ISSN No: 0976-5921

Research Article

Exploring the antiproliferative activity of Unani formulation *Itrifal Aftimoon* (IA) on MCF-7 breast cancer cells: An in-vitro screening

Salman Suhail Khan[1], Lahari Kuna[2], Sidama Gopal[3], Tasleem Ahmad[2], Uzma Viquar[1], Younis Iftikhar Munshi[4], Mohammad Zakir[1]*

1. Department of Ilmul Advia, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.
2. BCMB, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.
3. DSRU, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.
4. Deputy Director, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

Received: 16-05-2025 Accepted: 07-08-2025 Published: 30-09-2025

Abstract

Unani physicians provided a comprehensive elucidation of cancer, referred to as *Saraṭan*, along with several therapeutic approaches for its control. The canonical book of Unani medicine includes formulations composed of several compounds demonstrated to possess anti-cancer properties in humans. This research employed *Itrifal Aftimoon* (IA), a formulation including ten distinct drugs, to assess its antioxidant and anticancer properties on breast cancer cells. The DPPH assay was employed to assess antioxidant activity in MCF-7 breast cancer cells, while the MTT test was used to evaluate anticancer properties. The formulation was standardized, and HPTLC fingerprinting was conducted in anticipation of its potential future applications. The aqueous extract (AQ) exhibited the maximum activity, with an IC_{50} value of 61 µg/ml. Subsequently, the methanolic extract (ME) and the hydroethanolic extract (HE) demonstrated IC_{50} values of 71 µg/ml and 75 µg/ml, respectively while IC_{50} value for ascorbic acid was 21 µg/ml. The ME, HE, and AQ extracts exhibited anticancer activity with IC_{50} values of 64 µg/ml, 82 µg/ml, and 83 µg/ml, respectively. This contrasted with paclitaxel, which exhibited an IC_{50} value of 18 µg/ml. This study offers essential insights into the potential use of IA as a therapy for breast cancer.

Keywords: Antioxidant activity, Anticancer activity, Breast cancer cell line, MCF-7, Herbal formulation, Itrifal Aftimoon, Unani

Access this article online

Website: https://ijam.co.in

DOI: https://doi.org/10.47552/ijam.v16i3.6159

Introduction

Female breast cancer, with an estimated 2.3 million new cases, constitutes 11.7% of all cancer diagnoses, making it the most prevalent malignancy. Breast cancer is the most prevalent malignancy diagnosed in women and the primary cause of cancer-related mortality. Projections suggest that by 2040, breast cancer incidence would rise by 40%, while cancer-related mortality will escalate by 50% (1). The Arabic word "*Saraṭan*" translates to "crab." The term "crab" comes from the ancient Greek word καρκίνος (*karkinos*). Galen (*Jalinus*), a famous figure in ancient medicine (131-200 AD), drew an analogy between a disease and a crab. He described how the tendrils of the illness stretch like the claws of a crab (2). In Unani terminology, *Waram-o-Salaba* is defined by intense inflammation, lesions, and branches imbued with a melancholic humour (*Madda Sawdawi*) (3). This disorder generally arises in individuals who are overweight and possess a lax physique, leading to an increased prevalence of cancer, particularly in hollow organs where the etiological *Madda Sawdawi* (melancholic matter) can readily gather, such as the breast, lungs, cervix, oral cavity, and uterus (4,5). *Saraṭan* (cancer) presents as a solid, heated inflammation deeply embedded in the tissue, characterized by discomfort and dryness. The discomfort exacerbates due to pressure from the mass. Initially, the growth may be little, akin to a pea, although it has the potential to expand to the size of a watermelon. If the condition commences with excruciating agony, it is deemed untreatable (5,6).

In Unani medicine, *Saraṭan* (cancer) is classified as a *Sawdawi warm* (melanotic swelling). The onset and progression are influenced by multiple causes, particularly the excessive production of *Sawda* (black bile) and its anomalous transformations. Moreover, *Sawda* can also be generated from the combustion of *Safra* (yellow bile), especially referred to as *Mirra-i-Sawda* (bilious melancholy). Galen (Jalinus) posits that the elