in vivo evaluations are recommended to validate their therapeutic potential and mechanisms of action.

Keywords: Helminthiasis, Anthelmintic Activity, *Krimi*, *Annona squamosa, Murraya koenigii, Cassia tora*.

Introduction

Helminth infections is one of the most common parasitic infections that affect a large portion of the human population. In developing countries, the parasitic infection causes a larger threat to public health, which leads to anaemia, eosinophilia, malnutrition, and even death if untreated. Males are more prone to helminth infections than that of females. More than 24% of the total population depends on the native frameworks of medication such as Ayurveda, Unani & Sidha in India. (1)

Helminths the word is derived from the Greek meaning "worms".(2) Helminthiasis is an infectious disease caused by parasitic worms called helminths. These parasites are classified into tapeworms, roundworms and flukes. These worms live in the gastrointestinal tract or sometimes in other organs, inducing physiological damage. Present treatments for helminthiasis include drugs like albendazole, mebendazole, piperazine citrate, levamisole etc. However, these treatments exhibit side effects like dizziness, diarrhoea, undesirable neurological side effects etc. Therefore, herbal drugs are preferred over Allopathy. (3)

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Anthelminthic agents

In modern medicine, anthelmintic is a drug that kills or removes gastrointestinal worms. "Dewormer" or "wormer" are the more popular names. (4) Anthelmintics are deworming agents that either kill (vermicide) or expel (vermifuge) infesting helminths. (5) An anthelminthic drug can act by causing paralysis of the worm, or by damaging its cuticle, which lead to partial digestion or rejection by immune mechanisms (6).

In contrast, Ayurveda refers to worm infestations as *Krimiroga* and treats them with specific herbal drugs known as *Krimighna Dravyas*. These herbal formulations either destroy the *Krimi* (worms), similar to vermicidal action (*Krimivadha*), or facilitate their expulsion (*Kriminirharana*), much like modern vermifuges. Ayurvedic theory attributes the cause of *Krimiroga* to imbalances in *doshas*, especially *Kapha* and *Pitta*, and

impaired *Agni* (digestive fire), which create a favorable environment for parasites to thrive. (7)

Plants showing Anthelminthic properties

Several medicinal plants are said to be effective in the traditional medical system in killing worms. Some of them are as follows: (8, 9, 10, 11, 12, 13, 14, 15).

Table 1: List of plant possessing anthelmintic properties

Andrographis paniculata	*Pumica grantum Linn.*
Azadirachta indica	*Psoralea corylifolia*
Acacia auriculaeformis	*Piper longum Linn*
Adhatoda vasica	*Phyllanthus embilica*
Allium sativum	*Piliostigma thonningii (Schum.)*
Anacardium occidentale	*Plumbago indica*
Annona squamosa	*Ricinus communis*
Avicennia marina	*Suregada multiflora*
Butea monosperma	*Saraca asoca*
Cannabis sativa Linn.	*Semecarpus anacardium Linn*
Capparis decidua	*Strobilanthes discolour*
Capparis spinosa	*Trichilia emetic*
Carica papaya Linn	*Trifolium repens Linn*
Cassia tora	*Trachyspermum ammi*
Commiphora mukul	*Tinospora cordifolia*
Curcubita maxima	*Tamilnadia uliginosa*
Cleome icosandra Linn.	*Tamarindus indica L.*
Calatropis procera	*Terminalia catappa*
Evolvulus alsinoides Linn	*Trianthema portulacastrum*
Ficus religiosa	*Turraea vogelii*
Ferula asafoetida	*Terminalia arjuna*
Ficus carica	*Thymus vulgarisL.*
Gyanadropsis gynandra Linn.	*Uncaria gambier*
Murraya koenigii	*Urtica dioica*
Mimusops elengi Linn	*Vernonia anthelmintica*
Moringa oleifera	*Vernonia amygdalina*
Melia azedarach Linn.	*Valeriana officinalis*
Moghania vestita	*Withania somnifera*
Neolamarckia cadamba Roxb.	*Xylopia aethiopica*
Nyctanthes arbortristis	*Zingiber zerumbet*
Nicotiana tabacum	*Ziziphus mauritiana*
Ocimum sanctum Linn	*Zingiber officinale*
Oroxylum indicum	*Zanthoxylum armatum*

The plants mentioned above are known for its anthelmintic properties. The three plants *Annona squamosa, Murraya koenigii* and *Cassia tora* were selected for the present study.

Annona squamosa

Annona squamosa Linn belonging to family Annonaceae commonly known as Sugar apple has its origin from West Indies and is cultivated throughout Asia. It is a small, semi deciduous, much branched shrub or small tree about 3- 8 m tall. Leaves are singly, pale green on both surfaces and mostly hairless while flowers are solitary in nature, greenish yellow in color. (16)

In Traditional System of Medicine, the leaf is used as an insecticide, in skin infections, mucosae, laxative, diarrhea, dysentery, pregnancy, antiaborifacients, for treating cancerous tumors (1)

Murraya koenigii

Murraya koenigii commonly known as Kadi patta or curry leaves or as Meethi neem belongs to family Rutaceae. (17) It is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 m and are cultivated for ite aromatic leaves.

In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavouring agent in curries and chutneys. (18)

Cassia Tora L.

Cassia tora L., a seasonal weed, belongs to the Fabaceae family, traditionally reported to have medicinal properties, like laxative, antiperiodic, antihelmintic, ophthalmic, and effective for leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders, etc (7, 19).

Cassia tora is medicinal plant but it is known to us as weed because of lack of advance technology in our country to know the active chemical constituents of the easily available plant as their agricultural benefits to the medicinal point of view. It is an edible wild plant having remarkable nutritional as well as therapeutic property. This plant of seeds are roasted and dried then are used as substitute of coffee in many developing countries. (20) Thus in present study, the anthelmintic potential of three traditionally used Ayurvedic medicinal plants *Annona squamosa, Murraya koenigii,* and *Cassia tora* are explored as herbal alternatives to synthetic deworming agents. The anthelmintic efficacy of all the three plants was compared with standard.

Materials and methods

Collection and Authentication of Plant

The plant materials of *Annona squamosa and Murraya koenigii* were collected from the medicinal garden of Priyadarshini J. L college of Pharmacy, Nagpur and the seeds of *Cassia tora* were collected from cultivated plant of *Cassia tora* in farm of Yavatmal district. All the three plants were authenticated by Dr. N. M. Dongarwar, Head of Department of Botany, R. T. M. Nagpur University, Nagpur. The specimen herbarium sheets of *Annona squamosa, Murraya koenigii* and *Cassia tora* were submitted with the specimen number 67, 66 and 65 respectively.

Hydroalcoholic Extraction of Plant Material

The plant materials were extracted to obtain petroleum ether extract and hydroalcoholic extract. The petroleum ether extract was obtained by Soxhlet extraction method. The petroleum ether extract of each plant was evaporated to dryness and labelled as Petroleum extract of *Annona squamosa* (PEAS), Petroleum extract of *Murraya koenigii* (PEMK) and

Petroleum extract of *Cassia tora* (PECT). The marc obtained from Soxhlet extraction method was subjected to extraction by hydroalcoholic solvent. The dried marc of *Annona sqamosa, Murraya koenigii*, and seeds of *Cassia tora*, were refluxed with 750 ml of distilled water and 250 ml of Methanol for 30 minutes. It was filtered and evaporated to dryness to get Hydroalcoholic Extract of *Annona squamosa* (HAAS), Hydroalcoholic Extract of *Murraya koenigii (HAMK),* Hydroalcoholic Extract of *Cassia tora* (HACT). The percentage yield was calculated and the percentage yield of each is reported in table 2.

Phytochemical screening

The phytochemical screening of PEAS, PEMK, PECT, HEAS, HECT, and HAMK was conducted to identify presence of various phytoconstituents such as alkaloids, tannins, phenol, proteins, amino acids, flavonoids, glycosides and others. Phytochemical screening was carried according to the standard procedures.(21, 22) The results of phytochemical screening were shown in Table 3.

Evaluation of Antihelminthic activity

Anthelmintic activity was carried out on Indian adult earthworms (*Pheretima posthuma*) collected from and dump soil and washed with water to remove all foreign matter. Earthworms of 5-7 cm long and 0.1-0.2 cm wide were used throughout the experimental period due to their physical and physiological resemblance to human intestinal roundworm parasites. [4, 16] Earthworms were divided in eight groups containing 5 earthworms in each group. Earthworms were placed in I- VIII groups for mentioned treatment.

The petroleum ether extracts were dissolved in DMF, the hydroalcoholic extract were dissolved in distilled water and the Albendazole was dissolved in DMSO and then in saline water.

All the extracts, PEAS, PEMK, PECT, HAAS, HAMK AND HACT were dissolved in various concentrations such as 50, 100, and 150 mg/ml in saline water. The standard drug selected for this study was Albendazole.

- Group I - Earthworms were placed into 10 ml of the saline water in clean petri dish, It serves as control.
- Group II - Earthworms were placed in 10 ml of PEAS at concentrations of 50, 100, and 150 mg/ml separately.
- Group III - Earthworms were placed in 10 ml of PEMK at concentrations 50, 100, and 150 mg/ml.
- Group IV - Earthworms were exposed to 10 ml of PECT at concentrations 50, 100, and 150 mg/ml.
- Group V - Earthworms were placed in 10 ml of HAAS at concentrations 50, 100, and 150 mg/ml.
- Group VI - Earthworms were placed in 10 ml of HAMK at concentrations 50, 100, and 150 mg/ml.
- Group VII - Earthworms were placed in 10 ml of HACT at concentrations 50, 100, and 150 mg/ml.
- Group VIII - Earthworms were placed in 10 ml of standard drug Albendazole at concentrations 25 mg/ml.

The entire study was conducted in different petri dishes. Earthworms were observed for their movement. The time taken for paralysis and the time taken for death were monitored and documented in minutes. Paralysis was said to occur based on the behaviour of earthworms with no revival body state in the normal saline medium. Death was concluded after confirming that the earthworm neither moved when shaken vigorously nor when dipped in warm water (50 °C) with faded body colour. (23) The results of anthelmintic activity of each extract are reported in table 4.

Results and Discussion

The results of all the evaluations are depicted in below tables.

The percentage yield of all petroleum ether and hydroalcoholic extract of all the plant materials is reported in table 2.

Table 2: Percentage yield of Plant extract

SN.	Plant Extracts	% Yield
1	**PEAS**	8.05%w/w
2	**PEMK**	5.1%w/w
3	**PECT**	8.24%w/w
4	**HAAS**	24% w/w
5	**HAMK**	26% w/w
6	**HECT**	8.7% w/w

Table 2 shows that the percentage yield of HAMK is higher compared to the other extracts. This suggest that HAMK contains more quantity of polar components.

The preliminary phytochemical screening of petroleum ether and hydroalcoholic extracts of all the plant materials is reported in table 3.

Table 3: Preliminary phytochemical screening of extracts for various phytoconstituents

SN.	Phyto-constituents	Chemical test	Observation					
			PEAS	PEMK	PECT	HAAS	HAMK	HACT
1	Carbohydrate	Molisch's test	-	-	-	+	+	+
2	Protein	Biuret test	-	-	-	+	-	-
3	Flavonoid	Shinoda test	-	-	-	+	+	+
		Modified shinoda	-	-	-	+	+	+
		Sulphuric acid test	-	-	-	+	+	+
		Lead acetate test	-	-	-	+	+	+
		Alkali test	-	-	-	+	+	+
4	Amino acid	Ninhydrin test	-	-	-	+	+	+

5	Alkaloid	Dragendorff's	-	-	-	+	+	+
		Hager's reagent	-	-	-	+	+	+
		Mayer's reagent	-	-	-	+	+	+
		Wagner's reagent	-	-	-	+	+	+
6	Phenolic compounds	Ferric chloride test	-	-	-	+	+	+
		Lead acetate test	-	-	-	+	+	+
7	Steroid	Salkowski test	+	+	+	+	+	+
		Zimmermann test	+	+	+	+	+	+
		Pinus test	+	+	+	+	+	+
8	Triterpenoids	Noller's test	+	+	+	+	+	+

In table 3, + indicates Present is mentioned as, Absent

Table 3 illustrates the phytochemical screening results, which reveal that PEAS, PEMK, and PECT do not contain alkaloids, flavonoids, tannins, and phenolic compounds, while HAAS, HAMK, and HACT include carbohydrates, alkaloids, flavonoids, saponins, tannins and phenols.

Anthelmintic activity

The comparison between all the hydroalcoholic extracts of *Annona squamosa, Murraya koenigii,* and *Cassia tora* for anthelmintic activity was done. The plant extracts produced a significant anthelmintic activity in dose dependent manner as shown in below table 4.

Table 4: Anthelmintic activity with different extracts

SN.	Groups	Concentration	Time Taken in minutes	
			Paralysis (P)	Death (D)
1	Groups I- Control	-	-	-
2	Groups II- PEAS	50mg/ml	81.06 ± 0.30	113.69± 0.59
		100mg/ml	68.29 ± 0.18	98.85 ± 0.50
		150mg/ml	44.12 ± 0.06	70.99 ± 0.63
3	Groups III- PEMK	50mg/ml	72.35 ± 0.2	126.33 ± 0.20
		100mg/ml	55.01 ± 0.34	93.94 ± 0.40
		150mg/ml	43. 23 ± 0.21	82. 94 ± 0.52
4	Groups IV-PECT	50mg/ml	94.89 ± 0.40	132.9 ± 0.45
		100mg/ml	82.1 ± 0.39	111.77 ± 0.62
		150mg/ml	65.84 ± 0.36	87.01 ± 0.50
5	Groups V- HAAS	50mg/ml	52.42 ± 0.10	94.26 ± 0.19
		100mg/ml	35.26 ± 0.23	72.4 ± 0.1
		150mg/ml	30.21 ± 0.12	43.23 ± 0.21
6	Groups VI-HAMK	50mg/ml	44.77 ± 0.28	68.27 ± 0.17
		100mg/ml	37.19 ± 0.17	50.28 ± 0.17
		150mg/ml	24.19 ± 0.19	37.27 ± 0.18
7	Groups VII- HACT	50mg/ml	64. 86 ± 0.33	97.88 ± 0.33
		100 mg/ml	44.09± 0.38	84.93 ± 0.57
		150 mg/ml	29.21 ± 0.43	55.26 ± 0.17
8	GroupsVIII- Standard (Albendazole)	25 mg/ml	15.09 ± 0.40	25.24 ± 0.198

Results are expressed as Mean ± SD (n = 5)

Statistical analysis

F-value 9.420085 is greater than F- critical value 5.613591 and P (0.000956) < 0.05 indicates all of above three groups having different mean. Thus, the null hypothesis is that the mean anthelmintic effect is the same across all three groups i.e. standard (albendazole), petroleum ether extracts (PEAS, PECT, PEMK) and hydroalcoholic extracts (HAAS, HACT, HAMK). The alternative hypothesis is that at least one group has a different mean anthelmintic effect. Above data shows that hydroalcoholic extracts of all three plants found to have better anthelmintic activity when evaluated for paralysis time and death time upon increasing concentration of each extracts from 50 mg/ml to 150 mg/ml in comparison with petroleum ether extracts of all 3 plants. The standard albendazole dose of 25mg/ml shows mean paralysis time (min) and death time (min) 15.09 ± 0.4, 25.24 ± 0.19 respectively, whereas HAMK shows mean paralysis time (min) and death time (min) 24.19 ± 0.19 and 37.27 ± 0.18 respectively.

Conclusion

This research emphasizes the potential of three traditionally used medicinal plants—*Annona squamosa*, *Murraya koenigii*, and *Cassia tora* as natural and effective alternatives to synthetic deworming agents. The objective of this study was to compare hydroalcoholic extracts of these three medicinal plants for the anthelmintic activity. The results show that these plants possess dose-dependent anthelmintic activity, with hydroalcoholic extracts generally being more effective than petroleum ether extracts. The petroleum ether extract failed to show significant anthelmintic activity when compared to hydroalcoholic

extracts of all the three plants. The result suggested that as compared to HAAS and HACT, HAMK takes less time to paralyse the experimental animals. Among the plants studied, the hydroalcoholic extract of *Murraya koenigii* demonstrated the highest efficacy amongst other extracts. These findings support the traditional use of these plants for treating helminth infections and suggest they could serve as safer, more effective alternatives to synthetic anthelmintic drugs. However, further studies, including detailed quantitative analysis of phytoconstituents and in vivo testing, are needed to better understand their mechanisms of action and confirm their therapeutic potential. Given the rising concerns over drug resistance and side effects of synthetic treatments, these herbal remedies could offer a viable solution for managing helminthiasis, especially in developing regions.

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https://doi.org/10.47552/ijam.v16iS2.6173 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Mimusops elengi Phytoconstituents *In-silico* Prediction for Alzheimer Disease

Research Article

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Abstract

Objective: Alzheimer's disease (AD), which predominantly affects older adults, is the primary cause of dementia, impacting 46 million people worldwide. By 2030 and 2050, respectively, there will be 74.7 and 131.5 million persons with AD, as the incidence rises exponentially every five years. The goals of treatment are to control symptoms, lessen clinical decline, and manage the burden of illness. Our study included in-silico testing of the phytoconstituents of *Mimusops elengi* for immunosuppressive effectiveness in the treatment of Alzheimer's. Methods: Molecular docking is performed using Discovery Studio to evaluate the pattern of interaction between the crystal structure of the malarial proteins (PDB ID: 7UJQ & 7Q8V) and phytoconstituents from the *Mimusops elengi* plant. Swiss ADME and pkCSM were later used to test for both the pharmacokinetic profile and toxicity respectively. Result: According to the docking results, quercetin (-8.4 kcal/mol), myricetin (-8.5 kcal/mol), aesculin (-8.1 kcal/mol), and myricitrin (-9.3 kcal/mol) -9.7 kcal/mol for spinasterol Aesculin (-7.7 kcal/mol), Quercetin (-9.3 kcal/mol), Myricitrin (-9.9 kcal/mol), and Catechin (-8.2 kcal/mol) for 7UJQ macromolecule -8.2 kcal/mol of spinasterol The 7Q8V macromolecule's catechin (-9.1 kcal/mol) had the best binding to immunosuppressive action when compared to all other standards. Additionally, ADMET experiments showed that the pharmacokinetics and toxicity parameters were within acceptable bounds. Conclusions: The binding potential of phytoconstituents with an eye toward immunosuppressive function showed promising results. Along with providing important information on clinical treatment and pharmaceutical research, it encourages the use of *Mimusops elengi.*

Keywords: *Mimusops elengi,* In-silico study, Alzheimer disease, Docking, Immunosuppressant.

Introduction

Alzheimer's disease (AD), the leading neurological cause of dementia, affects more than 46 million people worldwide primarily older adults. According to estimates by 2030, 74.7 million people will have AD, and by 2050, 131.5 million as the disease's incidence rises exponentially every five years beyond the age of 65 (1).

Progressive memory loss, diminished cognitive function, and ultimately dementia is experienced by patients with AD. The neuropathological hallmarks of AD include intracellular neurofibrillary tangles of hyperphosphorylated tau protein, loss of synaptic connections, neuronal degeneration, with the development of senile plaques outside of cells as a result of amyloid-β (Aβ) aggregation, which is typically linked to local inflammation and neurite dystrophy/swelling (2). As of right now, AD has no known cure or treatment (3).

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Cognitive and behavioral decline in individuals over 65 is a hallmark of dementia. The most prevalent neurological condition, Alzheimer's disease (AD), affects about 24 million individuals globally, and by 2050, that number is expected to have tripled (4).

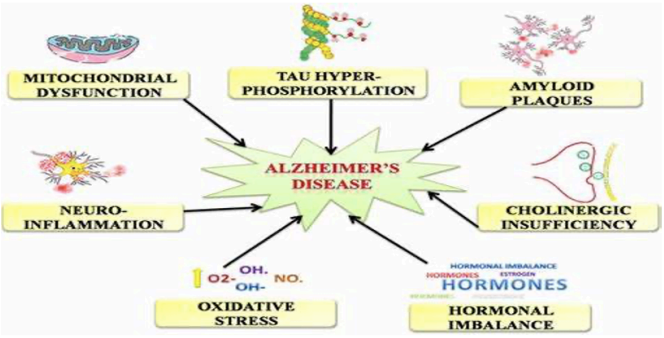
Acetylcholine (ACh) deficiency and elevated glutamatergic transmission resulting in oxidative damage are also linked to AD (2)(3)(4)(5)(6).

The pathogenesis of Alzheimer's

Progressive brain dysfunction that appears to be staged in a cell biology sequence—neuronal injury, synaptic failure, and neuronal death is a hallmark of AD and associated dementias. AD's pathological hallmarks are neuropil threads and neurofibrillary tangles (NFT). Under a microscope, amyloid plaques, also known as senile plaques, are amorphous aggregates of Abeta. Additionally, hyperphosphorylated Tau protein accumulates, suggesting the production of neurofibrillary tangles and widespread neuronal death. The pathophysiology of Alzheimer's disease can result from mitochondrial failure because they are engaged in many bodily cellular functions, including signal transmission and neural synapses. Neurodegeneration may result from the development of free radicals and an oxidative stress state within the cells. Given these functions of mitochondria in the pathophysiology of

Alzheimer's disease, the mitochondrial cascade theory was developed, which unambiguously links abnormalities in mitochondria to the etiology of Alzheimer's disease(7).

Figure 1: Pathophysiology of Alzheimer Diseases



Treatment of Alzheimer's Disease

There is no recognized cure for AD dementia. The complex and poorly understood pathological processes that cause AD dementia are increasingly recognized to begin decades before clinical symptoms manifest. By then, extensive and likely irreversible processes have likely caused extensive damage on a number of levels, including molecular, intracellular, network, and system. The current paradigm for treating AD includes many symptom management strategies to maintain quality of life, reduce the burden of illness, and avoid long-term clinical decline. efficient long-term treatment using FDA-approved AD medications. The establishment, application, and upkeep of a solid foundation of psychoeducation, behavioral and nonpharmacological care techniques, and clarity in care objectives and expectations all depend on a strong therapeutic relationship between the therapist and the patient-caregiver dyad. This involves cholinesterase inhibitor (ChEI) monotherapy at first, followed by additional dual-combination treatment with a ChEI and memantine (8).

Mimusops Elengi

The Sapotaceae family includes *Mimusops elengi*, often known as Spanish Cherry, Bullet Wood, Bunga Mengkula, or Mengkulah. Widely distributed throughout South Asian nations, this tropical evergreen shrub is significant in traditional medicine, particularly in Ayurveda, where different plant components are used for their therapeutic qualities. Due to its diverse pharmacological benefits, *Mimusops elengi* is a valuable resource in traditional healing practices (9). The World Health Organization (WHO) claims that approximately two-thirds of the global population relies on traditional medicine for treating various ailments, with Mimusops elengi, also known as the Indian Medlar Tree or Bakul tree, being a significant example (10). *Mimusops elengi* is regarded as a sacred plant by Hindus and holds significant importance in religious texts and ancient Sanskrit literature. Its aromatic flowers are praised in the Puranas and are even included among the flowers of Hindu paradise(11).

In Banda Aceh, Indonesia, *Mimusops elengi* serves as a source of agricultural residue biomass. This species has been in the archipelago for ages and is indigenous to India, Myanmar, and Sri Lanka(12)(13)(14). Currently, Banda Aceh has approximately 6,500 *Mimusops elengi* trees spread over 28.2 hectares(15). According to published research, the roots, bark, leaves, flowers, and foliage of the *Mimusops elengi* plant have all been thoroughly examined for potential therapeutic use with encouraging outcomes. It could also be applied to the manufacturing of biodiesel(16)(17).

Historically, *M. elengi* has been used as a tonic and astringent, particularly for the treatment of dysentery and diarrhea due to its ability to tighten and tone tissues. Recent studies have also identified antioxidant properties in its leaves, which are essential for combating harmful free radicals associated with various illnesses and the aging process. Additionally, investigation indicates that *M.elengi* might be beneficial because of its anti-diabetic properties. Diabetics regulate their blood sugar levels. Numerous pharmacological characteristics of *Mimusops elengi* point to both its usefulness in traditional medicine and its potential for use in contemporary medicine in the future. It is crucial to remember that additional research is necessary to completely examine its properties and possible uses in medicine. Before utilizing herbal treatments for health reasons, particularly if you already have health problems or are on additional prescription drugs, always get medical counsel(18).

Plant description

The medium- to large-sized *Mimusops elengi* tree can grow to a height of 25–30 m (82–98 ft). Its crown is straight and its trunk is upright. The evergreen leaves are simple and alternate. They can have an elliptical or oblong shape and are normally 2.4–4.7 inches (6–12 cm) in length. The leaves are glossy and dark green. One of the most notable characteristics of *Mimusops elengi* is its extremely scented blossoms. They resemble stars, have a cream to light yellow tone, and have a waxy touch. The flowers have a sweet and pleasant perfume and are often seen alone or in tiny groups. Blossoming typically occurs in the summer. *Mimusops elengi* produces a green, meaty berry as its fruit. *Mimusops elengi* produces meaty berries that are green while young and turn yellow or orange when ripe. Depending on the age of the tree, its grayish-brown bark may be smooth or rough. Its wood is used for many purposes, like as carving and furniture construction, and is prized for its tensile strength. Because of its extensive root system, the tree may thrive in a wide range of soil types. The Mimusops elengi is found in South and Southeast Asia is found in countries such as Thailand, Myanmar, Sri Lanka, India, and so forth. Throughout the world, in many tropical and subtropical areas, it is also grown as an ornamental tree. In addition to its usual medical uses, The wood of *Mimusops elengi* is prized, and aromatic blooms (19)(20)(21)(22). The different phytoconstituents in *M.elengi* are Myricitrin, Myricetin, Quercetin,

https://doi.org/10.47552/ijam.v16iS2.6173 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Coumarin, and phenolic acids like Aesculin, and Quinic acid. Steroid (Spinasterol), Tannis and Catechin.

Materials and technique

Platform for molecular docking

With Tau protein as the target and the N-methyl D-aspartate (NMDA) receptor as the ligand, a computational docking study of all the phytoconstituents selected as ligands was carried out using PyRx software (26).

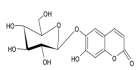
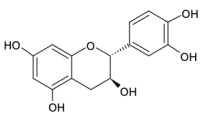
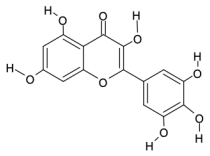
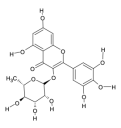
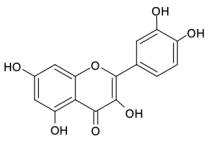
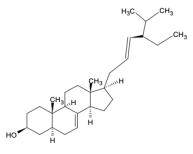
Preparing proteins

The macromolecule is 7UJQ and 7Q8V were examined using *in silico* methods for particular phytoconstituents. determination: 2.05 Å, R-Value Free: 0.244, R-Value Work: 0.194, R-Value Observed: 0.196), The protein data bank (https://www.rcsb.org) provided the information. 4RAC belongs to the group of drugs known as transferase/transferase inhibitors. All other molecules were removed, along with undesirable chains, co-crystallized water molecules, and nonstandard residues, utilizing Discovery Studio v.24 and PDB 7UJQ and 7Q8V macromolecule validation.

Ligand preparation

Avogadro software was used to extract the three-dimensional (3D) structures of all constituents from the NCBI website's PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Nonetheless, the geometrical 2D structure was drawn using the ChemSketch program. The Avogadro program was used to transform the two-dimensional (2D) ligand structures into three-dimensional (3D) models once they were saved in the PDB format. All of the chemical structures are shown in Figure 2.

Table 1: Chemical structures for each of the chosen phytoconstituents

Aesculin	Catechin	Myricetin
		
Myricitrin	**Quercetin**	**Spinasterol**
		

Standard Preparation

The standard is prepared steps like the two-dimensional structure of standard drug was made using chem sketch program, then the two-dimensional structure was converted Avogadro software was used to convert it into a 3D model, and the PDB file was saved. By utilizing PyRx molecular docking of N-methyl D-asparate (NMDA) receptor and Tau protein was done with 7UJQ and 7Q8V.

Docking of molecules

In order to forecast the ligand molecule's binding affinity, Protein-ligand interactions are evaluated by molecular docking, which also computes the scoring function according to the geometry (27, 28). The way certain phytoconstituents bind (Figure 1) and the standard medication, as well as the crystal structure of the immunosuppressive activity macromolecule (PDB ID: 7UJQ and 7Q8V), were examined using molecular docking experiments. Binding affinity was examined using the Vina wizard tool, while PyRx software was used to conduct the study of molecular docking as a standard, The 2020 Discovery Studio Client was utilized to analyse and illustrate the final data with bound ligands. Protein-ligand interaction visualization shows how many interactions and active residues are responsible for a considerable amount of binding at the active site of the target enzyme.

Predicting Absorption, distribution, metabolism, excretion, and toxicity (ADMET)

Applying the rule of Lipinski, the chosen Citations and phytoconstituents medication were investigated further for drug-likeness characteristics. Before being ingested by humans and animals, the acceptability of Phytochemicals needs to be predicted during the medication development process. The ligands' pharmacokinetic profile (ADME) and toxicity were predicted using SwissADME (http://www.swissadme.ch) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction), an online server database that uses graph-based signatures to predict small-molecule pharmacokinetic features. To examine the toxicological characteristics of ligands, PDB files or simplified molecular input line entry system (SMILES) notations were uploaded. After that, the appropriate models were selected to produce a multitude of data about effects associated with structures (29, 30).

Outcomes and Conversations

The current investigation sought to determine whether the phytoconstituents found in *Mimusops elengi* could reduce immunosuppressive function. In order to investigate the active ingredients inhibitory potentials, we first used Auto Dock Vina to undertake research using molecular docking of every phytoconstituent found in *Mimusops elengi*. Next, we looked at interacting amino acid residues. The servers pkCSM and SwissADME were utilized. To further assess the selected phytoconstituents' absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties showed the best fit.

Docking of molecules

Table 2 shows each chemical component's docking scores and binding energies. *Mimusops elengi* targets immunosuppressive activity (PDB ID: 7UJQ and 7Q8V) as well as binding relationships with residues of amino acids.

Table 2: Immunosuppressive activity-targeting ligand binding interaction from Mimusops elengi (PDB ID: 7UJQ and 7Q8V)

Sr.No.	Chemical constituent	Pub Chem ID	Docking score (binding affinity kcal/mol)	
			7UJQ	7Q8V
1	Myricitrin	330.00	-9.3	-9.9
2	Myricetin	205.16	-8.5	-8.8
3	Aesculin	328.23	-8.1	-7.7
4	Quercetin	179.64	-8.4	-9.3
5	Spinasterol	372.21	-9.7	-8.2
6	Catechin	261.03	-8.2	-9.1

7UJQ

The range of phytoconstituents' binding affinities was -9.7 to -8.1 kcal/mol. Compared to other docked compounds, such as Aesculin (−8.1 kcal/mol), Myrictrin (−9.3 kcal/mol), Myricetin (−8.2 kcal/mol), Quercetin (−8.4 kcal/mol), and Catechin (− 8.2 kcal/mol), it is clear from the docked results that spinasterol has the most favorable binding affinity (− 9.7 kcal/mol) and in complex with selected macromolecules (PDB ID: 7UJQ). demonstrated the substantial role of a variety of interactions, including hydrophobic interactions, π–π stacking, π–alkyl and alkyl interactions, and hydrogen bonding, in the stability of the phytoconstituents' binding to 7UJQ. Additionally, the number of closest amino acid residues, the binding energy of ligand 7UJQ stable complexes, and the number of intermolecular hydrogen bonds were ascertained. selected compound Myrictrin, Myricetin, Quercetin, Aesculin, Spinasterol, Catechin (Table 3).

An investigation of the interaction between the 7UJQ complex of proteins. Myrictrin, Myricetin, Quercetin, Aesculin, Spinasterol, Catechin ligand was also carried out, which demonstrated that the ligand molecule is orientated due to van der Walls interaction with amino acid residue GLU A:96, GLU A:139, GLY A:20, UZD A:301, LYS A:137, GLU B:139, LYS B:21, GLY B:20, ARG C:1300, GLN D:1301, HIS D:1302, ASN A: 135, GLU A: 139, GLY A:20, GLU A: 18, GLY B: 20, SER C: 1303, GLN C: 1301, ARG C: 1300, HIS D:1302, LYS A: 137, GLU A: 139, GLY A:20, UZD B: 301, GLY B: 20, HIS C: 1302, ARG D: 1300, HIS D: 1302, GLU A: 216, LEU A: 221, GLN A: 218, THR A:176, PRO A: 177, LEU B: 159, PHE B:24, LYS C:1297, ARG C: 1299, GLN C: 1301, HIS C: 1302, HIS D: 1302, TYR D: 1304, GLY A: 20, LYS A: 137, GLY B: 20, UZD B: 301, GLN C:1301, HIS C: 1302, HIS D: 1302, ARG D: 1300, GLU A: 139, LYS A: 137, ASN A: 135, ASP A: 156, UZD A: 301, GLY A: 20, GLY B:20, GLU B: 139, LYS B: 137, GLU B: 96, ASN B: 140, HIS C: 1302, ARG C:1300, GLN D:1301, ARG D: 1300, HIS D: 1302 were found (Fig.3).

Analysis of the interactions between the ligand Aesculin and the 7UJQ protein complex revealed that the ligand molecule is orientated as a result of pi-alkyl interactions with amino acid residues ARG D:1300 and the formation of traditional hydrogen bonds with LYS A:21,GLN C:1301, HIS C:1302, LYS B:137 residues and In addition, Eleven interactions between amino acid residues and van der Waals GLU A:96, GLU A:139, GLY A:20, UZD A:301, LYS A:137, GLU B:139, LYS B:21, GLY B:20, ARG C:1300, GLN D:1301, HIS D:1302, were found (Fig. 3).

Analysis of interactions between the ligand Catechin and the 7UJQ protein complex revealed that ligand molecule is orientated as a result Pi-alkyl interactions with amino acid residues LYS B:21, LYS A:21 and Pi-cation interaction with LYS A:137 and forming conventional hydrogen bonds with ASN A: 140, ASP A: 156, HIS C: 1302 residues and In addition, Nine interaction between amino acid residues and van der Waals ASN A: 135, GLU A: 139, GLY A:20, GLU A: 18, GLY B: 20, SER C: 1303, GLN C: 1301, ARG C: 1300, HIS D:1302 were found (Fig. 3).

Analysis of interactions between the ligand Myricetin and the 7UJQ protein complex revealed that ligand molecule is orientated as result Pi-alkyl interactions with amino acid residues ARG C:1300 and Pi-anion interaction with GLU A:96, Pi-sigma interaction LYS A:21 and forming conventional hydrogen bonds with LYS A: 21, UZD A: 301, LYS B:21, GLN C: 1301 residues and In addition, Eight interaction between amino acid residues and van der Waals LYS A: 137, GLU A: 139, GLY A:20, UZD B: 301, GLY B: 20, HIS C: 1302, ARG D: 1300, HIS D: 1302 were found (Fig. 4).

Analysis of interactions between the ligand Myricitrin and the 7UJQ protein complex revealed that the ligand molecule is oriented as a result of Pi-alkyl interactions with amino acid residues ARG C:1300, Pi-anion interaction with GLU A:96, and Pi-sigma interaction with LYS A:21. It also forms conventional hydrogen bonds with LYS A:21, UZD A:301, LYS B:21, and GLN C:1301 residues. In addition, eight van der Waals interactions were observed with amino acid residues LYS A:137, GLU A:139, GLY A:20, UZD B:301, GLY B:20, HIS C:1302, ARG D:1300, and HIS D:1302. Notably, non-amino acid interactions involving UZD moieties (UZD A:301 and UZD B:301) further stabilized the ligand binding, indicating the contribution of prosthetic group components or cofactors present in the protein complex. These interactions collectively contribute to the stabilization and specificity of ligand binding within the active site of the 7UJQ complex (Fig. 5).

Analysis of the interactions between the ligand Myricitrin and the 7UJQ protein complex revealed that the molecules is orientated as result of Pi–alkyl interaction with amino acid residues ALA B:23, Pi-anion interaction with LYS B:56,Pi-cation interaction with ASP D:1305 conventional hydrogen bonds interactions with the amino acid residues ASP A:215, TYR C:1304, SER D: 1303, ASP D: 1305. In addition, Thirteen van der Waals interactions with amino acid residues GLU A: 216, LEU A: 221, GLN A: 218, THR A:176, PRO A: 177, LEU B: 159, PHE B:24, LYS C:1297, ARG C: 1299, GLN C: 1301, HIS C: 1302, HIS D: 1302, TYR D: 1304 were found (Fig. 6).

Analysis of the interactions between the ligand Quercetin and the 7UJQ protein complex revealed that the molecules is orientated as result of Pi–alkyl

interaction with amino acid residues ARG C:1300, Pi-anion interaction with GLU A:96,Pi-sigma interaction with LYS A:21 conventional hydrogen bonds interactions with the amino acid residues GLU A: 139, UZD A: 301, LYS A: 21. In addition, Thirteen van der Waals interactions with amino acid residues GLY A: 20, LYS A: 137, GLY B: 20, UZD B: 301, GLN C:1301, HIS C: 1302, HIS D: 1302, ARG D: 1300 were found (Fig. 7).

An analysis of the interactions between the ligand Spinasterol and the 7UJQ protein complex revealed that the molecules is orientated as result of Pi–alkyl interaction with amino acid residue LYS A:21, LYS B:21 Unfavorable Donar Donar interaction with ASN A:140 Unfavorable acceptor-acceptor with SER C:1303. In addition, Sixteen van der Waals interactions with amino acid residues GLU A: 139, LYS A: 137, ASN A: 135, ASP A: 156, UZD A: 301, GLY A: 20, GLY B:20, GLU B: 139, LYS B: 137, GLU B: 96, ASN B: 140, HIS C: 1302, ARG C:1300, GLN D:1301, ARG D: 1300, HIS D: 1302 were found (Fig. 8).

7Q8V

The phytoconstituents' binding affinities varied from -9.9 to -7.7 kcal/mol. In comparison to other docked compounds, such as Aesulin (−7.7 kcal/mol), Spinasterol (−8.2 kcal/mol), Myricetin (−8.8 kcal/mol), Quercetin (−9.3 kcal/mol), and Catechin (− 9.1 kcal/mol), it is clear from the docked results that Myricitrin exhibits the most favorable binding affinity (− 9.9 kcal/mol) and in complex with selected macromolecules (PDB ID: 7Q8V). A visual inspection of the computationally docked optimal binding poses of phytoconstituents on selected macromolecules (i.e. 7Q8V). demonstrated that the stability of the phytoconstituents' binding to 7Q8V was significantly influenced by a variety of interactions, including hydrogen bonding and hydrophobic interactions, such as π–π stacking and π–alkyl and alkyl interactions.

Additionally, the number of closest amino acid residues, the binding energy of ligand 7Q8V stable complexes, and the number of intermolecular hydrogen bonds were ascertained. selected compound Myrictrin, Myricetin, Quercetin, Aesculin, Spinasterol, Catechin (Table 2).

An investigation of the interaction between the 7Q8V complex of proteins. Myrictrin, Myricetin, Quercetin, Aesculin, Spinasterol, Catechin ligand was also carried out, which demonstrated that the ligand molecule is orientated due to van der Walls interaction with amino acid residue LEU A: 179, GLY A: 178, VAL A: 73, PHE A: 45,GLU A: 77, LEU A: 74, GLY A: 46, GLU A: 65, LYS A:63, ASP A: 176, ILE A: 48, ASN A: 159 ASP A: 176, ILE A: 48, GLU A: 47, LEU A: 74, GLY A: 46, GLU A: 65, GLN A: 69, VAL A: 73, GLY A: 178, LYS A: 156, SER A: 158, ASN A: 159 GLY A:42, ILE A:48, VAL A:64, LEU A:74, GLU A:65, GLN A:69, VAL A:73, LEU A:179, GLY A:178, ASN A:159, LEU A:175, SER A:158, LYS A:156, LEU A:175, ASN A:113, LYS A:156, THR A:202, LEU A:179, GLY A:201, GLY A:44, GLY A:178, VAL A:73, GLU A:65, GLY A:46, LEU A:74, ILE A:48, ASN A:159, ASN A:113, LYS A:156, GLY A:178, LEU A:74, VAL A:73, GLY A:46, GLU A:65, GLU A:77, LEU A:74, VAL A:73, GLU A:65, GLY A:46, GLY A:43, PHE A:45, GLY A:42, ASN A:159, SER A:158, ARG A:119, ALA A:115, THR A:202, LYS A:156, ASP A:176, GLY A:178 were found (Fig No. 9)

Analysis of the interactions between the ligand Aesculin and the 7Q8V protein complex revealed that the ligand molecules are oriented due to one Pi–alkyl interaction with amino acid residues A:9IV402, Pi-cation interaction with LYS A:156, Pi-sigma interaction with A:9IV402 conventional hydrogen bonds interactions with the amino acid residues LYS A: 156, SER A: 158, VAL A: 64, GLU A: 47 U Carbon Hydrogen Bond interaction with GLU A:77, ASP A:176. In addition, ten interactions between amino acid and van der Waals LEU A: 179, GLY A: 178, VAL A: 73, PHE A: 45, LEU A: 74, GLY A: 46, GLU A: 65, LYS A:63, ILE A: 48, ASN A: 159 were found (Fig. 9).

An analysis of the interactions between the ligand Catechin and the 7Q8V protein complex revealed that the ligand molecule is oriented due to one Pi–alkyl interaction with amino acid residues A:9IV402, LYS A:63 Pi-anion interaction with GLU A:77 conventional hydrogen bonds interactions with the amino acid PHE A: 45, VAL A: 64 residues. In addition, twelve interactions between amino acids and van der Waals ASP A: 176, ILE A: 48, GLU A: 47, LEU A: 74, GLY A: 46, GLU A: 65, GLN A: 69, VAL A: 73, GLY A: 178, LYS A: 156, SER A: 158, ASN A: 159 were found (Fig. 10).

An analysis of the interactions between the ligand Myricietin and the 7Q8V protein complex revealed that the ligand molecule is oriented due to one Pi–alkyl interaction with amino acid residues A:9IV402, LYS A:63 Pi-anion interaction with GLU A:77 conventional hydrogen bonds interactions with the amino acid GLU A: 47, PHE A: 45, ASP A: 176 residues Carbon Hydrogen Bond interaction with GLY A:178, ILE A:48, GLY A:42. In addition, Twelve interaction between amino acid and van der Waals VAL A:64, LEU A:74, GLU A:65, GLN A:69, VAL A:73, LEU A:179, ASN A:159, LEU A:175, SER A:158, LYS A:156 were found (Fig. 11).

An analysis of the interactions between the ligand Myricitrin and the 7UJQ protein complex revealed that the ligand molecule is oriented due to one Pi–alkyl interaction with amino acid residues LYS A:63 A:9IV402, Pi-anion interaction with GLU A:77, ASP A:176, Pi-sigma interaction with PHE A:45 conventional hydrogen bonds interactions with the amino acid residues SER A:158, ASN A:159, VAL A:64, GLU A:47, ASP A:154 Unfavourable Acceptor-Acceptor interaction with ASP A:154. In addition, twelve interactions between amino acid and van der Waals LEU A:175, ASN A:113, LYS A:156, THR A:202, LEU A:179, GLY A:201, GLY A:44, GLY A:178, VAL A:73, GLU A:65, GLY A:46, LEU A:74 were found (Fig. 12).

An analysis of the interactions between the ligand Quercetin and the 7UJQ protein complex revealed that the ligand molecule is oriented due to one Pi–alkyl

interaction with amino acid residues LYS A:63 A:9IV402, Pi-anion interaction with GLU A:77, ASP A:176, conventional hydrogen bonds interactions with the amino acid residues VAL A:64, PHE A:45, SER A:158, Carbon Hydrogen Bond with interaction GLY A:178, Unfavourable Doner-Doner interaction with GLU A:47. In addition, Twelve interactions between amino acid and van der Waals ILE A:48, ASN A:159, ASN A:113, LYS A:156, LEU A:74, VAL A:73, GLY A:46, GLU A:65 were found (Fig. 13).

An analysis of the interactions between the ligand Spinasterol and the 7UJQ protein complex revealed that the ligand molecule is oriented due to one Pi–alkyl interaction with amino acid residues LYS A:63 A:9IV402 Alkyl interaction with ILE A:48. In addition, Sixteen interactions between amino acid and van der Waals GLU A:77, LEU A:74, VAL A:73, GLU A:65, GLY A:46, GLY A:43, PHE A:45, GLY A:42, ASN A:159, SER A:158, ARG A:119, ALA A:115, THR A:202, LYS A:156, ASP A:176, GLY A:178 were found (Fig.14).

Table 3: Binding interaction of ligand with the binding site 7UJQ and 7Q8V

S. No.	Inhibitors	Binding energy(kcal/mol)	H bond	Main amino acid interaction	
				Pi-sulfur, pi-alkyl, pi-sigma, Unfavorable acceptor-acceptor, Unfavorable doner-doner, Pi-cation, Pi-anion	Van der Waals
1	Aesculin	-8.1	LYS B: 137, LYS A: 21, HIS C: 1302, GLN C: 1301	ARG D: 1300	GLU A:96 (0.7A°), GLU A:139, GLY A:20, UZD A:301, LYS A:137, GLU B:139, LYS B:21, GLY B:20, ARG C:1300, GLN D:1301, HIS D:1302 (0.9A°)
2	Catechin	-8.2	ASN A: 140, ASP A: 156, HIS C: 1302	LYS A: 137, LYS A: 21, LYS B: 21	ASN A: 135 (0.7A°), GLU A: 139, GLY A:20, GLU A: 18, GLY B: 20, SER C: 1303, GLN C: 1301, ARG C: 1300, HIS D:1302 (0.6A°)
3	Myricetin	-8.5	LYS A: 21, UZD A: 301, LYS B:21, GLN C: 1301	GLU A: 96, ARG C: 1300	LYS A: 137 (0.7A°), GLU A: 139, GLY A:20, UZD B: 301, GLY B: 20, HIS C: 1302, ARG D: 1300, HIS D: 1302 (1.1A°)
4	Myricitrin	-9.3	ASP A:215, TYR C:1304, SER D: 1303, ASP D: 1305	TRP A: 214, ALA B: 23, LYS B: 56	GLU A: 216 (0.9A°), LEU A: 221, GLN A: 218, THR A:176, PRO A: 177, LEU B: 159, PHE B:24, LYS C:1297, ARG C: 1299, GLN C: 1301, HIS C: 1302, HIS D: 1302, TYR D: 1304 (1.3A°)
5	Quercetin	-8.4	GLU A: 139, UZD A: 301, LYS A: 21	GLU A: 96, LYS B: 21, ARG C: 1300	GLY A: 20, LYS A: 137, GLY B: 20, UZD B: 301, GLN C:1301, HIS C: 1302, HIS D: 1302, ARG D: 1300
6	Spinasterol	-9.7		ASN A: 140, LYS A: 21, LYS B: 21, SER C: 1303	GLU A: 139 (0.8A°), LYS A: 137, ASN A: 135, ASP A: 156, UZD A: 301, GLY A: 20, GLY B:20, GLU B: 139, LYS B: 137, GLU B: 96, ASN B: 140, HIS C: 1302, ARG C:1300, GLN D:1301, ARG D: 1300, HIS D: 1302 (0.2A°)
S. No.	**Inhibitor**	**Binding energy(kcal/mol)**	**H bond**	**Main amino acid interaction**	
				Pi-sulfur, pi-alkyl, pi-sigma, Unfavorable acceptor-acceptor, Unfavorable doner-doner, Pi-cation, Pi-anion Carbon Hydrogen Bond	**Van der Waals**
1	Aesculin	-7.7	LYS A: 156, SER A: 158, VAL A: 64, GLU A: 47	A:9IV402, LYS A:156, ASP A:176, GLU A:77	LEU A: 179, GLY A: 178, VAL A: 73, PHE A: 45, LEU A: 74, GLY A: 46, GLU A: 65, LYS A:63, ILE A: 48, ASN A: 159
2	Catechin	-9.1	PHE A: 45, VAL A: 64	GLU A: 77, LYS A: 63, A:9IV402	ASP A: 176, ILE A: 48, GLU A: 47, LEU A: 74, GLY A: 46, GLU A: 65, GLN A: 69, VAL A: 73, GLY A: 178, LYS A: 156, SER A: 158, ASN A: 159
3	Myricetin	-8.8	GLU A: 47, PHE A: 45, ASP A: 176	GLU A: 77, LYS A: 63, A:91V402, GLY A:178, ILE A:48, GLY A:42	VAL A:64, LEU A:74, GLU A:65, GLN A:69, VAL A:73, LEU A:179, ASN A:159, LEU A:175, SER A:158, LYS A:156

4	Myricitrin	-9.9	SER A:158, ASN A:159, VAL A:64, GLU A:47, ASP A:154	GLU A:77, ASP A:176, LYS A:63, A:91V402, PHE A:45, ASP A:154	LEU A:175, ASN A:113, LYS A:156, THR A:202, LEU A:179, GLY A:201, GLY A:44, GLY A:178, VAL A:73, GLU A:65, GLY A:46, LEU A:74,
5	Quercetin	-9.3	VAL A:64, PHE A:45, SER A:158,	GLU A:77, ASP A:176, GLU A:47, LYS A:63, A:91V402, GLY A:178	ILE A:48, ASN A:159, ASN A:113, LYS A:156, LEU A:74, VAL A:73, GLY A:46, GLU A:65
6	Spinasterol	-8.2		ILE A:48, LYS A:63, A:91V402	GLU A:77, LEU A:74, VAL A:73, GLU A:65, GLY A:46, GLY A:43, PHE A:45, GLY A:42, ASN A:159, SER A:158, ARG A:119, ALA A:115, THR A:202, LYS A:156, ASP A:176, GLY A:178

ADMET Research

When turning a chemical into a powerful medication, the ligands' pharmacokinetic profile (ADME) and toxicity projections are crucial considerations. pkCSM and SwissADME were utilized to assess these parameters in the current investigation. The topological polar surface area (TPSA) and the partition coefficient (Log P) are used to characterize the absorption potential and lipophilicity, respectively. A medication molecule's TPSA should be less than 140 Å for optimal cell membrane penetration. However, the drug target affects the value of Log P. For a number of medications, the optimal Log P value is 1.35 to 1.80 for oral and intestinal absorption, > 5 for sublingual absorption, and 31 for central nervous system (CNS) absorption (23). It is optimal for ligands to have an aqueous solubility between -6.5 and 0.5 (32). whereas the value ranges from -3.0 to 1.2 for the blood-brain barrier (BBB) (33). Drug resistance is also caused by P-glycoprotein which is not a substrate (34). There is no violation of the Lipinski rule as shown in Table 2.

Table 4 legend: *In-silico* kinetic prediction of phytoconstituent from *Mimosups elengii*

ADMET Properties	Formula	MW (g/ mol)	Log P	TPSA (A^2)	HB donor	Hb acceptor	Aqueous Solubility Log mol/L)	Human intestinal absorption (%)	Blood-brain barrier
Aesculin	$C_{15}H_{16}O_9$	340.28	-1.3227	149.82	5	9	-2.871	40.366	-1.186
Catechin	$C_{15}H_{14}O_6$	290.27	1.5461	110.38	5	6	-2.97	66.032	-1.219
Myricetin	$C_{15}H_{10}O_8$	318.24	1.6936	151.59	6	8	-2.979	67.622	-1.803
Myricitrin	$C_{21}H_{20}O_{12}$	464.38	0.1943	210.51	8	12	-2.949	56.935	-2.17
Quercetin	$C_{15}H_{10}O_7$	302.24	1.988	131.36	5	7	-3.18	74.634	-1.481
Spinasterol	$C_{28}H_{46}O$	398.66	7.5548	20.23	1	1	-6.56	97.539	0.814

Table 4 legend: *In-silico* kinetic prediction of phytoconstituent from *Mimosups elengii* (continued…)

ADMET Properties	P- glycoprotein substrate	Total clearance [Log mL(min.kg)]	Bioavailability score	AMES toxicity	Max tolerated dose [Log mg/(kg.d)]	hERG 1 inhibitor	hERG 2 inhibitor
Aesculin	Yes	0.751	0.55	No	0.424	No	No
Catechin	Yes	0.224	0.55	No	0.242	No	No
Myricetin	Yes	0.5	0.55	No	0.863	No	No
Myricitrin	Yes	0.501	0.17	Yes	0.662	No	Yes
Quercetin	Yes	0.547	0.55	Yes	0.951	No	No
Spinasterol	Yes	0.659	0.55	No	-0.915	No	No

Table 4 legend: *In-silico* kinetic prediction of phytoconstituent from *Mimosups elengii* (continued…)

ADMET Properties	Acute Oral rat toxicity, LD50 (mol/kg)	Oral rat chronic toxicity (Log mh/kg bw/day)	Hepatotoxicity	Skin sensitization	*T. Pyriformis* toxicity (Log µg/L)	Minnow toxicity (Log mmol/L)	Lipinski's rule violations
Aesculin	2.714	4.148	Yes	No	0.285	2.959	Yes (0)
Catechin	2.107	1.953	No	No	0.405	1.7	Yes (0)
Myricetin	2.854	2.811	No	No	0.288	0.942	Yes (1)
Myricitrin	2.753	3.551	No	No	0.285	2.972	No (2)
Quercetin	2.562	1.735	No	No	0.314	0.878	Yes (0)
Spinasterol	3.854	1.048	No	No	0.441	-1.156	Yes (1)

7UJQ

Figure 3: Aesculin's binding interaction and docking scores (PDB ID: 7UJQ)

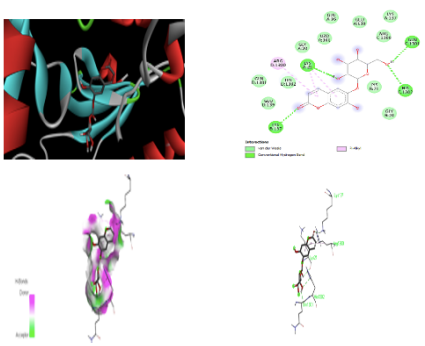


Figure 4. Catechin binding interaction and docking scores (PDB ID: 7UJQ)

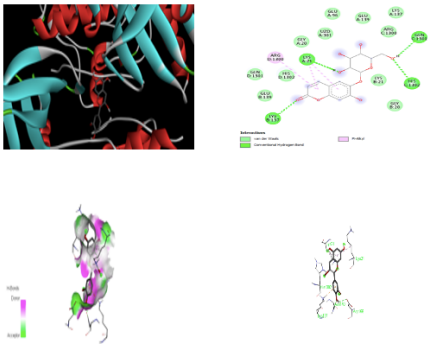


Figure 5: Myricetin binding interaction and docking scores (PDB ID: 7UJQ)

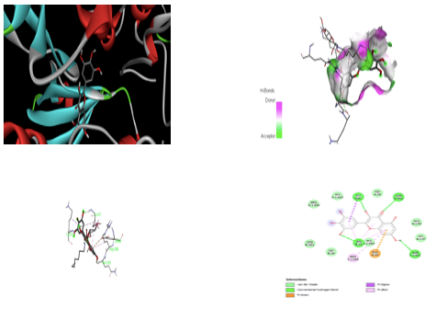


Figure 6. Myricitrin binding interaction and docking scores (PDB ID: 7UJQ)

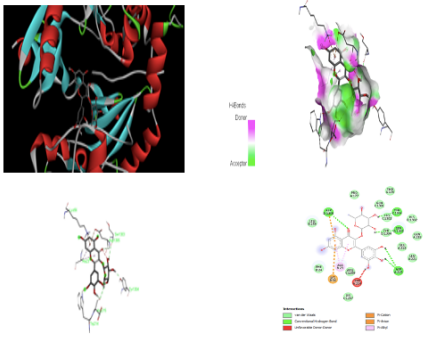


Figure 7. Quercetin binding interaction and docking scores (PDB ID: 7UJQ)

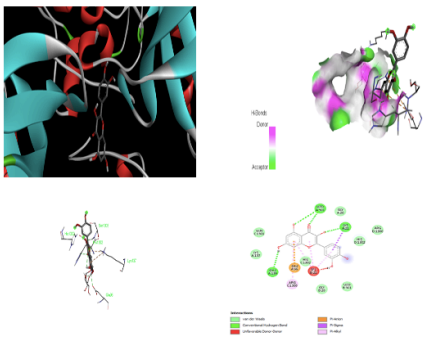
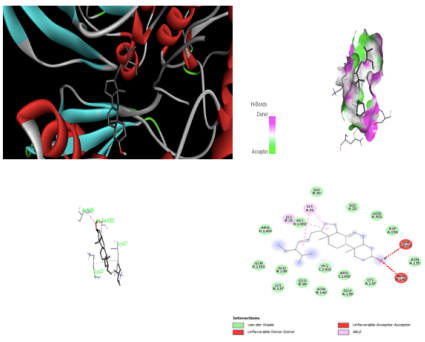


Figure 8. Spinasterol binding interaction and docking scores (PDB ID: 7UJQ)



7Q8V

Figure 9: Aesculin binding interaction and docking scores (PDB ID: 7Q8V)

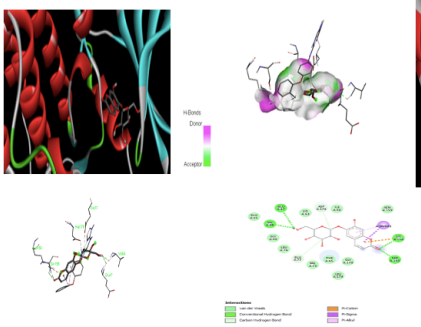


Figure 10. Catechin binding interaction and docking scores (PDB ID: 7Q8V)

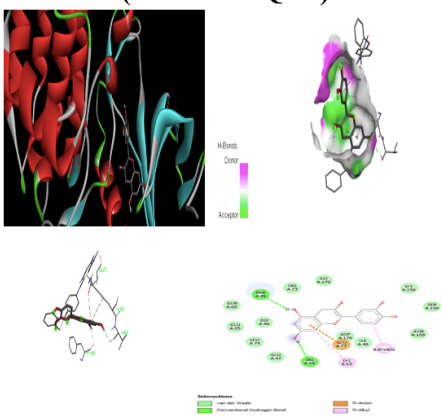


Figure 11. Myricetin binding interaction and docking scores (PDB ID: 7Q8V)

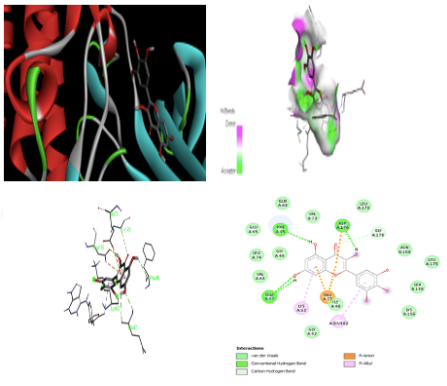


Figure 12. Myricetin binding interaction and docking scores (PDB ID: 7Q8V)

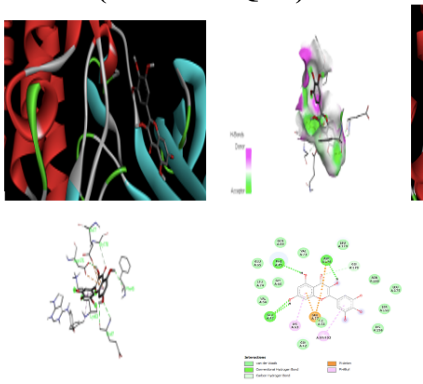


Figure 13. Quercetin binding interaction and docking scores (PDB ID: 7Q8V)

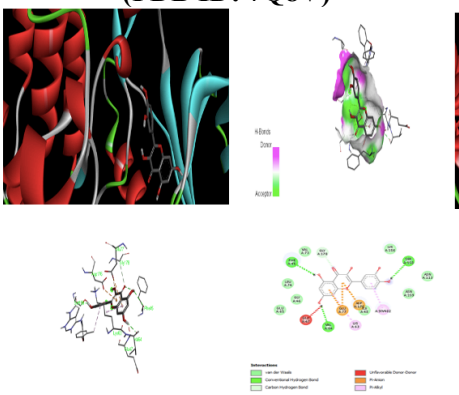
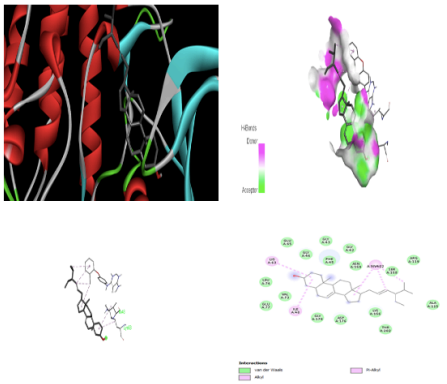


Figure 14. Spinasterol binding interaction and docking scores (PDB ID: 7Q8V)



https://doi.org/10.47552/ijam.v16iS2.6173 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Diagram of a Boiled Egg

Figure 15: A representation of all the phytoconstituents combined with a standard boiled egg.

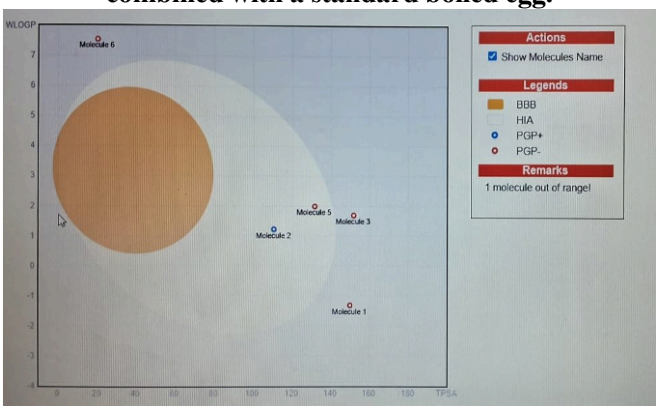


Table 4. Molecule names in boiled egg diagram

Molecule No	Phytochemicals
1	Aesculin
2	Catechin
3	Myricetin
4	Myricitrin
5	Quercetin
6	Spinasterol

The acronym for Brain or Intestinal Estimate D Permeation is Boiled the predictive model:

Two areas are depicted White and yellow in the diagram of a boiled egg. The physiochemical space of molecules likely to be absorbed via the whitish area is the gastrointestinal tract. while the physiochemical space of molecules most likely to enter the brain is the yellow region (yolk). Additionally, if the points are projected to be activated by P-gp (PGP+) efflux, they are colored blue. They become red if they are thought to be a non-substrate of P-gp (PGP).

Conclusion

In this study, we screened the phytoconstituents of the *Mimusops elengi* plant *in-silico*. Aesculin, catechin, myricetin, myricitrin, quercetin, and spinasterol are the six chemicals from the *Mimusops elengi* plant that were shown in this study. In 7UJQ and 7Q8V, the chosen phytocompounds had docking scores between -9.7 and -8.1 kcal/mol and between -9.9 and -7.7. In combination with 7UJQ & 7Q8V, spinasterol & myricitrin provided the highest binding energy (− 9.7 & -9.9 kcal/mol) of all. These ligands also show favorable ADMET characteristics. In conclusion, the phytoconstituents found in *Mimusops elengi* have potent anti-7UJQ and 7Q8V properties. They may also be further studied for their immunosuppressive properties and for the creation of less harmful pharmaceuticals used to treat Alzheimer's disease.

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https://doi.org/10.47552/ijam.v16iS2.6193 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Investigating the antioxidant and antiproliferative activity of *Mimusops elengi* Linn Extract against Hepatocellular Carcinoma

Research Article

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Abstract

The antioxidant profile of the methanolic extract of *Mimusops elengi* was measured using various antioxidant assays, and the cytotoxicity was evaluated using MTT and Alamar blue assay against a hepatocellular carcinoma cell line (Hep-G2). In the DPPH radical scavenging assay, IC50 values were 91.55±0.02 µg/ml. Superoxide Radical Scavenging IC50 Value is 57.14 ±0.088 µg/ml, Nitrogen Scavenging IC50 Value is 15.88 ±0.028 µg/ml, CUPRAC IC50 Value is 7.731±0.5 µg/ml, FRAP IC50 Value is 14.32 ±0.046 µg/ml, Hydroxyl radical scavenging IC50 value is 22.46 ±1.02 µg/ml, and ABTS IC50 value is 4.00±0.08 µg/ml. the cytotoxicity study by MTT Assay the IC50 Values 34.98± 0.24, Alamar Blue Assay, it was showed that when the HepG2 cell line was exposed to various concentrations of the sample on different treatment doses, MEME Mean Fluorescence intensity (MFI%) of Alamar blue was found increasing with decrease of Treatment Dose of sample MEME. The highest MFI was estimated at 105.20 % at a 3.601 µg/ml dose of sample MEME, while the Lowest MFI% % (99.24%) was observed at a higher treatment dose of 14.405 µg/ml for Sample MEME concerning the control.

Keywords: *Mimusops elengi,* Antioxidant, Antiproliferative, Hep G2 Cell lines.

Introduction

Mimusops elengi belongs to the Sapotaceae family and is commonly referred to as Bakula in Ayurvedic medicine. Our study examined the possible presence of proteolytic activity in an aqueous Mimusops elengi leaf extract about the traditional and Ayurvedic medicinal usage of *M. elengi* leaves, particularly in wound healing and dental care (1). It is a well-known plant in Indian traditional medicine (2). An indigenous herb from India, *M. elengi,* has long been utilized in medicine. The plant parts strong potential therapeutic usefulness led to extensive research on it in most parts of the world. All of this plant's parts, such as root, fruit, seed, leaf, flower, and bark, have traditionally been used to treat a variety of illnesses. The information gathered here will help advance the current examination of various medical studies on *M. elengi.* (3) The tropical region of Aceh is frequently covered in a variety of medicinal plants, including *M. elengi*. Triterpene and phenolics, the primary constituents of *M. elengi* flower extract, possess antibacterial, antifungal, antioxidant, and antineoplastic properties. Maceration typically extracts chemical compounds, including essential oils, yielding modest amounts of chemical compounds (4). To varying degrees, the *M. elengi* extracts demonstrated cytotoxicity towards CCRF-CEM leukemia cells (5). The study aimed to assess the phytochemical makeup, antioxidant capacity, and cytotoxic effects of *M. elengi* Linn extract (ME) compared to normal human cultured adult gingival fibroblasts (HGFs) (6). Traditionally used to treat anxiety and panic attacks and as a brain tonic in several nations. In Wistar rats, the impact of a standardized hydroalcoholic extract of *M. elengi* flowers (ME) against excitotoxicity and oxidative stress caused by MSG was assessed (7). The identification, measurement, antioxidant, and possible biofunctional characteristics of lesser-known (8). In recent years, there has been increasing awareness of the potential anticancer, antioxidant, and apoptosis-inducing properties of some polyphenolic compounds derived from plants. Therefore, the role of plant-produced polyphenols in cancer chemoprevention has emerged as an intriguing area of research. (9).

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Materials and Methods

Plant Material

The plant *mimusops elengi* L. (Leaves) was obtained from the Botanical Garden of Nagpur (Maharashtra) and authenticated by the botanist, and the herbarium sheet was deposited in the Botany Department, voucher specimen no. 102. The second part of July 2022 was the time for plant collection.

Extraction of Plant Material

After thoroughly rinsing the Leaves in distilled water, they were allowed to air dry. Dried Leaves were

pulverized to a coarse powder (100 g) and macerated with methanol for 7 days. After the maceration of the extract was concentrated by a rotary evaporator and allowed to dry in a Lyophilizer, 20 g (20%) of *mimusops elengi* L. methanolic extract was obtained.

Phytochemical study

The Phytochemical screening was carried out on methanolic extracts of *Mimusops elengi* L. leaves. identify the active phytoconstituents, including terpenoids, alkaloids, glycosides, flavonoids, steroids, saponins, etc., present in the ethanolic extract, using the following standard phytochemical tests.

Test for alkaloids

1. Dragendroff's reagent test: 2 mL of extract was heated with 2% H 2 SO 4. A small amount of Dragendroff's reagent was added, and orange-red precipitate was observed.
2. Mayer's test: 1-2 mL of ethanolic extract was taken into a test tube, then 1-2 drops of Mayer's reagent were added. The result was positive; a creamy white precipitate formed.
3 Wagner's tests: 1-2 mL of extract solution was taken, and 1- 2 drops of Wagner's reagent were added. then, a brown precipitate formed.

Test for glycoside

Borntrager test: 1- 2 mL of extract solution was taken, 2-3 mL of Chloroform was added, then shaken, and the chloroform layer was separated. 10% ammonia solution added. The result was positive, a red coloured solution formed.

Test for flavonoids

Shinoda test: 1- 2 mL of extract was taken, and 5 mL of ethanol added, then added few grains of magnesium, turnings, with a few drops of conc. HCL, the sample was positive for flavonoids, red to pink coloured

Test for Phenolic compounds

Gelatin test: The ethanolic extract was dissolved in 5 mL of distilled water, followed by the addition of a 1% gelatin solution and a 10% sodium chloride solution, resulting in the formation of a white precipitate.

Test for terpenoids

Salkowski's test: The Alcoholic extract solution was taken, and a few drops of concentrated Sulphuric acid was added to the sample was positive for steroids when a red colour formed in the lower layer (29), (30).

Test for tannins

Nitric acid test: 2- 3 mL of the ethanolic extract was taken in a test tube, a few drops of dil. nitric acid was added, then a reddish yellow colour formed (29).

Test for fixed fats and oils

Spot test/stain test: a small quantity of plant extract is pressed between to filter papers, then an oil stain appears on the paper.

Test for saponin

Foam formation test: 2 mL of aq. the solution was taken into a test tube and shaken vigorously. If foam was formed and did not disappear for 5 min. (29)

Antioxidant assays

DPPH Assay

Various stock solutions of the test sample, each measuring 5 µl, were combined with 0.1 ml of 0.1 mM DPPH (2,2-diphenyl-1- 1picrylhydrazyl) solution in 96-well plates. The solution mixture was prepared in triplicate, and two blank samples were created using 0.2 ml of DMSO (Dimethyl sulfoxide) and 5 ml of the sample at different concentrations. Wells without any treatment served as controls. The plate was kept in the dark for 30 minutes. Following the incubation period, a microplate reader (iMark, BioRad) was used to measure the decolorization at 517 nm. A control reaction mixture containing 20 microliters of deionized water was employed. The scavenging activity was expressed as '% inhibition' relative to the control. Software Graph Pad Prism 6 was utilized to calculate the IC50. A graph was constructed with the sample concentration on the X-axis and the % inhibition compared to the control on the Y-axis. (10), (11), (12).

CUPRAC Assay

In designated wells of 96-well plates, 10µl of various test sample concentrations were deposited. Following this, 200µl of a reagent mixture was added to each well. The solution was prepared in triplicate for samples and duplicate for blanks, consisting of 200µl Methanol and 10µl of a compound at different concentrations for both sample and standard (Trolox – Ottokemi). This mixture underwent a 30-minute incubation period in dark conditions. Untreated wells served as controls. After incubation, a microplate reader (iMark, BioRad) was used to measure the absorbance and evaluate decolorization at 490 nm. The control reaction mixture replaced the sample or standard with 20µl of deionized water. Scavenging activity was reported as "% inhibition" about the control. IC50 was calculated using GraphPad Prism 6 software. A graph was plotted with the percentage of inhibition compared to the control on the Y-axis and sample concentration on the X-axis. (13), (14).

FRAP Assay

The reducing power of the test samples was determined using absorbance values obtained from a microplate reader. A higher absorbance value indicated greater reducing power, with ascorbic acid used as the positive control. GraphPad Prism 6 software was employed to calculate IC50 values, which represent the concentration needed for 50% reduction, allowing for comparison across various samples. The experimental procedure involved adding 10 microliters of different test sample stocks and ascorbic acid standard (SRL) to a mixture containing 0.04 ml of 0.2 M sodium phosphate (Rankem) buffer (pH 6.6) and 0.05 ml of 1% potassium ferricyanide (SRL) solution. After thorough vortexing, the reaction mixture was incubated at 50°C for 20

minutes. Wells without treatment served as controls. Following incubation, 0.5 ml of 10% trichloroacetic acid (SRL) was introduced to the mixture. Subsequently, 50µl of deionized water and 50 microliters of 0.1% ferric chloride solution (Fischer Scientific) were added. The resulting colored reaction solution was measured at 700 nm against a blank using a microplate reader (iMark, BioRad). IC50 values were calculated using GraphPad Prism 6 software. (13), (14), (15).

Hydroxyl free radical scavenging Assay

A mixture comprising 66µl Reagent Mixture, 10µl EDTA 0.5 M (HiMedia), 24.14 mg Deoxyribose (SRL), 88µl Ferric chloride (Fischer Scientific), 28 µl H 2 O 2 of 6 % (Neurochem Laboratories), and water was prepared. To this, 10µl of plant extract, 24 µl of phosphate buffer (pH 7.4), and 10 µl of ascorbic acid (SD Fine) were sequentially added to the wells of a 96-well plate. The mixture was then incubated at 37°C for 1 hour. Wells without treatment served as controls, and Gallic Acid (SRL) was utilized as the standard. Post-incubation, 50µl of 10% TCA (Fischer Scientific) and 50µl of 1% TBA (HiMedia) were introduced to each well, resulting in the formation of a pink chromogen. Subsequently, absorbance was measured at 540 nm using a microplate reader (iMark, BioRad). IC50 was determined using GraphPad Prism software, with a graph plotted between Sample Concentration (X-axis) and % inhibition relative to control (Y-axis). (14), (15), (16), (17), (18). The IC50 value denotes the sample concentration needed to inhibit 50% of the enzymatic activity. This metric is frequently employed to assess the efficacy of potential inhibitors or drug candidates. The graphical representation of sample concentration versus percentage inhibition offers a visual insight into the dose-response relationship, facilitating easy interpretation of the compound's inhibitory effects.

Super Oxide Anion Radical Scavenging Assay

Different concentrations of extract and standard were combined with riboflavin solution and incubated for 30 minutes in 96-well plates under ambient light conditions. The reaction mixture was subsequently added to the pre-incubated solution and thoroughly mixed. Wells without treatment served as controls. Following this, absorbance measurements were taken at 560 nm using an ELISA plate reader (iMark, BioRad). The IC50 was determined using GraphPad Prism 6 software. A graph was constructed with sample concentration on the X-axis and percentage inhibition relative to the control on the Y-axis. (11), (19), (20). The absorbance data were utilized to calculate the half-maximal inhibitory concentration (IC50) of the sample, which denotes the sample concentration needed to inhibit 50% of the measured biological or biochemical function. The resulting graph offers a visual depiction of the relationship between sample concentration and inhibition percentage, facilitating easy interpretation of the sample's efficacy.

Antioxidant assay by ABTS methods

The preparation of ABTS (2,2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic) acid) (SRL-Chemicals) radicals involved combining APS (2.45 mM) and ABTS (7 mM) solutions, followed by a 100-fold dilution to create the ABTS free radical reagent. In 96-well plates, 10 microliters of various Ascorbic Acid (SD Fine) stocks, serving as standards and samples, were added to 200 microliters of the ABTS free radical reagent. The mixture was then incubated at room temperature for 10 minutes in the dark. Wells without treatment served as controls. Following incubation, a microplate reader (iMark, BioRad) was used to measure the absorbance of the decolorization at 750 nm. Results were compared to the negative control. The IC50 was determined using the Software GraphPad Prism 9.5.1. A graph was constructed with the sample concentration on the X-axis and the percentage inhibition relative to the control on the Y-axis. (9) (21), (22).

Antioxidant assay by Reactive Nitrogen Oxide Scavenging methods

A reaction solution was formulated by combining 50 microliters of 10 mM sodium nitroprusside (Fisher Scientific), 40 microliters of distilled water, and 10 microliters of gallic acid (SRL). The untreated reaction mixture served as a control. This solution underwent pre-incubation for 15 minutes at ambient temperature in the presence of light. Subsequently, 100 microliters of Griess reagent were introduced to both the test and control wells, followed by an additional 5-10 min incubation at room temperature to allow for chromophore formation and stabilization. A microplate reader (iMark, BioRad) was employed to measure absorbance at 540 nm and 660 nm. The IC 50 value was determined using Software GraphPad Prism 6. (10), (23), (24).

Antiproliferative assay

In Vitro Cytotoxicity assessment of the compound by MTT

The cytotoxicity of MEME was evaluated on the HepG2 cell line using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells (10000 cells/well) were cultivated in 96-well plates for 24 hours at room temperature with 5% carbon dioxide in DMEM medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotic solution. The following day, cells were exposed to various concentrations of the compound, with untreated cells serving as controls. After a 24-hour incubation period, the MTT solution was introduced to the cell culture and incubated for an additional 2 hours. Upon completion of the experiment, the culture supernatant was removed, and 100 microliters of DMSO (dimethyl sulfoxide) were used to dissolve the cell layer matrix. Data was measured using an ELISA plate reader (iMark, Biorad) at 540 and 660 nm. The IC50 was determined using GraphPad Prism-6 software. An inverted microscope (Olympus eK2) and an Amscope digital camera (10 MP Aptima CMOS) were employed

to capture images. Cell Proliferation Studies with Alamar Blue Assay on HepG2 (25), (26).

Alamar Blue Assay

To assess cell proliferation in HepG2 cell line samples, the Alamar blue test was utilized. A 96-well plate was prepared with 5000-8000 cells per well and incubated for 24 hours at 37 °C with 5% carbon dioxide in DMEM (Dulbecco Modified Eagle Medium) enriched with 10% FBS (Flavin Bovine Solution) and 1% antibiotic solution. The cells were then subjected to final concentrations of 1, 10, and 25µM for a full day. Following these 24 hours, 10 microliters of Alamar Blue reagent were introduced to every 100 microliters of media, and the plate was incubated for an additional 3-4 hours. The final measurement was conducted using an Agilent BioTek Epoch 2 fluorescence ELISA plate reader at a wavelength of 540 nm.(28)

Results

Phytochemical screening (Table 1) revealed that the leaves of the methanolic extract of *M. elengi* L. are rich in secondary metabolites, particularly flavonoids, alkaloids, glycosides, tannins, terpenoids, saponins, fixed oils, and phenolic compounds.

Table 1: Phytochemical screening of the leaf extract of *M. elengi*

Sr. No	Test	Observation	Results
1	**Alkaloids** Dragendroff's test Mayer's test Wagner's test	 A reddish-brown precipitate White precipitated Brown precipitated	 + ve + ve + ve
2	**Glycosides** Borntrager test	 The red colour solution formed	 + ve
3	**Tannins** Ferric chloride test	 The greenish blue colour formed	 + ve
4	**Phenolic compound** Gelatin test	 White precipitated	 + ve
5	**Flavonoids** Shinoda test	 Red to pink precipitated	 + ve
6	**Terpenoids** Salkowski's test	 Red colour formed at a lower layer	 + ve
7	**Saponin** Foam test	 Foam formed	 + ve
8	**Fixed Fats and oils** Spot test	 Oil spot observed	 + ve

+ve = Positive test, - ve = Negative

The methanolic extracts of *M. elengi* L. leaves were subjected to various antioxidant assays, with the results displayed in Table 2. The DPPH assay revealed an IC50 value of 110.53±1.20 µg/ml, while the Superoxide Radical Scavenging assay showed 64.32±0.10 µg/ml. Nitrogen Scavenging and CUPRAC assays yielded IC50 values of 37.10±0.5 µg/ml and 7.002±0.5 µg/ml, respectively. The FRAP assay demonstrated an IC50 value of 9.585±0.097 µg/ml, Hydroxyl radical scavenging registered 23.16±1.29 µg/ml, and the ABTS assay recorded 4.00±0.10 µg/ml. Table 3 further illustrates the efficacy of these extracts, presenting their antioxidant activity and inhibition percentages across different assays.

Table 2: In vitro antioxidant activity and IC50 Values

Sr. No	Antioxidant Assay	Standard	IC50 Values
1	DPPH	Ascorbic acid	91.55 ±0.02 µg/ml
2	ABTS	Ascorbic acid	4.62±0.08 µg/ml
3	CUPRAC	Trolox	7.731 ±0.5 µg/ml
4	FRAP	Ascorbic acid	14.32±0.046 µg/ml
5	NOSA	Gallic acid	15.88 ±0.028 µg/ml
6	SOARSA	Gallic acid	57.14±0.10 µg/ml
7	HFRSA	Gallic acid	22.46 ±1.29 µg/ml

Table 3: In vitro antioxidant activity and % inhibition Values

Sr. No	DPPH	ABTS	CUPRAC	FRAP	NOSA	SOARSA	HFRSA
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	-1.05	14.66	-2.78	-1.92	20.14	24.52	20.51
3	5.48	72.79	56.67	19.27	42.57	44.03	32.69
4	27.14	84.81	118.33	57.44	64.32	47.74	51.28
5	51.78	95.76	337.22	102.16	73.32	53.23	86.22

https://doi.org/10.47552/ijam.v16iS2.6193 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

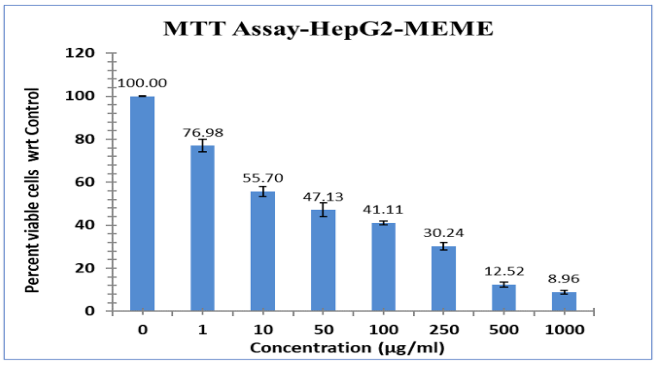
6	88.50	97.00	588.33	274.45	81.31	59.35	102.88
7	90.67	96.82	786.67	390.36	85.64	64.84	120.19
8	93.51	107.95	1320.00	446.14	92.52	63.39	145.19

Table 4: The antiproliferative capacity of MEME

Antiproliferative assay	Celline	IC50 Values	MFI %
1. MTT Assay	Hep-G2	34.98 ± 0.25 µg/ml	----
2. Alamar Blue Assay	Hep-G2	-------	
a. Dose 3.61 µg/ml			105.20
b. Dose 14.40 µg/ml			99.24

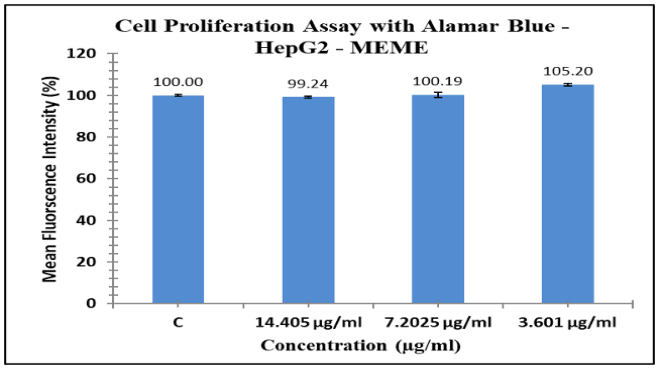
The cytotoxic activity(Table 4) of the sample MEME was demonstrated through MTT test results, which revealed an IC50 value of 34.98 ± 0.25 µg/ml when the HepG2 cell line was exposed to various sample concentrations. The IC50, as illustrated in Figure 1, denotes the sample concentration that reduces cell viability by 50%. Figure 1 presents the MTT assay findings for the methanolic extract of *Mimusops elengi* L. on Hepatocellular carcinoma cell lines (Hep-G2), confirming the cytotoxic nature of the sample MEME.

Figure 1: MTT Assay of MEME against HepG2 Celline



The Alamar Blue Assay results revealed that the Mean Fluorescence Intensity (MFI%) of Alamar blue in the HepG2 cell line exhibited an inverse relationship with the treatment dose of sample MEME. As the concentration of the sample MEME decreased, the MEME MFI% increased. The peak MFI was recorded at 105.20% when the sample MEME dose was 3.61 µg/ml, while the lowest MFI% (99.24%) was observed at a higher treatment dose of 14.60 µg/ml for Sample MEME, in comparison to the control, as depicted in Figure 2.

Figure 2: Cell proliferation assay with Alamar blue assay of MEME on HepG2 Cell line



Discussion

Coarse powder (100 g) of *M. elengi* leaves was macerated with methanol for 7 days. After the maceration of the extract was concentrated and dried by a rotary evaporator, 20 g (20%) of methanolic extract was obtained. The Phytochemical screening (Table 1) shows that the leaves of the methanolic extract of *M. elengi* L. are rich in secondary metabolites, such as flavonoids, glycosides, tannins, terpenoids, saponins, fixed oils, alkaloids, and phenolic compounds. Methanol was utilized as an extractant due to its established efficacy in obtaining extracts for antioxidant and antiproliferative activity studies of M. elengi L. plants. This study demonstrated that extracts from M. elengi L. leaves exhibited antioxidant properties through various assays to determine IC50 values. A comparative analysis based on different antioxidant assays revealed that the ABTS method displayed potential antioxidant scavenging activity, with an IC50 of 4.62±0.08 µg/ml compared to ascorbic acid, as shown in Table 1. Additionally, in vitro antioxidant activity and % inhibition values were assessed, with the DPPH assay demonstrating better scavenging activity, as presented in Table 2. Cytotoxic properties against hepatocellular carcinoma (Hep-G2) cell lines were evaluated using the MTT test. Results indicated that the MEME sample (IC 50 = 34.98 ± 0.25 µg/ml) showed cytotoxic activity when HepG2 cells were exposed to varying doses, as illustrated in Table 3. The IC50, defined as the concentration at which the number of viable cells is reduced by half, is depicted in Figure 1. Furthermore, the Mean Fluorescence Intensity (MFI%) of Alamar blue for MEME was observed to increase as the treatment dose decreased. The highest MFI was recorded at 105.20% with a 3.61 µg/ml dose of MEME, while the lowest MFI% (99.24%) was noted at a higher treatment dose of 14.60 µg/ml, as shown in Table 3 and Figure 2, respectively. The MEME extracts demonstrated antiproliferative qualities. In conclusion, the investigated extracts of M. elengi L. exhibited both antiproliferative and antioxidant effects, attributed to the combined action of their phytoconstituents.

Conclusion

Research findings demonstrated that extracts from *M. elengi* L. leaves exhibited cytotoxicity towards Hep-G2 hepatocellular cancer cell lines, inducing apoptosis. The antiproliferative and antioxidant effects observed were likely attributed to the phytoconstituents

present in the examined extracts, particularly flavonoids, alkaloids, glycosides, tannins, terpenoids, saponins, fixed oils, and phenolic compounds. These results lend credence to the traditional belief that *M. elengi* (L.), a perennial herb belonging to the Sapotaceae family, possesses cancer-preventive properties.

Acknowledgements

The authors would like to thank Aakar Biotech Limited in Lucknow for their support in conducting antiproliferative studies. We also extend our gratitude to BARTI Fellowship, Pune, MH, India, and the Department of Pharmaceutical Sciences, RTMNU, Nagpur, MH, India.

Conflict of Interest

The authors declare that there is no conflict of interest.

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https://doi.org/10.47552/ijam.v16iS2.6194 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Mahua Oil as a Promising Antipsoriatic Agent: Insights from In Vitro Studies

Research Article

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Abstract

Psoriasis, a chronic autoimmune skin condition, aligns with the Ayurvedic classification of *Kitibha*, which results from vitiated Vata and Kapha doshas and the accumulation of *Ama*. This study investigates the therapeutic potential of *Madhuca longifolia* (Mahua) oil in the management of psoriasis. Organoleptic Evaluation, Physicochemical analysis (Acid Value, Saponification Value, Iodine Value) and Gas Chromatography-Mass Spectrometry (GC-MS) were performed To check authenticity and purity of the oil. In vitro evaluations on HaCaT keratinocyte cell lines were conducted to assess antiproliferative and anti-inflammatory activities. Results revealed that Mahua oil significantly reduced pro-inflammatory cytokines (IL-1β, TNF-α) and nuclear transcription factor NF-κB ($p < 0.05$). Furthermore, Mahua oil demonstrated inhibitory effects on COX-2 pathway, confirming its efficacy in modulating inflammatory responses. These findings support its traditional use and propose Mahua oil as a viable natural treatment for psoriasis.

Keywords: Psoriasis, Mahua oil, GCMS, HaCaT cells, IL-1β, TNF-α, NF-κB, COX-II.

Introduction

Psoriasis is a chronic, immune-mediated dermatological condition characterized by hyperproliferation and abnormal differentiation of keratinocytes, accompanied by inflammation and immune dysregulation. It affects approximately 2–3% of the global population and significantly impairs quality of life, often leading to psychological distress, including anxiety and depression [1]. Conventional treatments primarily involve immunosuppressants, corticosteroids, and biologic agents; however, these are often associated with adverse effects, long-term dependency, and economic burden, especially in resource-limited settings [2].

In Ayurvedic literature, psoriasis is closely associated with *Kitibha*, a type of *Kshudra Kushtha*, which arises from the vitiation of *Vata* and *Kapha* doshas, along with the accumulation of *Ama* (toxins) and impairment of *Rakta dhatu* (blood tissue) [3]. Vata contributes to dryness and scaling, Kapha to thickening and plaque formation, while Pitta may exacerbate inflammation. The traditional Ayurvedic approach to treating *Kitibha* includes dosha pacification, detoxification (*shodhana*), and topical or systemic application of herbal oils known for their emollient, anti-inflammatory, and detoxifying properties.

Madhuca longifolia (Mahua) commonly known as the "Tree of Life" among tribal populations of India due to its diversified uses as nutrient, medicine and as fodder, Mahua oil has been used traditionally for managing a wide range of conditions including skin disorders, rheumatism, and inflammation [4]. Mahua oil contains an abundance of fatty acids such as linoleic, palmitic, and stearic acids, along with bioactive phytosterols that contribute to its skin conditioning, moisturizing, and anti-inflammatory effects [5]. Despite its extensive traditional usage, scientific validation of Mahua oil's efficacy in treating psoriasis is limited.

Recent advances in dermatological research emphasize the role of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and nuclear factor kappa B (NF-κB) in the pathogenesis of psoriasis [6–8]. These molecular mediators contribute to the hyperproliferation of keratinocytes and perpetuate chronic inflammation. The HaCaT keratinocyte cell line has emerged as a reliable in vitro model for investigating antipsoriatic activity due to its preserved differentiation characteristics and responsiveness to inflammatory stimuli [9].

In this study, we evaluate the physicochemical and phytochemical characteristics of cold-pressed Mahua seed oil and investigate its antiproliferative and anti-inflammatory effects on HaCaT cells. Through MTT cytotoxicity assay, cytokine quantification via ELISA, and cyclooxygenase (COX) enzyme inhibition assay, we aim to scientifically substantiate the traditional claims regarding the antipsoriatic potential of Mahua oil.[10-12]

Materials and Methods

Materials

Culture Media and Reagents: Dulbecco's Modified Eagle Medium (DMEM, AT149-1L, HiMedia), Fetal Bovine Serum (FBS, RM10432, HiMedia), Penicillin-

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Streptomycin (Sigma-Aldrich, P0781), Dimethyl Sulfoxide (DMSO, Cat. No. 67685, SRL), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich), Bovine Hemin Chloride (SRL-78372), Phenol (Fisher Scientific-35953), Arachidonic acid (SRL-20975), TMPD solution (17 mM, HiMedia, GRM445), LOX Enzyme (Sisco Research Laboratories Pvt. Ltd, Cat. No. 9029-60-1).

ELISA Kits: Human IL-1β ELISA Kit (GENLISA™, Cat. No. KB1063), Human TNF-α ELISA Kit (GENLISA™, Cat. No. KB1145), Human NF-κB ELISA Kit (GENLISA™, Cat. No. KBH14016).

Others: Tris-Cl buffer (pH 8.0, 100 mM), iron sulphate (Merck), ammonium thiocyanate (SRL), Celecoxib (positive control, TCI-C2816), sterile double-distilled water.

Procurement and Preparation of Mahua Oil

Cold-pressed seed oil of *Madhuca longifolia* was obtained from Cit Spray Aroma Science, Nagpur, Maharashtra, India.

Organoleptic Evaluation, Physicochemical analysis (Acid Value, Saponification Value, Iodine Value) and Gas Chromatography-Mass Spectrometry (GC-MS) were performed To check authenticity and purity of the oil.

Organoleptic Evaluation

The oil was examined for its characteristic color, odor, and consistency.

Physicochemical Characterization of Oil

The acid value, saponification value, and iodine value of Mahua oil were determined as per the methods outlined by the Food Safety and Standards Authority of India (FSSAI). All analyses were performed in triplicate and the results were expressed as mean ± standard deviation. [13]

GC-MS Analysis of Fatty Acid Composition

Gas Chromatography–Mass Spectrometry (GC-MS) was conducted using a SHIMADZU GCMS-QP-2010 Plus instrument equipped with an Rtx-5MS capillary column (30.0 m × 0.32 mm × 0.50 µm). Helium was used as the carrier gas with a flow rate of 41.0 mL/min. The oven temperature was programmed from 45°C to 270°C in controlled ramps. The injection port temperature was 270°C and ion source temperature was 200°C. Mass spectra were interpreted using integrated software.

In Vitro Cytotoxicity (MTT Assay)

Cell Line Culture

HaCaT human keratinocyte cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10% FBS and 1% Penicillin-Streptomycin, maintained at 37°C in a humidified incubator with 5% CO_2.

MTT Assay

Cells (10,000 cells/well) were seeded in 96-well plates and incubated for 24 h. Subsequently, cells were treated with Mahua oil at concentrations ranging from 0.078 µl to 5 µl (diluted in incomplete medium). After 24 h, MTT solution (5 mg/mL) was added and incubated for 2 h. The formazan crystals were solubilized with 100 µl DMSO, and absorbance was measured at 540 nm using a microplate reader (iMark™, Bio-Rad, USA). Percentage viability was calculated as:

$$\% \text{ Viable cells} = (A_{test} / A_{Control}) * 100$$

(A_{test} = Absorbance of test sample)
($A_{Control}$ = Absorbance of Control)

Protein Expression Analysis via ELISA

Protein expressions of IL-1β, TNF-α, and NF-κB were quantified using specific sandwich ELISA kits as per manufacturer protocols. Briefly, treated and untreated HaCaT cells (control and IC_{50} dose of Mahua oil) were used for analysis. Samples were incubated with capture and detection antibodies, followed by streptavidin-HRP and TMB substrate. The reaction was stopped and absorbance was recorded at 450 nm using an ELISA plate reader.

COX Enzyme Inhibition Assay

Cyclooxygenase (COX) Inhibition

Reaction mixtures containing Tris-Cl buffer, enzyme reagents (Bovine Hemin, Phenol), and Mahua oil dilutions were prepared in 96-well plates. The reaction was initiated by adding arachidonic acid and TMPD, incubated for 10 minutes, and read at 595 nm. Celecoxib (25 µM) was used as the positive control.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA).

Results and Discussion

Organoleptic Evaluation and Physicochemical Properties of Mahua Oil

Table 1: Organoleptic Evaluation of Mahu oil.

Parameter	Observation
Color	pale to dark yellow liquid
Odor	Pleasant, sweet fragrance
Consistency	Viscous, smooth oily texture

Physicochemical Properties of Mahua Oil

The physicochemical parameters of Mahua oil were analyzed to confirm its purity and suitability for topical application. As shown in *Table 2*, the observed values were within acceptable ranges: acid value (24.10), saponification value (188.3), and iodine value (68.01). These values correlate well with literature findings and indicate the oils unsaturation and emollient potential and purity of oil. [5].

https://doi.org/10.47552/ijam.v16iS2.6194 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Table 2. Physicochemical properties of Mahua oil.

Parameters	Reported	Observed
Acid value (mg of KOH/g)	20-25	24.10
Saponification value (mg of	187-197	188.3
Iodine value (I_2/100g)	55-70	68.01

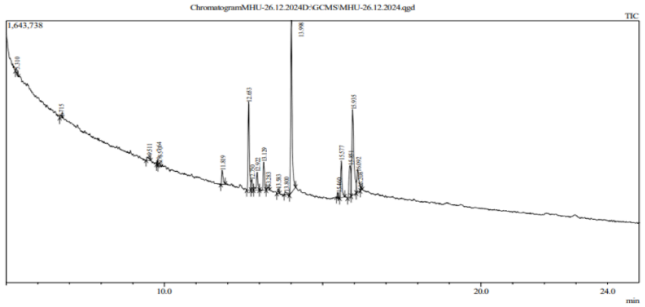
Fatty Acid Profile

GC-MS analysis identified major fatty acid components in Mahua oil. Notable constituents included palmitic acid (28.98%), methyl linolenate (21.35%), behenic acid (5.61%), and phytol (5.88%), each known for their skin conditioning and anti-inflammatory properties [4]. These constituents are crucial in barrier repair and inflammation control. (*Table 2*)

Table 3: Fatty acid composition of Mahua oil

Sr. no.	Chemical Name	Common Name	Area %	Role
1	Hexadecanoic acid	Palmitic Acid	28.98%	Skin conditioning Agent, emollient
2	Docosanoic acid	Behenic Acid	5.61%	Emollient, Moisturizing agent
3	2 heptadecanone	Stearic acid	1.83%	Moisturizing agent, Emollient
4	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	Phytol	5.88%	Antioxidant, Precursor for Vitamin E
5	9,12,15-Octadecatrienoic acid, methyl ester	Methyl Linolenate	21.35%	Anti-inflammatory property, antioxidant
6	11,14-Eicosadienoic acid, methyl ester	Methyl Eicosadienoate	6.69%	Reduces inflammation and supports skin barrier, Anti-inflammatory

Figure 1. GC-MS chromatogram of Mahua oil showing major constituents.



Antiproliferative Effects on HaCaT Cells

The skin is a continuously self-renewing organ that actively participates in the host defenses. Under homeostatic conditions, keratinocyte differentiate and mature from proliferating nucleated basal cells to the highly differentiated, nucleus-free corneocytes.

In Psoriais keratinocyte proliferation is induced by the cytokines that contributes to thickened skin, a scaly surface appearance, epidermal hyperplasia, hyperkeratosis, and parakeratosis. Altered homeostasis of proliferation and differentiation of cell act on the immune cells to continue the inflammatory response. An ideal agent for treating psoriasis should have the role in antiproliferation, anti-inflammation, and immunomodulation. [14,15]

Figure 2 A. Antiproliferative Effects of Mahua oil on HaCaT cells at various concentrations measured using MTT assay

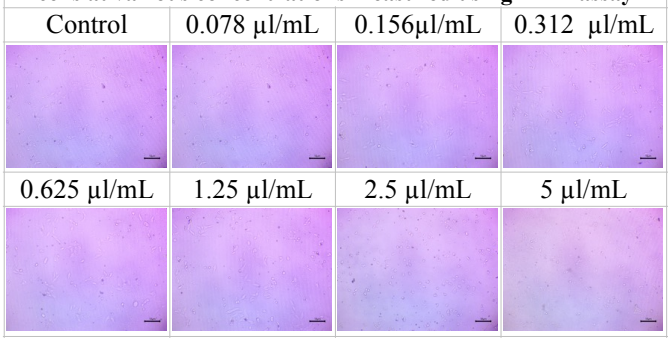
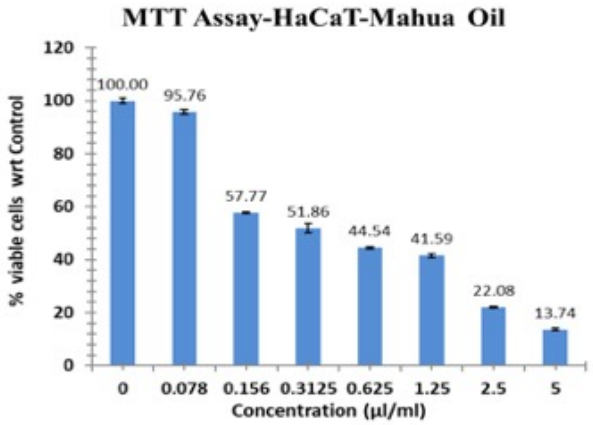


Figure 2 B: Antiproliferative Effects of Mahua oil on HaCaT cells at various concentrations measured using MTT assay



The cytotoxic effect of Mahua oil on HaCaT human keratinocyte cells was evaluated using the MTT assay. Cells were exposed to various concentrations of Mahua oil (0.078–5 µl/mL) for 24 hours, and cell viability was assessed by measuring absorbance at 540 nm. The results demonstrated a concentration-dependent reduction in cell viability (Figure 2A and 2B).

At the lowest concentration (0.078 µl/mL), cell viability was maintained at 95.76%, which was comparable to the control (100%). A significant decline in viability was observed at higher concentrations: 57.77% at 0.3125 µl/mL, 51.86% at 0.625 µl/mL, 44.54% at 1.25 µl/mL, and 22.08% at 2.5 µl/mL. The highest dose (5 µl/mL) resulted in only 13.74% cell viability. These findings indicate a dose-dependent cytotoxic effect of Mahua oil on keratinocytes.

On microscopic observations (Figure 2A) untreated control cells showed a typical characteristic appearance of epithelial cells. They are well-differentiated and growing in a healthy state and

confluency, whereas cells treated with higher concentrations of Mahua oil exhibited rounding, shrinkage, and detachment, suggestive of cytotoxicity.

All results are presented as mean ± SEM of triplicate experiments. Statistical analysis using one-way ANOVA revealed that reductions in cell viability at concentrations ≥0.3125 µl/mL were statistically significant ($p < 0.05$) when compared to the control group.

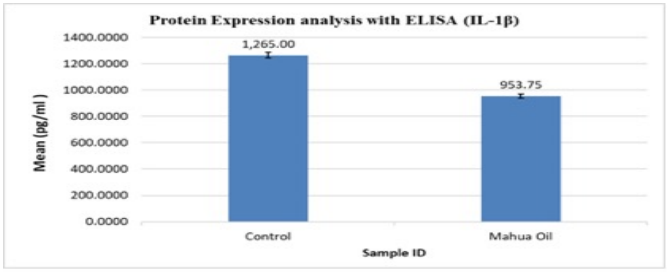
These findings suggest that Mahua oil effectively inhibits HaCaT cell proliferation, indicating its potential antipsoriatic activity via suppression of keratinocyte overgrowth.

The MTT assay demonstrated a dose-dependent reduction in keratinocyte viability. The IC_{50} value was calculated as 0.4816 ± 0.1002 µl/mL, indicating effective inhibition of cell proliferation (*Figure 2 A and B*). Morphological changes, such as cytoplasmic shrinkage and cell rounding, were observed at higher concentrations, aligning with keratinocyte cytotoxicity profiles of known antipsoriatic agents [16].

Inhibition of Inflammatory Cytokines

Mahua oil significantly downregulated the expression of pro-inflammatory cytokines:

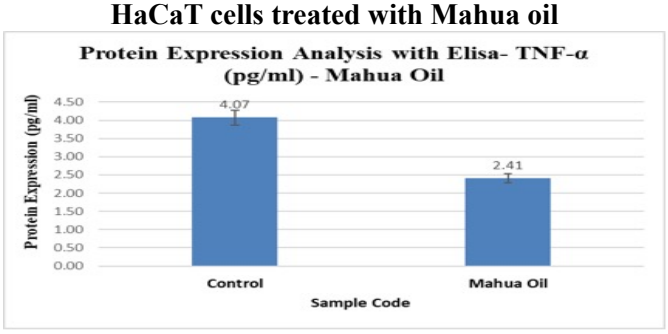
Figure 3: Downregulation of IL-1β expression in HaCaT cells treated with Mahua oil



IL-1β, a crucial pro-inflammatory cytokine, plays a central role in the pathogenesis of psoriasis by promoting the activation of keratinocytes, recruitment of inflammatory cells, and the amplification of the immune response within the skin. [17]

In this study, the mean IL-1β concentration in the untreated control group was 1265.00 ± 45.08 pg/ml, while treatment with Mahua oil significantly reduced it to 953.75 ± 29.17 pg/ml (**$p < 0.01$**). This statistically significant reduction underscores the efficacy of Mahua oil in downregulating IL-1β inflammatory cytokine expression.[6,18].

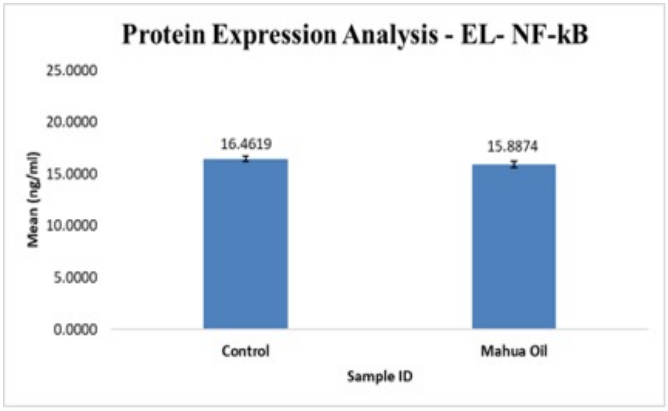
Figure 4: Downregulation of TNF expression in HaCaT cells treated with Mahua oil



TNF-α is a pivotal cytokine in the inflammatory cascade of psoriasis, playing a key role in activating keratinocytes, dendritic cells, and endothelial cells, and in promoting leukocyte infiltration into psoriatic plaques [19, 20].

The ELISA results showed that TNF-α levels dropped from 4.07 pg/ml in the control group to 2.41 pg/ml in the Mahua oil-treated group, indicating a statistically significant suppression **($p < 0.05$).** Given that TNF-α is a therapeutic target in conventional psoriasis treatments (e.g., biologics like etanercept and infliximab), Mahua oil could represent a cost-effective, natural alternative or adjunctive therapy with fewer side effects. [7]

Figures 5: Down regulation of NF-κB expression in HaCaT cells treated with Mahua oil



NF-κB plays a central role in regulating genes associated with inflammation, cell proliferation, and apoptosis. Its dysregulation has been implicated in chronic inflammatory diseases. [21]

As illustrated in Figure 5, NF-κB expression was reduced in the Mahua Oil-treated group (15.8874 ng/ml) compared to the control group (16.4619 ng/ml). Mahua Oil may exert a modest modulatory effect on NF-κB activity.

COX Inhibition

Figure 6: COX-2 enzyme inhibition by Celecoxib (positive control)

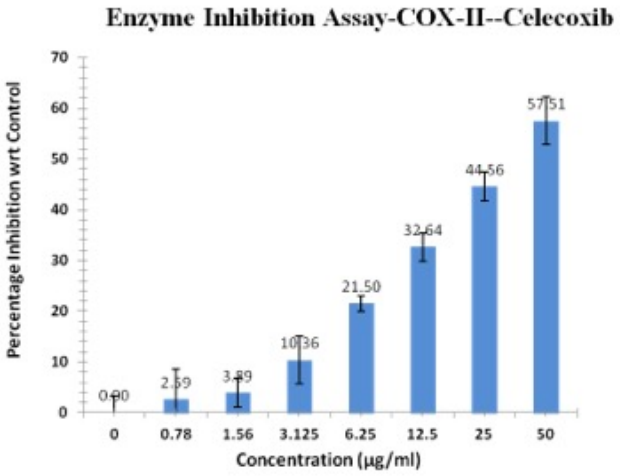
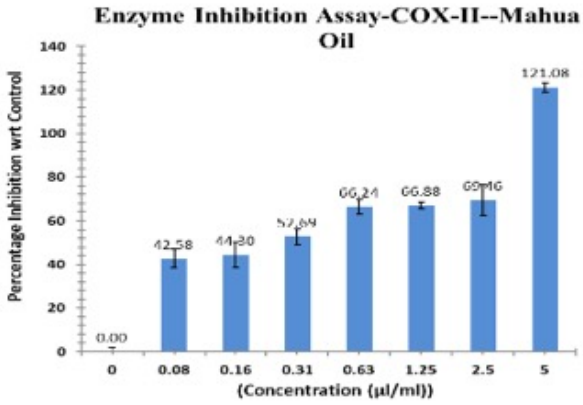


Figure 7: COX-2 enzyme inhibition by Mahua oil compared to Celecoxib (positive control)



The current study investigated the COX-II inhibitory potential of Mahua oil in comparison with the standard anti-inflammatory drug Celecoxib, using an in vitro enzyme inhibition assay. The results demonstrate a dose-dependent inhibition of COX-II by both agents, with Mahua oil exhibiting notably high inhibitory activity at higher concentrations.

Celecoxib, a selective COX-II inhibitor widely used in the management of inflammatory conditions, exhibited up to 57.51% inhibition at 50 µg/ml. In contrast, Mahua oil showed remarkable COX-II inhibition, reaching 121.08% inhibition at 5 µl/ml, suggesting potent activity potentially exceeding that of the standard drug under the conditions tested.

The observed effects of Mahua oil are particularly significant in the context of psoriasis, a chronic inflammatory skin disorder characterized by overexpression of COX-II, increased prostaglandin production, and aberrant keratinocyte proliferation. Inhibition of COX-II can attenuate the inflammatory cascade by reducing prostaglandin E2 (PGE2) levels, thus offering symptomatic relief and slowing disease progression.[22] The data were statistically analyzed using one-way ANOVA, confirming that the differences in percentage inhibition between treatment groups were statistically significant ($p < 0.05$).

Plant-derived oils are increasingly recognized in dermatology for their role in hydration, barrier restoration, and anti-inflammatory activity [23]. The results of our study align with traditional Ayurvedic principles where *Madhuca longifolia* is applied topically for skin disorders. The oil's emollient properties are enhanced by its unsaturated fatty acid content, and phytosterol likely contribute to its immunomodulatory activity.

In comparison to conventional therapies, Mahua oil offers a natural, cost-effective alternative with multifaceted actions—cytokine inhibition, enzyme blockade, and keratinocyte regulation—making it a compelling candidate for further clinical evaluation.

Conclusion

This study support the antipsoriatic potential of Mahua oil, an Ayurvedic remedy traditionally used for skin ailments. The oil exhibited significant anti-proliferative effects on HaCaT keratinocyte cell lines, with an IC_{50} of 0.4816 ± 0.1002 µl/mL, indicating strong efficacy in inhibiting abnormal skin cell growth —a hallmark of psoriasis.

Mahua oil also demonstrated down regulation of key inflammatory mediators implicated in psoriasis pathogenesis, including IL-1β, TNF-α, and NF-κB. In addition, the oil effectively suppressed COX-2 enzyme activities, further confirming its anti-inflammatory properties. The phytochemical constituents of Mahua oil, including unsaturated fatty acids and phytosterols, likely contribute to these effects through moisturization, skin barrier restoration, and immune modulation.

These findings support the traditional use of Mahua oil in Ayurvedic medicine for treating skin diseases and further advocate for its potential as a complementary therapeutic agent in managing psoriasis. However, further in vivo and clinical studies are warranted to validate its therapeutic utility and standardize dosage forms for dermatological applications.

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https://doi.org/10.47552/ijam.v16iS2.6195 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Extraction and Profiling of Fatty Acids obtained from different Marine Seaweeds/Macro Algae

Research Article

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Abstract

Seaweeds are acknowledged as potentially abundant sources of bioactive molecules with diverse applications in food, nutraceutical, and pharmaceutical industries. This study investigated the fatty acid (FA) composition of selected seaweed species collected from the Indian coastline, encompassing representatives from the Chlorophyta, Phaeophyta, and Rhodophyta phyla. Using gas chromatography, approximately 20 FAs were identified and quantified, including polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). Among SFAs, palmitic acid (16:0) was the most prevalent across all species, followed by oleic acid (18:1) among MUFAs and linoleic acid (18:2) and eicosapentaenoic acid (20:5) among PUFAs. The variation in FA profiles among species suggests species-specific metabolic pathways influenced by environmental conditions. Additionally, the availability of essential FAs and notable PUFA/SFA ratios indicates the nutritional value of these seaweeds. These findings not only enhance our understanding of the biochemical diversity in Indian seaweeds but also support their potential utilization in health-promoting and therapeutic formulations.

Keywords: Seaweeds, Extraction, Physiochemical analysis, Fatty acids profiling, Nutraceuticals.

Introduction

Global food security has become a major problem in recent years because of factors including climate change, population growth and diversifying terrestrial food sources to meet energy demands (1). Furthermore, the geographical divisions between states and the boundaries between human races are becoming less distinct as a result of the globalisation of markets and the resulting increased globalisation of foods. Additionally, there has been an effort to improve and augment the nutritional value of human diets by investigating and using foods from unconventional sources, both terrestrial and marine. Subsequently, this relieves the increasing strain on traditional meals. Seaweeds, sometimes referred to as marine macroalgae, are one of the oceans' living, renewable resources that can be utilized for its potential food applications (2).

The pharmaceutical sector is very interested in the structurally unique and physiologically active metabolites found in marine species (3). As significant biological resources, seaweeds are an essential component of marine ecosystems. As primary producers, they contribute significantly to the diversity and productivity of marine populations. Moreover, they provide food and refuge to a variety of marine species at different stages of life (4). In an investigation conducted by Sohrabipour et al. (5), importance of seaweeds from 3 different species - red(Rhodophyta), green(Chlorophyta) and brown(Phaeophyta) - was assessed depending on their FA composition and medical applications in the treatment of specific human ailments. The study investigated the possibility that certain algae species may be useful resources for therapeutic uses.

The FA content of seaweeds exhibits certain traits. Their fatty acids usually consist of one or more double bonds and linear chains of carbon atoms (even number) (6). Essential FAs, particularly PUFAs like ω-3 and ω-6, are prevalent in seaweeds and are crucial for both human and animal nutrition. The significant quantities of ω-3 and ω-6 FA in seaweeds maintains the ω-6/ω-3 ratio in accordance with WHO dietary guidelines (5).

Seaweeds have a low lipid content but a high proportion of PUFAs with a distinctive FA pattern, however they are underutilized irrespective of their abundance. A few bioactive metabolites that may be beneficial to human health are also present in them, including amino acids, carotenoids, chlorophylls, phenolics, polysaccharides and sterols. Algal blooms and invading seaweeds are examples of low-cost biomass that shows great promise (7) (8). Seaweeds are preferred because of their high PUFA content, quick growing rate, and simpler structure. However, studies are currently in progress to use seaweeds for FA, most likely because of this resource's abundance (9). Hence

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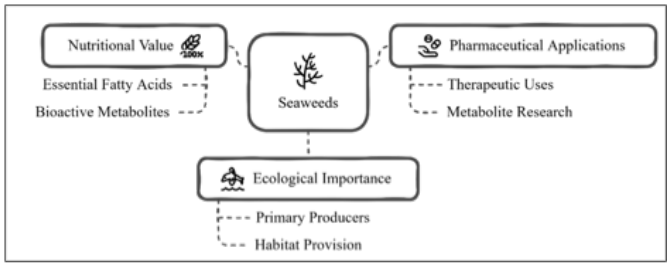
this study's goal is to determine the qualitative and quantitative content of total FA as well as their profiles in a few seaweeds that were gathered from the Indian coastlines.

Figure 1: Nutritional and pharmaceutical potentials of seaweeds



Materials and methods

Seaweeds collection and preparation

The three different species of seaweeds viz, *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera* were procured and authenticated from Annakkili Amma Research Institute (AARI), Medavakkam, Chennai. To get rid of epiphytes, dirt, and other foreign objects, the freshly harvested seaweeds were first cleaned with seawater. The seaweeds were thoroughly cleaned with filtered water to remove any remaining salt and sand particles. In a shady location, the samples were allowed to air dry at ambient temperature (25–30°C) for 3-5 days. After that, they were dried at 38±2°C until their weight remained constant. After the samples had dried fully, they were broken up into tiny pieces and crushed into a coarse powder. Impurity removal for further extraction and analysis was guaranteed by this preparation technique.

Extraction of total lipids

The modified Folch technique (10) was used to extract the lipid, but with minor changes. The sample of dried seaweed that had been roughly powdered was extracted using a solvent combination of chloroform and methanol (2:1, v/v). The combination was maintained at room temperature on a rotatory shaker set to 120 rpm for 48 hours. Whatman filter paper (No. 1) was used to filter the homogenate in order to extract the liquid phase. To create phase separation, the same amount of water and chloroform (1:1 v/v) combination was added. After being collected in a glass vial that had been previously weighted, the organic phase of the lipid extract was dried at 40–45°C until its weight remained constant. By this technique, the lipid extract was prepared for further examination.

Phytochemical evaluation

- Alkaloids: Small quantity of sample was combined with Mayer's reagent. Presence of alkaloids were indicated by a cream-colored precipitate.
- Steroid: Conc. sulfuric acid and acetic anhydride (2ml each) were mixed with the sample. Change in colour to bluish green from violet confirms the steroids test.
- Terpenoids: Conc. sulfuric acid and chloroform (1ml each) are incorporated into the sample. Terpenoids are indicated by a reddish-brown interface.
- Flavonoids: Magnesium was introduced into the solution of sample and then overlaid with strong hydrochloric acid; flavonoids are indicated by intense red coloration.
- Phenolic Compounds: Addition of ferric chloride reagent to the sample was done; blue-green or violet color indicates the phenolic compounds.
- Tannins: The lead acetate solution was combined with the sample. Presence of tannins was confirmed by an intense white solution.
- Cardiac Glycosides: Ferric chloride solution, glacial acetic acid, and conc. sulfuric acid was carefully added to the sample. Deoxy-sugar was indicated by a brown ring.

Physicochemical tests

Determination of density

A 25 ml empty density bottle's weight was determined with an electronic weighing scale. Then it was determined how much the bottle and sample weighed when it was completely filled to the brim with sample. The following formula was used to get the density.

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Saponification value:

A measured portion (2g) of sample was taken in 250 mL round-bottom flask, to which 0.5 M ethanolic KOH (25ml) was added. After that, a reflux condenser was utilized, and the mixture was subjected to heat in a water bath for half an hour while being constantly swirled until it began to gently boil. While still hot, the remaining unreacted KOH was back titrated with 0.5 M HCl after adding phenolphthalein which acts as indicator. Under the same circumstances, a blank titration was also carried out using distilled water. The saponification value was subsequently calculated using the obtained titration data.

$$\text{Saponification value} = \frac{28.05 * (\text{Blank titration reading} - \text{Sample titration reading})}{\text{Weight of oil sample taken}}$$

Acid value

A combination of equal parts ethanol and ether was taken in a 250 ml round-bottom flask along with the sample (10g). With regular shaking, the flask's content were gradually warmed by a reflux condenser until the oil sample was fully dissolved. After that, 0.1 N KOH was used to titrate the content, with phenolphthalein serving as indicator to obtain the faint pink colour after shaking for 30 sec.

$$\text{Acid value} = \frac{5.61 * \text{Vol. of potassium hydroxide required}}{\text{Weight of oil sample taken}}$$

Iodine Value

The sample (1g) was placed in a 250ml iodine flask and stored in the drawer for exactly 30 minutes. To wash away any iodine that could have been on the

https://doi.org/10.47552/ijam.v16iS2.6195 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

stopper, a 15% W/V potassium iodine solution (10ml) were introduced to the flask. This was titrated until the sodium became bright yellow against 0.14 M Na_2SO_3. Following this the starch indicator was added and the titration continued until the blue color simply disappeared. For a blank determination, the same conditions were applied using distilled water. The titre reading was noted and utilised to determine the iodine value as shown below.

$$\text{Iodine value} = \frac{1.269 * (\text{Blank titration reading} - \text{Sample titration reading})}{\text{Weight of oil sample taken}}$$

Hexabromide Test

Measure the sample (1g) into a wide-mouthed boiling tube with a 50 ml capacity. Add chloroform (5ml) gradually, then continue to add bromine (1ml), until a deep red coloration develops. Cool the tube by submerging it in cold water. While gently shaking the mixture, slowly introduce rectified spirit dropwise (1.5ml) until any initial precipitate dissolves completely. Then, add diethyl ether (10ml) and mix thoroughly. Put the tube back in the cold water and let it there for 20 mins. At this point, the development of precipitates suggests that PUFAs are present.

Fatty Acid Methyl Ester (FAME) analysis

Preparation of FAME

The transmethylation of lipids obtained from the seaweeds (*Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera*) and extraction of FAME was done (11). 200µL of sample was mixed with 1 mL Hexane, shaken for 10 seconds. 200µL of 2N Methanolic NaOH was added to the mix and vortexed. 200µL 2N Methanolic HCl was added and vortexed. The top layer was collected, passed through a nylon 13 mm 0.2µm syringe filter, and then injected into the GCMS.

GC-MS analysis of FAME

FAMEs were analyzed using Agilent 7890A GC paired with a 5975C MS instrument, equipped with DB-WAX capillary column (30 m length; 0.25 mm internal diameter; 0.25 µm film thickness). 2 µL sample was inserted using split ratio of 300:1. High-purity helium (99.9995%) was incorporated as carrier gas, flowing at a steady rate of 0.6 mL/min. The instrument operated in electron impact (EI) mode with an ionization energy of 70 eV. The injector temperature was kept constant at 250°C. The temperature schedule for the column oven as follows:

Oven	Rate (°C/min)	Value (°C/min)	Hold Time (min)
Beginning		80	5
Level 1	5	150	5
Level 2	2	175	1
Level 3	10	250	3

The chemical compounds were identified by matching the spectrum configurations with those available on mass spectral database (NIST -08 spectrum DATA).

Results

Phytochemical evaluation

Table 1: Phytochemical evaluation parameters

Test	*Chaetomorpha linum*	*Padina pavonica*	*Gracillaria foliifera*
Alkaloids	×	✓	✓
Steroids	×	✓	✓
Terpenoids	✓	×	✓
Flavonoids	✓	×	×
Phenolic compounds	✓	✓	×
Tannins	×	✓	✓
Cardiac Glycosides	×	×	×

Physicochemical tests

Table 2: Physicochemical test parameters

Test	*Chaetomorpha linum* (Chlorophyta)	*Padina pavonica* (Phaeophyta)	*Gracillaria foliifera* (Rhodophyta)
Density (g/ml)	0.930	0.864	0.892
Saponification Value (mg KOH/g)	185.2	286.2	221.6
Acid Value (mg KOH/g)	2.13	4.66	3.42
Iodine Value (mg I/g)	107.6	86.8	81.9
Hexabromide Test	+	+	+

Fatty acid analysis

GC Spectra and results

Figure 2: GC profile of *Chaetomorpha linum*

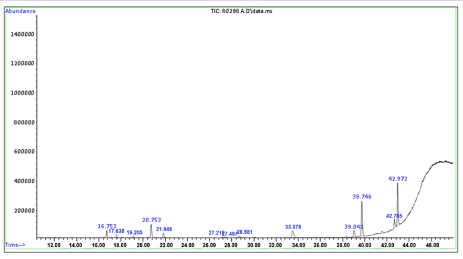


Figure 3: GC profile of *Padina pavonica*

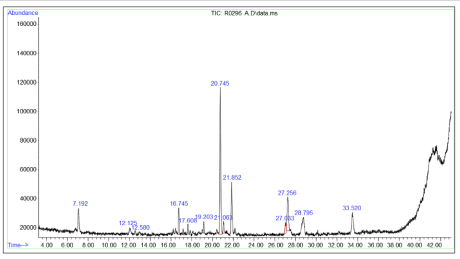


Figure 4: GC profile of *Gracilaria foliifera*

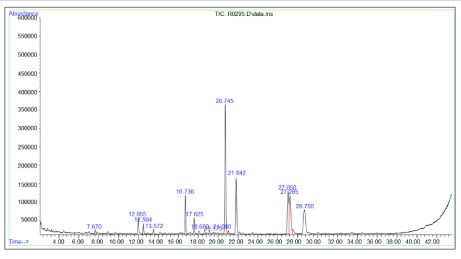


Table 3: Fatty acid profile of *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera*

FAME	C:D	*Chaetomorpha linum*	*Padina pavonica*	*Gracilaria foliifera*
Capric acid	10:0	-	6.71%	1.35%
Undecylic acid	11:0	-	2.24%	4.05%
Lauric acid	12:0	-	1.58%	2.70%
Tridecylic acid	13:0	-	-	1.35%
Myristic acid	14:0	4.82%	6.84%	9.46%
Myristoleic acid	14:1	2.07%	3.16%	4.05%
Pentadecylic acid	15:0	-	-	1.22%
Pentadecyloleic acid	15:1	1.38%	3.68%	1.08%
Palmitic acid	16:0	8.28%	34.47%	31.08%
Palmitoleic acid	16:1	-	3.68%	0.95%
Margaric acid	17:0	3.45%	12.63%	13.78%
Stearic acid	18:0	1.38%	3.55%	10.27%
Oleic acid	18:1	0.69%	9.21%	9.46%
Linoleic acid	18:2	1.04%	4.60%	5.95%
Arachidic acid	20:0	4.14%	5.79%	-
Hexadecatetraenoic acid	16:4	4.48%	-	-
Ecosapentaenoic acid	20:5	22.07%	-	-
Docosapentaenoic acid	22:5	11.38%	-	-
Docosahexaenoic acid	22:6	32.76%	-	-

Discussion

The three different species of seaweeds viz., *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera* were procured and authenticated. Total lipid was extracted from these seaweeds by modified Folch method employing a chloroform/methanol (2:1 v/v) solvent mixture as the extraction solvent. The portion of the extract that was subjected to phytochemical evaluation, according to the research findings, included terpenoids, flavonoids and phenolic compounds in *Chaetomorpha linum*; alkaloids, steroids, saponins, phenolic compounds and tannins in *Padina pavonica*; alkaloids, steroids, terpenoids and tannins in *Gracilaria foliifera*.

During the physicochemical evaluation, the physical attribute i.e. density was obtained in the range of 0.85-0.95 g/ml. The Lorentz–Lorenz relation may provide a good description of the RI-density relationship, according to a combination of theoretical and empirical investigation. Since more electric dipoles are produced by the applied electric field, a denser material usually has a higher refraction index (12).

The extracts' saponification values (SV) ranged from 180 to 290 mg KOH/g, indicating that the triglycerides present in the extract had a low molecular weight of FA (both saturated and unsaturated). The outcome showed a favorable comparison with soybean oil [13]. Thus the length of the FA chains generated from triacylglycerols determines the SV. Triacylglycerols with shorter FA chains are indicated by a high SV; conversely, a lower SV shows long chain FA on the glycerol backbone (13).

The acid value of seaweeds *Chaetomorpha linum*, *Padina pavonica* and *Gracilaria foliifera* are 2.13 mg KOH/g, 4.66 mg KOH/g and 3.42 mg KOH/g respectively. Given that solvent extraction reduced the acid content, this low acid value highlights the advantages of solvent extraction versus mechanical extraction (14). This acid value indicates how well lipase action can break down the constituent glycerides.

The iodine value of the extracts was 80-110 mg I/g which is similar to those of sesame seed oil [115 mg I/g] and sunflower oil [124 mg I/g] (15). Usually, the high unsaturation is depicted by the higher iodine value and the more likely it is to experience oxidative rancidity (16).

A hexabromide test is used to measure the amount of unsaturation present in the sample. The presence of unsaturated FA is indicated by the production of a hexabromide precipitate when FA, such as extract containing linolenic acid, are treated with bromine in chloroform and subsequently with alcohol and ether (17).

Overall, there were notable differences in quantities of various kinds of FA across all species that were studied. FA composition of algal lipids changes greatly depending on the species, salinity, pollution, light, habitat and environmental factors (18). The FA composition was determined by capillary GC method of three different seaweeds and it indicates the presence of around 20 compounds. The FA content in the examined seaweeds was roughly the same but *Chaetomorpha linum* contains significant quantity of unsaturated FAs, subsequently *Padina pavonica*, and finally *Gracilaria foliifera*.

The current findings indicate distinct FA profiles across the studied seaweed species, characterized by notable amounts of SFAs, including stearic acid (18:0), margaric acid (17:0), palmitic acid (16:0) and myristic acid (14:0); as well as MUFAs such as oleic acid (18:1), pentadecyloleic acid (15:1) and myristoleic acid (14:1). These FAs together made up over half of the total FA composition in the examined samples. Among them, palmitic and oleic acids were particularly abundant in red and brown seaweeds. This trend is consistent with

earlier reports that identified palmitic acid as the dominant FA in various seaweed species (19).

Seaweed samples contain notable quantities of oleic acid, palmitoleic acid and palmitic acid, which could be valuable for use in nutritional products or as dietary supplements. These fatty acids have demonstrated significant antimicrobial effects against key oral pathogens, including *Fusobacterium nucleatum*, *Streptococcus mutans*, *Candida albicans* and *Porphyromonas gingivalis*. In addition, MUFAs derived from C16 and C18 chains are believed to play a protective role in various health conditions, especially those that have an effect on the cardiovascular system (20) (21).

In the case of Chaetomorpha linum, analysis of its FA profile revealed a predominance of PUFAs. The largest concentration among these was docosahexaenoic acid (DHA; 22:6), which was followed by docosapentaenoic acid (DPA; 22:5), eicosapentaenoic acid (EPA; 20:5), and hexadecatetraenoic acid (HDA; 16:4). Research by Moustafa and Batran (22) highlights the importance of PUFAs in the diets of humans and other vertebrates, as these organisms are unable to synthesize them on their own. Additionally, Erkkilä et al. (23) noted that an increased ratio of PUFAs to SFAs in the diet is linked with a reduced risk of cardiac diseases, supporting the recommendation to substitute SFAs with PUFAs for better heart health.

Conclusion

This study offers a thorough examination of the FA profiles of several seaweed species that were collected along the Indian coastline, highlighting their potential nutritional and pharmaceutical value. The predominance of saturated FA, particularly palmitic acid, along with significant levels of MUFAs and PUFAs such as linoleic acid, oleic acid and eicosapentaenoic acid, underscores the rich lipid diversity of these marine macroalgae. These bioactive compounds are not only essential for human health but also exhibit promising antimicrobial properties, suggesting their possible application as natural food supplements and therapeutic agents. Furthermore, the variation in fatty acid composition among different species and phyla emphasizes the need for species-specific investigations to fully exploit their functional properties. In broad terms, this research adds to the expanding corpus of information demonstrating the valorization of seaweeds as sustainable and valuable resources for the food, nutraceutical, and pharmaceutical industries.

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https://doi.org/10.47552/ijam.v16iS2.6203 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

In-Silico Analysis Bioactive Compounds from *Carica papaya* and *Triticum aestivum* for Androgen Receptor Modulation in Polycystic Ovary Syndrome (PCOS)

Research Article

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Abstract

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder that affects a large population of women in their reproductive years. A key feature of PCOS is hyperandrogenism, which contributes significantly to the clinical manifestations of the syndrome, including irregular menstrual cycles, infertility, and metabolic disturbances. The androgen receptor (AR), a nuclear transcription factor that mediates the biological effects of androgens such as testosterone and dihydrotestosterone (DHT), has become a critical molecular target in efforts to develop effective therapeutic strategies for PCOS. With the growing interest in computational drug discovery, in-silico techniques such as molecular docking and virtual screening have gained prominence for identifying promising compounds that interact favourably with target receptors. These approaches provide valuable insights into the structural compatibility and binding affinity of ligand-receptor complexes. The present study focuses on exploring natural therapeutic alternatives by investigating the binding potential of phytoconstituents derived from two traditionally significant medicinal plants—Carica papaya and Triticum aestivum. *C. papaya* is rich in bioactive molecules like flavonoids, alkaloids, and papain, known for their anti-inflammatory and hormonal balancing properties. *T. aestivum*, or wheatgrass, contains chlorophyll, phenolic compounds, and micronutrients reputed for detoxification and endocrine modulation. Molecular docking simulations were performed using UCSF Chimera for ligand preparation, AutoDock Vina for docking, and Discovery Studio for interaction analysis. The findings revealed that several compounds from these herbs showed strong binding affinities to the androgen receptor, suggesting their potential as natural therapeutic agents for the management of PCOS.

Keywords: Polycystic Ovary Syndrome, Androgen receptor, *Carica papaya, Triticum aestivum*, Dihydrotestosterone

Introduction

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder that significantly impacts women's reproductive, metabolic, and psychological health. It is characterized by hormonal imbalances, particularly elevated levels of androgens (male hormones), which disrupt normal ovarian function, leading to anovulation, irregular menstrual cycles, and the formation of ovarian cysts.(1) Central to this condition is the over activation of the androgen receptor (AR), a ligand-dependent nuclear transcription factor that mediates the physiological actions of testosterone and DHT in target tissues.(2) The androgen receptor is considered a pivotal node in the hormonal network that influences PCOS, making it a compelling target for drug development.

Modern drug discovery has been revolutionized by the advent of computational techniques, especially molecular docking and virtual screening. These in silico approaches allow researchers to predict the binding mode and affinity of ligands within the active site of a target protein, bypassing the need for expensive and time-consuming wet-lab experimentation.(3) Molecular docking tools like AutoDock Vina, UCSF Chimera, and Discovery Studio provide robust platforms for visualizing ligand-receptor interactions, optimizing ligand geometry, and evaluating binding affinities. These tools have become indispensable in preclinical drug development and are now being increasingly applied to herbal and natural product research.(4)

The present study investigates the potential of natural phytochemicals derived from Carica papaya(5) and Triticum aestivum(6) to modulate the androgen receptor through computational docking methods. By comparing their performance with standard drugs such as clomiphene citrate(7) and DHT(8), we aim to identify novel therapeutic candidates that could serve as effective anti-androgens in the management of PCOS. These phytochemicals were selected based on their reported antioxidant, anti-inflammatory, and hormone-modulating activities in literature. A summary of traditional and experimental uses, along with PubChem CIDs, is provided in Table X to enhance reproducibility.

Plant Profile

Carica papaya

Carica papaya, more commonly known as papaya, is a tropical fruit-bearing plant that belongs to

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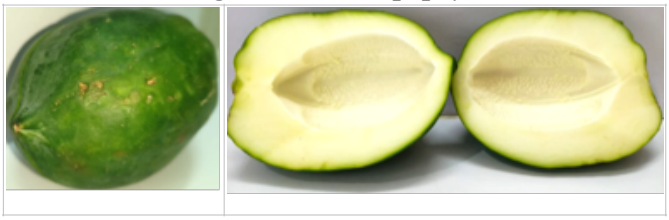
the Caricaceae family. Native to Central America and southern Mexico, it is now cultivated widely in tropical and subtropical climates around the world due to its nutritional and medicinal value.(9) This fast-growing plant resembles a tree but is actually a large herbaceous species, typically growing up to 10 meters tall. It features a straight, hollow stem topped with a canopy of large, lobed leaves and produces soft, sweet fruits that range in color from yellow to orange when ripe.(10) Inside the fruit are numerous black seeds, which are also known to have medicinal properties. Papaya is rich in several beneficial compounds, including the enzyme papain, which helps in protein digestion, and a range of phytochemicals like flavonoids, alkaloids, tannins, and carpaine. Traditionally, different parts of the plant—leaves, seeds, fruit, and latex—have been used in folk medicine for treating a variety of ailments such as digestive issues, skin disorders, parasitic infections, and inflammation.(11) Modern studies have confirmed many of these uses, highlighting papaya's anti-inflammatory, antioxidant, antimicrobial, and even hepatoprotective effects.(12) Its diverse therapeutic properties and ease of cultivation make *Carica papaya* an important plant in both traditional healing systems and modern herbal medicine.(13)

Figure 1: *Carica papaya*



Triticum aestivum

Triticum aestivum, commonly known as wheat, is one of the most important cereal grains in the world and belongs to the Poaceae family.(14) Believed to have originated in the Fertile Crescent of the Middle East, it has been a staple crop in human diets for thousands of years and continues to be a primary food source for billions.(15) Wheat is an annual grass that grows upright with slender green leaves and golden spikes containing grains that are harvested for flour.(16) The plant is valued not only for its high carbohydrate content but also for providing protein—especially gluten—along with fiber, B-vitamins, and a host of antioxidants and phytochemicals.(17) While the grain is essential for food production, the young green shoots, commonly known as wheatgrass, have also gained attention for their health benefits. Wheatgrass juice is widely consumed for its detoxifying properties, rich chlorophyll content, and its ability to boost energy and immunity.(18) Scientifically, *Triticum aestivum* has been found to exhibit several beneficial properties including antioxidant, anti-inflammatory, antidiabetic, and lipid-lowering effects. Whether as a dietary staple or as a functional food in alternative medicine, wheat holds a unique place in both agriculture and health traditions across the globe.(6)

Figure 2: *Triticum aestivum*

Table 1: Features of Carica papaya L. Triticum aestivum L

Feature	Carica papaya	Triticum aestivum
Scientific Name	*Carica papaya* L.	*Triticum aestivum* L.
Family	Caricaceae	Poaceae (Gramineae)
Common Names	Papaya, Pawpaw, Papita (Hindi)	Wheat, Gehun (Hindi)
Major Phytoconstituents	Papain, chymopapain, carpaine, alkaloids, flavonoids, tannins, saponins, phenolic compounds.	Starch, gluten, dietary fiber, B-complex vitamins, phenolic acids, flavonoids, lignans.
Pharmacological Properties	Anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, antihelminthic, wound healing.	Antioxidant, anti-inflammatory, antidiabetic, hypolipidemic, antibacterial.
Traditional Uses	Used in digestive issues, skin diseases, wound healing, and as a vermifuge; latex used for papain.	Staple food crop; wheatgrass juice used for detoxification, digestion, and boosting immunity.
Economic/Medicinal Significance	Valuable in herbal medicine and food industry; therapeutic and nutritional applications.	Essential in global nutrition; also recognized for medicinal benefits of wheatgrass.

Materials and Methods

Extract characterization

The *Carica papaya* was purchased from the market and authenticated by botanist from RTMNU, Nagpur. The method of extracting the *C.Papaya juice* was followed by placing the fresh papaya pulp in grinder also wheatgrass juice was obtained by placing the fresh crude drug into a grinder immediately after cutting from the field and crushing it well. Then it was wrapped in muslin cloth and juice was strained out of it. Then the obtained juice was oven dried to obtain an extract in powder form at 55± 5 degree celcius.(16)(19)

Table 2: Identification test

Sr. No.	Test	Procedure
1	Alkaloid Test (Mayer's Test)	To the extracts, 1% hydrochloric acid and six drops of Mazyer's reagent were added. The appearance of an organic precipitate indicates the presence of alkaloids in the sample. Detection of Flavonoids The extracts were treated with conc.H_2SO_4 and observed for a yellowish orange color for the presence.
2	Terpenoid Test (Salkowiski Test)	Five ml of the extracts were mixed with 2 ml of chloroform and 3 ml of conc.H2SO4 solution. A reddish brown color at the interphase indicates the presence of terpenoids.
3	Phenols (Ferric Chloride Test)	Two ml of diluted extracts were treated with dil.FeCl3 solution. The appearance of a violet color indicates the presence of phenol-like compounds.
4	Sugar Test	One ml of Benedict's solution is added to the extract. Sample is incubated in water for 2-4 mins. Red, orange, blue or green color represents presence of sugar.
5	Saponins (Foam Test)	Two ml of the extracts were diluted with 20 ml of distilled water, shaken vigorously and was observed for a stable persistent froth.
6	Flavonoids	The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow color was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.
7	Proteins (Biuret Test)	One ml of 40% NaCl and two drops of 1% CuSO4 were added to the leaf extracts. Appearance of a violet color confirms the presence of proteins.

Validation of Docking Protocol:

To ensure the reliability of the docking protocol, re-docking of the co-crystallized ligand into the androgen receptor (PDB ID: 2PIU) was performed. The root mean square deviation (RMSD) between the original and re-docked pose was found to be <2.0 Å, indicating accurate reproduction of the binding conformation. Dihydrotestosterone (DHT) served as the positive control.

Docking study

To carry out this study, a combination of advanced computational tools was employed. The main software platforms used were AutoDock Vina (v1.1.2), UCSF Chimera (v1.14 and v1.15), and Discovery Studio Visualizer 2021. AutoDock Vina is renowned for its efficiency and accuracy in predicting ligand binding poses, while UCSF Chimera was utilized for both visualization and structural preparation of the proteins and ligands. Discovery Studio was used to analyse the molecular interactions in detail through 2D and 3D interaction maps.(20)

Protein structures related to PCOS—specifically Interleukin-6(20) (IL6, PDB ID: 1P9M), Tumor Protein p53(4) (TP53, PDB ID: 1AIE), and Catalase (4) (CAT, PDB ID: 6BO9)—were initially retrieved from the RCSB Protein Data Bank. However, the androgen receptor (PDB ID: 2PIU)(21) was selected as the primary target for molecular docking due to its critical role in PCOS pathology. Before docking, the proteins were prepared by removing water molecules, adding polar hydrogens, and assigning appropriate charges using AutoDock Tools. The processed protein files were saved in .pdbqt format for compatibility with AutoDock Vina.

Ligands for this study included both phytochemicals and standard drugs. Phytochemicals such as luteolin(14) (PubChem CID: 5280445), quercetin(22) (CID: 5280343), apigenin (22), p-coumaric acid (23), and benzo[a]pyrene (14) (CID: 2336) were selected based on their known antioxidant and anti-androgenic properties. Standard drugs included clomiphene citrate (24) (CID: 2800) and testosterone(25) (CID: 6013). The ligand structures were downloaded from the PubChem database and energy minimized using the Amber ff14SB force field in UCSF Chimera. The ligands were then saved in .mol format for docking purposes(26).

The docking procedure involved defining the active site of the androgen receptor based on known binding pockets and centroids. Grid coordinates were set to X = 27.104, Y = 2.449, and Z = 5.000, which ensured that the ligands were docked precisely within the receptor's ligand-binding domain.(27) Docking simulations were run using AutoDock Vina, and the best binding poses were selected based on their binding energy (in kcal/mol). The interaction analysis of docked complexes was carried out using ViewDock and Discovery Studio Visualizer (4).

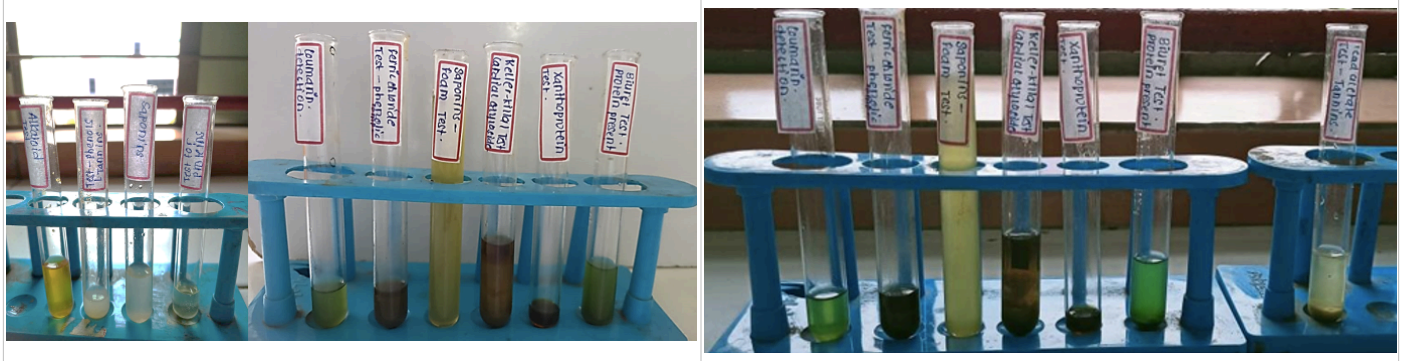
Results

Results of Phytochemical screening of crude drug

Table 3: Results of Phytochemical screening of cruds drug

Sr. No	Test	Reagent/Test Used	Result/Observation	*Carica*	*Triticum*
1	Alkaloid	Mayer's reagent	Brown precipitate	+	+
2	Terpenoid	Salkowski test	Reddish-brown color	+	+
3	Phenols	Ferric Chloride	Blue, green, purple, or red-brown	+	+
4	Saponins	Foam test	Frothy layer	+	+
5	Flavonoids	Shinoda test	Blue color	+	+
6	Protein	Biuret test	Purple color	+	+
7	Sugar	Benedict's solution	Red, orange, blue or green	+	+

Fig 03: Results of Phytochemical screening of crude drug



a. Carica papaya **b. *Triticum aestivum***

ADMET and Pharmacokinetics Prediction Details:

The pharmacokinetic profiling of drug candidates plays a pivotal role in the early stages of drug discovery and development. In the present study, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of selected bioactive compounds from Carica papaya and Triticum aestivum were evaluated to predict their pharmacokinetic behavior and drug-likeness for potential androgen receptor modulation in the management of polycystic ovary syndrome (PCOS).

ADMET analysis serves as a predictive model to understand how compounds behave within a biological system, reflecting their pharmacokinetic potential. Absorption characteristics, including human intestinal absorption and Caco-2 permeability, provide critical insight into the oral bioavailability of the phytoconstituents. Compounds demonstrating high gastrointestinal absorption are considered favorable for systemic therapeutic effects.

The distribution profile, particularly blood-brain barrier (BBB) permeability and volume of distribution, aids in predicting the ability of the compound to reach target tissues, including peripheral organs and possibly the central nervous system, depending on the mechanism of androgen receptor interaction. Such information is essential to understand the extent of compound dispersion post-absorption.

Metabolism, assessed through cytochrome P450 (CYP450) enzyme interactions, is a crucial determinant of compound biotransformation. Inhibition or induction of CYP isoenzymes can significantly alter the plasma concentration of therapeutic agents, influencing both efficacy and safety. The in-silico data obtained indicates whether the selected compounds are likely to undergo phase I metabolic transformation, and whether they may pose a risk of drug-drug interactions.

Excretion parameters, including total clearance and renal transport prediction, provide insight into the duration of action and potential accumulation of the compounds in the system. A compound with balanced clearance is desirable to maintain therapeutic levels without causing toxicity.

Although toxicity is not a direct component of pharmacokinetics, it profoundly impacts the compound's therapeutic viability. Toxicological predictions such as hepatotoxicity, AMES toxicity, and carcinogenicity were evaluated to ensure the safety of the lead compounds.

Docking results:

ADMET and Pharmacokinetics Prediction

SwissADME and pkCSM online tools were used to assess pharmacokinetics and toxicity. Parameters such as GI absorption, blood-brain barrier permeability, CYP450 inhibition, and AMES toxicity were predicted. Most phytochemicals showed high oral bioavailability, low toxicity, and no significant CYP inhibition, suggesting suitability for further investigation.

A total of 25 ligands, including both phytochemicals and standard compounds, were docked against the androgen receptor (PDB ID: 2PIU) using AutoDock Vina. The docking scores were assessed based on binding energy (kcal/mol), with more negative values indicating stronger binding affinity. Among all the compounds screened, dihydrotestosterone (DHT) exhibited the highest binding affinity with a docking score of –11.2 kcal/mol, confirming its natural high-affinity interaction with the androgen receptor. The carcinogenic compound benzo[a]pyrene followed closely with a docking score of –10.8 kcal/mol, though its toxicity excludes it from therapeutic consideration. Among the phytochemicals, luteolin demonstrated the strongest binding affinity, with a score of –8.9 kcal/mol, followed by quercetin (–8.8 kcal/mol) and apigenin (–8.7 kcal/mol). These compounds interacted with key residues in the receptor's ligand-binding domain. For instance, luteolin formed hydrogen bonds with LEU (A:873), and apigenin showed hydrogen bonding with GLN (A:711). Other compounds, such as p-coumaric acid and vanillin, also showed moderate binding affinities in the range of –7.0 to –7.5 kcal/mol.In contrast, the standard ovulatory drug clomiphene citrate displayed a relatively lower binding affinity of –6.1 kcal/mol, suggesting that certain natural compounds may have superior binding capabilities in silico. The pharmacokinetic analysis revealed that all the key phytochemicals adhered to Lipinski's Rule of Five, with appropriate molecular weights, hydrogen bond donors/ acceptors,, indicating potential drug-likeness and oral bioavailability.

https://doi.org/10.47552/ijam.v16iS2.6203 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Figure 4: Structure of 2PIU and DHT

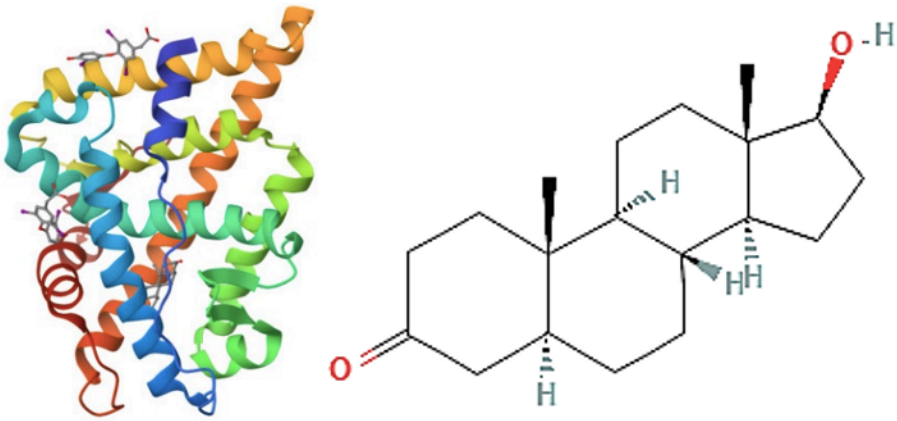
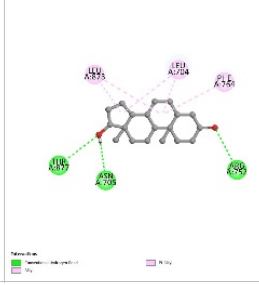
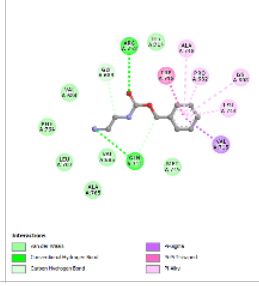
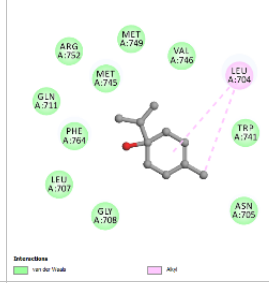
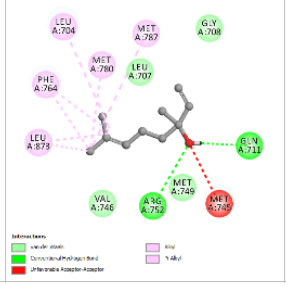
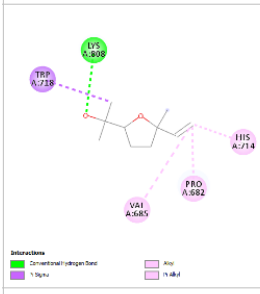
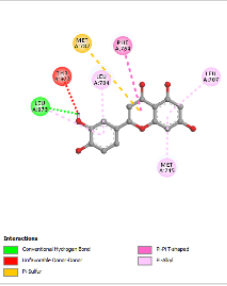
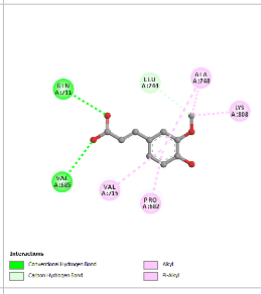
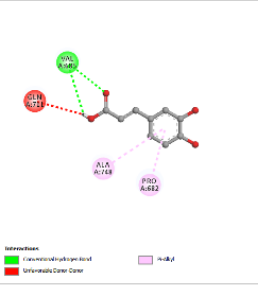
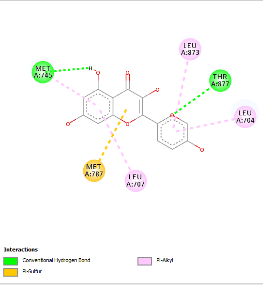


Table 4: Docking results

Sr. no	Ligand	Docking score (kcal/mol)	MW (g/mol)	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	Follow lipinski	Violations
1	Clomiphen Citrate	-6.1	405.96	9	2	0	12.47	Yes	1
2	DHT	-11.2	290.44	0	2	1	37.30	Yes	0
3	Benzyl (cyanomethyl)	-7.3	50.05	5	3	1	62.12	Yes	0
4	4-terpineol	-6.0	154.25	1	1	1	20.23	Yes	0
5	Linalool	-5.8	154.25	4	1	1	20.23	Yes	0
6	linalool oxide	-6.5	170.25	2	2	1	29.46	Yes	0
7	Leuteolin	-8.9	286.24	1	6	4	111.13	Yes	0
8	Ferulic Acid	-6.8	194.18	3	4	2	66.76	Yes	0
9	Caffeic Acid	-6.9	180.16	2	4	3	77.76	Yes	0
10	Quercetin	-8.8	302.24	1	7	5	131.36	Yes	0
11	benzyl	-6.3	149.21	2	1	0	44.45	Yes	0
12	Glucosinolates	-7.2	333.34	5	10	5	199.79	Yes	0
13	pantothenic acid	-6.2	219.23	7	5	4	106.86	Yes	0
14	p-coumaric acid	-6.7	164.16	2	3	2	57.53	Yes	0
15	Syringic acid	-5.5	198.17	3	5	2	75.99	Yes	0
16	Caffeic acid	-6.9	180.16	2	4	3	77.76	Yes	0
17	Gallic Acid	-6.0	170.12	1	5	4	97.99	Yes	0
18	Ferulic acid	-6.7	194.18	3	4	2	66.76	Yes	0
19	Benzo[a]pyrene	-10.8	252.31	0	0	0	0.00	Yes	0
20	4-Hydroxybenzoic	-5.9	138.12	1	3	2	57.53	Yes	0
21	Luteolin	-8.9	286.24	1	6	4	111.13	Yes	0
22	Quercetin	-5.7	302.24	1	7	5	131.36	Yes	0
23	Apigenin	-8.7	270.24	1	5	3	90.90	Yes	0
24	Alkyl Resorcinols	-5.4	110.11	0	2	2	40.46	Yes	0
25	Syringic acid	-5.6	198.17	3	5	2	75.99	Yes	0

Table 5: 2D Structure Interaction Analysis

Clomiphen Citrate	DHT	Benzyl (cyanomethyl) carbamate	4-terpineol	Linalool
				

linalool oxide	Leuteolin	Ferulic Acid	Caffeic Acid	Quercetin
				
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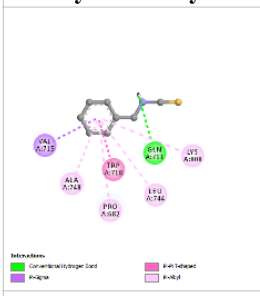	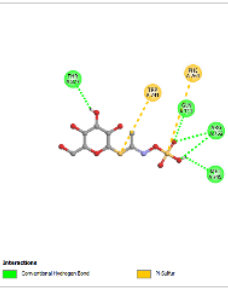	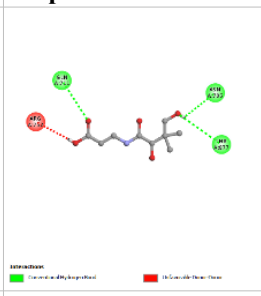	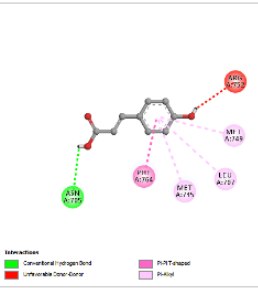	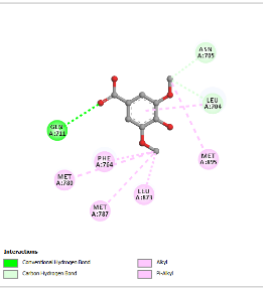
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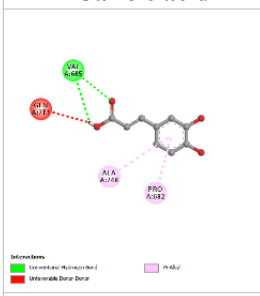
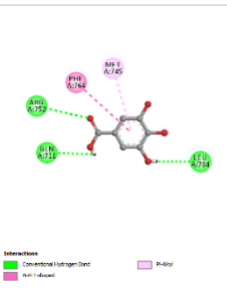
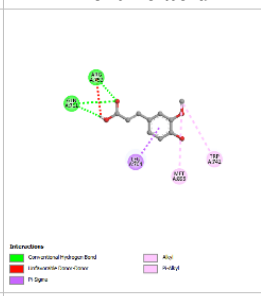
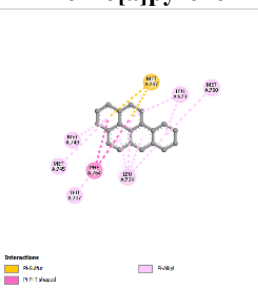
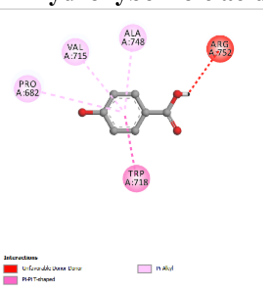
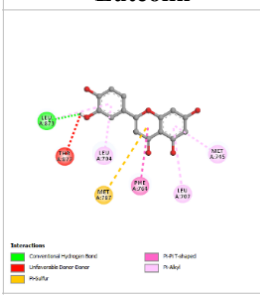
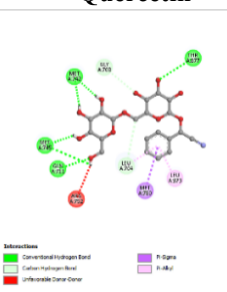
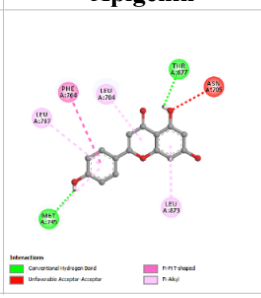
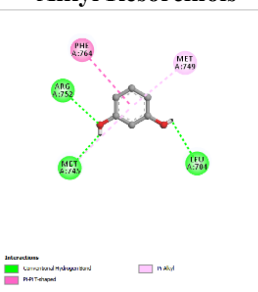
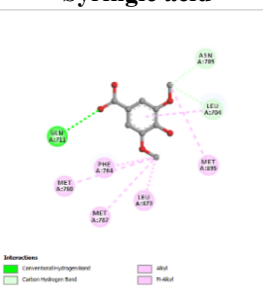
				
Luteolin	**Quercetin**	**Apigenin**	**Alkyl Resorcinols**	**Syringic acid**
				

Discussion

The docking results highlight the potential of natural phytochemicals in modulating androgen receptor activity, a key pathological factor in PCOS. The highest binding affinity of dihydrotestosterone aligns with its known physiological role as a potent endogenous ligand for AR, reinforcing the validity of the docking protocol. The strong performance of luteolin, quercetin, and apigenin in docking simulations suggests that these compounds could effectively compete with androgens for AR binding sites, thereby acting as potential antagonists or modulators.

Luteolin's interaction with LEU (A:873) and quercetin's hydrogen bonding with residues such as GLN (A:711) and ASN (A:705) demonstrate that these flavonoids can establish stable interactions within the androgen receptor's ligand-binding pocket. These findings support previous reports that these compounds possess anti-androgenic, anti-inflammatory, and antioxidant properties, which may contribute to alleviating PCOS symptoms. The ability of these compounds to bind strongly to AR also implies potential inhibition of androgen-mediated gene transcription, offering a plausible mechanism for their therapeutic role.

Interestingly, clomiphene citrate, though widely used in PCOS treatment, showed lower docking scores, indicating that while it is effective clinically, it may not bind to the androgen receptor as efficiently as certain phytochemicals in silico. However, it's important to note that in vitro efficacy also depends on receptor specificity, metabolic stability, and cellular uptake, which are not captured in docking alone.

The study also confirms that all selected ligands, especially the key phytochemicals, possess favourable ADME properties. Their compliance with Lipinski's rule enhances their candidacy as orally bioavailable agents. These results warrant further exploration through in vitro receptor binding assays and in vivo models to validate the biological relevance of these in- silico findings.

Conclusion

The present in-silico molecular docking study was conducted to evaluate the binding affinities and drug-likeness of various synthetic and naturally derived ligands targeting the androgen receptor, a key therapeutic target in the management of Polycystic Ovary Syndrome (PCOS). The docking scores, along with parameters such as molecular weight, rotatable bonds, hydrogen bond donors and acceptors, topological polar surface area (TPSA), and Lipinski's rule of five, were analysed to assess the pharmacokinetic suitability of each compound.

Among all ligands, Dihydrotestosterone (DHT) showed the strongest binding affinity with a docking score of -11.2 kcal/mol, followed by Benzo[a]pyrene (-10.8 kcal/mol). However, due to the toxic and carcinogenic nature of Benzo[a]pyrene, it is not a suitable therapeutic candidate. Among the natural compounds, Luteolin (-8.9 kcal/mol), Quercetin (-8.8 kcal/mol), and Apigenin (-8.7 kcal/mol) demonstrated strong binding interactions, suggesting their potential role in modulating androgenic activity and offering therapeutic benefits in PCOS.

Most of the evaluated ligands followed Lipinski's rule of five, indicating good oral bioavailability and drug-likeness. Particularly, natural compounds such as flavonoids and phenolic acids showed promising docking results while maintaining favourable pharmacokinetic profiles. These findings indicate that such compounds could be explored further as alternative or adjunct therapies for PCOS.

PCOS is characterized by hyperandrogenism, anovulation, and metabolic disturbances. Therapeutic strategies often aim to reduce androgen levels or block androgen receptors. Several natural compounds in this study, especially flavonoids, possess antioxidant, anti-inflammatory, and hormone-modulating properties. Their ability to reduce oxidative stress and regulate androgen biosynthesis positions them as promising agents for PCOS treatment.

Although Clomiphene Citrate shows one violation of Lipinski's rule due to its high molecular weight and number of rotatable bonds, it is still widely used in clinical practice for the treatment of anovulatory infertility in PCOS. This is because:

- It has proven pharmacological efficacy as a selective estrogen receptor modulator (SERM), effectively inducing ovulation.
- It has a well-established safety and tolerability profile.
- Lipinski's rule serves as a guideline, and many clinically approved drugs have minor violations without compromising efficacy or safety.
- Clomiphene is effective at low oral doses, mitigating bioavailability concerns.

In conclusion, this study presents an in-silico evaluation of phytochemicals from Carica papaya and Triticum aestivum, demonstrating promising interactions with the androgen receptor relevant to PCOS treatment. Compounds such as luteolin, quercetin, and apigenin displayed superior docking scores and favorable ADMET profiles. The docking protocol was validated using RMSD analysis and DHT as a positive control, confirming methodological accuracy. These findings support further experimental validation and pharmacological screening of these natural agents in vitro and in vivo.

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https://doi.org/10.47552/ijam.v16iS2.6204 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Exploring the Anti-inflammatory Potential of Blue-Green Algae: Formulation and Evaluation of Spirulina Ointment

Research Article

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Abstract

Blue-green algae, also known as cyanobacteria, are a diverse and ancient group of photosynthetic microorganisms that have been of great interest to scientists due to their nutritional, medicinal, and industrial applications. These microbes, some of the oldest organisms on our planet, are currently being discovered as a rich reservoir of bioactive compounds with applications ranging from nutrition to drug discovery. Spirulina and other cyanobacterial genera, in specific, have exhibited strong anti-inflammatory, antioxidant, and immunomodulatory activities and are potential drugs for topical and systemic therapy. Bioactives like phycocyanin, polysaccharides, and carotenoids are key players in exerting these properties and have been effectively added to ointments for better delivery and efficacy. Cyanobacteria exhibit significant utility in promoting human health and possess extensive applications in the field of cosmeceuticals due to their photoprotective properties and skin-regenerative capabilities. Furthermore, they are employed in bioremediation, biofuel generation, and nutraceutical synthesis, thereby constituting a vital component of sustainable biotechnological innovations. Despite these advantages, challenges such as the occurrence of cyanotoxins like microcystins, variability in bioactive compound content, and constraints associated with cultivation underscore the imperative for additional research and standardization efforts. The current investigation aimed to examine the anti-inflammatory properties of the blue-green algae Spirulina, alongside the formulation and evaluation of Spirulina-based ointments. This review endeavors to highlight recent advancements in the anti-inflammatory potential of blue-green algae, with particular focus on the formulation of topical ointments.

Keywords: Spirulina, Blue-Green Algae, Anti-inflammatory, Ointment, Microalgae, Skin Protection.

Introduction

Blue-green algae, also known as cyanobacteria, are among the oldest and most versatile microorganisms on Earth, with a history dating back over 3.5 billion years. These photosynthetic prokaryotes play a vital role in global ecological processes, such as nitrogen fixation and oxygen production, contributing significantly to the planet's biosphere and sustaining a wide range of life forms. In recent decades, their pharmacological and biotechnological potential has attracted increasing scientific interest, particularly for their production of diverse bioactive compounds.

One of the most prominent genera within cyanobacteria is Spirulina (Arthrospira platensis), renowned for its exceptional nutritional value, including high protein content, essential amino acids, vitamins, minerals, and pigments such as phycocyanin and chlorophyll. Beyond its dietary benefits, Spirulina has demonstrated significant therapeutic properties, especially in addressing inflammation, oxidative stress, immune modulation, and skin regeneration.

The anti-inflammatory potential of Spirulina is primarily attributed to phycocyanin, a blue pigment-protein complex known for inhibiting pro-inflammatory enzymes like COX-2 and reducing cytokine activity. Additionally, polysaccharides and essential fatty acids derived from Spirulina contribute to immune regulation and tissue repair. These bioactives make Spirulina a compelling candidate for topical use in dermatology, particularly in treating conditions such as eczema, psoriasis, and UV-induced skin damage.

With proven safety in toxicological studies, Spirulina is increasingly incorporated into pharmaceutical and cosmetic products. Its cultivation is cost-effective and sustainable, further supporting its use in large-scale biotechnological applications. Recent advancements have enabled the development of ointments and creams that utilize Spirulina extracts to deliver targeted therapeutic effects while also enhancing skin barrier function and photoprotection. (26, 27).

The anti-inflammatory effects of blue-green algae are mainly ascribed to their phytochemical diversity. Phytochemicals such as C-phycocyanin, polysaccharides, and essential fatty acids have been documented to modulate well the inflammatory response. C-phycocyanin, a blue pigment-protein

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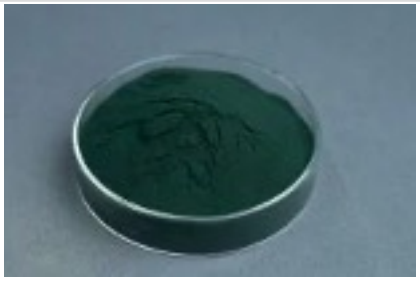
complex from Spirulina platensis, has been intensely studied for its effects on the inhibition of pro-inflammatory mediators such as cytokines and nitric oxide and antioxidants (27, 28, 31)

Figure 1. Spirulina powder



History and Significance of Blue-Green Algae

Blue-green algae or *cyanobacteria* are one of the earliest living organisms on our planet. Their history goes back more than 3.5 billion years, and they are one of the earliest groups of organisms to have evolved oxygenic photosynthesis a phenomenon that revolutionized the Earth's atmosphere and paved the way for the development of complex aerobic forms of life (1). Fossilized stromatolites composed of layered mats of *cyanobacteria* constitute direct proof of their existence and ecological role in the Precambrian period.

The name "blue-green algae" is due to the characteristic pigmentation of these microorganisms—specifically their phycocyanin (blue pigment) and chlorophyll-a (green pigment) content, which are responsible for their color and photosynthetic activity. *Cyanobacteria* are prokaryotic rather than true algae, with no membrane-bound organelles, but they occupy many of the same ecological niches as eukaryotic algae (1).

Historically, *cyanobacteria* not only contributed significantly to the creation of Earth's biosphere but were also pivotal in the establishment of contemporary agriculture and aquaculture. In premodern farm production, particularly in Asia, species like Nostoc and Anabaena contributed to soil fertility enrichment through nitrogen fixation in rice paddies long before the creation of synthetic fertilizers.

In the last few decades, scientific interest in blue-green algae has escalated because of their bioactive attributes. Among them, Spirulina (*Arthrospira platensis*) is the most economically important species. It came into international focus in the 20th century when NASA suggested that it be used as a nutritional additive for space explorers because of its higher protein content, rich vitamin composition, and higher biomass production (7).

Cyanobacteria synthesize a broad range of secondary metabolites such as toxins, pigments, polysaccharides, and fatty acids, most of which have anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory activities (4,5,10).

Cyanobacteria also have a central role in environmental sustainability. Their capacity to survive under adverse conditions, absorb heavy metals, and produce biofuels has made them a critical component in bioremediation and green energy studies (12,20). Their capability for carbon sequestration, nutrient recycling, and wastewater treatment is also a key contribution towards the fight against climate change and minimizing ecological footprints.

Blue-green algae are becoming potential agents in topical preparations in the dermatological and medical sciences because of their skin-rejuvenating, sun-protection, and anti-inflammatory activities (8,17). Topical preparations using Spirulina extracts are now being researched for treatments ranging from eczema and psoriasis to photoaging of the skin.

Modern Applications

As a result of the development in biotechnology, blue-green algae emerged as an important element in nutrition. Their potential as a source of bioactive compounds leads to their diverse applications in pharmaceuticals, cosmetics, and environmental sustainability. They particularly show great significance in their ability to produce natural anti-inflammatory, antioxidant, and antimicrobial substances relevant to health and skincare industries (29, 30).

Ecological and Industrial Significance

Cyanobacteria play a role in fixing nitrogen in aquatic and terrestrial ecosystems, making the soil fertile and promoting agricultural productivity. They are also important in carbon sequestration as it prevents the worsening effects of climate change because cyanobacteria absorb carbon dioxide in the process of photosynthesis (31, 32).

In recent decades, there has been an increasing industrial interest in blue-green algae. These organisms are considered a significant potential sustainable resource for biofuels, bioplastics, and high-value nutraceuticals. Moreover, their cultivation does not need much land and water compared to other crops; therefore, they are a promising candidate for addressing global food and energy demands (33, 34)

Materials and Methods

Formulation of Spirulina ointment

Table 1: Composition of Spirulina ointment

Ingredients	Quantity	Percentage
Spirulina powder	1.5 gm	10.02%
Petrolatum (Vaseline)	1.5 gm	10.02%
Lanoline	4.5 gm	30.05%
Beeswax	1.5 gm	10.02%
Steric acid	0.75 gm	5.01%
Cetyl alcohol	0.75 gm	5.01%
Glycerin	1.5 gm	10.02%
Methyl paraben	0.02 gm	0.13%
Vitamin E	0.5 gm	3.34%
Ascorbic acid	0.2 gm	1.34%
Mint oil	1 ml/0.9 gm	6.68%
Xanthan gum	0.3 gm	2.00%
Phenoxyethanol	1.0 gm	1.0%

https://doi.org/10.47552/ijam.v16iS2.6204 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Formulation of Spirulina ointment

Spirulina powder was solubilized in distilled water. The ointment base was formulated by melting together petrolatum, lanolin, and beeswax. The spirulina powder slurry was subsequently incorporated into the molten base progressively. Glycerin was introduced to enhance the emollience of the formulation. The preparation was then allowed to cool to 40°C. and melting point is petrolatum 35° C -45° C, lanolin 35° C -40° C , and beeswax 60° C Finally, methyl paraben and phenoxyethanol were incorporated into the mixture. Mint oil was added to impart fragrance. Upon reaching ambient temperature, the ointment was meticulously transferred into sterilized containers and stored in a cool and dark place.

Figure 2: Spirulina powder

Physical evaluation parameters

Preliminary assessment of formulation was conducted on various parameters such as;

- The organoleptic attributes, including color and odor of the formulation, were evaluated through visual inspection.
- pH: The pH levels of the different formulations were measured utilizing a digital pH meter. Specifically, 0.5 g of the formulation was dissolved in 50 ml of distilled water, and the pH value was recorded.
- Homogeneity: Each of the formulated ointments was assessed for homogeneity via visual examination, ensuring that the products exhibited no lumps.
- Washability: A quantity of 0.5 g of the formulated preparation was applied to the skin, and subsequently washed off with lukewarm water. The duration required for the complete removal of the preparation was noted.
- Viscosity: The viscosity of the prepared ointments was quantified using a Brookfield Viscometer. Each formulations viscosity was measured in triplicate, and the average values were subsequently reported.
- Spreadability: The assessment of spreadability was performed using a specialized apparatus consisting of a wooden block equipped with a pulley at one end. Through this methodology, spreadability was evaluated based on the slip and drag characteristics of the ointments. An excess quantity of approximately 2 g of the ointment under investigation was placed on the lower glass slides. The ointment was then sandwiched between this slide and an additional glass slide, which matched the dimensions of the fixed lower slide and included a hook. A weight of one kilogram was positioned on top of the slides for five minutes to eliminate air and ensure a uniform film of the ointment between the slides. Any surplus ointment was removed from the edges. The upper slide was then subjected to a force of 80 g via a string attached to the hook, and the time (in seconds) required for the upper slide to traverse a distance of 7.5 cm was recorded. A shorter duration signifies superior spreadability. It was calculated using the formula S = M x L/T, where S represents spreadability, M denotes the weight applied to the upper slide, L represents the length of the glass slide, and T represents the time required to completely separate the slides from one another.
- Stability study: Physical stability study of the spirulina herbal ointment was carried out for four weeks at different temperature conditions: 15°C, 25 °C, 37 °C. The herbal ointment was found to be physically stable at different temperature i.e., 15°C, 25°C, 37°C. for four weeks.

Results

Table 2: Evaluation parameters for spirulina herbal ointment

EVALUATION	OBSERVATION
Colour	Dark Green
Odour	Pleasant
Consistency	Smooth
pH	7
Spreadibility	Evenly spreadable
Washability	Easily washable
Irritancy	Non irritant
Stability study (15°C to 37°C)	Stable
Homogeneity	Homogenous
Microbial contamination	No growth of fungi and yeast was seen until one month
Storage condition	At room Temperature

The emulsifying ointment was used as the substrate to create herbal ointments in the current investigation. The formulations physical characteristics were then assessed. These physicochemical characteristics were acceptable. The formulations appeared to be stable based on the stability analyses that were conducted. In tabular form, the maximum dosages of compounded ointments are displayed (Table No. 2).

Discussion

The current investigation was conducted to formulate and evaluate a herbal ointment. The herbal formulation was prepared by incorporating spirulina extract into a prepared ointment base. It exhibited stability throughout the storage period. The physicochemical properties of the ointment were analyzed, yielding satisfactory results concerning extrudability, washability, spreadability, solubility, and loss on drying, among others. Stability assessments were performed under varying temperature conditions of 2°C, 25°C, and 37°C over a four-week duration. No

alterations were detected in spreading ability, diffusion characteristics, or any irritant effects.

Conclusion

The study successfully highlights the potential of Spirulina-based ointments as a natural and effective alternative for managing inflammation. The findings encourage further exploration, including clinical trials and product development. With minor refinements in methodology presentation and expanded comparative analyses, this research could form the foundation for a new class of algae-derived topical therapies in the pharmaceutical and cosmeceutical industries. The article requires minor revisions for publication in the IJAM

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https://doi.org/10.47552/ijam.v16iS2.6205 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Investigation of Phytochemicals and Pharmacognostic Features of *Feronia Limonia* Fruit and Its In Vivo Effects on Oxidative Stress

Research Article

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Abstract

Feronia limonia, commonly known as wood apple, is a medicinal plant widely used in traditional medicine for its therapeutic properties. This study investigates the phytochemical constituents, pharmacognostic characteristics, and in vivo antioxidant potential of *F. limonia* fruit to provide a scientific foundation for its medicinal applications. Phytochemical screening revealed the presence of key bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Pharmacognostic and physicochemical analyses were performed to establish quality control and standardization parameters for the fruit. In addition, the methanolic extract was evaluated for its antioxidant activity using a carbon tetrachloride (CCl_4)-induced oxidative stress model in Wistar rats. The extract significantly reduced malondialdehyde (MDA) levels and restored antioxidant enzyme activities (SOD, CAT, GSH) in a dose-dependent manner, supported by histopathological findings of hepatoprotection. These results validate the traditional use of *F. limonia* and highlight its potential as a natural source of antioxidant agents for pharmaceutical and nutraceutical development.

Keywords: *Feronia Limonia*, Phytochemical Constituents, Pharmacognostic Properties, Medicinal Plant, Antioxidant activity, Herbal Medicine.

Introduction

Medicinal plants have been a cornerstone of traditional healing systems for centuries, providing essential bioactive compounds for drug development and therapeutic applications. *Feronia limonia* (commonly known as wood apple or elephant apple) is a lesser-explored medicinal fruit belonging to the Rutaceae family, widely distributed in South and Southeast Asia. (1)

The fruit has been traditionally used for its diverse pharmacological properties, including antimicrobial, antioxidant, hepatoprotective, and antidiabetic activities. Despite its widespread ethnomedicinal use, comprehensive scientific investigations on the phytochemical and pharmacognostic properties of *Feronia limonia* remain limited. Phytochemical analysis is essential to identify bioactive constituents such as flavonoids, alkaloids, tannins, and phenolic compounds, which contribute to the fruit's medicinal potential. Meanwhile, pharmacognostic studies provide critical information on the macroscopic, microscopic, and physicochemical characteristics of plant materials, ensuring their authenticity, purity, and quality in herbal medicine formulations. (2)

This study aims to explore the phytochemical constituents and pharmacognostic properties of *Feronia limonia* fruit, providing a scientific basis for its medicinal value. By analysing its bioactive compounds and standardising its pharmacognostic profile, this research seeks to contribute to the validation and potential pharmaceutical application of *Feronia limonia* in modern medicine. (3,4)

Plant Profile

Kingdom	- Plantae
Division	- Magnoliophyta
Class	- Magnoliopsida
Order	-Sapindales
Family	- Rutaceae
Subfamily	- Aurantioideae
Genus	- *Feronia*
Species	- *F. limonia* C.

Materials and Methods

Collection of Plant Material

The plant material was procured and authenticated from Shree Shail Herbs PVT. LTD. Nagpur.

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Figure 1. *Feronia limona* fruit

Organoleptic evaluation (5)

The methanolic and petroleum ether extracts of *Feronia limona* fruit were evaluated for their organoleptic properties, including colour, and weight.

Physicochemical Evaluation (5)

Determination of Extractive Value

A coarse powder of dried plant material was placed in a Soxhlet thimble in the appropriate amount. The plant material was then extracted with 100-250 ml of two different solvents at 60^{0}c °C, in order of polarity, i.e., Methanol and Petroleum ether. Extraction was carried out until 50 cycles were completed for the solvents. The percentage practical yield of each extract was calculated.

Figure 2: Soxhlet Extraction Method

Determination of Total Ash Content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread evenly in a layer and ignited to a constant weight by gradually increasing the heat to 500-600°C until it turned white, indicating the absence of carbon. The remaining ash was allowed to cool in a desiccator. The content of total ash (in mg/g) of air-dried material was calculated as follows:

% Total ash = weight ash/ weight of sample × 100

Determination of Acid-Insoluble Ash

HCl (2 N; 25 ml) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water, and the rinsed contents were added to the crucible. The acid-insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid-insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid-insoluble ash (in mg/g) of air-dried material was calculated as follows:

% Acid-insoluble ash = weight ash/ weight of sample ×100

Determination of Water-Soluble Ash

Water (25 ml) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and added to the crucible. The water-insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water-insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water-soluble ash content was calculated using the following equation

% Water soluble ash = total ash content-water insoluble residue in total/ ash weight of sample ×100

Foreign Matter Analysis

Foreign matter presence may be due to faulty collection of crude drugs or deliberate mixing. It was separated from the drug so that the results obtained are important parts of the morphology of a particular drug.

Determination of pH

The pH of the 1 % solution of extract was determined by making an appropriate concentration of powdered drug in an aqueous solution, filtering, and checking the pH of the filtrate. A digital pH meter was utilised to ascertain the pH of the mixtures.

Preliminary Phytochemical Screening of *Feronia limonia* Fruit Extracts

Qualitative chemical tests were performed for extracts of plants - Dragondroff's test, Mayer's test, Molish test, Fehling's test, Benedict test, Bortanger test, Saponin Foam test, Sulphuric acid test, etcThe extracts were shown to contain active phytochemical elements such as alkaloids, carbohydrates, glycosides, tannins, and saponins.

Quantitative Estimation of Total Phenolic Content

The Folin – Ciocalteu colorimetric colorimetry method was used to determine the total phenolic content, based on the procedure of Azlim Almey (2010), using Gallic acid as a standard phenolic compound. (7,9,10)

Reagents: Folin Ciocalteu reagent: Dilute the Folin Ciocalteu reagent with an equal volume of distilled

water; 20% sodium carbonate: 20 g sodium carbonate in water; Gallic acid.

Procedure

- Prepare a calibration curve of standard Gallic acid (10-100 µg/ml in water).
- Prepare 1 mg/mL of extract solutions.
- Mix 1 ml of each sample with 0.25 ml of Folin-Ciocalteu's reagent and 1.25 ml of 20% sodium carbonate solution.
- Allow the mixture to react for 40 minutes. At room temperature.
- After the reaction period, the contents are mixed, and the blue colour is at 725 nm in comparison with standards. Calculate the number of total phenols from the calibration curve as a Gallic acid equivalent by the following formula:

$$T = \frac{C.V}{M}$$

Where T = total content of phenolic compounds, milligram per gram plant extract, C the concentration of gallic acid established from the calibration curve, milligram per milliliter, V the volume of extract, milliliter, and M the gram weight of plant extract

Quantitative Estimation of Total Flavonoid Content

The Aluminium Chloride colorimetry method was used to determine the total flavonoid content, based on the procedure of Azlim Almey (2010), using Quercetin as a standard flavonoid compound.

Reagents: Quercetin, ethanol, Aluminium chloride, potassium acetate.

Procedure

- Prepare the calibration curve of standard Quercetin (10- 100 µg/ml in methanol).
- Mix 0.5 ml standard solution with 1.5 ml of 95% ethanol, 0.1 ml of 10% aqueous Aluminium chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water.
- Incubate for 30 min at room temperature. Measure the absorbance of the reaction mixture at 415nm with a UV spectrophotometer.
- To prepare a blank solution, substitute 10% Aluminium chloride with an equal amount of distilled water.
- Similarly, treat 0.5 ml of plant extract samples with Aluminium chloride for the determination of flavonoid content from the calibration curve.

In vitro antioxidant activity of *Feronia limonia*

In the present study, the methanolic extract was tested for free radical scavenging activity at various concentrations using different in vitro methods.

Hydrogen Peroxide Scavenging Assay

Principle: It uses a colour reagent that contains xylenol orange dye in an acidic solution with sorbitol and ammonium iron sulfate that reacts to produce a purple colour in proportion to the concentration of H_2O_2 in the sample being tested.

Reagents

- **Phosphate Buffer Saline (pH 7.4):** Prepare 800 ml of distilled water in a suitable container. Add 20.214 g of Sodium Phosphate Dibasic Heptahydrate and 3.394 g of Sodium Phosphate Monobasic Monohydrate to the solution. Adjust the solution to the final desired pH using HCl or NaOH. Add distilled water until the volume is 1 L.
- **H_2O_2 solution:** Procedure: In this test, varying concentrations of the test substance (50 to 800 µg/ml) were assayed. Test solution: H_2O_2 solution (40 mmol/l in phosphate buffer): phosphate buffer (pH 7.4) at 1:0.6:3.4 ml was added to the test tube. The absorbance of the reacting solution versus blank, including the extract solution plus phosphate buffer (1:4, ml), was checked spectrophotometrically at 230nm. The control consisted of phosphate buffer: H_2O_2 solution (3.4:0.6, ml). The equation was used for % H_2O_2 inhibition.

In vivo antioxidant activity of *Feronia limonia*

The in vivo antioxidant study was designed based on the methodology outlined by Jain et al. (2018), with minor modifications. Wistar rats weighing between 150 and 200 grams were randomly assigned to one of four groups (n = 6 per group): Control, Positive Control (Ascorbic acid at 100 mg/kg), *F. limonia* extract low dose (200 mg/kg), and *F. limonia* extract high dose (400 mg/kg). Oxidative stress was induced by administering carbon tetrachloride (CCl_4) intraperitoneally at a dose of 0.5 mL/kg, diluted in olive oil (1:1 v/v), twice a week for two weeks. Treatment with either the extract or ascorbic acid was given orally, once daily, for a total of 14 days. On day 15, blood and liver samples were collected to evaluate antioxidant markers, including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), following standard biochemical protocols.

Experimental Design and Treatment Groups

- Group I: Control (vehicle only)
- Group I: CCl_4 + Positive control (Ascorbic acid, 100 mg/kg/day, orally)
- Group III: CCl_4 + *F. limonia* extract (Low dose: 200 mg/kg/day, orally)
- Group IV: CCl_4 + *F. limonia* extract (High dose: 400 mg/kg/day, orally)

Results

Organoleptic evaluation

Organoleptic evaluation for both the methanolic and petroleum ether extracts was done.

Physicochemical characteristics

The plant extracts were subjected to various evaluation parameters.

Phytochemical screening

The phytochemical screening of the extracts with different chemical tests was performed, and the results are as follows:

Table 1: Organoleptic properties of plant extracts

Sr. No	Solvent used	Colour of the extract	Weight of the extract	The weight of the crude plant taken	Extractive value
1	**Petroleum ether**	Dark green	0.6 gm	50 gm	1.19 % w/ w
2	**Methanol**	Dark brown	4.0375 gm	50 gm	8.05 % w/ w

Table 2: Physicochemical characteristics

Physicochemical Character	*Feronia limonia* crude drug
Total ash (%w/w)	9.47
Acid-insoluble ash (%w/w)	1.24
Water insoluble ash (%w/w)	8.23
Foreign organic matter (%w/w)	1.63
Loss on drying ash (%w/w)	16.28
pH	5.92

Table 3: Phytochemical screening of Methanol and Petroleum ether extract

Sr. No.	Chemical test	Methanol extract	Petroleum ether extract
1	**Alkaloid**		
	Dragondroff's test	+	+
	Mayers test	+	+
2	**Carbohydrate**		
	Molish test	+	+
	Fehlings test	+	+
	Benedict test	+	-
3	**Glycosides**		
	Bortanger test	+	-
4	**Saponin Foam test**	+	+
5	**Tannin**	+	+
6	**Phenolic**	+	+
7	**Flavonoid**	+	-
8	**Saponin**	+	-
9	**Mucilage**	-	-
10	**Lipids/Fats**	-	+

Quantitative estimation of Total Phenolic Content

A linear calibration curve of gallic acid with an R_2 value of 0.9765 was obtained. Figure 3 shows the mean TPC of the plant extract measured using the GAE equation of Y = 0.0018x +0.0061 (R^2 = 0.9765), whereby Y = absorbance at 765nm and X = concentration of total phenolic compounds in mg per ml of the extract. The Methanol extract showed the GAE of (11.23 ±0.13 mg/g), and the petroleum ether extract showed the GAE of (3.74 ±0.13 mg/g).

Quantitative estimation of Total flavonoid content

A linear calibration curve of Quercetine with an R_2 value of 0.9628 was obtained. Figure 4 shows the mean TFC of the plant extract measured using the TFC equation of y = 0.0009x +0.002 (R^2 = 0.9628), whereby Y = absorbance at 415nm and X = concentration of total flavonoid in mg per ml of the extract. The Methanol extract showed the flavonoid content of (4.86 ±0.13 mg/g), and the petroleum ether extract showed the flavonoid content (4.064 ±0.13 mg/g).

Figure 3: Calibration Curve of Gallic Acid

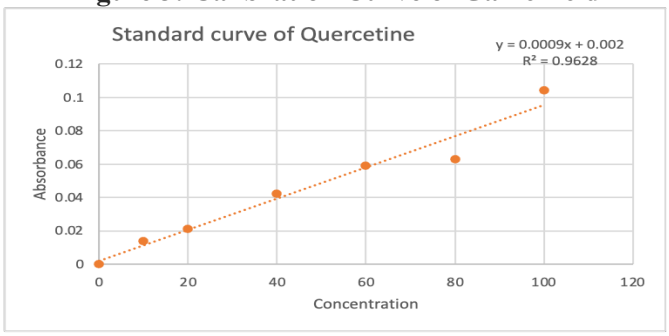
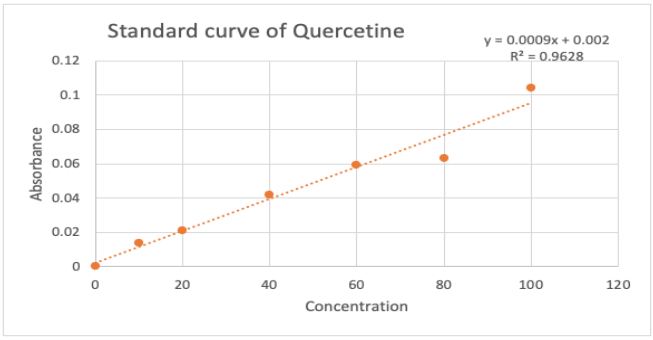


Figure 4: Calibration curve of Quercetine as standard



In vitro antioxidant activity of *Feronia limonia*

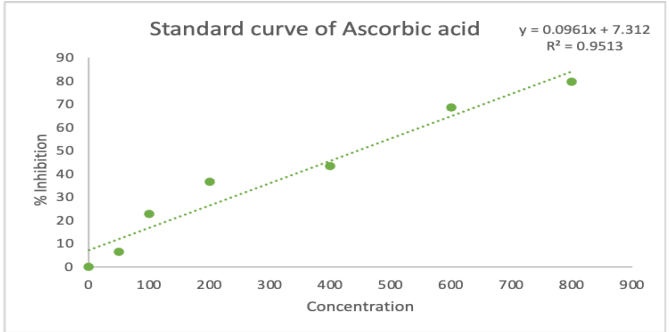
Hydrogen Peroxide Scavenging Assay

In the present study, methanolic extract of *Feronia limonia* in various concentrations was tested for its free radical scavenging activity in different in vitro methods. It was concluded that free radicals were scavenged by the test extract.

Table 4: Antioxidant Activity (Hydrogen Peroxide) of Ascorbic Acid

Sr. No.	Concentration (µg)	Absorbance	% Inhibition	IC 50 Value
1	0	0	0	444.20 µg/ ml
2	50	0.589	6.50794	
3	100	0.486	22.8571	
4	200	0.399	36.6667	
5	400	0.356	43.4921	
6	600	0.198	68.5714	
7	800	0.128	79.6825	

Figure 5: Graph of Hydrogen peroxide radical scavenging assay of Ascorbic acid



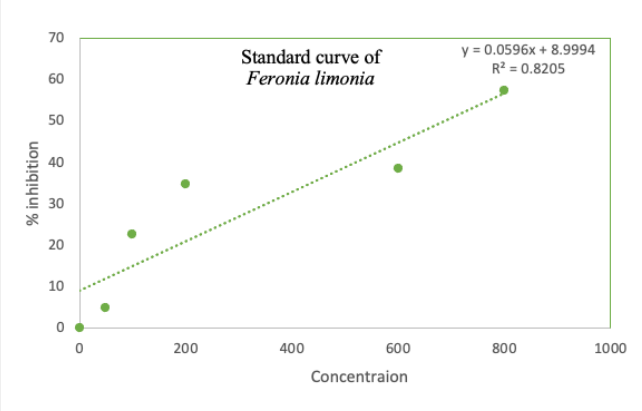
https://doi.org/10.47552/ijam.v16iS2.6205 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Table 5: Antioxidant Activity (Hydrogen Peroxide) of *Feronia limonia*

Sr. no.	Concen-tration (µg)	Control	Sample	% Inhibition	IC 50
1	0	0	0	0	687.93
2	50	0.63	0.599	4.92063	
3	100	0.63	0.487	22.6984	
4	200	0.63	0.411	34.7619	
5	400	0.63	0.387	38.5714	
6	600	0.63	0.269	57.3016	
7	800	0.63	0.183	70.9524	

Figure 6: Graph of Hydrogen peroxide radical scavenging assay of *Feronia limonia*



In Vivo Antioxidant Study

Effect of *F. limonia* Extract on Lipid Peroxidation (MDA Levels)

The MDA levels were significantly elevated in the CCl_4 group compared to the control group ($p < 0.001$), indicating increased lipid peroxidation. Treatment with *F. limonia* extract at both 200 mg/kg and 400 mg/kg significantly reduced MDA levels in a dose-dependent manner ($p < 0.01$ and $p < 0.001$, respectively), comparable to the standard ascorbic acid group.

Table 6: Effect of *F. limonia* Extract on Lipid Peroxidation (MDA Levels)

Sr. No.	Group	MDA (nmol/mg protein)
1	Control	2.3 ± 0.15
2	CCl_4	6.8 ± 0.24 (↑↑↑ vs. control)
3	CCl_4 + Ascorbic Acid	3.0 ± 0.18 (↓↓ vs. CCl_4)
4	*F. limonia* (200 mg/kg)	4.1 ± 0.21 (↓)
5	*F. limonia (400 mg/kg)*	3.2 ± 0.17 (↓↓)

Antioxidant Enzyme Activities

Superoxide Dismutase (SOD) Activity

CCl_4 significantly reduced SOD activity compared to the control group ($p < 0.001$). Both doses of *F. limonia* significantly increased SOD activity, with the higher dose showing results nearly equivalent to the standard.

Catalase (CAT) Activity

CAT activity was markedly decreased in the CCl_4 group. Treatment with *F. limonia* extract restored CAT levels in a dose-dependent manner ($p < 0.05$ and $p < 0.01$).

Reduced Glutathione (GSH) Levels

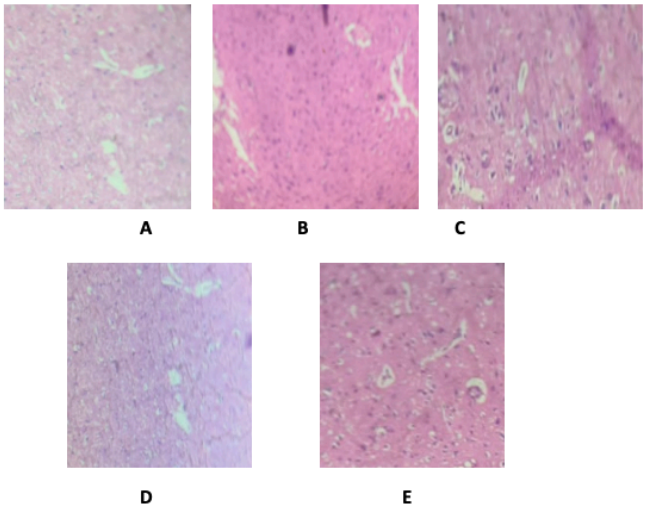
GSH levels were significantly depleted in the CCl_4 group. Both *F. limonia* doses significantly replenished GSH, with the high dose showing the strongest effect ($p < 0.001$).

Table 7: Antioxidant Enzyme Activities

Sr. No.	Group	Group SOD (U/mg protein)	CAT (U/ mg protein)	GSH (U/mg protein)
1	Control	8.2 ± 0.34	62.3 ± 2.4	48.2 ± 1.7
2	CCl_4	3.9 ± 0.29 (↓↓↓)	3.4 ± 0.93 (↓↓↓)	4.5 ± 1.73 (↓↓↓)
3	CCl_4 + Ascorbic Acid	6.32 ± 0.78 (↑↑↑)	7.2 ± 0.88 (↑↑↑)	7.8 ± 0.23 (↑↑↑)
4	*F. limonia* (200 mg/kg)	5.63 ± 0.30 (↑)	5.86 ± 0.36 (↑)	6.01 ± 0.48 (↑)
5	*F. limonia* (400 mg/kg)	7.36 ± 1.26 (↑↑)	7.65 ± 0.67 (↑↑)	6.96 ± 1.83 (↑↑)

Note: Values are presented as mean ± SEM (n = 6). Significance vs. CCl_4 group: $p < 0.05$ (↑/↓), $p < 0.01$ (↑↑/↓↓), $p < 0.001$ (↑↑↑/↓↓↓).CCl_4 group: Hepatocytes showed ballooning degeneration, centrilobular necrosis, and dense inflammatory infiltration.

Figure 7: Representative H&E micrographs from liver tissues collected from rats under a 100X microscope



A- control, B- CCL_4, C- CCL_4 +Ascorbic acid, D- F. *limonia* (200 mg/kg), E- *F. limonia* (400 mg/kg)

A: Control group showing normal hepatic architecture with intact hepatocytes and central veins

B: CCl_4-treated group exhibiting severe hepatocellular necrosis, ballooning degeneration, and inflammatory cell infiltration.

C: CCL_4 +Ascorbic acid treated group exhibiting severe hepatocellular necrosis, ballooning degeneration, and inflammatory cell infiltration.

D: The treatment with *F. limonia* at a dosage of 200 mg/kg demonstrated moderate improvement, resulting in reduced necrosis and inflammation.
E: The treatment with F. limonia at a dosage of 400 mg/kg showed near-normal liver architecture, with only minimal histopathological changes observed.

Statistical Analysis

All data are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey's post hoc test was used for group comparisons. Differences were considered statistically significant at $p < 0.05$. Statistical software used: [GraphPad Prism v9].

Discussion

The phytochemical constituents and pharmacognostic properties of *Feronia limonia* fruit were evaluated using various standard parameters. The powdered fruit was extracted using methanol and petroleum ether. Phytochemical screening revealed the presence of several bioactive compounds, including flavonoids, alkaloids, tannins, saponins, and phenolic compounds. These phytochemicals are well-documented for their antioxidant and hepatoprotective properties. The quantitative estimation of total phenolic content in the methanolic extract yielded a value of 4.064 ± 0.13 mg/g, suggesting that phenolic compounds significantly contribute to the extract's biological activity. The methanolic extract demonstrated promising antioxidant potential in vitro, as assessed by the hydrogen peroxide scavenging assay. This observed activity can be attributed to the presence of polyphenolic constituents, particularly flavonoids and tannins, which are known to neutralise free radicals by donating electrons and inhibiting lipid peroxidation. Building on these in vitro findings, an in vivo study was conducted using a carbon tetrachloride (CCl_4)-induced oxidative stress model in Wistar rats to further evaluate the extract's antioxidant potential under physiological conditions. Administration of carbon tetrachloride resulted in a significant increase in malondialdehyde (MDA) levels and a concurrent decrease in endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), indicating oxidative liver damage. Treatment with *F. limonia* extract significantly reduced these biochemical alterations in a dose-dependent manner. The high-dose group (400 mg/kg) exhibited a protective effect comparable to that of the standard antioxidant, ascorbic acid. Histopathological examination further supported the biochemical data, showing substantial preservation of liver architecture in treated groups compared to the CCl_4 group, which displayed significant necrosis and inflammation. These in vivo findings corroborate earlier reports, including those by Jain et al. (2018), who observed similar hepatoprotective and antioxidant effects of *F. limonia* stem bark extract in a comparable model. The presence of flavonoids and phenolic compounds, identified in the phytochemical analysis, likely underlies the antioxidant mechanism through free radical scavenging, inhibition of lipid peroxidation, and enhancement of endogenous defence enzymes. Overall, the combination of in vitro and in vivo data strongly supports the antioxidant efficacy of *F. limonia* methanolic extract. These results provide a scientific basis for the traditional use of the plant in managing oxidative stress-related disorders and highlight its potential as a natural therapeutic agent.

Conclusion

The study of *Feronia limonia* fruit reveals its potential medicinal applications due to various bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties. Quantitative analysis showed significant phenolic content, aiding in quality control and standardisation for herbal formulations. An in vivo study indicated that the methanolic extract reduces carbon tetrachloride-induced oxidative stress in Wistar rats, significantly decreasing lipid peroxidation and restoring antioxidant enzymes, especially at a dose of 400 mg/kg. These findings suggest that *Feronia limonia* fruit may serve as a valuable natural resource for pharmaceutical applications. Further research, including chronic toxicity assessments and clinical trials, is necessary to confirm its efficacy and safety.

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https://doi.org/10.47552/ijam.v16iS2.6206 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Unlocking the Anticancer Potential of Metal-Curcumin Complexes: Docking Insights with EGFR

Research Article

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Abstract

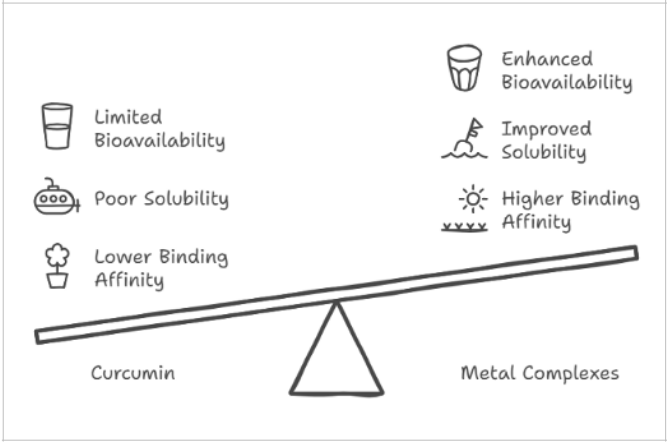
The Epidermal Growth Factor Receptor (EGFR) plays a pivotal role in cervical cancer progression by driving uncontrolled cell proliferation and survival. Given its oncogenic significance, EGFR remains a prime therapeutic target for cancer drug development. Curcumin, a bioactive polyphenol, exhibits promising anticancer properties but suffers from poor solubility and bioavailability, limiting its clinical application. To enhance its pharmacological potential, metal complexation has been explored as a strategy to improve its pharmacokinetics and bioactivity. This study employed molecular docking to assess the binding affinities of curcumin and its metal complexes (Ca, Cu, Mg, Na, Zn) with two EGFR conformations: the active kinase domain (PDB ID: 1M17) and the extracellular ligand-binding domain (PDB ID: 4ZSE). Docking analyses revealed that Ca-Bicurcumin (-11.1 kcal/mol), Na-Bicurcumin (-11.0 kcal/mol), and Cu, Mg, and Zn-Bicurcumin complexes (-10.8 kcal/mol each) exhibited strong binding affinities toward 1M17, outperforming the reference ligand AQ4 (-7.0 kcal/mol) and native curcumin (-7.3 kcal/mol). Similarly, Cu-Bicurcumin (-11.0 kcal/mol), along with Ca, Mg, and Na-Bicurcumin complexes (-10.9 kcal/mol each), displayed superior interactions with 4ZSE, exceeding the binding of ANP (-9.4 kcal/mol) and curcumin (-7.2 kcal/mol). These enhanced interactions resulted from strong hydrogen bonding, hydrophobic interactions, and electrostatic forces, improving receptor complementarity. The findings suggest that metal-curcumin complexes hold promise as EGFR-targeted therapeutics, potentially overcoming curcumin's pharmacokinetic limitations while enhancing its anticancer efficacy. Further, in vitro kinase inhibition assays and in vivo tumor regression studies are necessary to validate their therapeutic potential in EGFR-driven cancers.

Keywords: EGFR, Molecular Docking, Metal-Curcumin Complexes, Kinase Inhibition, Targeted Anticancer Therapy.

Introduction

Cancer remains among the most difficult problems in modern healthcare, with its multifactorial etiology and intricate molecular pathways making things complicated to combat effectively. (1) It remains one of the most challenging diseases in modern medicine, and significant efforts are directed toward discovering novel and effective therapeutic agents.(2) Among various molecular targets, the Epidermal Growth Factor Receptor (EGFR) has garnered substantial attention because it encourages tumor proliferation, angiogenesis, and metastasis.(3) EGFR is a transmembrane tyrosine kinase receptor that, upon ligand binding, triggers downstream signaling pathways, namely the PI3K-AKT, RAS-RAF-MEK-ERK, and JAK-STAT pathways, that leads to uncontrolled cell proliferation, angiogenesis, and metastasis.(4,5) Overexpression and abnormal activation of EGFR have been linked to various malignancies, including lung, breast, and colorectal cancers, making it a prime target for anticancer drugs development. (6)

Figure 1: Compairing Curcumin and its metal Complexes in EGFR Targeting



Curcumin, a bioactive polyphenol extracted from Curcuma longa's rhizome, has long been studied for its multifaceted pharmacological properties, including anti-inflammatory, antioxidant, and anticancer activities. (7,8) However, poor bioavailability, rapid metabolism, and limited solubility have hindered its clinical

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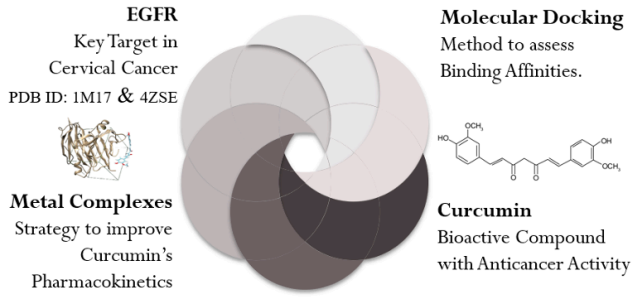
translation.(9) With the ultimate goal to get beyond these restrictions, researchers have looked into the potential of curcumin-metal complexes, which exhibit enhanced stability, solubility and bioactivity.(10,11) In recent years, the integration of bioactive natural compounds with metal ions has gained considerable attention for enhancing therapeutic efficacy in cancer treatment. Curcumin's coordination with transition metal ions, like copper (Cu), zinc (Zn), and platinum (Pt) has shown promising improvements in its anticancer efficacy. (10,12)

Figure 2: Strategy to Overcome Curcumins Limitation



Molecular docking analyses have provided valuable insights into the binding interactions between these complexes and the EGFR tyrosine kinase domain, revealing greater affinity for binding than free curcumin.(13) These findings suggest that metal coordination stabilizes the curcumin structure and facilitates stronger interactions for their anticancer potential. They also propose novel therapeutic candidates for EGFR-targeted cancer treatment. The insights gained from this study may pave the way to rationally develop metal-curcumin compounds as effective anticancer agents, offering new avenues in targeted cancer therapy.(14)

Materials and Methods

Platform for molecular docking

The computational docking assessment of native curcumin and metal-curcumin complexes was conducted using Chimera (version 1.17.3) integrated with AutoDock Vina (version 1.5.7). (15)

Selection of Target Protein Structure

The molecular docking study was conducted using two crystallized structures of the EGFR: **PDB ID: 1M17** and **PDB ID: 4ZSE**. (16,17) These structures are sourced from the Protein Data Bank (PDB).

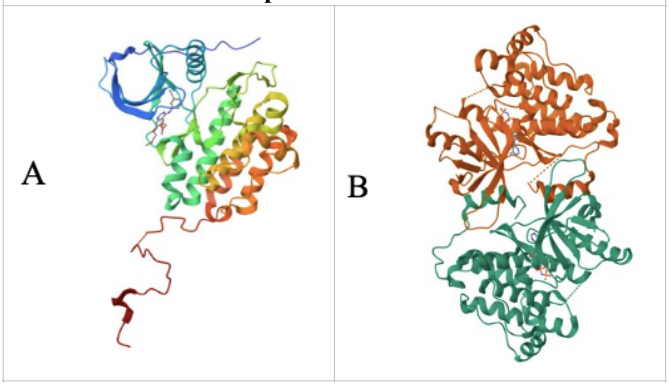
- **PDB ID: 1M17** represents EGFR tyrosine kinase bound [S1] with 4-anilinoquinazoline inhibitor erlotinib.(18)
- **PDB ID: 4ZSE**[S2] [SS3] represents the EGFR 696-1022 T790M/V948R Crystal structure form II. (19)

Both structures were chosen to evaluate the selectivity and binding affinity of **native curcumin and metal-curcumin complexes** against different forms of EGFR, allowing for a comprehensive assessment of their potential as EGFR inhibitors

Preparation of Protein Structures

To assure appropriate docking simulation conditions, the selected EGFR protein structures (1M17 and 4ZSE) were preprocessed using AutoDockTools. This included eliminating water molecules, incorporating polar hydrogens, assigning charges to optimize electrostatic interactions, and defining a grid box to ensure that the ligand was positioned at the functionally relevant location. (20)

Figure 3: Ribbon Representations of EGFR Structures – (A) 1M17 Complexed with AQ4 and (B) 4ZSE Complexed with ANP.



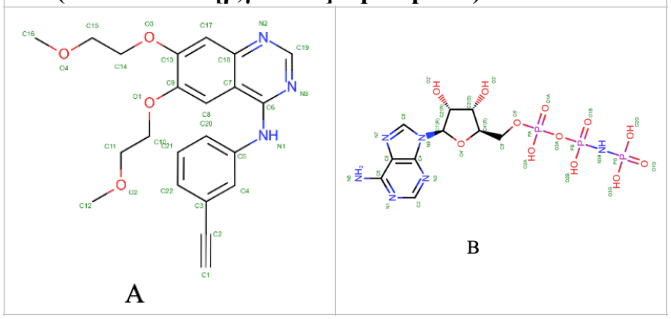
Ligand Preparation

The ligands were made by adding partial charges and polar hydrogens to the compounds to bring them to the necessary protonation state at physiological pH.

Native Ligands: AQ4 and ANP

The native ligands AQ4 and ANP, which are co-crystallized inhibitors of EGFR (PDB IDs: 1M17 and 4ZSE, respectively), were extracted directly from their respective crystal structures. These ligands were pre-processed by eliminating water molecules, allocating Gasteiger charges, and adding polar hydrogens to maintain their biologically active form. Energy minimization was performed using UCSF Chimera with the Amber ff14SB force field to ensure an optimal starting geometry before docking analysis.(21)

Figure 4: Chemical Structures of Native Ligands – (A) AQ4 (4-Anilinoquinazoline Derivative) from 1M17 and (B) ANP (Adenosine-5'-[β,γ-imido]triphosphate) from 4ZSE.



Native Curcumin

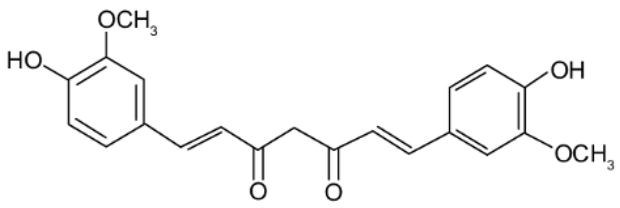
We used the PubChem database to obtain Curcumin's 3-D structure (CID: 969516).(22) The ligand was optimized using the Amber ff14SB force

https://doi.org/10.47552/ijam.v16iS2.6206 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

field UCSF Chimera software to ensure an accurate conformational state.

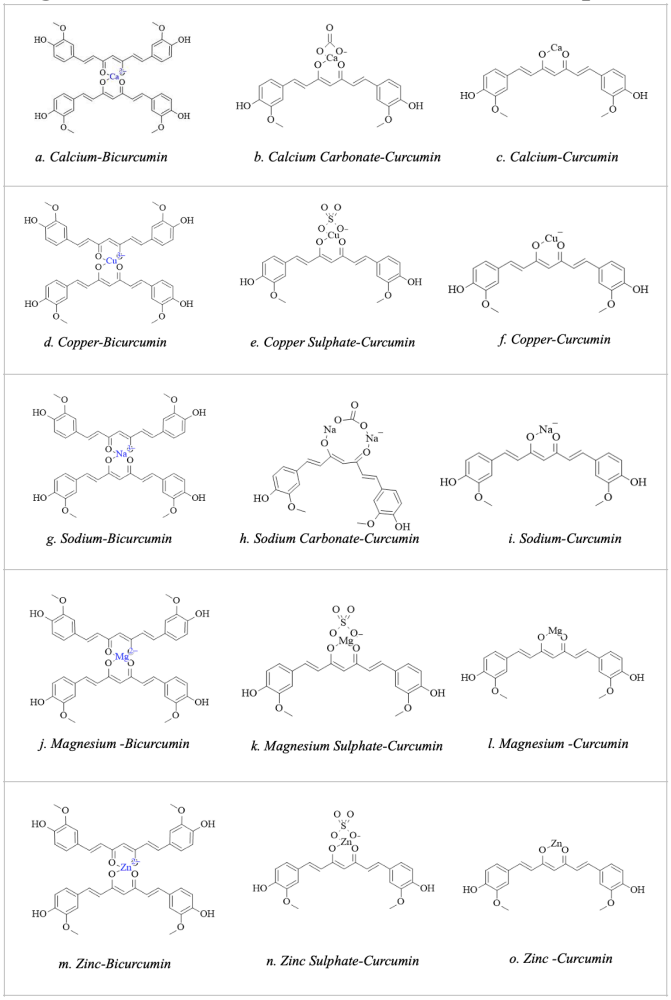
Figure 5: Structure of Curcumin



Metal-Curcumin Complexes

To investigate the role of metal coordination in EGFR inhibition, curcumin-metal complexes were modeled using Chemsketch, optimized using Avagadaro, and transformed into 3D structures with Open Babel. (23) The following metal-curcumin complexes were considered:

Figure 6: Structures of Metal Curcumin Complexes



The optimized geometries were converted into PDBQT format using AutoDock Tools for docking.

Molecular Docking Studies

Docking studies used the EGFR, (PDB IDs: 1M17 and 4ZSE) as target proteins, with each ligand appropriately prepared. Based on complex geometry and binding interactions, the predicted binding energy was analyzed using AutoDock Vina integrated with UCSF Chimera (v1.17.3). Default parameter values were applied for all docking simulations. The grid box coordinates were defined based on the binding site of the co-crystallised ligand AQ4 in PDB ID: 1M17 and ANP in PDB ID: 4ZSE. This ensures that all docking simulations were focused on the experimentally validated active site, allowing for more accurate and meaningful predictions of ligand–receptor interactions. The docking grid box for 1M17 was set to (25 × 25 × 25) Å, centered at (22.014, 0.253, 52.794) Å, while for 4ZSE, the grid box was set to (25 × 25 × 25) Å, centered at (2.367, 20.251, -33.251) Å. The docking results were examined using the "View Dock" tab for further analysis.(24)

Results

Analysis of Molecular Docking of Curcumin and Metal-Curcumin Complexes with EGFR

Figure 7: Color Scale for Docking Scores – Green (strongest binding) to Red (weakest binding)

Table 1: Docking Scores of Native ligand, Curcumin and Metal-Curcumin Complexes Against EGFR (PDB IDs: 1M17 & 4ZSE).

PDBID		1M17	4ZSE
Ligand		AQ4	ANP
Docking Score		-7.0	-9.4
Curcumin	Cur	-7.3	-7.2
Ca BICURCUMIN	Ca-1	-11.1	-10.9
$CaCO_3$	Ca-2	-9.4	-9.4
Ca. Curcumin	Ca-3	-8.5	-8.8
Cu BICURCUMIN	Cu-1	-10.8	-11.0
CuSO4	Cu-2	-9.8	-9.7
Cu. Curcumin	Cu-3	-8.7	-8.9
Mg BICURCUMIN	Mg-1	-10.8	-10.9
MgSo4	Mg-2	-9.7	-9.4
Mg. Curcumin	Mg-3	-8.4	-8.8
Na. BICURCUMIN	Na-1	-11.0	-10.9
Na2Co3	Na-2	-10.0	-9.1
Na. Curcumin	Na-3	-8.4	-8.9
Zn BICURCUMIN	Zn-1	-10.8	-10.8
ZnSO4	Zn-2	-9.6	-10.1
Zn. Curcumin	Zn-3	-8.5	-8.8

Molecular docking studies have been performed to assess the binding interactions of native curcumin and metal-curcumin complexes with EGFR using the crystal structures 1M17 (wild-type EGFR) and 4ZSE (mutant EGFR). The binding affinities, represented as docking scores (kcal/mol), are summarized in the Table 1. Lower docking scores indicate stronger binding interactions between the ligand and the receptor.

Discussion

Comparing the Binding Affinity of Curcumin and Native Ligands (AQ4 and ANP)

The reference ligands AQ4 (for 1M17) and ANP (for 4ZSE) exhibited docking scores of -7.0 kcal/mol and -9.4 kcal/mol, respectively. These values serve as benchmarks for Assessing the efficacy of f curcumin and its metal complexes in EGFR binding.

Native curcumin showed binding scores of -7.3 kcal/mol (1M17) and -7.2 kcal/mol (4ZSE), which are comparable to AQ4 but significantly weaker than ANP. This suggests that while curcumin has some affinity for EGFR, it is not as potent as the native ligand ANP in targeting the mutant EGFR (4ZSE).

Enhanced Binding of Metal-Curcumin Complexes Compared to Curcumin and Native Ligands

The introduction of metal ions significantly enhanced the binding affinity of curcumin derivatives,

with several metal-curcumin complexes outperforming both AQ4 and ANP. The most notable improvements were observed in metallic bicurcumin complexes (BICURCUMIN derivatives), particularly those containing calcium (Ca-1: -11.1 kcal/mol for 1M17, -10.9 kcal/mol for 4ZSE), sodium (Na-1: -11 kcal/mol for 1M17, -10.9 kcal/mol for 4ZSE), copper (Cu-1: -10.8 kcal/mol for 1M17, -11 kcal/mol for 4ZSE), magnesium (Mg-1: -10.8 kcal/mol for 1M17, -10.9 kcal/mol for 4ZSE), and zinc (Zn-1: -10.8 kcal/mol for both 1M17 and 4ZSE).

Among all complexes, all BICURCUMIN Complexes exhibited the strongest binding affinity for 1M17 and 4ZSE, surpassing both AQ4 and ANP. This indicates that BICURCUMIN complexes may serve as a more potent EGFR inhibitor than the native ligand.

Effect of Metal Salts on Binding Affinity

Curcumin complexes with metal salts ($CaCO_3$, $CuSO_4$, $MgSO_4$, Na_2CO_3, $ZnSO_4$) exhibited moderate binding affinities, with scores ranging between -9.4 and -10.1 kcal/mol. These values are still significantly lower (better binding) than native curcumin (-7.3 kcal/mol) and AQ4 (-7 kcal/mol), but slightly weaker than ANP (-9.4 kcal/mol).

For instance, while Cu-BICURCUMIN (Cu-1) demonstrated -10.8 kcal/mol (1M17) and -11 kcal/mol (4ZSE), $CuSO_4$-Curcumin (Cu-2) exhibited slightly weaker binding scores of -9.8 kcal/mol (1M17) and -9.7 kcal/mol (4ZSE). A comparable pattern was noted for $MgSO_4$-Curcumin (-9.7 kcal/mol for 1M17, -9.4 kcal/mol for 4ZSE) compared to Mg-BICURCUMIN (-10.8 kcal/mol for 1M17, -10.9 kcal/mol for 4ZSE). This indicates that free metal ions contribute more effectively to EGFR binding than metal salts.

Comparison of Binding Affinities Across EGFR Variants

Overall, all metal-curcumin complexes exhibited stronger binding to EGFR than both native curcumin and AQ4. Interestingly, the docking scores for mutant EGFR (4ZSE) were slightly better than those for wild-type EGFR (1M17) across most complexes, suggesting that metal-curcumin derivatives may be particularly effective against EGFR-driven drug resistance.

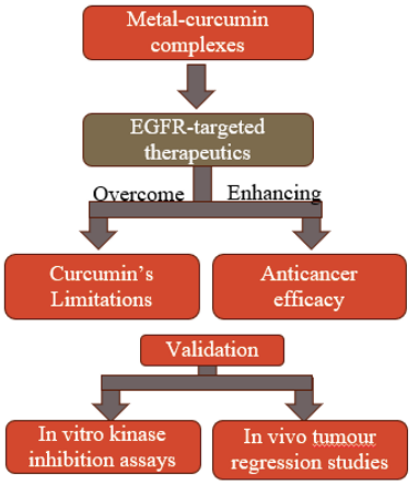
For instance, Cu-BICURCUMIN (-11 kcal/mol) and $ZnSO_4$-Curcumin (-10.1 kcal/mol) showed higher affinity for 4ZSE than ANP (-9.4 kcal/mol), indicating their potential as superior inhibitors for mutant EGFR.

Role of Individual Metal Ions in Binding Strength

- **Copper (Cu)**: Exhibited the strongest interactions, particularly in Cu-BICURCUMIN (10.8 kcal/mol for 1M17, -11 kcal/mol for 4ZSE), highlighting its strong chelation effects with EGFR active site residues.
- **Calcium (Ca)** and **Sodium (Na)**: Also demonstrated high docking scores (-11.1 kcal/mol for Ca-1 and -11.0 kcal/mol for Na-1) for 1M17 and (-10.9 kcal/mol for both Ca-1 and Na-1) for 4ZSE, suggesting significant contributions from electrostatic and coordination interactions.
- **Magnesium (Mg)** and **Zinc (Zn)**: Showed similar trends, with Mg-BICURCUMIN (-10.8 kcal/mol for 1M17, -10.9 kcal/mol for 4ZSE) and Zn-BICURCUMIN (-10.8 kcal/mol for both 1M17 and 4ZSE), reinforcing the importance of metal incorporation in curcumin derivatives.

Conclusion

Figure 8: Flowchart Representation of the Conclusion and Validation Strategy for Metal-Curcumin Complexes as EGFR-Targeted Therapeutics.



The molecular docking studies of curcumin and metal-curcumin complexes with the EGFR provide compelling evidence that metal coordination significantly enhances binding affinity when compared to native curcumin. Among the tested complexes, Cu-BICURCUMIN (-11 kcal/mol for 4ZSE) exhibited the strongest binding affinity, outperforming the native ligand ANP (-9.4 kcal/mol), indicating its potential as a more potent EGFR inhibitor. Additionally, Ca-BICURCUMIN and Na-BICURCUMIN also demonstrated exceptional binding interactions, reinforcing the role of metallic coordination in improving curcumin's anticancer properties.

A comparative analysis of docking scores between wild-type EGFR (1M17) and mutant EGFR (4ZSE) suggests that metal-curcumin complexes may be particularly effective against EGFR mutations associated with drug resistance. This finding is crucial, as EGFR mutations are a major cause of resistance to conventional tyrosine kinase inhibitors (TKIs) used in cancer therapy.

Overall, this study highlights the therapeutic potential of metal-curcumin complexes as novel EGFR inhibitors, with Cu, Ca, Na, Mg, and Zn-based curcumin derivatives exhibiting significantly improved binding affinities over native curcumin. These results provide a strong basis for further preclinical and experimental studies aimed at developing metal-curcumin-based anticancer drugs.

The promising docking results of metal-curcumin complexes against EGFR highlight their potential as next-generation anticancer agents. Additional validation by molecular dynamics simulations, in vitro as well as

vivo research, and mechanistic investigations is necessary. Structural modifications, QSAR modeling, and combination therapy with TKIs could enhance efficacy and overcome resistance. Additionally, Nanoparticle-based delivery methods may improve bioavailability and targeted action. With continued research, these complexes hold significant promise for clinical translation as novel EGFR inhibitors.

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https://doi.org/10.47552/ijam.v16iS2.6213 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Herbo Glow: Formulation and Evaluation of a Botanical Face Pack

Research Article

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Abstract

Aim/Objectives: The main goal of this research was to create and test a herbal face pack that would improve the health and appearance of the skin by utilising all-natural components. The goal of the research is to create a face pack out of all-natural herbs as a substitute for manufactured cosmetics. Materials: As for the contents, we formulated it using a blend of popular herbal herbs that are well recognised for their beneficial effects on the skin. The skin-nourishing, antibacterial, and brightening characteristics of turmeric, neem, sandalwood, orange peel, and rose petals were among them. Multani mitti, also known as Fuller's Earth, was one of them. Methods: A fine face pack formulation was created by drying, powdering, and blending the chosen herbs in the suitable amounts. Testing for organoleptic properties, particle size, pH, smoothness, spreadability, grittiness, and stability were all part of the physicochemical assessment of the finished product. Furthermore, patch testing was used to assess irritancy in the participants. Results: The physical properties of the herbal face pack were found to be desirable, including a fine texture, an appropriate pH, decent spreadability, and the absence of grittiness. After 30 days, the product's physical and chemical properties had not changed. While testing on volunteers, we did not find any indications of skin irritation or negative consequences. Discussions: The findings indicate that the herbal face pack offers the intended cosmetic advantages, such nourishing the skin, enhancing its radiance, and washing it, without the hazards of synthetic ingredients. Using only pure herbal powders guarantees that the composition is safe, biocompatible, and effective. Conclusion: In conclusion, the results show that it is possible to create and test a cosmetic face pack that is completely herbal. To keep skin healthy and radiant, the mixture provides an alternative to synthetic cosmetic face packs that is safe, natural, and effective.

Keywords: Herbal Face Pack, Natural Ingredients, Skin Nourishment, Cosmetic Formulation, Glowing Skin.

Introduction

Cosmetics are described as items designed for washing, beautifying, or augmenting one's look. Although synthetic cosmetics prevail in the market, apprehensions over chemical-induced skin irritation, chronic toxicity, and environmental consequences have generated interest in herbal alternatives. Historically, herbs have been used in cosmetics for their natural bioactive chemicals, providing therapeutic advantages including anti-inflammatory, antibacterial, and antioxidant properties. Contemporary dermatological concerns, including acne, blackheads, pimples, and dark circles, are often ascribed to variables such as oxidative stress, microbial activity, and inadequate skin cleanliness (2).

Ayurvedic teachings assert that several skin illnesses originate from blood impurities, often caused by inadequate food and lifestyle choices. Herbs like as Manjistha (Rubia cordifolia) and Chandana (Santalum album) are conventionally acknowledged for their blood-purifying and skin-calming attributes. These botanical substances are the foundation of "mukhalepa" - an Ayurvedic facial treatment that employs the topical use of herbal pastes to address skin ailments and improve complexion (6).

This study's selection of herbal constituents was informed by both traditional Ayurvedic knowledge and their pharmacological significance. Ingredients were selected for their proven effectiveness in treating prevalent skin issues and their capacity to provide vital nutrients via transdermal absorption. The objective was to develop a face pack that nourishes the skin, improves microcirculation, and fosters a natural radiance without the negative effects of synthetic substances (8).

Herbal face packs operate via many mechanisms: cleaning skin pores, tightening tissues, absorbing excess oil, and delivering a calming and revitalising impact. Upon drying, these formulations create a film that compresses and eliminates collected debris and dead cells upon removal. Due to its transient effects, consistent application (2–3 times weekly) is advised for prolonged skin health and aesthetics. Consequently, the present formulation seeks to integrate scientifically substantiated herbs into a synergistic composition to provide a safe and effective option for regular skincare (8, 11, 12, 14).

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Materials and Methods

The crude drugs used in this study of our research were procured from the nearby local area. The entire ingredient used were cleaned, washed, dried and powdered finely for preparation of face pack. The following ingredients were used for preparation of this herbal face pack formulation.

Multani Mitti (Calcium Bentonite)

Multanimitti helps skin in different way like diminishing pore size, removing blackheads and whiteheads, soothing sunburns, cleaning skin, improving blood circulation, complexion, reducing acne and blemishes and gives a glowing effect to a skin as they contain healthy nutrients. Its cooling action soothes the skin, and give relief to inflammation caused by aggravated pitta. It removes dirt and dead skin cells accumulated and replace with fresh radiant and glowing skin.

Figure 1: Multani Mitti (Calcium Bentonite)

Neem (*Azadirachta Indica*)

Neem has antibacterial, anti-inflammatory, antiseptic and highly beneficial for acne prone skin and oily skin. An Antiacneproperty is due to the antioxidant, anti-inflammatory and anti-microbial activity of various phyto-constituents of neem.

Neem is very popular as a medicinal plant. And its leaves and extracts are commonly used for their antiseptic, anti-inflammatory, antioxidant, and healing properties. It is a great source of fatty acids, vitamins, and minerals that are all needed for healthy skin. Moreover, neem contains active constituents like nimbidin, nimbolide and azadirachtin that can help to get rid of many skin problems.

The anti-bacterial and anti-fungal properties of neem protect the skin and help in lightening the blemishes or scars left behind by acne or pimples.

Figure 2: Neem and its powder

Sandal wood (*Santalumalba*)

Sandalwood has an anti-tanning and anti-aging property. It also helps skin in many ways like toning effect, emollient, antibacterial properties, cooling astringent property, soothing and healing property. (12) Sandalwood protects the skin against the impact ofenvironmental pollution and keep the skin cool, fair and healthy. Sandalwood is helpful Ayurvedic herb withantimicrobial properties is used for healing various skin problems and removes scars.

Fig. 3: Sandal wood and coarse powder

Rose petals powder

Rose is rich with anti-bacterial potential. It also have good amount of antioxidants. Helpful to get a radiant and glowing skin. Dried rose petals or rose water is one of the age-old magic blessings for beauty and cosmetics as a very herbal and natural approach. Amazing anti-bacterial properties of rose petals can reduce skin irritations, and redness and help to reduce acne troubles. It's also having powerful antiseptic properties which is why many medicines have rose petal extractions. Its sweet-smelling rose scent actually has antidepressant and antianxiety properties, which can help as a mood enhancer and relax you from anxiety when you apply it as a mask on the face or hair.

It also helps to sleep better. It hydrates and moisturizes the skin, delivering a rejuvenated and radiant face and skin. It has anti-aging properties, which is able to decrease the glances of wrinkles upon daily usage. Rose petal powder is extremely safe to use with no threats, except if you are allergic to roses. (7)

Fig. 4: Dried rose petals powder

Table 1: Composition of the herbal face pack formulation, detailing the ingredients, their respective quantities for a 50 g batch, and associated dermatological activities (6)

Sr. No.	Name of Ingredients	Quantity (For 50gm)	Activity
1	Multanimitti powder	24gm	Complexion
2	Neem leaves powder	12gm	Antiacne, antimicrobial
3	Sandal wood powder	7gm	Anti-tanning, Soothing agent
4	Rose petals powder	7gm	Anti-aging agent

https://doi.org/10.47552/ijam.v16iS2.6213 ISSN No: 0976-5921

Figure 5: Formulation ingredients

Method

Concentration of each ingredient was mentioned in Table 1. The accurate quantity of ingredients was weighed and ground into fine powder by using sieve no.120. Then all the ingredients were mixed uniformly. Then the prepared face pack was stored and labelled for further studies or tests.

Procedure for Face Pack Application

- Formulation was prepared according to Table no.1.
- Take prepared face pack powder in a bowl as per requirement, add water (rose water) to mix it well up-to forming a smooth paste.
- Apply this paste over a face skin which covers acne, blackheads and whiteheads.
- Keep it for 20-30 Min and then wash the face with cold water.

Evaluation (6, 11)

To evaluate the goodness of our prepared face pack we performed following evaluation parameter.

Organoleptic Evaluation

Physical Parameter of our face pack such as colour, odour, appearance and texture were checked visually.

Physical Evaluation

Total Ash

Place about 2 g of ground air dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). Spread the material in an oven layer and ignite it by gradually increasing the heat to 500-600 °C until itis white, indicating the absence of carbon. Cool in a dessicator and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrateR. Dry on a water-bath, then on a hot-plate and ignite to constant weigh. Allow the residue to cool in a suitable dessicator for 30 min ad then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

Determination of Moisture Content

Weigh about 2 gm of powdered face pack into a weighed flat and thin porcelain dish. Dry it in Hot Air Oven at 100 °C-105°C, until two consecutive weighing do not differ by more than 0.5 mg. Cool in dessicator and weigh the loss in weight is usually recorded as moisture.

Particle Size

Particle size is a parameter, which affect various properties likespread ability, grittiness, etc., particle size was determined bysieving method by using I.P. Standard sieves by mechanicalshaking for 10 min.

Angle of Repose

The angle of repose is used to quantify the flow properties of powder because it influences cohesion among the particles. The fixed funnel cone method employs the calculation of height (H) above the glass paper that is placed on a flat tabular surface. The powdered was carefully poured through the funnel till the peak of the conical heap just touched the tip of the funnel.

For the above method, the angle of repose (¢) can be calculated by using the formula.

$$¢ = \tan^{-1}(H/R)$$

Where, ¢ - Angle of repose, H - Height of the heap, R - Radius of the base

Bulk Density

Bulk Density is the ratio between the given mass of a powder and its bulk volume. Required amount of the powder is dried and filled in a 50 ml measuring cylinder up to 50 ml mark. Then the cylinder is dropped onto a hard wood surface from a height of 1 inch at 2 sec intervals. The volume of the powder is measured. Then, the powder is weighed. This is repeated to get average values. The Bulk Density is calculated by using the below given formula.

$$\text{Bulk Density} = \text{Mass/Volume}$$

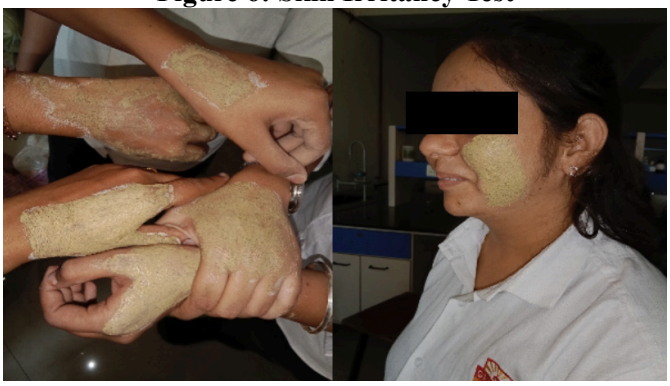
Tapped Density

Tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped for 1min and volume or mass readings are taken until little further volume or mass change was observed. It was expressed ingrams per cubic centimetre (g/cm3).

Irritancy test

Mark an area (1sq.cm) on the left hand dorsal surface. Definite quantities of prepared face packs were applied to the specified area and time was noted. Irritancy, erythema & edema, was checked and reported.

Figure 6: Skin Irritancy Test



Stability studies

Stability testing of prepared formulation was conducted by storing at different temperature conditions for the period of one month. The packed glass vials of formulation stored at different temperature conditions using stability chamber viz., Room temperature, 35ºC and 40ºC and were evaluated for physical parameters like Colour, Odor, pH, Consistency and feel.20

Washability

This is the common method for checking the washability of the formulation were applied on the skin and then ease and extent of washing with water were checked manually by using 1 litre of water is used to remove all content of the formulation were applied on the surface.

Figure 7: Formulation (Herbal face pack)

Results and Discussion

The result of evaluation test carried out of a face pack which includes nature, colour, odour, taste, texture, ash values, mixture, contents and pH of dried powder provide information about organoleptic and physiochemical evaluation.

Table 2: Evaluation of Herbal Face Pack

Sr. No.	Evaluation Parameter	Observation
Organoleptic evaluation		
1	Appearance	Powder
2	Colour	Yellowish brown
3	Odour	Pleasant
4	Taste	Characteristic
5	Texture	Fine
Physicochemical evaluation		
1	Total ash	1.58 gm
2	Acid insoluble ash	0.21 gm
3	Moisture content (LOD)	0.38
4	pH	7.3
General powder characteristics		
1	Particle size by SEM (Scanning Electron Microscopy)	25 -30 µm
2	Angle of Repose	1.125^0
3	Bulk density	10 gm/ml
4	Tapped density	2.7 gm/ml
5	Grittiness	No gritty particles were found when mixed with water
6	Nature of face after wash	Soft and fresh, Clean from dirt.
Irritancy test		
1	Irritation	No irritation observed
2	Redness	No Redness observed
3	Swelling	No swelling observed

Conclusion

This study's findings indicate that the developed herbal face pack provides several skin advantages due to the bioactive phytochemicals included in the chosen components. The face pack demonstrated advantageous physicochemical characteristics, including a smooth texture, acceptable pH for facial skin, homogenous particle size, and excellent spreadability. No indications of irritation were noted during patch testing, demonstrating its safety for topical use. Anecdotal response from participants indicated improvements in skin smoothness, luminosity, and a decrease in acne severity with consistent use.

The discovered benefits, including antibacterial, anti-acne, and anti-wrinkle capabilities, may be scientifically linked to the active compounds found in the herbal components. Neem and turmeric are known for their antibacterial and anti-inflammatory properties, whilst Multani mitti aids in oil absorption and exfoliation. These results correspond with the known dermatological characteristics of the used herbs.

The research confirms that the formulated herbal face pack is cost-efficient, non-toxic, and beneficial in improving skin health by restoring shine, boosting texture, and preventing acne. The formulation satisfied all assessed criteria, indicating its viability as a safe and natural substitute for synthetic cosmetic items.

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https://doi.org/10.47552/ijam.v16iS2.6241 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Comparative Evaluation of In-House Prepared and Marketed Chaturbeeja Churna

Research Article

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Abstract

The standardization and quality control of herbs and herbal dosage forms with proper integration of modern scientific technique and traditional knowledge is important. The term 'standardization' refers to all actions undertaken during manufacturing and quality control to ensure consistent and reproducible product quality. The present study focuses on the comprehensive comparative evaluation of in-house prepared and marketed Chaturbeeja Churna. Chaturbeeja Churna is a classical ayurvedic formulation traditionally used for managing metabolic disorders. The formulation, comprises of four seeds viz. *Trigonella foenum-graecum* (Fenugreek), *Nigella sativa* (Kalonji), *Lipidium sativum* (Chandrasura), and *Trachyspermum ammi* (Ajwain). Each plant material were subjected to rigorous physicochemical, phytochemical, and quantitative estimation of secondary metabolites. These evaluation helps to assess quality, safety, and therapeutic potential of all the four seeds to be used in churna preparation. The in-house chaturbeeja churna was prepared according to the ayurvedic text. Standardized laboratory protocols were employed on Churna to determine parameters such as organoleptic, micromeritics, physicochemical and phytochemical properties. High-performance thin-layer chromatography (HPTLC) profiling was performed to evaluate chemical fingerprinting and ensure batch-to-batch consistency. The in-house formulation demonstrated superior physicochemical properties and a richer phytochemical profile, indicating better therapeutic efficacy. The marketed sample showed variability in certain physicochemical parameters, suggesting the need for stringent quality control practices. This study underscores the importance of standardization and quality assessment in the preparation of Ayurvedic formulations and provides a validated framework for future quality control of Chaturbeeja Churna and similar polyherbal products.

Keywords: *Chaturbeeja churna,* Ayurvedic formulation, *Methika, Chandrasura, Kalonji, Yavanika*.

Introduction

Chaturbeeja Churnam is a unique Ayurvedic formulation composed of four herbs that are Methika, Chandrashur, Kalonji and Yavanika mentioned in the ancient Ayurvedic text, *Bhavaprakasha Nighantu*. These herbs known for treating menstrual disorders, showcasing its curative properties in traditional medicine practices. It is primarily utilized for treating *Vataroga* (disorders related to the Vata dosha), as well as a range of conditions including *Ajeernam* (indigestion), *Soola* (pain), *Adhmanam* (abdominal distention), *Parshwashulam* (flank pain), and *Katishulam* (low back pain). (1)

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Evidence-Based Approach

The relevant literature for this study was sourced from the *Bhavaprakasha Nighantu* as well as databases like Google Scholar, PubMed, Web of Science, and the Ayush Portal. This evidence-based approach helps in understanding the formulation's effects in light of both classical and contemporary research.

Methika

Trigonella foenum-graecum, commonly known as fenugreek, belongs to the *Fabaceae* family and is cultivated extensively across India. While the name "Methika" may not be directly mentioned in the classical *Brihat Trayees*, fenugreek seeds have long been recognized in Ayurvedic medicine for their wide-ranging therapeutic properties. Traditionally, fenugreek has been used as a carminative, demulcent, expectorant, laxative, and stomachic agent.The seeds contain several bioactive compounds, including steroidal saponins such as diosgenin and gitogenin, essential oils, and proteins. These constituents contribute to its broad pharmacological effects, which include antioxidative, antineoplastic, anti-inflammatory, anti-ulcerogenic,

antipyretic, immunomodulatory, and anti-tumor properties.(2) Notably, fenugreek has been shown to interact with key proteins that activate the EGFR/AKT/mTOR signaling pathway, indicating its potential in managing hyperglycemia and hyperlipidemia.(3) Furthermore, polysaccharides like galactomannans have demonstrated anti-diabetic effects,(4) while the amino acid 4-hydroxy isoleucine possesses insulin-mimetic properties.(5)

Chandrasura

Lepidium sativum, or cress, is an annual herb belonging to the *Cruciferae* family. It is commonly used in Arab countries for treating respiratory conditions such as bronchitis and asthma.(6) The herb exhibits a variety of therapeutic properties, including antibacterial, aphrodisiac, diuretic, expectorant, gastrointestinal stimulant, gastroprotective, and laxative effects. Additionally, it is used for managing conditions like hemorrhoids, constipation, and swelling.(7) Chemical constituents like lepidine, imidazole, oleic acid, linoleic acid, and ascorbic acid contribute to its diverse pharmacological profile. Studies have demonstrated its antihypertensive, anti-inflammatory, analgesic, anticoagulant, antirheumatic, and hypoglycemic activities, among others. These findings support the traditional use of *Chandrasura* in a wide range of ailments.(8)

Kalonji

Nigella sativa, known as black cumin, is an annual herb from the *Ranunculaceae* family, renowned for its numerous therapeutic effects. The seeds are rich in essential oils containing compounds such as thymoquinone, cymine, nigellone, and carvone. Black cumin has been widely studied for its antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties. It also possesses diuretic, antihypertensive, anticancer, and hepatoprotective activities. The therapeutic properties of *Kalajaji* are attributed largely to thymoquinone, a major active constituent, which has shown promise in the treatment of conditions such as asthma, bronchitis, rheumatism, gastrointestinal disorders, and skin diseases. Its wide range of effects also includes its use as an appetite stimulant, anti-diarrheal, and for managing parasitic infections.(9)

Yavanika

Trachyspermum ammi, or ajwain, belongs to the *Umbelliferae* family and is commonly used both as a culinary spice and for its medicinal properties. In Ayurveda, it is classified under *Sulaprasamanam*, which refers to remedies for alleviating pain, especially abdominal discomfort. *Yavanika* is known for its effectiveness in treating digestive issues such as indigestion, flatulence, and abdominal cramps.(10) This herb has a variety of therapeutic benefits, including bronchodilatory, antitussive, and antimicrobial properties. It is also used for its carminative effects, helping to relieve bloating and gas, and for treating gastrointestinal disorders like acid reflux, abdominal tumors, and infections caused by *Helicobacter pylori*. (11) Additionally, *Yavanika* has been shown to possess anti-carcinogenic, diuretic, and antidiarrheal effects.(12)

Comparison Between Modern Medicine and Ayurveda

The Ayurvedic and modern medical perspectives on the therapeutic uses of the herbs in *Chaturbeeja Churnam* align in many ways, though the mechanisms may be described differently. While modern science highlights the specific biochemical compounds and their effects on disease pathways, Ayurveda provides a broader framework that incorporates the balance of doshas and the qualities (guna) of each herb. Herbs like *Methika* are primarily used to manage *Kapha* disorders with additional effects on *Vata*, while *Chandrasura*, *Kalajaji*, and *Yavanika* are similarly effective in treating conditions associated with *Kapha* and *Vata* imbalances. (13-16)

This comparison between modern research and Ayurvedic principles opens the door for further exploration and integration of these healing systems. Therefore, in the present study the chaturbeeja churna was prepared in the laboratory as per the standard procedure from traditional literature and was evaluated simultaneously by comparing it with the marketed chaturbeeja churna. The evaluation of the sample was done by various analytical methods.

Materials and Methods

Procurement of plant materials and formulation:

The plant materials methika, chandrashur, kalonji and yavanika were purchased from Shivshankar Ayurvedic Pharmacy. All the plant materials were cleaned and dried properly. They were powdered using Wiley mill (HICON), passed through a #80 mesh sieve and stored separately in air tight containers. The marketed Chaturbeeja churna of brand Shri. Navjeevan Rasayanshala was purchased from amazon.

Figure 1: Plant Materials

Methika Chandrashur Kalonji Yuvanik MCC

The evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of adulteration if any. The evaluation of each plant material is mandatory before using them in formulations. Therefore, as per WHO guidelines, the evaluation of quality and purity of plant materials has been carried out.(17)

Preliminary Phytochemical Screening (18)

The dried powder of each plant material was extracted with 70% ethanol at room temperature and evaporated under vacuum. The extract of plant material were labeled. All the extracts were subjected to preliminary phytochemical screening for testing various phytoconstituents such as carbohydrate, tannins, alkaloids, proteins, sterols, amino acids etc.

https://doi.org/10.47552/ijam.v16iS2.6241 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Quantitative estimation of Phytoconstituents (19)

The quantitative estimation is carried out for amount determination of secondary metabolites in samples. The various estimations such as total alkaloid content, total flavonoid content and total phenol content were performed on selected plant materials extract by the standard procedure. (19) Estimation of Total alkaloids content by using Bromocresol Green reagent, Total phenolic content by Folin- Ciocalteu reagent, Total flavonoid content by Aluminium Chloride method and Total Saponin content were performed by n-butanol method.

Preparation and Evaluation of Chaturbeeja churna

The Chaturbeeja churna was prepared in laboratory by using the plant materials which were evaluated for assessing its quality and purity.

Preparation of Chaturbeeja Churna

Each ingredient were accurately weighed (10g). These powders were separately passed through sieve # 80 to prepare churna and then mixed together in 1:1:1 proportion. The prepared churna was labelled as LCC and evaluated for organoleptic characters, and micromeritic properties.

Table 1: Composition of Chaturbeeja Churna

Sr. No.	Ingredients	Quantity (g)
1	**Methika Seeds -MS**	10g
2	**Chandrashur Seeds - CS**	10g
3	**Kalonji Seeds - KS**	10g
4	**Yavanika Seeds - YS**	10g

The in-house Chaturbeeja Churna (LCC) was prepared which is shown in fig no 2 a. and the marketed Chaturbeeja Churna (MCC) in fig 2 b. and were selected for the further study.

Figure 2: In-house Churna and marketed Churna

a. In- house Chaturbeeja Churna (LCC)

b. Marketed Chaturbeeja Churna (MCC)

Organoleptic evaluations

The organoleptic evaluation of LCC and MCC was conducted for the characteristics such as color, odour, taste, texture.

Micromertic Evaluation:

Both the formulations, LCC and MCC were evaluated for the micromeritic properties such as, Bulk density, Tapped density, Angle of repose, Carr's index and Hausner's ratio. (18)

Physicochemical Evaluation

LCC and MCC were evaluated for their physicochemical properties such as, total ash value, acid in soluble ash value, water soluble ash value, water soluble extractive value and loss on drying.**(17)**

Preliminary Phytochemical screening:(19)

The various chemical tests were performed on LCC and MCC for the presence of various secondary metabolites such as carbohydrates, proteins, tannins, alkaloids, saponin, flavonoids, steroids, etc.

High Performance Thin Layer Chromatography (21)

The methanolic extracts of both LCC and MCC were subjected to High Performance Thin Layer Chromatographic (HPTLC) studies, to determine the probable number of compounds present. The standard procedure for developing the chromatographic profile of the sample was followed. The chromatographic fingerprinting of MCC and LCC formulations were done by using HPTLC method. Camag HPTLC system comprising of CAMAG Nanomat 4 sample applicator and TLC scanner 4 controlled by win CATS software version 3.1.5 was used for HPTLC method. Stationary phase used was MERCK precoated TLC Aluminium foil silica gel 60 F254 and the mobile phase Toluene: ethyl acetate: formic acid (6:4:0.3). Samples LCC and MCC were applied as spots with 10mm distance between two tracks. Tank saturation and plate equilibrium was given with filter paper for 20 min. Ascending development for a distance of 80 mm in a twin trough chamber was completed in 15 min. Samples i.e MCC and LCC was optimized for fingerprinting.

Observations and Results

Physicochemical Evaluation of crude drugs:

The results of all the physicochemical evaluation method are reported in table no.2.

Table 2: Results of Physicochemical evaluation of plants materials- MS, CS, KS, YS

Sr. No	Parameters	Methika - MS	Chandrashur - CS	Kalonji -KS	Yavani -YS
1	**Total ash value**	3.9%	2.5%	41%	5.6%
2	**Acid insoluble ash**	0.8 %	0.6%	1.9%	0.45%
3	**Water soluble ash**	2.73%	0.82%	2.1%	3.2%
4	**Petroleum soluble extractive value**	11.2%	12.5%	19.2%	17.4%
5	**Water soluble extractive value**	7.2%	9.4%	8.1%	21%
6	**Loss on drying**	0.9%	5%	1.95%	2.4%

All the physicochemical parameters determined for plant materials (MS, CS, KS, YS), complies the standard findings reported in various official literature. These findings suggest that all the four plant materials selected and evaluated were of good quality.

Preliminary Phytochemical Screening

The results of all the Preliminary Phytochemical Screening are reported in table no.3.

Table 3: Results of Preliminary phytochemical screening of plant materials

S. N.	Chemical test	Methika - MS	Chandrashur - CS	Kalonji -KS	Yavani -YS
1	Carbohydrates	+	+	+	+
2	Proteins	+	+	+	+
3	Tannins	-	-	+	-
4	Alkaloids	+	+	+	+
5	Saponins	+	-	+	+
6	Flavonoids	+	+	+	+
7	Steroids	-	-	-	-

Where, (-) represents absences of phytoconstituents, (+) represents presences of phytoconstituents.

Thus, table 3 reveals that MS contain carbohydrates, alkaloids, saponin and flavonoids. CS contains carbohydrates, alkaloids and flavonoids. KS contains carbohydrates, proteins, tannins, alkaloids, saponin and flavonoids. YS contains carbohydrates, proteins, alkaloids, saponins and flavonoids.

Quantitative Estimation of Phytoconstituents:

The results of all the Quantitative estimation are reported in table no.4.

Table 4: Quantitative estimations of plant extract

SN	Plant Extract	Total Alkaloid Content (mg atropine Equivalent /g extract)	Total Phenolic Content (mg Gallic Acid equivalent / g extract)	Total Flavanoid Content (µg/mg of quercetin /g extract)	Total Saponin Content (%)
1	MS	0.67	77.44	38.34	18-21%
2	CS	1.17	121.86	44.61	-
3	KS	0.35	98.84	42.65	5-7%
4	YS	0.65	76.05	39.61	1-2%

Thus, the table no. 4 reveals that the total alkaloid present in extracts of MS, CS, KS and YS found to be 0.6mg/g, 1.17mg/g, 0.35mg/g and 0.65mg/g respectively. The total polyphenol content in MS extract was found to be 77.44µg/mg, CS extract was found to be 121.86µg/mg, in KS extract it was found to be 98.84 µg/mg and in YS extract it was found to be 76.05µg/mg**.** The total flavonoid content in MS extract was found to be 38.34. µg/mg, in CS extract it was found to be 44.61 µg/mg and in KS extract it was found to be 42.65 µg/mg and total flavonoid content in YS extract was found to be 39.61 µg/mg, The total flavonoid content of CS was significantly higher than that of the other extracts i.e. CS, KS and YS. The total saponin content in MS was found to be 18-21 %, in KS it was found to be 5-7% and in YS it was found to be 1-2%.

After evaluating the plant extracts the formulation was prepared. This evaluation is important for standardization and assuring the quality of plant materials. The formulation was further studied for its organoleptic evaluations, micromeritic properties, physicochemical evaluations, phytochemical screening, and chromatographic fingerprinting by TLC and HPTLC.

Organoleptic Evaluations

Organoleptic evaluation of both marketed and in-house chaturbeeja churna shows that colour of LCC shows light brown and MCC shows dark brown, odour of LCC is aromatic and characteristic while odour of MCC is characteristic, taste of both LCC and MCC was bitter in taste and texture of LCC was fine and MCC shows coarse texture.

Table 5: Organoleptic Evaluation

S.N	Observations	LCC	MCC
1	Colour	Light brown	Dark brown
2	Odour	Aromatic and characteristic	Characteristics
3	Taste	Bitter	Bitter
4	Texture	Fine	coarse

Micromeritics Evaluation

For evaluating the flow properties of the powdered plant material selected in this study the parameters such as Bulk density, Tapped density, Carr's index, Angle of repose, Hausner's ratio were performed on in- house as well as marketed chaturbeeja churna.

Table no. 6: Micromeritics evaluation

Sr No.	Parameters	LCC	MCC
1	Bulk density	0.33 gm/cm^3	0.49 gm/cm^3
2	Tapped density	0.49 gm/cm^3	0.66 gm/cm^3
3	Carr's index	32.6 %	25.75%
4	Hausner's ratio	1.48	1.34
5	Angle of repose	25.20◦	22.47◦

The results depicted in Table no. 6 reveals that there was no significant difference between results of in house as well as marketed churna. Bulk density of chaturbeej churna was found to be 0.33 gm/cm^3 (F1), 0.49 gm/cm^3 (F2). Tapped density was found to be 0.49 (F1), 0.66 gm/cm^3 (F2). Carr's index was found to be 32.6 % (F1), 25.75 % (F2). Hausner's ratio was found to be 1.48, 1.34 and Angle of repose was found to be 25.20◦, 22.47◦. From this values both powders we understand chaturbeeja churna powder possess good flow properties.

https://doi.org/10.47552/ijam.v16iS2.6241 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Physicochemical Evaluation

The results of Physicochemical evaluations are depicted in table no. 7.

Table no. 7 Physicochemical evaluations

Sr. No	Parameters	LCC	MCC
1	Total ash value	0.16g	0.19g
2	Acid insoluble ash	0.6g	0.8g
3	Water soluble ash	0.90g	0.95g
4	Water soluble extractive value	2.63g	2.60g
5	Loss on drying	1.4g	1.8g

The results of physicochemical evaluation of chaturbeeja churna are repored in Table no.7. based on the result, it was observed that the total ash value of marketed chaturbeeja churna was slightly more than laboratory chaturbeeja churna. Almost every parameter shows that the LCC was found to be of better quality.

Preliminary Phytochemical Screening

The result of chemical evaluation of in-house LCC and marketed *chaturbeej churna* MCC are reported in table no. 8.

Table 8: Preliminary phytochemical Screening of

Sr No.	Chemical	LCC	MCC
1	Carbohydrat	+	+
2	Proteins	+	+
3	Tannins	-	-
4	Alkaloids	+	+
5	Saponins	+	+
6	Flavonoids	+	+

Thus, the results of table no. 8 reveals that the Chaturbeeja formulations shows presence of Carbohydrates, Proteins, Alkaloids, Saponins and Flavonoids.

Chromatographic fingerprinting of LCC and MCC

The chromatographic fingerprints of both LCC and MCC obtained from HPTLC method are shown in fig 3. The distinct spots were observed on track 1 and 2 . The samples had shown much similarity in peaks under UV 364 scanner.

The HPTLC results indicate that 13 peaks were detected on track 1 and 12 peaks were detected on track 2 under the specified conditions. The Rf values and Area in % are presented in figure 5 and 6. This suggests that these particular components are common among the Methanolic extract of LCC and MCC. Thus, it can be concluded that the same compounds are present in the samples LCC and MCC on track 1 and 2 respectively.

Figure no. 3. Chromatogram of LCC and MCC on track 1 and 2 respectively in solvent system B under UV 364.

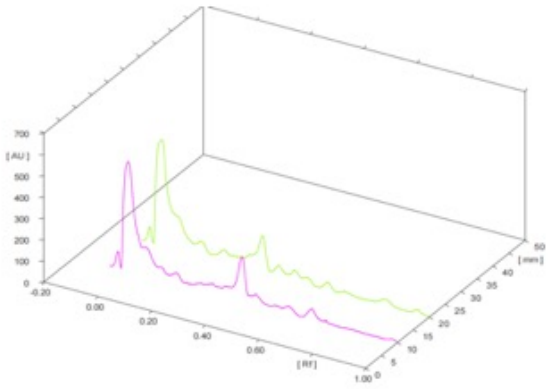
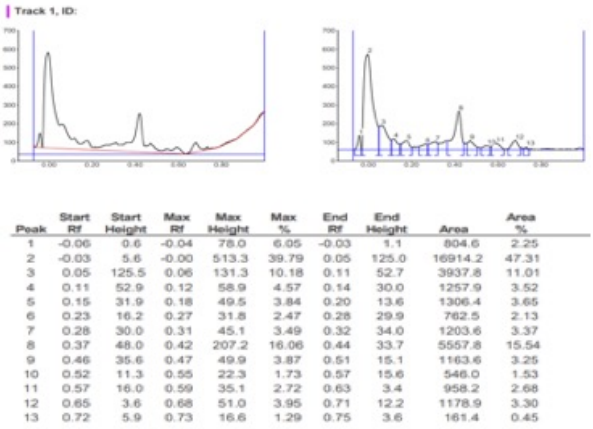
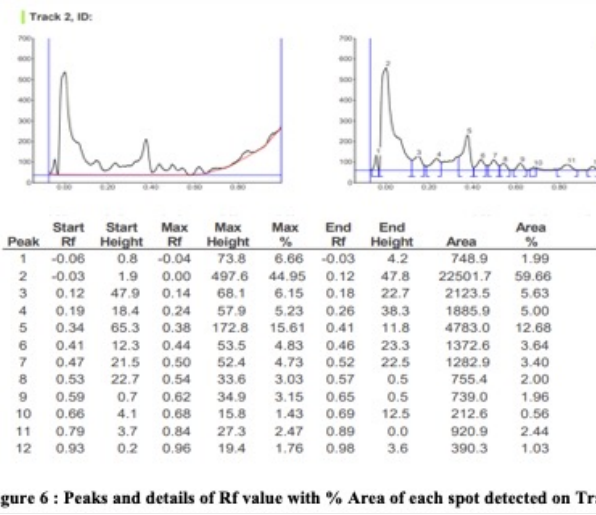


Figure no. 4. 3D representation of HPTLC of LCC and MCC



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	0.6	-0.04	78.0	6.05	-0.03	1.1	804.6	2.25
2	-0.03	5.6	-0.00	513.3	39.79	0.05	125.0	16914.2	47.31
3	0.05	125.5	0.06	131.3	10.18	0.11	52.7	3937.8	11.01
4	0.11	52.9	0.12	58.9	4.57	0.14	30.0	1257.9	3.52
5	0.15	31.9	0.18	49.5	3.84	0.20	13.6	1306.4	3.65
6	0.23	16.2	0.27	31.8	2.47	0.28	29.9	762.5	2.13
7	0.28	30.0	0.31	45.1	3.49	0.32	34.0	1203.6	3.37
8	0.37	48.0	0.42	207.2	16.06	0.44	33.7	5557.8	15.54
9	0.46	35.6	0.47	49.9	3.87	0.51	15.1	1163.6	3.25
10	0.52	11.3	0.55	22.3	1.73	0.57	15.6	546.0	1.53
11	0.57	16.0	0.59	35.1	2.72	0.63	3.4	958.2	2.68
12	0.65	3.6	0.68	51.0	3.95	0.71	12.2	1178.9	3.30
13	0.72	5.9	0.73	16.6	1.29	0.75	3.6	161.4	0.45

Figure 5 : Peaks and details of Rf value with % Area of each spot detected on Track 1



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	0.8	-0.04	73.8	6.66	-0.03	4.2	748.9	1.99
2	-0.03	1.9	0.00	497.6	44.95	0.12	47.8	22501.7	59.66
3	0.12	47.9	0.14	68.1	6.15	0.18	22.7	2123.5	5.63
4	0.19	18.4	0.24	57.9	5.23	0.26	38.3	1885.9	5.00
5	0.34	65.3	0.38	172.8	15.61	0.41	11.8	4783.0	12.68
6	0.41	12.3	0.44	53.5	4.83	0.46	23.3	1372.6	3.64
7	0.47	21.5	0.50	52.4	4.73	0.52	22.5	1282.9	3.40
8	0.53	22.7	0.54	33.6	3.03	0.57	0.5	755.4	2.00
9	0.59	0.7	0.62	34.9	3.15	0.65	0.5	739.0	1.96
10	0.66	4.1	0.68	15.8	1.43	0.69	12.5	212.6	0.56
11	0.79	3.7	0.84	27.3	2.47	0.89	0.0	920.9	2.44
12	0.93	0.2	0.96	19.4	1.76	0.98	3.6	390.3	1.03

Figure 6 : Peaks and details of Rf value with % Area of each spot detected on Track 2

Discussion

The standardization and quality control of herbal dosage form with proper integration of modern scientific technique and traditional knowledge is important. The route methods of herbal drug standardization addresses quality related issues using several evaluation parameters of crude drugs and chromatographic profiling assisted characterization with spectroscopic techniques. In case of the herbal dosage form the validated assays of the content of constituents are required along with details of analytical procedure.

The assays of marker substances or other justified determinations are required.

The present investigation involves evaluation of the plant materials used to prepare formulations, preparation of churna and evaluation of all the prepared as well as marketed chaturbeeja formulations.

All the physicochemical parameters determined for the plant materials (MS, CS, KS, YS), complies the standard findings reported in various official literature. Thus the findings suggest that all the four plant material selected and evaluated were of good quality. The preliminary phytochemical screening reveals that MS contain carbohydrates, alkaloids, saponin and flavonoids. CS contains carbohydrates, alkaloids and flavonoids. KS contains carbohydrates, proteins, tannins, alkaloids, saponin and flavonoids. YS contains carbohydrates, proteins, alkaloids, saponins and flavonoids.

These evaluated crude drug materials were used to prepare LCC. Organoleptic evaluation of both marketed (MCC) and in-house chaturbeeja churna (LCC) shows that colour of LCC was light brown and MCC was dark brown, odour of LCC is aromatic and characteristic odour of MCC is characteristic, taste of both LCC and MCC was bitter in taste and texture of LCC was fine and MCC was found to be course as compared to LCC. The flow properties such as Bulk density, Tapped density, Carr's index, Angle of repose, Hausner's ratio were performed for in house as well as marketed churna. There was no significant difference between results of LCC well as MCC. Bulk density of chaturbeej churna was found. The preliminary phytochemical screening reveals that the both chaturbeeja formulations LCC and MCC mainly contains carbohydrates, Protein, Tannin, Alkaloids, Saponins and Flavonoids. The chromatographic fingerprinting of the churna samples were obtained by HPTLC. The results indicate that each sample, LCC and MCC on track 1 and 2 share compounds with specific RF value, suggesting a common components in these samples. This suggests that these particular components are common among the Methanolic extract of LCC and MCC. Thus, This HPTLC method can be further utilized by the researchers for standardization of the chaturbeeja churna.

Conclusion

Standardization of *chaturbeeja churna* formulations was done using pharmacognostical and physicochemical parameters, chemical evaluation, HPTLC fingerprinting and proximate analysis of active constituents. Marketed sample was also evaluated and compared with in- house formulation. There was variation between marketed and in house formulations regarding ash values, extractive values and total phenolics. These variations may be due to change in the quality of raw materials. It is generally realized that for monitoring quality the standardization is needed which is performed here. Hence, the results of these compound can be kept as a standard for comparison and evaluation of other commercial samples of chaturbeeja churna available in the market. The method was found to be useful in detecting the genuineness of the herbal formulations.

Future scope

In future the Quantitative estimation by HPTLC can be done by using various markers.

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https://doi.org/10.47552/ijam.v16iS2.6242 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Pharmacognostic Exploration, Formulation and Evaluation of *Caesalpinia bonduc* seeds syrup and candy formulation

Research Article

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Abstract

The pharmacognostic exploration and formulation development of *Caesalpinia bonduc* seeds for anthelmintic activity. *Caesalpinia bonduc*, a member of the Fabaceae family, is traditionally used in various indigenous systems of medicine for its diverse therapeutic effects. The present study focuses on the detailed pharmacognostic investigation of the seeds, including their morphological, microscopic, and physicochemical properties. Formulation development efforts involved creating herbal formulations incorporating *Caesalpinia bonduc* seed extracts, followed by an evaluation study. The formulation of herbal syrup and herbal candies containing *C. bonduc* not only offers a practical and enjoyable method of ingestion but also paves the way for the incorporation of traditional medicinal practices into contemporary lifestyles. These formulations enhance the compliance of pediatric patients towards anthelmintic medication. The findings emphasize the need for further clinical studies to confirm the safety and efficacy of *Caesalpinia bonduc* in treating helminthic infections, which could lead to the development of novel, affordable treatments for worm-related diseases.

Keywords: *Caesalpinia bonduc*, Anthelmintic activity, Herbal formulation, Phytochemical screening.

Introduction

Medicinal herbs have been used by humans for centuries as a natural remedy for various ailments and disorders. Numerous pieces of evidence, especially through scientific studies, illustrate the immense potential of medicinal plants utilized in various traditional systems (1). Medicinal plants have emerged as a major problem in the world due to a lack of data on the safety and efficacy of medicinal plants used in treatment. The plant *Caesalpinia bonduc* (Family Caesalpiniaceae) is a medicinally important, it is wild, thorny Dicotyledon plant distributed in hotter parts, coastal areas, Deltaic, eastern, western, southern parts of India, and in other Tropics, subtropics of the World (2). The plant has been reported to possess anxiolytic, antinociceptive, antidiarrhoeal, antidiabetic, adaptogenic, anthelmintic, antiestrogenic, anti-inflammatory, antimalarial, antimicrobial, antifungal, antispasmodic, antioxidant, antiproliferative, antipsoriatic, antitumor, larvicidal, muscle contractile, hepatoprotective, anticonvulsant, and antifilarial activities (3). *Caesalpinia bonduc* is popularly known as African nutmeg or fever nut. The kernel obtained from the seed is a popular condiment used as a spicing agent in both African and continental Food in Nigeria (4). They are mostly used as a condiment and flavouring agent. In powdered form, it acts as a stimulant and relieves constipation. The seed is bitter but has no toxic effect on the human body when it's consumed. The root of the plant is used in the treatment of fever, cough, and asthma, while the leaves have great value in the treatment of elephantiasis, intestinal worms, and fever (5).

Figure 1:

***Caesalpinia bonduc* seed**	***Caesalpinia bonduc* plant**	***Caesalpinia bonduc* plant**
		

Taxonomic Classification

- Kingdom: Plantae
- Phylum: Magnoliophyta
- Division: Magnoliopsida
- Class: Angiospermae
- Order: Fabales
- Family: Fabaceae (or Caesalpiniaceae)
- Genus: *Caesalpinia*
- Species: *bonduc*

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Botanical description

Caesalpinia bonduc is characterized by its bitter taste and evergreen foliage. It has deep taproots and a hard, woody stem. The leaves are bipinnately compound, elliptical to ovate, and arranged alternately along the stem. The leaf color is green, with a glossy surface. The plant produces dicot seeds and has a characteristic odor (6).

Synonyms

Caesalpinia bonduc in Hindi called Kantkarej, Kanti Karanja, or Sagargota; in English, it is referred to as Fever nut, Bonduc nut, Nicker nut, or Nicker seed; and in Marathi, it is known as Gajaga (3).

Methods and Materials

Collection and Authentication of Plant Material

Seeds of *Caesalpinia bonduc* were gathered from a natural habitat of the Sewagram region, and the sample was authenticated by the Botanical Department of RTMNU Nagpur University, receiving the specimen number 104217.

Preparation of Plant Extract

The seeds underwent a thorough washing process, were subsequently air-dried at ambient temperature for 7 to 10 days, and were then subjected to pulverization using a mechanical grinder. Seeds were first defatted with petroleum ether in a Soxhlet apparatus and then extracted with a Hydroalcoholic extract (ethanol:water = 70:30 v/v) through sonication. The [ss3] [JD4] resultant extract was concentrated *via* a rotary evaporator and subsequently desiccated to yield a semi-solid mass. The extract was preserved in an airtight container maintained at a temperature of 4°C.

Figure 2. Seeds outercoat

Figure 3. Seeds

Figure 4. Seeds kernel

Figure 5. Seed powder

Pharmacognostic Evaluation

Organoleptic Characteristics like shape, size, color, surface characteristics, and odor of seeds were studied (Table 3).

Physicochemical Parameters

The physicochemical parameters, such as Total ash, acid-insoluble ash, water-soluble ash, extractive values, Loss on drying, swelling index, and foaming index, were studied (Table 3) (7).

Phytochemical Evaluation

The bioactivity of the herbal product was determined by the phytoconstituent present in it. The hydroalcoholic extract of *Caesalpinia bonduc* seed was screened to determine the presence of phytoconstituents by using various chemical tests as per standard procedures (Table 4) (7).[ss5] [JD6]

The confirmatory qualitative phytochemical analysis of plant extracts was conducted to ascertain the predominant classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts in accordance with standard procedure (7).

- **Protein Test: Biuret Test:** Two drops of 0.1% copper sulphate solution and 10% sodium hydroxide solution were added to the test solution, and the production of a violet or pink color was monitored.
- **Test for Free Amino Acids: Ninhydrin Test:** This test solution forms a purple color when it is heated with a 0.2% solution of Ninhydrin, indicating the presence of free amino acids.
- **Test for Carbohydrate: Benedict's test:** A small amount of Benedict's reagent (an alkaline solution containing cupric citrate complex) was added to the test solution, which was then boiled in a water bath. The formation of a reddish-brown precipitate indicated the presence of carbohydrates.
- **Glycoside test (Salkowski's test):** 2 ml of concentrated H^2SO^4 acid was added to the aqueous plant crude extract. A reddish brown color formed which indicated the presence of the steroidal aglycone part of the glycoside.
- **Test for Tannins:** Approximately 200 mg of the plant extract was subjected to boiling with 10 mL of distilled water; subsequently, 0.1% Ferric chloride was introduced to the mixture, which was then scrutinized for the development of a blue-black coloration, thereby indicating the presence of tannins.
- **Test for Alkaloids:** The plant extract was initially dissolved in 100 mL of water, filtered, and subsequently heated in a water bath with 2 mL of the filtrate and three drops of 1% HCl. This mixture was used for the following chemical test.
- **Meyer's test:** To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of cream color precipitate indicated the presence of alkaloids.
- **Dragendroff's reagent test:** 2 mL of extract was heated with 2% H^2SO^4. Few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids.
- **Test for Saponins:** Approximately 0.5 milliliters of the extract was mixed with 5 mL of distilled water and mixed. Then, foam formation indicates the presence of saponins.
- **Test for Flavonoids:** Two hundred milligrams of the botanical extract was combined with 10 mL of ethanol and subsequently subjected to filtration. Following this, 2 mL of the filtrate was mixed with concentrated hydrochloric acid and a magnesium ribbon. The emergence of a pink or red color signifies the existence of flavonoids.
- **Test for Steroids:** Approximately 1 mL of the crude extract was mixed with 10 mL of chloroform and 10 mL of sulfuric acid; the formation of a bilayer (characterized by a red upper layer and a greenish lower layer) indicates the presence of steroids.
- **Test for Terpenoids:** The assessment of terpenoids was conducted by observing the development of a reddish-brown coloration during the test for terpenoids, which involved the combination of 0.5 mLof the crude extract with 2 mL of chloroform.

https://doi.org/10.47552/ijam.v16iS2.6242 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Formulation Development

The *Caesalpinia bonduc* seed shows anthelmintic activity (8). The primary goal of this study was to create a formulation with anthelmintic activity that is appropriate for use in children. The pediatric patient facing problems taking bitter drugs and swallowing, so attempts were made to mold this into a candy and syrup formulation.

Herbal Syrup

Table 1: Composition of *Caesalpinia bonduc* syrup

Ingredients	Quantity	Figure 6: *C. bonduc* syrup
Caesalpinia bonduc seed extract	2 gm	
Sucrose	66.7gm	
Coloring Agent	5 mg	
Flouring Agent	2 to 3 drops	
Water	q.s up to 100ml	

Preparation Method

Seeds of *Caesalpinia bonduc* collected, cleaned, and dried. Seeds were ground and pulverized, and extracted by Soxhlet extraction. Seeds were first defatted with petroleum ether later extracted by hydroalcoholic Solvent (ethanol: water) (20:80).

Preparation of simple syrup IP

66.7 g of sucrose was dissolved in sufficient distilled water to obtain 100 ml of concentrated simple syrup. The solution was boiled, cooled, filtered, and the simple syrup was used as the vehicle (9).

Preparation of (medicated) *Caesalpinia bonduc* syrup

The powdered *Caesalpinia bonduc* extract was added to the prepared syrup base and heated until the volume was reduced to half, cooled and filtered.

Preparation of final syrup

Caesalpinia bonduc extract syrup should be added to the sugar syrup by simple stirring to obtain a uniform and consistent syrup. Coloring agents and flavoring agents were incorporated into the mixture. The resultant syrup was then transferred into an amber-colored bottle, securely sealed, and stored in a cool, dry environment. The final syrup formula is stipulated in Table 1.

Evaluation parameters

- Organoleptic study: The organoleptic attributes, including color and odor of the formulation, were evaluated through visual inspection Table 5.
- Determination of pH: Take 10 mL of the final syrup in the volumetric flask and make up the volume to 100 mL with distilled water. The pH was measured by using a digital pH meter.
- Determination of viscosity: The Viscosity of syrup can be determined by using an Ostwald viscometer. The determination of viscosity was executed employing the following formula: Density of the syrup (d_s) multiplied by the time required for the syrup (t_s) to flow, divided by the density of water (d_w) and the time required for water to flow (t_w).
- Determination of density: The assessment was conducted utilizing the formula delineated below. The Density of the liquid under examination (syrup) is defined as the weight of the syrup divided by the volume of the syrup, represented mathematically as w_s/v_s.
- Determination of specific gravity: The specific gravity of the liquid under examination (syrup) is expressed as the weight of the syrup divided by the weight of water, denoted as w_s/w_w.

Stability testing involved the assessment of the prepared herbal syrup under accelerated temperature conditions. The final syrup was allocated into culture tubes and subjected to accelerated temperatures at 4°C, room temperature, and 47°C, respectively. The samples underwent evaluation for all physicochemical parameters, turbidity, and homogeneity at intervals of 24 hours, 36 hours, and 72 hours to monitor any potential alterations (10).

Herbal Candy

Table 2: Composition of *Caesalpinia bonduc* candy formulation

Ingredients	Quantity per candy	Figure 7: *C. bonduc* candy
Sugar	1.2 g	
Drug (hydroalcoholic extract)	120 mg	
Lemon juice	0.4 mL	
Colouring agent	0.5 mg	
Flavoring agent (cardamon)	0.1mg	
Propyl Paraben	0.02%	
Water	q. s	

Preparation method

The sugar was dissolved in a small volume of water and subsequently heating the mixture until complete dissolution occurred, resulting in a brownish hue. Extract was added to the resultant sugar solution. Furthermore, a small amount of lemon juice was integrated into the preparation to prevent excessive boiling of the sugar solution. Subsequently, beetroot juice was introduced as a coloring agent, while cardamom powder was added as a flavoring agent. Preservatives are incorporated into the blend to enhance its longevity. The concocted mixture is then transferred into a mold and permitted to cool at ambient temperature until it solidifies. Upon cooling, it is imperative to store the product appropriately at the designated temperature (Table 2) (11).

Evaluation parameters

- Physico-chemical parameters: The evaluation of curcumin candies was conducted based on various physicochemical parameters, including color, odor, and taste (Table 6).

- Measurement of pH: The candies were introduced into a 100 ml flask containing 100 ml of distilled water and subjected to sonication for approximately fifteen minutes, after which the pH was determined utilizing a digital pH meter.
- Hardness: To acquire the values pertinent to the candy, the force (N) requisite for the rupture of the candy was quantified utilizing the hardness tester (Pfizer Hardness Tester). Ten candies corresponding to each batch were employed, allowing for the computation of their average breaking force.
- Thickness: The thickness and diameter of the formulated candy were assessed using Vernier calipers. Herbal candies were designed to maintain a uniform thickness to ensure optimal dissolution within the oral cavity.
- Weight Variation: The formulated lozenges underwent an evaluation for weight uniformity, wherein both collective and individual weights of ten lozenges were determined. The average weight was subsequently derived from the combined weight, and the weight of each lozenge was compared against this average to ascertain compliance with permissible limits.
- Friability: The Roche friability test apparatus was employed to ascertain the friability of the lozenges. Ten pre-weighed lozenges were introduced into the apparatus, which was subjected to 100 revolutions, after which the lozenges were reweighed.
- Moisture Content: The sample was weighed and subsequently pulverized in a mortar. One gram of the sample was then measured and placed within desiccators for 24 hours. Following this period, the sample was weighed again. The moisture content was determined by subtracting the final weight from the initial weight of the lozenges.
- Stability Test: A physical stability assessment of the Candy was performed under varied temperature conditions of 2°C, 25°C, and 37°C over four weeks. The Candies exhibited physical stability across the different temperatures, specifically at 2°C, 25°C, and 37°C, throughout the four-week duration (11-13).

Results

In the present study, efforts were made to formulate and evaluate the *Caesalpinia bonduc* herbal syrup. The formulated syrup passes all physicochemical and phytochemical parameters, and the formulation can be further used for anthelmintic activity.

Phytochemical Evaluation

The results of the phytochemical study were presented in Table 4. Phytocompounds are highly present in the hydroalcoholic extract than petroleum extracts. Among all the phytocompounds, alkaloids, flavonoids, polyphenols, saponins, steroids, and tannins show higher concentrations in the hydroalcoholic extract. The hydroalcoholic extract shows the presence of amino acids, carbohydrates, alkaloids, flavonoid glycosides, proteins, polyphenols, and tannins (7).

Table 3: Physicochemical parameters of *Caesalpinia bonduc* seeds

Sr. No.	Parameter	% Values (w/w)
A.	**Ash Value**	
1	Total ash value	33.47
2	Acid insoluble ash value	7.86
3	Water soluble ash value	18.92
4	Sulphated ash value	34.08
B.	Extractive value	
5	Petroleum ether	2.42
6	Chloroform	13.45
7	Ethanol	7.30
8	Water	15.37
9	Moisture content	31.5
C.	Swelling index	5.64
D.	Foaming index	166.67

(% w/w = Percent weight by weight)

Table 4: Phytochemical screening of *Caesalpinia bonduc* seeds

Sr. No.	Phytoconstituents	Petroleum ether extract	Hydroalcoholic extract
1	Carbohydrates	-	+
2	Amino acids	-	+
3	Proteins	-	+
4	Tannins	-	+
5	Flavonoids	-	+
6	Terpenoids	-	-
7	Triterpenoids	-	-
8	Alkaloids	-	+
9	Saponin	-	+
10	Polyphenols	-	+
11	Glycosides	-	-
12	Sterols	+	-

(-) = Negative test; (+) = Positive test

Table 5: Evaluation parameters of herbal syrup

Evaluation Parameters	Results
Colour	Yellowish
Odour	Lemon like
Taste	Sweet
pH	6.2
Specific Gravity	1.06 gm
Density	1.05 gm/ml
Viscosity	1.614 Cps

Table 6: Evaluation parameters of herbal candy

Evaluation Parameters	Results
Colour	Reddish brown
Odour	Lemon like
Taste	Sweet
pH	5.6
Thickness	12.57 ± 0.5
Hardness	6.5 ± 0.25
Friability	0.31± 0.07
Weight Variation	7.26 ± 0.078
Moisture content	0.8%
Stability	Stable at 2°C, 25°C, and 37°C

Discussion

The Caesalpinia bonduc seed shows anthelmintic activity. The primary aim of the present investigation was to formulate and evaluate the polyherbal syrup and polyherbal candy of the hydroalcoholic extract of *Caesalpinia bonduc* seeds used as an anthelmintic agent for paediatric use. This research examines the formulation and evaluation of Polyherbal candies that do not presently exist in the commercial marketplace. The fundamental aspects concerning herbal medicines encompass purity, safety, potency, and efficacy for consumers. Consequently, the standardization and quality control of herbal pharmaceuticals and their raw materials are invariably essential before formulation. The seeds of *Caesalpinia bonduc* exhibit anthelmintic properties (14). The principal objective of the current study was to formulate a preparation that is suitable for pediatric application while eliciting anthelmintic activity. Pediatric patients often encounter difficulties in ingesting bitter medications and swallowing; thus, it was transformed into a syrup and candy formulation for better compliance.

Conclusion

In conclusion, the integration of *C. bonduc* in confectioneries and syrups presents substantial potential for improving adherence of pediatric patients to anthelminthic preparations. *C. bonduc* functions as an antioxidant, serving as an anthelminthic agent that bolsters the body's innate defense mechanisms against pathogenic infections. The formulation of herbal candies containing *C. bonduc* not only offers a practical and enjoyable method of ingestion but also paves the way for the incorporation of traditional medicinal practices into contemporary lifestyles. Further empirical investigations are essential to clarify the specific mechanisms of action, optimal dosages, and the long-term implications of *C. bonduc* in confectionery preparations. Overall, the application of *C. bonduc* in candies signifies a promising domain of inquiry and innovation, presenting a natural and palatable dosage form as an anthelminthic. Furthermore, it underscores the extensive potential of botanical substances in the advancement of novel therapeutic agents characterized by improved efficacy and minimal side effects.

Acknowledgement

The authors express their gratitude to the Principal Agnihotri Institute of Pharmacy, Wardha,

Maharashtra, India, for the facilities and encouragement in carrying out research work.

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https://doi.org/10.47552/ijam.v16iS2.6278 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

In-Silico Prediction of Phytoconstituents from *Manilkara hexandra* for Antidiabetic Activity targeting LRH-1

Research Article

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Abstract

Objective: A complex metabolic condition known as diabetes mellitus is caused by either inadequate or dysfunctional insulin. Once more, medicinal plants are being researched for the treatment of diabetes. Prototypical compounds found in medicinal plants have been the source of many conventional medications. *In-silico* testing of *Manilkara hexandra* phytoconstituents for antidiabetic efficacy was a part of our investigation. Design: Utilizing Discovery studio, molecular docking is done to assess the pattern of interaction between the phytoconstituents from the *Manilkara hexandra* plant and the crystal structure of the antidiabetic proteins (PDB ID: 4DOS). Later, SwissADME and pkCSM were used to screen for toxicity as well as the pharmacokinetic profile. Results: The docked results suggest that Quercetin (-8.8 kcal/mol),Kaempferol (-8.2 kcal/mol), P-coumaric acid (-6.3 kcal/mol) and cinnamic acid (-6.2 kcal/mol), for 4DOS macromolecule has best binding affinity towards LRH-1 for antidiabetic activity as compared to the standard drug metformin (-4.8 kcal/mol). Furthermore, pharmacokinetics and toxicity parameters were within acceptable limits according to ADMET studies. Conclusion: Results from the binding potential of phytoconstituents aimed at antidiabetic activity were encouraging. It promotes the usage of *Manilkara hexandra* and offers crucial details on pharmaceutical research and clinical care.

Keywords: In-silico, *Manilkara hexandra*, Antidiabetic Activity, 4DOS, Discovery studio.

Introduction

Manilkara hexandra (Roxb.) Dubard, synonym: *Mimusops hexandra* Roxb, which is widely distributed over central India and the Deccan Peninsula. It is grown in all of India's principal regions. It is compared to Khirni as well. One of the most underutilized fruits in the state of Gujarat is the fruit of the plant. It is commonly referred to as Rayan.(1)

It is indigenous to India and is primarily found growing wild in the country's south and north. Our efforts are focused on gathering important data regarding the morphology, microscopy, phytoconstituents, and pharmacological aspects of the plant, which is widely distributed in Gujarat, Rajasthan, Madhya Pradesh, Andhra Pradesh, Kerala, and Maharashtra. Protobassic acid, 16-ahydroxyprotobassic acid, taraxerol, a triterpene ketone, alpha and beta-amyrin, cinnamates, alpha-spinasterol, beta-sitosterol, its beta-D-glucoside, quercetin, and its dihydroderivatives, ursolic acid, are only a few of the important phytoconstituents found The entire plant is traditionally used as an astringent, aphrodisiac, alexipharmic, stomachic, anthelmintic, and to relieve symptoms of fever, flatulence, colic, dyspepsia, helminthiasis, hyperdipsia, and burning sensation. All of these substances asserted to have a range of pharmacological activities, including those of an antioxidant, an antiulcer, an anti-inflammatory, an antidiuretic, and others.(2)

A series of metabolic illnesses known as diabetes mellitus are characterised by chronic hyperglycemia brought on by deficiencies in insulin secretion, insulin action, or both. The significance of insulin as an anabolic hormone leads to metabolic irregularities in carbohydrates, lipids, and proteins. These metabolic abnormalities are brought on by insufficient insulin levels to produce an adequate response and/or insulin resistance of target tissues, primarily skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes. The kind and length of diabetes affect the severity of symptoms. Patients with diabetes can experience polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Some patients with diabetes are asymptomatic, particularly those with type 2 diabetes in the early stages of the disease. However, patients with severe hyperglycemia and, particularly in children, those with absolute insulin deficiency, can experience these symptoms. If untreated, uncontrolled diabetes can cause coma, stupor,

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and, in rare cases, death from nonketotic hyperosmolar syndrome or ketoacidosis.(3-5)

The human nuclear receptor liver receptor homolog-1 (LRH-1) has an important role in controlling lipid and cholesterol homeostasis and is a potential target for the treatment of diabetes and hepatic diseases. LRH-1 is known to bind phospholipids, but the role of phospholipids in controlling LRH-1 activation remains highly debated.

Liver receptor homolog 1 (LRH-1; NR5A2) is a nuclear hormone receptor (NR)3 that controls expression of a diverse set of genes important both in normal physiology and disease. In addition to a vital role during development (6,7) LRH-1 regulates many genes related to metabolism, proliferation, and cell survival. In the liver, LRH-1 regulates bile acid biosynthesis (8) and reverse cholesterol transport (9,10), affecting hepatic and circulating cholesterol levels. Glucose metabolism is also regulated by LRH-1 at several points, including GLUT-4-mediated transport (11) and glucose phosphorylation, the latter of which is essential for proper postprandial glucose sensing, flux through glycolysis and glycogenesis pathways, and *de novo* lipogenesis (12). LRH-1 is a key mediator of the cell stress response through control of genes involved in the hepatic acute phase response(13) and in the cytoprotective resolution of endoplasmic reticulum stress(14). Additionally, LRH-1 can be aberrantly overexpressed in certain cancers and can promote tumor growth through estrogen receptor and β-catenin signaling (15-21).

There are, however, limited investigations on *M. hexandra's* phytoconstituents for the antidiabetic. A molecular docking technique was used in the current work to find possible phytochemicals of *M. hexandra* that are resistant to 4DOS while keeping the aforementioned information in mind.

Materials and Methods

Platform for molecular docking

Using AutoDock Vina software, a computational docking analysis of all the phytoconstituents chosen as ligands with antidiabetic action as the target was carried out. (22)

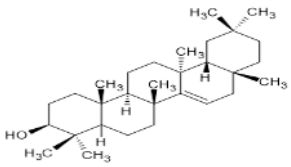
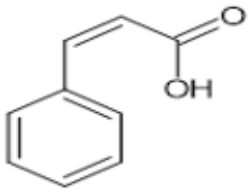
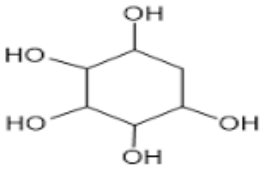
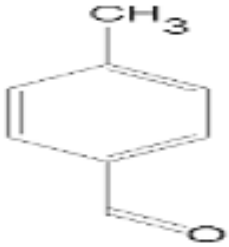
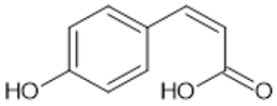
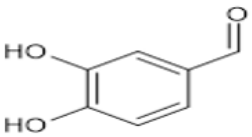
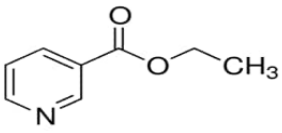
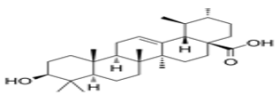
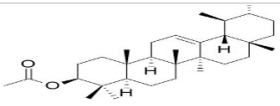
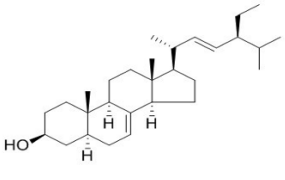
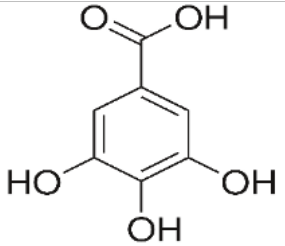
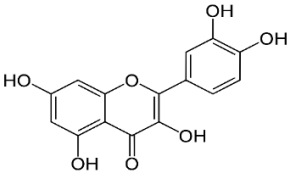
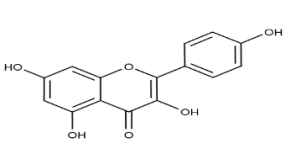
Protein preparation

The 2.00 crystal structure of antidiabetic with inhibitor, (PDB ID:4DOS, having resolution: 2.00, R-Value Free: 0.238, R-Value Work: 0.188, R-Value Observed: 0.191), which was retrieved from the protein data bank (https://www.rcsb.org), was subjected to *in-silico* analysis of a few phytoconstituents. 4DOS is used to treat diabetes. Using Discovery Studio, all additional molecules were eliminated, including undesirable chains, nonstandard residues, and co-crystallized water molecules.(23)

Ligand preparation

Using the Avogadro programme, all constituents' three-dimensional (3D) structures were extracted from the PubChem database on the NCBI website (https://pubchem.ncbi.nlm.nih.gov/). However, the ChemSketch application was used to sketch the geometrical 2D structure. The ligand structures were saved in the PDB format and the two-dimensional (2D) structures were converted into 3D models using the Avogadro software.

Figure 1: Chemical structures of all selected phytoconstituents in the molecular docking studies

1.Taraxerol	2. Hentriacontane
	
3. Cinnamic acid	**4. Quercitol**
	
5. 4-Methyl benzaldehyde	**6. *p*-Coumaric acid**
	
7. 3,4-Dihydroxy benzaldehyde	**8. Ethyl nicotinate**
	
9. Ursolic acid	**10. α-Amyrin acetates**
	
11. A-Spinasterol	**12. Gallic Acid**
	
13. Quercetin	**14. Kaempferol**
	

Molecular docking

In order to determine the scoring function based on geometry and forecast the binding affinity of the ligand molecule,(24,25) molecular docking analyses the interactions between the protein and the ligand. We used molecular docking experiments to examine the interactions between specific phytoconstituents (Fig.1), the conventional medication, and the crystal structure of a macromolecule with antidiabetic activity (PDB ID: 4DOS). PyRx software was used to carry out the molecular docking investigation, and the Vina wizard tool was used to investigate binding affinity. With bound ligands as the benchmark, the final data were analysed and presented using Discovery Studio 2020 Client.(26) The number of contacts and active residues responsible for significant binding at the target enzyme's active site are reflected in the protein-ligand interaction visualisation.

Absorption, distribution, metabolism, and excretion (ADME) and toxicityprediction

The chosen phytoconstituents and the reference medication were then examined for drug-like characteristics in accordance with Lipinski's rule. The tolerability of phytochemicals must be predicted during therapeutic development before they are consumed by people and animal models. SwissADME (http://www.swissadme.ch) and pkCSM (an online server database predicting small-molecule pharmacokinetic features using graph-based signatures, http://biosig.unimelb.edu.au/pkcsm/prediction) were used to determine the pharmacokinetic profile (ADME) and toxicity predictions of ligands. Simplified Molecular Input Line Entry System (SMILES) notations or PDB files were uploaded to examine the toxicological qualities of ligands, and then the necessary models were chosen to generate a wealth of information regarding effects associated to structure.(27,28)

Standard Preparation

The most often given medication for type 2 diabetes mellitus is metformin.(29)

A series of metabolic illnesses known as diabetes mellitus cause blood glucose levels to be higher than usual due to inadequate insulin release or inappropriate cell responses to insulin, which raises blood pressure. Serious difficulties are brought on by the ensuing hyperglycemia. The medicine metformin has been found to reduce the majority of diabetic complications and prevent diabetes in persons who are at high risk. Recent findings on metformin not only show some implications, such as reno protecting characteristics, but some reports also suggest its negative consequences, which are minor when its positive effects are taken into consideration.(30)

The standard is created in a series of phases, such as creating the 2D structure of the standard medicine using the chemsketch tool, then converting the 2D structure into a 3D model using the Avogadro Software, and finally saving it in PDB format. Metformin's molecular docking with 4DOS was carried out utilising PyRx.

Results and Discussion

The objective of the current study was to investigate the phytoconstituents found in *M. hexandra's* antidiabetic activity's inhibitory capacity. Using PyRx, we conducted molecular docking studies of all the phytoconstituents present in *M. hexandra* for this investigation. We next looked at the interactions between the amino acid residues and how they affected the inhibitory potentials of the active components. Using SwissADME and pkCSM servers, selected phytoconstituents with the best fit were further assessed for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics.

Molecular docking

The docking scores and binding energies of all chemical constituents of *M. hexandra*targeting antidiabetic activity (PDB ID: 4DOS) and binding interactions with amino acid residues are presented in Table 1.

Table 1: Binding interaction of ligands from *M. hexandra* targeting antidiabetic activity (PDB ID: 4DOS)

Sr. No.	Chemical constituent	PubChem ID	Docking Score 4DOS
1	Taraxerol	92097	-9.3
2	Hentriacontane	12410	-4.3
3	Cinnamic acid	444539	-6.2
4	Quercitol	441437	-5.4
5	4-methyl benzaldehyde	7725	-5.5
6	P-coumaric acid	322	-6.3
7	3,4-dihydroxy benzaldehyde	8768	-5.5
8	Ethyl nicotinate	69188	-5.5
9	Ursolic acid	64945	-9.3
10	A-amyrin acetates	92842	-8.9
11	A-spinasterol	5281331	-10.1
12	Gallic acid	370	-6
13	Quercetin	5280343	-8.8
14	Kaempferol	5280863	-8.2
Standard Drug			
15	Metformin	4091	-4.8

The binding affinities of phytoconstituents ranged from -10.1 to -4.3 kcal/mol for 4DOS macromolecule. From the docked results, it is evident that the compounds, A-spinasterol, ursolic acid, *taraxerol*, A-amyrin acetates and quercetin for 4DOS exhibit the most favourable binding affinity (-10.1, -9.3, -9.3, -8.9, -8.8 kcal/mol respectively)in complex with antidiabetic activity, as compared to other docked compounds i.e., kaempferol(-8.2 kcal/mol), p-coumaric acid (-6.3 kcal/mol), cinnamic acid (-6.2 kcal/mol), *gallic acid* (-6 kcal/mol), quercitol(-5.4 kcal/mol), 3,4-dihydroxy benzaldehyde (-5.5 kcal/mol),ethyl nicotinate (-5.5 kcal/mol),4-methyl benzaldehyde (-5.5 kcal/mol) and hentriacontane (-4.3 kcal/mol).

The binding affinity of the standard (metformin) for 4DOS is -4.8 kcal/mol.(31)

In addition, an analysis of the interactions of the 4DOS protein complex and ligand metformin was performed, which showed that the ligand molecule is oriented due to one salt bridge with ASP 389(A) amino acid and ten van der waals interactions with amino acid residues PHE 448(A), LYS 452(A), ILE 356(A), TRP 359(A), PRO 313(A), SER 355(A), VAL 318(A), VAL 406(A), GLU 315(A), ARG 393(A) were also found. (Fig.2)

In addition, an analysis of the interactions of the 4DOS protein complex and ligand quercetin was performed, which showed that the ligand molecule is oriented due to two Pi-Alkyl interaction with ALA 513(A)LEU 517(A), two Pi-Sigma interactions with ALA 349(A), LEU 532(A), one conventional hydrogen bond with ASP 389(A), two unfavourable donor-donor interaction with LEU 386(A), ARG 393(A) and nine van der waals interaction with TYR 516(A), GLU 514(A), SER 383(A), TRP 382 (A), HIS 390(A),MET 345(A), THR 352(A), MET 348(A), VAL 406(A) were also found. (Fig.3.a)

In addition, an analysis of the interactions of the 4DOS protein complex and ligand kaempferol was performed, which showed that the ligand molecule is oriented due to one Pi-sigma interaction with ALA 513(A), two alkyl interaction with LEU 386(A), ALA 349(A), two conventional hydrogen bond with THR 352(A), HIS 390(A), one carbon hydrogen bond with SER 383(A), and eleven van der waals interaction with SER 510(A), GLU 514(A), LEU 532(A), LEU 517(A), ILE 387(A), LEU 427(A), TRP 382(A), LEU 405(A), MET 345(A), VAL 406(A), MET 345(A) were also found. (Fig.3.b)

In addition, an analysis of the interactions of the 4DOS protein complex and ligand p-coumaric acid was performed, which showed that the ligand molecule is oriented due to oneconventional hydrogen bond interaction with TYR 413(A), one attractive charge interaction with HIS 390(A), two Pi-Alkyl interaction with ILE 416(A), MET 428(A), and seven van der waals interaction with ILE 403(A), GLN 432(A), VAL 435(A), ALA 431(A), GLN 394(A), ALA 417(A), ASN 425(A) were also found. (Fig.3.c)

In addition, an analysis of the interactions of the 4DOS protein complex and ligand cinnamic acid was performed, which showed that the ligand molecule is oriented due to onePi-Sigma interaction with ALA 513(A), one carbon hydrogen bond with CYS 346(A), three Pi-Alkyl interaction with LEU 532(A), LEU 386(A), LEU 517(A), and nine van der waals interaction with GLU 514(A), TRP 382(A), SER 383(A), ILE 387(A), ALA 349(A), ASN 530(A), TYR 516(A), PHE 342(A), MET 345(A) were also found. (Fig.3.d)

ADMET study

Pharmacokinetic profile (ADME) and toxicity predictions of the ligands are important attentive parameters during the transformation of a molecule into a potent drug. In the present study, these parameters were assessed using SwissADME and pkCSM. The absorption potential and lipophilicity are characterized by the partition coefficient (Log *P*) and topological polar surface area (TPSA), respectively. For better penetration of a drug molecule into a cell membrane, the TPSA should be less than 140 Å. However, the value of Log *P* differs based on the drug target. The ideal Log *P* value for various drugs are as follows: oral and intestinal absorption, 1.35 − 1.80; sublingual absorption, > 5; and central nervous system (CNS). The aqueous solubility of ligands ideally ranges from –6.5 to 0.5 , while the blood brain barrier (BBB) value ranges between –3.0 and 1.2 (32). In addition, non-substrate P-glycoprotein causes drug resistance (33).

In our study, all the selected ligands followed the TPSA parameter, P-glycoprotein non-inhibition, thereby showing good intestinal absorption and an acceptable range of BBB values. All the compounds showed aqueous solubility values within the range. Further, it was predicted that the selected ligands do not show AMES toxicity, hepatotoxicity, and skin sensitivity. In addition, it did not inhibit hERG-I (low risk of cardiac toxicity). Lipinski's rule violations, *T. pyriformis* toxicity, minnow toxicity, maximum tolerated dose, rat acute oral toxicity, and chronic toxicity are depicted in table 2.(34)

Standard Drug

Figure 2: Docking scores and binding interaction of metformin (PDB ID: 4DOS).The ligand is shown in line and stick representation along with its 2D diagram and hydrogen bond interaction.

1. Metformin, 4DOS

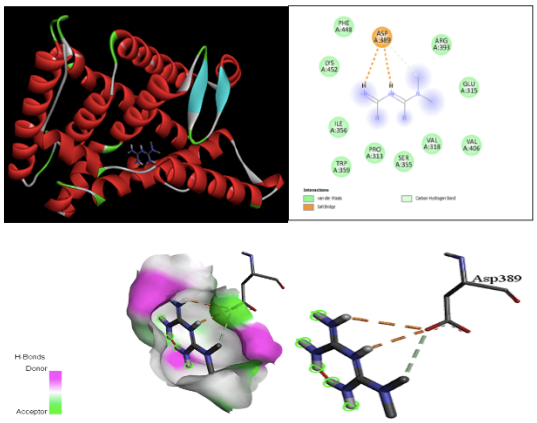


Table 2: ADME and toxicity predicted profile of ligands with superior docking scores

ADMET Properties	Formula	MW (g/mol)	Log P	TPSA (Å²)	HB donor	Hb acceptor	Aqueous solubility (Log mol/L)	Human intestinal absorption (%)	Blood-brain barrier
Taraxerol	C30H50O	426.72	8.17	20.23	1	1	-6.87	97.652	0.715
Hentriacontane	C31H64	436.84	12.34	0.00	0	0	-6.09	85.891	1.222
Cinnamic Acid	$C_9H_8O_2$	148.16	1.68	37.30	1	2	0.65	94.35	-0.312

https://doi.org/10.47552/ijam.v16iS2.6278 ISSN No: 0976-5921

Quercitol	$C_6H_{12}O_5$	164.16	-2.81	101.15	5	5	0.10	38.499	-1.082
4-Methyl Benzaldehyde	C_8H_8O	120.15	1.81	17.07	0	1	-1.71	97.33	0.394
P-Coumaric Acid	$C_9H_8O_3$	164.16	1.38	57.53	2	3	-2.01	93.512	-0.184
3,4-Dihydroxy Benzaldehyde	$C_7H_6O_3$	138.12	0.91	57.53	2	3	-0.75	77.745	-0.306
Ethyl Nicotinate	$C_8H_9NO_2$	151.16	1.26	39.19	0	3	-0.75	98.458	-0.236
Ursolic Acid	$C_{30}H_{48}O_3$	456.70	7.09	57.53	2	3	-4.65	97.503	-0.379
A-Amyrin Acetates	$C_{32}H_{52}O_2$	468.75	8.60	26.30	0	2	-7.04	100	0.662
A-Spinasterol	$C_{29}H_{48}O$	412.69	7.80	20.23	1	1	-7.10	95.981	0.805
Gallic Acid	$C_7H_6O_5$	170.12	0.50	97.99	4	5	-2.17	42.498	-0.958
Quercetin	$C_{15}H_{10}O_7$	302.24	1.99	131.36	5	7	-3.13	69.235	-1.372
Kaempferol	$C_{15}H_{10}O_6$	286.24	2.28	111.13	4	6	-3.30	74.567	-1.218
Metformin	$C_4H_{11}N_5$	129.16	-1.03	88.99	4	2	-2.67	57.273	-1.117

Table 2 Continued

ADMET Properties	P-glycoprotein substrate	Total clearance (Log ml/(min.kg))	Bio availability score	AMES toxicity	Max tolerated dose (Log mg/(kg.d))	hERG I inhibitor	hERG II inhibitor
Taraxerol	NO	-0.081	0.55	NO	-0.066	NO	YES
Hentriacontane	NO	2.188	0.55	NO	-0.254	NO	YES
Cinnamic Acid	NO	0.869	0.85	NO	1.17	NO	NO
Quercitol	NO	0.595	0.55	NO	2.461	NO	NO
4-Methyl Benzaldehyde	NO	0.265	0.55	NO	1.121	NO	NO
P-Coumaric Acid	NO	0.682	0.85	NO	1.089	NO	NO
3,4-Dihydroxy Benzaldehyde	NO	0.552	0.55	NO	0.739	NO	NO
Ethyl Nicotinate	NO	0.782	0.55	NO	1.122	NO	NO
Ursolic Acid	YES	0.079	0.85	NO	-0.65	NO	NO
A-Amyrin Acetates	NO	0.029	0.55	NO	0.423	NO	YES
A-Spinasterol	NO	0.611	0.55	NO	-0.318	NO	YES
Gallic Acid	YES	0.527	0.56	NO	1.414	NO	NO
Quercetin	YES	0.502	0.55	NO	0.779	NO	NO
Kaempferol	YES	0.538	0.55	NO	0.935	NO	NO
Metformin	YES	0.332	0.55	YES	0.364	NO	NO

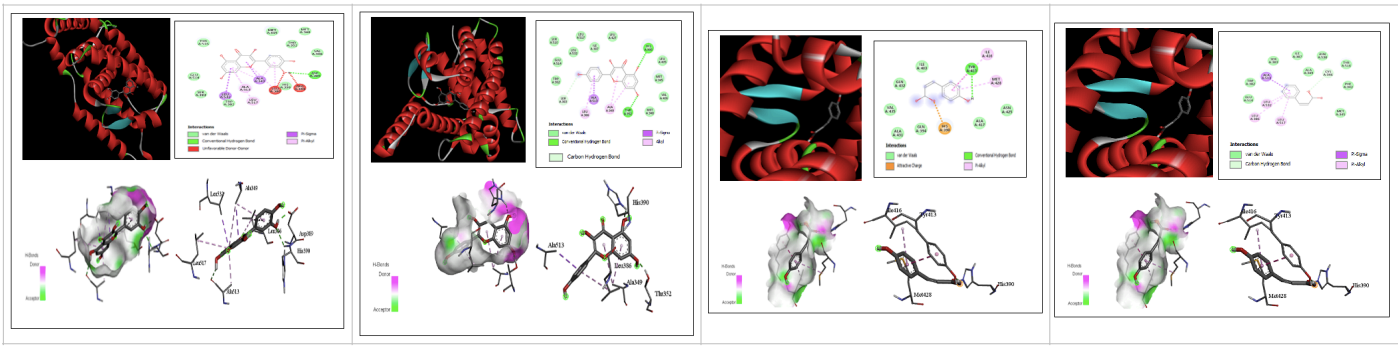
Table 2 Continued

ADMET Properties	Acute oral rat toxicity, LD50 (mol/kg)	Oral rat chronic toxicity (Log mg/kg bw/day)	Hepato-toxicity	Skin sensitisation	*T. Pyriformis* toxicity (Log µg/L)	Minnow toxicity (Log mmol/L)	Lipinski's rule violations
Taraxerol	2.828	1.288	NO	NO	0.41	-1.741	YES (1)
Hentriacontane	1.86	0.848	NO	YES	0.287	-5.021	YES (1)
Cinnamic Acid	2.05	2.549	NO	NO	-0.944	2.705	YES (0)
Quercitol	1.385	3.506	NO	NO	0.283	3.837	YES (0)
4-Methyl Benzaldehyde	1.731	1.959	NO	YES	-0.059	1.453	YES (0)
P-Coumaric Acid	1.912	2.953	NO	NO	0.223	1.79	YES (0)
3,4-Dihydroxy Benzaldehyde	1.865	2.149	NO	NO	-0.17	2.336	YES (0)
Ethyl Nicotinate	2.093	2.534	NO	YES	-0.39	2.187	YES (0)
Ursolic Acid	4.086	2.043	YES	NO	0.315	-0.596	YES (1)
A-Amyrin Acetates	2.261	2.187	NO	NO	0.37	-4.263	YES (1)
A-Spinasterol	2.454	1.125	NO	NO	0.56	-2.141	YES (1)
Gallic Acid	1.987	2.773	NO	NO	0.285	2.64	YES (0)
Quercetin	2.513	2.636	NO	NO	0.374	1.776	YES (0)
Kaempferol	2.329	2.616	NO	NO	0.448	1.034	YES (0)
Metformin	2.322	2.162	NO	YES	0.205	4.157	YES (0)

Drugs considered for 4DOS macromolecule

Figure 3: Docking scores and binding interaction for antidiabetic activity (PDB ID: 4DOS). The ligand is shown in line and stick representation along with its 2D diagram and hydrogen bond interaction.

a. Quercetin	b. Kaempferol	c. P-Coumaric Acid	d. Cinnamic Acid



Combine Boiled Egg Diagram

BOILED means **B**rain **O**r **I**ntestina**LE**stimate**D** permeation predictive model.

The boiled egg diagram shows two regions white and yellow.

The white region is the physicochemical space of molecules with highest probability ofbeing absorbed by the gastrointestinal tract, and the yellow region (yolk) is the physicochemical space of molecules with highest probability to permeate to the brain.

In addition, the points are coloured in blue if predicted as actively effluxed by P-gp(PGP^+) and in red if predicted as non-substrate of P-gp(PGP^-).

Conclusion

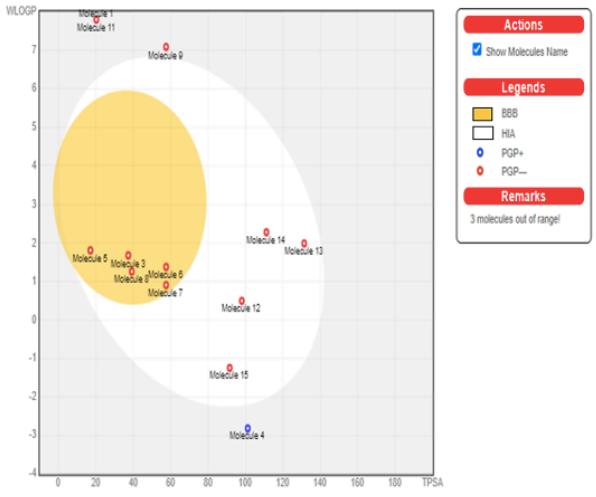
Glucose metabolism is also regulated by LRH-1 at several points, including GLUT-4-mediated transport and glucose phosphorylation, the latter of which is essential for proper postprandial glucose sensing, flux through glycolysis and glycogenesis pathways, and *de novo* lipogenesis and played important role in diabetes management. In this study, we have carried out an *in-silico* screening of the phytoconstituents of *Manilkara Hexandra*. This study demonstrate that thirteen compounds from selected phytoconstituents showed docking results from -10.1 to -5.4 kcal/mol. Among all, quercetin gave the lowest binding energy (-8.8 kcal/ mol) with 4DOS macromolecule, whereas the reference compound, metformin showing a docking score with a binding energy -4.8 kcal/mol.

To summarize, phytoconstituents present in *Manilkara Hexandra* possess strong inhibitory effects against 4DOS and could be further evaluated for their antidiabetic activity.

Table 3: Molecule names in boiled egg diagram

Molecule No.	Drug Name
1	Taraxerol
2	Hentriacontane
3	Cinnamic acid
4	Quercitol
5	4-methyl benzaldehyde
6	P-coumaric acid
7	3,4-dihydroxy benzaldehyde
8	Ethyl nicotinate
9	Ursolic acid
10	A-amyrin acetates
11	A-spinasterol
12	Gallic acid
13	Quercetin
14	Kaempferol
15	Metformin

Figure 4: Combined boiled egg diagram of all phytoconstituents with standard.



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https://doi.org/10.47552/ijam.v16iS2.6287 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Ethanopharmacology, Pharmacognostic Exploration, Formulation and Evaluation of A Topical Gel Containing *Euphorbia Hirta* Linn Plant Extract

Research Article

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Abstract

The medicinal plant *Euphorbia hirta* Linn, commonly referred to as asthma weed or pill-bearing spurge, can be found across tropical and subtropical regions. Its bioactive elements, which include flavonoids, alkaloids, tannins, and saponins, have been accountable for its medicinal qualities and have been utilised in traditional medicine for quite a while. A wide range of pharmacological actions, including anti-inflammatory, antibacterial, antifungal, antioxidant, and antidiabetic properties, have been demonstrated for *E. hirta*. Because of its broncho-dilatory and expectorant qualities, studies have also shown that it may help with respiratory ailments like cough, bronchitis, and asthma. *E. hirta* exhibits promise in the treatment of gastrointestinal issues, skin ailments, and a number of inflammatory conditions in addition to respiratory health. In order to fully investigate *E. hirta's* medicinal potential, it is crucial to combine traditional knowledge with contemporary scientific study. To gain a greater understanding of the plant's significance in modern medicine. In the current investigation, the ethnopharmacological assessment of the plant has been conducted. The formulation and subsequent evaluation of a topical anti-inflammatory gel incorporating Euphorbia hirta Linn. extract has been performed.

Keywords: *Euphorbia hirta,* Traditional medicine, Phytochemicals, Anti-inflammatory, Topical gel.

Introduction

Euphorbia hirta L. belongs to family *Euphorbiaceae* (1). It is commonly called as Asthma weed in English, Dugdhika, Kshirini, Ksheerani, Svaduparni in Sanskrit, Dudhi in Hindi and Marathi. It is also called as Australian asthma her (2, 3).

The Indo-Pakistan subcontinent is home to it, and it is primarily found in roadside waste areas (1). These plants are distinguished by the presence of a milky white, mostly poisonous latex (4). Throughout several tropical continents, *Euphorbia hirta L.* is used as an annual medicinal herb to treat a variety of illnesses, including infections the anti-fungal infections, gastrointestinal tract, pulmonary problems, wound healing, urinogenital disorders, malignancies, and breastfeeding in women (5,6).

Ethnopharmacological Study

Plant Description:

Taxonomy of plant:

Kingdom – Plantae.
Subkingdom – Viridaeplantae.
Division – Tracheophyta.
Subdivision – Spermatophytina.
Infradivision – Angiosperms.
Class – Magnoliopsida.
Superorder – Rosanae.
Order – Malpighiales.
Family – *Euphorbiaceae.*
Genus – *Euphorbia.*
Species – *hirta* (7,8)

Figure 1: Wild plant of *E.hirta*

Morphology

The plant is a common herb found in World Wide Australia, Northern Territory, Queens land, New South Wales, Central America, Africa, Indomalesia, Philippines, China and India *Euphorbia hirta* is usually grow up to height 40 cm tall. *Euphorbia hirta* is a slender- stemmed reddish and purplish color, with many branches from the base to summit (9). Covered with yellowish hair especially young parts. The leaves 1-1.5cm long is opposite, elliptic-long to oblong-lanceolate, unequal base, cuneate one side, round other side, dark green above, pale beneath, purple both in middle. when leaves were injured, they release a strong, aromatic minty scent. Flowers are unisexual male flowers are sessile linear, bracteoles, single stamen and perianth is absent. Female flowers are short pedicles, rimmed perianth superior ovary, covered with short hair. The fruits are yellow, three-celled, hairy, keeled capsules, 1 - 2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds (10).

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Histological Character

- Leaf: Leaf is dorsoventral and with uniformly thin lamina. It is 300µm thick and 390µm wide. The epidermal layer consists of fairly thick cylindrical thin-walled cell which are 10µm thick. Adaxial part of the xylem strand occurs on one of the dilated cells with dense chloroplast, there cells are called 'Krantz tissue' (11,12).
- Epidermal cell and Stomatal type: Epidermal cell are thin walled, the anticlinal walls are highly wave with deep folds so that this cell appear amoeboid in outline. Stomata occur only on the adaxial site of lamina that is hypostomatic they are anomocytic type and have no subsidiary cells. The guard cells are small and elliptical in shape they have wide stomatal pore (13,14).
- Venation pattern: The venation is densely reticulate, the major and minor veins are equally thick. The veins islets are wide rectangular or squares in outline. The veins boundaries are thick and distinct. The vein termination is either unbranched or branched twice or more. Single layer of Kranz cell forming thick sheath all around the veins (14).
- Petiole: It is semicircular and planoconvex in sectional view. It is 950µm wide and 750µm thick, has a thin less prominent epidermal layer of small cell. There is shallow arc of four vascular bundle with narrow gap in between (15).
- Powder microscopy: Powder characteristic revealed the presence of starch granules, scalariform vessels, covering trichomes, lignified trichomes, pericyclic fibers, epidermal cell with trichomes and Kranz tissues (16).
- Quantitative microscopy: Vein islet number, vein termination number, stomatal number, stomatal index (Table 1) (16,17).

Table 1: Quantitative evaluation of the crude drug of leaf of *Euphorbia hirta*.

Sr.no.	Standardization parameters	Inference
1	Vein islet no.	8 /sqmm
2	Vein termination no.	6 /sqmm
3	Stomatal no. (upper)	16.66
	Stomatal no. (Lower)	28.66
4	Stomatal index (upper)	6.319
	Stomatal index (Lower)	8.835

Traditional uses

The plant used as anti-inflammatory, antioxidant, antitumor, antidiabetic and free radical scavenging, anti-allergic, analgesic and antianaphylaxis, anxiolytic, sedative, antiarthritic, antidiarrheal, spasmogenic, anti-thrombocytopenic, diuretic, GI tract, burn wound healing, immune stimulatory, sperm motility, genotoxic, synergic, antiviral, anthelmintic, immunoprophylactic, antimalarial, antimicrobial, herbicidal, antimolluscidal, larvicidal property (18). In South India it is used as Eardrops in treatment of boils, score and wounds the plats are also eaten has vegetable. The latex of plant is often used as warts and cuts to prevent pathogenic infection (19). The decoction of leaves induces milk flow and leave chewed with palm kernel for restoration of virility (20). It is also effective in treating ulcer (21).

Pharmacological Action

- **Antidiabetic activity:** Significant antidiabetic action has been shown in studies on flavonoids extracted from *Euphorbia hirta*, especially in novel prenylated flavonoids, quercetin, 3'4'-dimethoxy quercetin, and hirta flavonoid B. The α-glucosidase enzyme, which lowers intestinal absorption of glucose and aids in regulating postprandial blood sugar levels, is strongly inhibited in vitro by these substances. These flavonoids improved lipid profiles, reduced blood glucose levels, and shown anti-inflammatory and antioxidant properties in rats with alloxan-induced diabetes. Additionally, histopathological investigations demonstrated that pancreatic β-cells were protected. These flavonoids may improve insulin sensitivity, trigger AMPK signalling, and lower inflammation and oxidative stress, providing a comprehensive strategy for diabetes management. Although more clinical research is required to validate their effectiveness, these findings show promise as natural treatment agents for diabetes (21).
- **Antiviral activity:** Herpes simplex virus (HSV), influenza, hepatitis B and C, and may be HIV are among the viral diseases that myricitrin, a flavonoid that was isolated using 50% ethanolic or methanolic solutions, has demonstrated encouraging antiviral qualities against. Inhibiting viral reproduction, preventing viral attachment and entrance into host cells, and modifying the immune response are the main mechanisms responsible for its antiviral action. Antioxidant qualities also seen in myricitrin aid in lowering oxidative stress during viral infections. Its effectiveness and low toxicity are demonstrated by in vitro and preliminary in vivo experiments (21).
- **Anti-inflammatory activity:** The terpenoid component, β-amyrin, and the ethanolic extract of *Euphorbia hirta* have notable anti-inflammatory properties, especially in the mouse model of TPA-induced ear inflammation. Prostaglandins and cytokines (TNF-α, IL-1β, and IL-6) are among the important pro-inflammatory mediators that β-amyrin suppresses by altering pathways such as NF-κB and COX enzymes. In addition, it has anti-inflammatory qualities because it scavenges free radicals and lowers oxidative stress, both of which contribute to its antioxidant activity (22).
- **Anticancer activity:** *Euphorbia hirta* possesses a variety of bioactive compounds with notable anticancer effects, including a newly identified cyclopentanone derivative found in the ethanolic extract, along with flavonoid glycosides such as afzelin, quercetin, and myricitrin extracted through methanol. The cyclopentanone derivative demonstrates cytotoxic properties by hindering the proliferation of cancer cells, triggering apoptosis, and diminishing metastasis. The flavonoid glycosides,

especially afzelin, quercetin, and myricitrin, exhibit strong anticancer effects against human epidermoid carcinoma by facilitating apoptosis, causing cell cycle arrest, and preventing tumor migration and angiogenesis. These compounds operate through diverse mechanisms, including the activation of caspases, the inhibition of crucial cancer-related proteins, and the reduction of oxidative stress, positioning *Euphorbia hirta* as a valuable candidate for anticancer drug innovation (21).

- **Antimicrobial activity:** The antimicrobial activity of *Euphorbia hirta* is primarily attributed to its bioactive compounds, including flavonoids, alkaloids, terpenoids, and saponins. These compounds exhibit strong activity against Gram-negative bacteria such as Salmonella typhi and Escherichia coli, which cause typhoid fever and urinary tract infections, respectively (22).
- **Allergic activity:** In experimental models, the 90% ethanolic extract of *Euphorbia hirta* has shown significant anti-allergic activity by inhibiting mast cell degranulation. During allergic reactions, mast cells release histamine and inflammatory mediators; by stabilizing these cells, *Euphorbia hirta* prevents the release of these substances, reducing allergic symptoms. The extract most likely acts through its bioactive compounds, such as flavonoids, saponins, triterpenoids, and alkaloids, which aid in modulating immune responses. This activity suggests that *Euphorbia hirta* could be useful in treating allergic conditions like asthma, rhinitis, and urticaria by supplementing conventional therapies (22).
- **Antiasthmatic activity:** By preventing histamine-induced bronchoconstriction in guinea pigs, paraxarol, a tri-terpene that was extracted from the ethanolic extract of *Euphorbia hirta* stems, has anti-asthmatic properties. Paraxarol reduces airway constriction, inflammation, and hyper-responsiveness by blocking the effects of histamine, a major mediator in asthma that promotes airway constriction. Because of its activity, paraxarol has the potential to be used as a therapeutic drug to manage asthma, reducing symptoms including wheeze and dyspnea (22).
- **Anti-snake venom:** Pyrogallol, a triphenolic molecule, and quercetin, a flavonoid, are both present in the methanolic extract of *Euphorbia hirta* and have both demonstrated strong anti-snake venom efficacy. The protease activity of Naja naja venom, which causes tissue damage and disrupts blood coagulation during envenomation, is inhibited by these substances. Pyrogallol and quercetin help stop the toxic consequences of venom. As a result, *Euphorbia hirta* is a potentially effective natural treatment for snake bites that may be used in conjunction with conventional antivenoms to treat envenomation (22).
- **Immunomodulatory activity:** Remarkable immunomodulatory action is demonstrated by the methanolic extract of *Euphorbia hirta*, which can both boost immune responses and reduce inflammation. By inducing immunological cells such as T-lymphocytes, B-lymphocytes, and macrophages, it strengthens the body defense system. Furthermore, the extract reduces inflammation by boosting phagocytic activity and blocking pro-inflammatory mediators. According to these results, *E. hirta* may help cure immune-related disorders such infections, autoimmune illnesses, and chronic inflammation (23).
- **Antifungal activity:** Remarkable antifungal behaviour is demonstrated by the methanolic extract of *Euphorbia hirta* leaves, flowers, stems, and roots, especially against yeast species with names like Candida albicans. By compromising the integrity of the cell membrane, interfering with vital metabolic functions, and blocking important enzymes, the extract prevents fungal development. Because of these characteristics, *E. hirta* shows promise as a natural treatment for fungal infections (22).
- **Antianxiety activity:** The hydro alcoholic extract of *Euphorbia hirta* aids in lowering stress and anxiety reactions. In order to promote serenity and relaxation, it probably modulates neurotransmitters like serotonin, GABA, and dopamine. It may also increase GABAergic activity. In animals, including those under long-term immobilization stress, the extract reduces anxiolytic behavioral and physiological manifestations. According to these findings, *E. hirta* may be a promising natural medication that can be used as a supplement or alternative for anxiety disorders and chronic stress (23).
- **Antiarthritic activity:** The water-soluble extract of *Euphorbia hirta* reduces joint inflammation and cartilage degradation in arthritis, showing good antiarthritic efficacy, even at modest dosages. Joint mobility is enhanced, pro-inflammatory mediators are inhibited, and cartilage is protected by the extract. With its potential to alleviate symptoms and enhance joint health, *E. hirta* may prove to be a valuable adjunctive treatment for osteoarthritis and rheumatoid arthritis (23).
- **Antimalarial activity:** *Euphorbia hirta* methanolic extract exhibits strong antimalarial activity. Plant extract prevent the Plasmodium parasite from growing, which lowers parasitemia and lessens the symptoms of malaria. The extract has potential as a supplemental therapy or natural alternative for malaria, especially in regions where antimalarial medication resistance is an issue (23).
- **Analgesic and antipyretic activity:** With notable analgesic and antipyretic properties, the lyophilized aqueous extract of *Euphorbia hirta* holds promise for the treatment of fever and pain. The extract successfully lowers yeast-induced hyperthermia in rats and mice. Its antipyretic effect is ascribed to its capacity to work on the hypothalamus to regulate body temperature, while its analgesic activity is probably caused by the inhibition of pro-inflammatory cytokines and prostaglandins, which are implicated in pain perception. This shows that *E. hirta* may be used as a supplementary or natural remedy for ailments like headaches, arthritis, and fever brought on by inflammation or infections (23).

Materials and Methods

Pharmacognostic Study

Organoleptic Properties of *E.hirta* Plant

Euphorbia hirta Linn has been extensively studied for its organoleptic properties; The organoleptic properties of the plant are important for determining its quality and potency.

Physico-Chemical Evaluation of *E.hirta* Plant

Loss on drying (LOD): A technique used to determine the moisture content of a substance. The process involves heating a sample until it's completely dry, measuring its weight before and after, and calculating the weight loss. This method can also measure the loss of other volatile components, such as alcohol or fat.

Ash value determination

Ash values are helpful to determine the quality as well as purity of a crude drug. Especially when drug is present in the powder form. The object of ashing crude drug is to remove the trace of organic matter may be interferes in an analytical determination (24).

Total ash value:

3g of powder was taken in a dried attired silica crucible and incinerated at a temperature not exceeding than 400^{o}C until free from carbon. The resultant ash was cooled and weighed.

Water soluble ash

3g of powder was boiled for 5min with 25 ml of water and the insoluble matter was collected on ashless filter paper. It was washed with hot water, ignited and weighed.

Acid insoluble ash

3g powder was boiled for 5 min with 25 ml of Dil. HCL and the insoluble matter collected on ashless filter paper. It was washed with hot water, ignited and weighted.

Alcohol soluble extract

4g of powder, add 100 ml of distilled water, shake occasionally for 6 hour and stand for 18 hours. Filter the solution and pipette out 25ml filtrate in 100 ml beaker and evaporate to dryness on water bath. (Keep it in oven at 105°C for 6 hours cool in desiccators for 30 min and weighted) (24).

Water soluble extract

4g of powder, add 100 ml of distilled water, shake occasionally for 6 hour and stand for 18 hours. Filter the solution and pipette out 25ml filtrate in 100 ml beaker and evaporate to dryness on water bath. (Keep it in oven at 105°C for 6 hours cool in desiccators for 30 min and weighted) (24).

Preliminary phytochemical testing of drug powder

The bioactivity of herbal constituents was evaluated based on the phytoconstituents they contain. The methanolic, and hydroalcoholic extracts of *Euphorbia hirta* foliage were subjected to screening procedures to verify the presence of phytoconstituents through various chemical assays in accordance with established protocols.

Method of Extraction

The aerial components of Euphorbia hirta were collected from local areas, subjected to thorough cleaning, and subsequently permitted to undergo desiccation in a shaded environment. Upon achieving complete dryness, a mechanical grinder was employed to reduce the plant material into a fine powder. A sterile conical flask was then filled with approximately 50 grams of the processed plant material. The extraction solvent, specifically 500 mL of methanol, was introduced while maintaining a weight-to-volume ratio of 1:10 by immersing the flask in an ultrasonic bath, the resultant mixture was subjected to sonication for a duration of 30 to 60 minutes at ambient temperature. The extraction of phytoconstituents was significantly enhanced by the ultrasonic waves, which facilitated the dissolution of plant cell walls. To eliminate any residual plant matter, the mixture was allowed to settle post-sonication, followed by the extraction being filtered through Whatman filter paper. The filtrate was concentrated utilizing a rotary evaporator under reduced pressure at temperatures ranging from 40 to 45°C to ensure the removal of methanol. The concentrated extract was subsequently dried to attain either a semi-solid or a dry mass. It was then stored in an airtight container at a temperature of 4°C for prospective application in gel formulation. Following sonication, the mixture was permitted to settle before being filtered through Whatman filter paper to eliminate any remaining plant residues.

Table 2: Formulation of Batches of Gel Formulation

Sr.No	Ingredients	F1	F2	F3	F4
1	Distilled water	3 ml.	3.5 ml.	5 ml.	5 ml.
2	Glycerine	2.0 gm.	2.5 gm.	1.5 gm.	2.0 gm.
3	Tea tree oil	0.5 gm.	0.4 gm.	0.3 gm.	0.3 gm.
4	Guar gum	1.5 gm.	0.8 gm.	1.2 gm.	1.0 gm.
5	Drug extract	1.2 gm.	1.8 gm.	1.5 gm.	1.5 gm.
6	Aloe Vera gel	q.s.	q.s.	q.s.	q.s. to 20.0 gm.
7	Reasons For Failure	Too viscous due to high Guar gum Leads to poor spreadability.	Excess glycerine leads to greasy texture and reduced aesthetics	High Aloe Vera and Guar gum leads to overly thick and stiff gel	Final Formulation

https://doi.org/10.47552/ijam.v16iS2.6287 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Formulation of Gel

With constant stirring, 1.0g of Gaur gum was added to 5 mL of distilled water. After that, it was left for half an hour. In a beaker with hydrated guar gum, 1.5g of the *E. hirta* plants methanolic extract was added, and the mixture was gently stirred. It was well blended with 0.3g of tea tree oil. Aloe vera gel was weighed and its volume was adjusted to 20g moved the gel into a sterile container and kept it in dry and cool place (25, 26).

Evaluation of Gel

Clarity and Appearance

- Purpose: Colour, transparency, homogeneity, and the presence of any suspended particles.
- Procedure: Visually inspect the gel against a black and white background and in normal light was examined.

Measurement of pH

- Purpose: To obtained skin compatibility.
- Procedure: Weighed one gram of gel and dispersing it in ten millilitres of distilled water. Use a digital pH meter that has been calibrated to determine the pH.

Spreadability

- Purpose: To determine how simple it is to apply to the kin.
- Procedure:
  - Sandwich one gram of gel between two 20 x 20 cm glass plates.
  - For one minute, apply a given weight on the top plate.
  - Determine the spread circle's diameter.

Formula: $S = M \times L/T$

Where, S = Spreadability coefficient. M= Mass
L = Length moved on the glass slide. T = Time taken.

Figure 2: *E. hirta* topical gel

Figure 3: pH detection

Figure 4: Spreadability

Extrudability

Purpose: Determines the amount of force needed to remove gel from the tube.
Procedure: Involves filling a collapsible tube with gel. Measure the amount extruded in ten seconds after applying force to the crimped end.

Uniformity

Purpose: To obtain consistency and even dispersion of active ingredients. Procedure: Visually examine and handle small samples.

Skin Irritation Test (Patch Test)

- Purpose: To assess skin application safety.
- Procedure: Apply a tiny bit of gel to a 1 cm^2 section of three to five healthy volunteer forearms. Over a 24-hour period, keep an eye out for redness, itching, or inflammation.

Microbial assay

Table 3: Microbial assay

Sr.no	Microorganism	Result	Inference
1	*Aspergillus niger*	+	No growth
2	*Candida albicans*	+	No growth
3	*Streptococcus aureus*	+	No growth

Zone of Inhibition

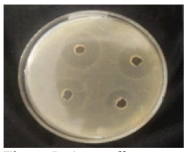

Figure 5: *Aspergillus niger*

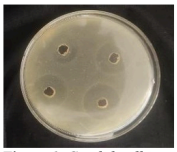

Figure 6: *Candida albicans*

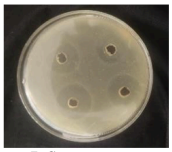

Figure 7: *Streptococcus aureus*

Results

Pharmacognostic study

Shade-dried leaves of the *E. hirta* plant were evaluated for organoleptic, microscopic, and physical characteristics. The organoleptic characteristics of the *E. hirta* plant are given in Table 4.

Table 4: Organoleptic characterestics of *E.hirta*

Organoleptic characters	Description
Color	Leaves: green with purplish undertones. Stems: Round, red stems with long, white hairs lining them.
Odor	Characteristic odor that is slightly pungent and bitter.
Texture	Hairy texture on its stems and leaves
Taste	Bitter and astringent, with a cooling effect.
Size	Leaf length (2-7 cm), leaf width (0.5-1 cm), flower size (0.5-1.5 cm).
Shape	Oblong, paired leaves have a sharp leaf border

Physicochemical Studies

The ash values, extractive values of powdered leaves were investigated. The information congregated from the previous studies is presented in Table 5. The physicochemical analysis performed in this study will help to identify plant adulteration with other species.

Table 5: Parameters of physicochemical studies

Sr.no.	Parameters	% value (w/w)
1	Loss on drying	14.14
2	Total ash value	12.66
3	Water-soluble ash value	10.26
4	Acid insoluble ash value	2.41
5	Sulphated ash value	14.21
6	Alcohol soluble extractive value	8.37
7	Water-soluble extractive value	8.55

(% w/w = Percent weight by weight)

Extraction Yield

The extraction yields of the *E. hirta* plant were quantified for methanolic, and hydroalcoholic extracts. The extraction yield was determined by employing the equation that relates the weight of the extract to the weight of the desiccated plant material. The hydroalcoholic extracts of the foliage demonstrated a superior yield (23.36 % w/w), followed closely by the methanolic extract (19.76 % w/w).

Phytochemical analysis

The phytochemical examination of the extracts was conducted according to a standard method delineated in the literature (Khandelwal, 2008). The findings of the phytochemical investigation are presented in Table 6. Phytocompounds were found to be predominantly concentrated in the methanol extract in comparison to the hydroalcoholic. Among the various phytocompounds, alkaloids, flavonoids, polyphenols, saponins, exhibited higher concentrations in the methanolic extract. The hydroalcoholic extract revealed the presence of amino acids, proteins, carbohydrates, polyphenols, and tannins.

Table 6: Phytochemical screening of *E. hirta*

Sr. No.	Phytoconstitue	Methanolic	Hydroalcoholic
1	Carbohydrates	-	+
2	Proteins	-	+
3	Amino acid	-	+
4	Alkaloids	+	-
5	Flavonoids	+	-
6	Saponin	+	-
7	Glycosides	-	-
8	Polyphenols	+	+
9	Sterols	-	-
10	Terpenoids	-	-
11	Tannins	-	+

(+) = Positive test; (-) = Negative test

Table 7: Evaluation Parameter of topicl gel

Sr.no	Evaluation parameter	Result
1	Clarity and Appearance	Clear
2	Measurement of pH	5.41
3	Spreadability	120 g.cm/s
4	Extrudability	Pass
5	Uniformity	Pass
6	Skin Irritation Test	Pass
7	Microbial assay	pass

Discussion

The study conducted a comprehensive analysis on ethnomedicinal, phytochemical, and physico-chemical properties of *Euphorbia hirta,* commonly referred to as Asthma Plant or Garden Spurge. The ethnopharmacological studies revealed that the *E. hirta* plant traditionally used as anti-inflammatory, antioxidant, antitumor, antidiabetic, anxiolytic, as well as its utility in various respiratory condition, expectorants and digestive problems. The plant powders under investigation exhibited a moisture loss of 14.14% w/w. This water content level is known to impede oxidation reactions and fermentation processes. The total ash content of the *E. hirta* powder was determined to be 12.66% w/w. The sulphated ash, water-soluble and alcohol-soluble ash values were noted as 14.21%, 10.26 % w/w and 2.41% w/w, respectively. Furthermore, the extractive values for both alcohol and water-soluble compounds were found to be 8.37%w/w and 8.55%w/w, respectively. The study delineates several active phytoconstituents present in *Euphorbia hirta*, including flavonoids, alkaloids, saponins, terpenoids, and tannins. These bioactive compounds are attributed to the diverse pharmacological effects exhibited by the plant. The gel formulation proved to be safe, stable, and effective for topical application. This research substantiates the medicinal potential of *Euphorbia hirta* and supports its integration into natural therapeutic products, encouraging further clinical studies and product development in phytopharmaceuticals.

Conclusion

The present study reviewed the botanical description, medicinal uses, active phytoconstituents and pharmacological activities of an invasive plant *E.hirta*. It has a wide and significant therapeutic activity. It is a popular herb among practitioners of traditional medicines in China and other countries. It has been used for treatment of various ailments in the form of decoction or infusion. It is used in intestinal disorders, diarrhea, amoebic dysentery, peptic ulcers, and asthma, bronchitis and skin diseases. The parts have various anti-inflammatory, antidiabetic, anti-tumor, antioxidant and antimicrobial properties. More research is being carried out to isolate and describe particular active constituents for various purposes. The gel formulation prepared for anti-inflammatory activity proved to be safe, stable, and effective for topical application. Further investigation is necessary to understand the relationship between phytochemicals and their activities through various analytical studies.

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https://doi.org/10.47552/ijam.v16iS2.6294 ISSN No: 0976-5921

Int J. of Ayurvedic Med, "Natural to Synthetic: The Convergence of Traditional Medicine to Modern Medicine" Supplement 2025

Formulation And Evaluation of Herbal Emulgel for Treatment of Psoriasis

Research Article

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Abstract

One of the most prevalent skin conditions in humans, psoriasis is thought to have strong genetic roots. It is characterized by abnormal keratinocyte differentiation and excessive development, however with the right treatment, it is completely curable. The development of psoriatic plaques is associated with environmental triggers and other factor such as streptococcal infection, physical trauma (such as tattoos and surgical incisions), smoking and alcohol abuse, as well as certain medications such as antidepressant drugs, anti-hypertensive drugs, anti-cytokine medication. The formulation goal is to develop an emulgel infused with a combination of herbs such as Neem oil, Coconut oil and Aloevera gel, that can help to alleviate the symptoms of Psoriasis and also to cure the condition. The prepared gel formulations were evaluated for pH, viscosity, drug content uniformity, physical characterization, phytochemical screening and by also evaluating it's effect on psoriasis affected skin. Aloevera and neem oil, and coconut oil containing emulgel exhibited the drug content within the optimum range 87.56%-90.45% which concluded efficient drug loading in the formulation.

Keywords: Psoriasis, Emulgel, Aloevera, Neem oil, Coconut oil.

Introduction

One of the most prevalent skin conditions in humans, psoriasis is thought to have strong genetic roots. It is characterized by abnormal keratinocyte differentiation and excessive development, however with the right treatment, it is completely curable (1).The development of psoriatic plaques is associated with environmental triggers and other factor such as streptococcal infection, physical trauma (such as tattoos and surgical incisions), smoking and alcohol abuse, as well as certain medications such as antidepressant drugs, anti-hypertensive drugs, anti-cytokine medication (2).

Psoriasis is a chronic, genetically influenced, remitting and relapsing scaly and inflammatory skin disorder that affects 1 to 3 percent of the world's population. For best treatment, specific immune therapeutics are necessary for the unique auto inflammatory process of generalized pustular psoriasis (GPP). Clarifying the immune processes and genetic variations (especially those pertaining to the IL-36 signaling axis) that contribute to illness pathogenesis can improve our comprehension of the immune responses, the course of the disease, and the resolution of inflammation. (1,3).

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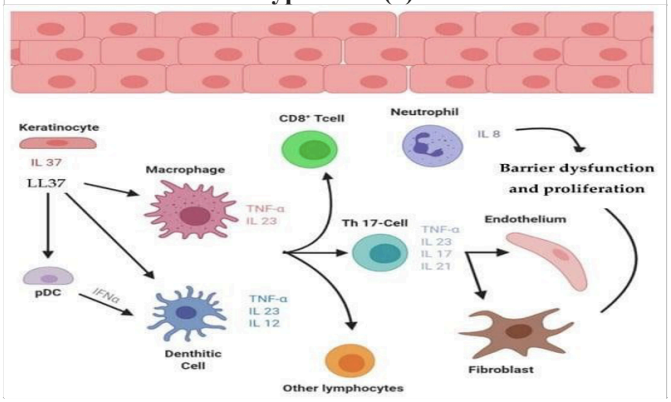
The study of the pathophysiology of psoriasis has significantly advanced our understanding of skin biology in general. Over the last fifteen years, advances in our knowledge of the pathophysiology of psoriasis have resulted in very successful, targeted therapies that have given us a better understanding of the pathophysiology of chronic inflammatory illnesses that are dominated by the IL-23/Th17 axis. (4)

Pathogenesis of Psoriasis

Figure 1: Plaque-type psoriasis pathogenesis principal hypothesis (5)



Activation of the adaptative immune response through T cell subsets drives the maintenance phase of psoriatic inflammation. Two distinct mechanisms contribute to the proliferation of keratinocytes in the epidermis: inflammation caused by TNF-α, IL-17, and IFN-γ, and LL-37 complexing with DNA to increase the synthesis of type I IFNs. By generating LL-37, proinflammatory cytokines (TNF-α, IL-1β, and IL-6), chemokines, and S100 proteins, all of these mediators

further sustain keratinocyte activation and spread chronic inflammation. When taken as a whole, these encourage keratinocyte growth and the synthesis of AMPs and chemokines, which in turn encourage the recruitment of neutrophils and maintain skin inflammation. The primary hypothesis on the development of plaque-type psoriasis is illustrated in Figure 1. Plaque-type psoriasis is characterized by the TNFα–IL-23–Th17 inflammatory cascade. Hematopoietic cells, including CD8+ T cells (Tc17), invariant NKT cells, γδ T cells, non-T non-B lymphocytes (also known as type 3 innate lymphoid cells), and neutrophils, are among the cell types that produce the various forms of IL-17. IL-17A-F cytokines control inflammatory reactions [20]. It has been established that a receptor that may be activated by two distinct cytokines, IL-17A and IL-17F, with IL-17A having a larger effect, mediates the most significant signaling in psoriasis. (5,6).

New comorbidities related to Psoriasis

a) Psoriatic Arthritis

Alibert in 1818 observed the occurance of simultaneous Arthritis and Inflammatory Joint Disease in patients of Psoriasis. The arthritis was discovered as a different form i.e. a distinct one from Rheumatoid Arthritis. The major difference between the Rheumatoid and Psoriatic Arthritis was the absence of (RF) Rheumatoid Factor, lack of B-cell activation and also the presence of HLA- B27 and a continuous of enthesitis (7). Since some medical professionals believed that psoriasis was a chronic wound-healing reaction, a subsequent model emphasized the possible involvement of fibroblasts. By this theory, fibroblasts activated in the dermis were viewed as the genesis of psoriatic plaques as by driving keratinocyte proliferation . Defects in neutrophils have been proposed. Mast cells have been noted to have increased interferon-gamma (IFN-γ) in psoriatic patients (8).

b) Osteoporosis

Bone microarchitecture change resulting in decreased strength is called as osteoporosis which came out as a recent comorbid condition related to Psoriasis. Current advancement in the research described the involvement of certain cytokines such as Interferon-gamma, TNFalpha and Interleukin-6. The research suspected that the patient of Psoriasis (9).

c) Chronic Obstructive Pulmonary Disease

COPD affects approximately 10% of the population and encompasses chronic obstructive bronchitis and emphysema. Th-1 and Th-17 cells are disrupted in psoriasis, and IL-1, IL-6, IL-8, and TNF-α are elevated along with systemic inflammation markers including C-reactive protein (CRP). Both of these cytokines and CRP are elevated in COPD and are linked to the severity of the illness. Moreover, sputum and bronchoalveolar lavage fluid from COPD patients had elevated levels of neutrophils, TNF-α, IL-6, and IL-8.40 IL-17, which is already linked to psoriasis through the Th-17 response, was recently linked to lung conditions like COPD (6,9).

d) Obstructive sleep apnea

In addition to daytime symptoms brought on by excessive sleepiness, OSA is a common type of sleep disorder that affects 2-4% of the general population. It is characterized by recurrent episodes of partial or complete obstruction of the upper airway during sleep, which causes intermittent hypoxia and frequent awakenings. OSA patients have inflammation of the upper airways, as well as increased oxidative stress and systemic inflammation indicated by elevated levels of TNF- α, IL-6 and C-reactive protein (5,9).

Table 1: Marketed formulations for the treatment of Psoriasis

Sr.no.	Chemical Constituents	Adverse effects
1	Triamcinolone (Trianex)	itching, scaling, severe redness, soreness, or swelling of the skin. Blistering, burning, crusting, dryness, or flaking of the skin, irritation, redness and scaling around the mouth
2	Clobetasol (Clobex)	High doses or long-term use of clobetasol can lead to thinning skin, easy bruising, changes in body fat (especially in your face, neck, back, and waist), increased acne or facial hair, menstrual problems, impotence, or loss of interest in sex.
3	Coal tar based shampoos (Neutrogena T-Gel and MG217 Psoriasis Medicated Conditioning Shampoo)	Skin/scalp irritation or staining of skin/hair (especially in patients with blonde, bleached, dyed, or gray hair) may occur
4	DHS Tar lotion and shampoo, Doak Tar lotion and shampoo	DHS Tar lotion and shampoo, Doak Tar lotion and shampoo long term use can cause tar acne.
5	Calcipotriol (Cal) and Betamethasone dipropionate (BDP)	Calcipotriol can cause itching, stinging, dryness, and rash, while BDP can lead to thinning of the skin, stretch marks, and skin infections.

Tachyphylaxis, more often observed local cutaneous NB in the face and intertriginous areas include purpura, folliculitis, acne, striae distensiae, telangiectasia, and skin atrophy could make co-existing dermatoses worse: tinea, perioral dermatitis, and roseacea could result in contact dermatitis.Systemic (rare): glaucoma, cataracts, Cushing's disease, femoral head osteonecrosis, and HPA axis support (10).

Use of Neem oil in the treatment of Psoriasis

Neem possesses anti-inflammatory, antibacterial, analgesic, antiviral, antifungal, immune modulatory and antioxidant activities which substantiate its use as skin therapy. Various novel formulations and associated

patents that improved the permeability of neem based products across skin could be found in literature (11). An indigenous medication consisting of an aqueous extract of Neem leaves was tested in 50 cases of uncomplicated psoriasis under a conventional coal tar regimen in a double-blind clinical drug trial. Patients who took the medication in addition to coal tar responded more quickly and effectively than those who took a placebo, and no adverse effects were observed during the trial period (11).

Use of Aloevera gel in treatment of Psoriasis

Aloevera pulp is made up of 98.5% water and 1.0–1.5% proteins, organic acids, enzymes, phenolic compounds, polysaccharides, and minerals. Hepatoprotective, immunosuppressive, anti-diabetic, analgesic, anti-inflammatory, and antioxidant qualities are all present in aloe vera gels and extracts. The preclinical and clinical benefits of A. vera in the treatment of psoriasis have been shown in many research. Antioxidant, antinociceptive, and anti-inflammatory effects have also been reported. Aloe vera modulates immune response by activating macrophages with an increase in lymphocyte response to alloantigens, releasing nitric oxide and cytokines, and activating the maturation of immature dendritic cells. A double-blind, placebo-controlled, randomized study was conducted to assess the potential adjuvant activity and tolerability of an Aloe vera extract in a hydrophilic cream for the treatment of psoriasis vulgaris. For four weeks, the interventions were applied topically three times a day for five days in a row. Patients were clinically assessed once a week up to 16 weeks, and afterward followed up once a month for the following 8 months (12).

Use of coconut oil in the treatment of Psoriasis

Fatty acids also have skin-soothing benefits for treating psoriasis. Coconut oil might help soften the skin, due to containing lauric, capric, and caprylic acids, which are all types of fatty acid. Lauric acid also has antimicrobial activity, which helps to reduce the risk of skin infections and irritation. The biggest benefit of the oil is its ability to moisturize the scalp. In fact, it's sometimes used as a conditioner to hydrate dry scalp and skin. This possibility brings hope to people experiencing dry scales that itch relentlessly (13).

Method of Preparation

Collection of Aloevera

Aloevera was collected from the herbal garden. It was cleaned properly with sterile water to remove the yellowish fluid. The sap was scraped out of aloevera leaves. The fibers were broken down uniformly by mechanical stirrer at 4000 rpm for 1 hour. It was filtered out and strained using the muslin cloth. The filtrate was refrigerated and stored until it's use. Neem oil was purchased from an online store that was manufactured and marketed by Baidyanath.

Preparation of standard solution

1 ml of aloevera gel and neem oil were weighed accurately and each were dissolved in methanol in separate volumretric flask, sonicated, filtered and analyzed.

Formulation table

Method of preparation of emulgel

The gel base (aqueous phase) was prepared by dispersing carbopol in a mixture of propylene glycol and distilled water. Triethanolamine was added dropwise until the pH of the solution becomes 7.6 suitable for skin. The aloevera gel was added to the mixture dropwise and the solution was placed on a magnetic stirrer.On the other hand the oil phase was prepared when neem oil is added to the mixture of coconut oil and PEG (Polyethylene Glycol). A small quantity of methyl paraben was added to water and heated until it completely dissolved. The methyl paraben was then added to the aqueous phase. A small quantity of Propyl paraben was added to the oil phase. Both aqueous and oil phase was heated on a hot plate maintaining the temperature upto 50 degree Celsius. The oil phase was added dropwise to the aqueous phase. 1% of Zinc oxide was added to the prepared solution. The prepared emulgel was placed on a magnetic stirrer for 3 hours. The prepared gel was then filled into a containor, labelled and refrigerated.(14,15)

Table 2: Formulation table of emulgel formulation

Sr. No.	Ingredients	F1	F2
1	Aloevera(ml)	40	40
2	Neem oil (ml)	3.5	3.5
3	Coconut oil (ml)	4.0	4.0
4	Carbopol (gm)	1.0	2.0
5	Triethanolamine (ml)	1.0	1.0
6	Propylene glycol (ml)	7.5	7.5
7	Polyethylene glycol (ml)	10	10
8	Methyl Paraben(gm)	1.0	1.0
9	Propyl Paraben(gm)	1.0	1.0
10	Zinc Oxide(gm)	1.0	1.0
11	Water(ml)	q.s	q.s

Evaluation of the herbal components

Characteristic of neem oil and aloevera gel used

The characteristics such as physical state colour, odour and taste were checked and the results are depicted into table no.3 (15,16).

Construction of Calibration curve

Calibration curve of aloevera and neem oil: Preparation of standard solution: 1 ml of aloevera gel and neem oil were weighed accurately and each were dissolved in methanol in separate volumetric flasks .

Evaluation of the formulation

Physical characteristics of the emulgel

Physical appearance

Physical parameters such as colour and appearance were checked visually and the results are depicted in the table 3.

Determination of the pH

The developed formulations were evaluated for pH using a digital pH meter (Elico LI 617), the pH meter probe was immersed in the formulation for 5 minutes and then the readings were taken.

Spreadability test

Two glass plates of 5x20 cm and a weight of 30 gm were used for the study. About 100 mg of the test formulation was placed over one glass plate. Then the second glass plate was placed over the first glass plate in such a way that the formulation is sandwiched between the 2 glass plates. An extra weight of about 10gm was placed over the sandwiched glass plate for uniform spreading, then after five minutes, the diameter of the spread formulation was measured using as scale. An average of these readings were taken.

The formula for calculating the spreadability of a gel is

S=M x L/T where ;

S : spreadability

M: the weight in the pan, tied to the upper slide

L: Is the length moved by the glass slide T: Is the time taken to separate the slide completely from each other (17).

Viscosity determination

The viscosity of is an important factor to determine the rheological properties of the emulgel. The viscosity of emulgel can be determined by using the Brookfield Viscometer at 25 degree Celsius. The measurement over the whole range of speed setting from 10 rpm to 100 rpm with 30 sec between two successive speed (17).

Drug content determination

Each formulation was weighed 1ml accurately and transferred into a 10 ml of volumetric flask and volume was made up by using methanol as a solvent. The content was filtered out using a suitable filter paper and sonicated for 5 minutes 0.1 ml of solution was taken up from the above mixture and again the dilution was made upto 10 ml by using a volumetric flask.1 ml filtrate was taken and the drug content was estimated by using UV/Visible Spectrophotometer at 250 nm for neem and 205 nm for aloe.

Results and Discussion

Evaluation of the herbal components use formulation

Physical Characteristics of neem oil, aloevera gel

The physical characteristics such as colour, odour and taste of neem oil and aloevera gel were observed and the results were stated in table no.3

Table 3: Characteristics of herbal components

Sr. no.	Characteristic	Observation (Neem oil)	Observation (Aloevera gel)
1	Physical State	Liquid	Semisolid
2	Colour	Brown	Translucent
3	Odour	Pungent	No odour
4	Taste	Bitter	Characteristic

Construction of Calibration Curve

The construction of calibration curves of neem oil and aloevera in methanol were constructed so that the absorbance results collected through experimentation could be directly converted into concentration by extrapolating through the linearity range.

Figure 3: Calibration curve of neem oil in methanol at 250 nm

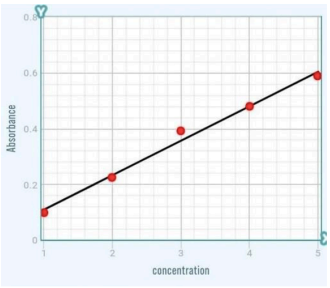
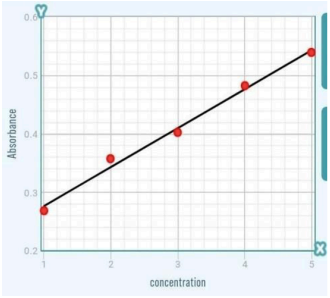


Figure 4: Calibration curve of aloevera gel in methanol at 205nm



Evaluation of the formulation

Figure 5: Formulation batch F1 and F2

Table 4: Characteristics of herbal components

Sr. No.	Physical Characteristics	Batch F1	Batch F2
1	Physical State	Semisolid	Semi-solid
2	Colour	Creamish brown	Creamish
3	Texture	Smooth	Smooth
4	Odour	Slightly pungent	Slightly
5	pH	6.88±0.88	6.95±0.97
6	Viscosity(cps)	4600±1.08	4097±1.12
7	Spreadability (gm cm/sec)	11.97±0.95	11.34±1.02
8	Drug Content	90.34%±1.14	91.48%±1.04
9	Phase Separation	Not observed	Not observed
10	Flocculation	Not observed	Not observed

Emulgel formulation

The emulgels were prepared by incorporation method. Polyethylene glycol and Propylene glycol were used as surfactants, coconut oil and neem oil were used as oil phase and water was used as external phase. To prepare the emulgel formulation oil phase was incorporated into the aqueous phase and then mixed by stirring continuously using magnetic stirrer. Samples in which no signs of phase separation, flocculation and sedimentation were visually observed and were selected as stable formulations. The emulgel prepared showed other physical characteristics such as physical state, colour, texture, odour as stated in table 4.

Physical characterization of Emulgel formulation

- **pH determination:** The pH values of 2 developed formulae was in the range 5-6 which is considered acceptable to avoid risk of irritation upon application to the skin and the results are tabulated in table no 4.
- **Viscosity determination:** The viscosity of the emulgel formulation was in the range 1,134-21,000 centipoises (cps) which is also considered acceptable as it displays the excellent rheological properties of a formulation, the results are tabulated in table no.4
- **Spreadability:** Spreadabilty is very important because it shows the behavior of the emulgel that comes out of the tube. The spreadability values shown in the table no. shows that all the polymers used gave gels spreadable with a little shear. The diameters of the extended circle ranged from 11.97 and 11.34 gm cm/sec for formulation F1 and F2. The data in the table no.4 revealed that increase in the spreadability was always associated with decrease in concentration of gelling agent

Conclusion

In this study, aloevera and neem oil containing emulgel was developed as a carrier for topical delivery containing the combination of different herbal drugs was successfully formulated.The emulgel was prepared by incorporation method using Carbopol as a gelling agent and surfactants such as Polyethylene glycol and propylene glycol and zinc oxide was also added for UV protection.The magnetic stirrer and sonicator showed to be a simple and efficient techniques for mixing and size reduction, it was concluded that the system exhibited uniformity and enhanced diffusion rate due to these techniques and the parameters effecting performance of the formulation were optimized.The emulgel was characterized by a pH range of 5-6 which was concluded as an optimized pH range to avoid irritation on skin surface.The viscosity of the formulation F1 and F2 shown optimized range for the excellent rheological properties of the formulation. The spreadability values characterized that the prepared emulgels are easily spraeadable with the application of little shear, it was concluded that the spreadability values are within the optimized range of 11-12. The emulgel exhibited the drug content within the optimum range 87.56-90.45% which cocluded efficient drug loading in the formulation.It was also observed that no phase separation and flocculation was exhibited by the formulation which showed that the formulation exhibits optimized uniformity. It was also concluded that formulation batch F2 was developed, as low viscosity was exhibited by formulation batch F1, the viscosity was improved by increasing the concentration of Carbopol as mentioned in table no. 1.It was also observed that introduction of the coconut oil to the formulation affected the gel's strength, gelling point and oil binding capacity of the formulation and also improved the viscosity of the formulation.

Acknowledgement: Authors are thankful to Dr. Saleemuddin Farooqui, Principal of Shree Sainath College of Pharmacy, Nagpur, to provide all essential chemicals and laboratories facilities. The authors are thankful to Gurunanak College of Pharmacy, Nagpur, to provide lab facilities for evaluation of final product.

Conflict of interest: The authors have no conflict of interest.

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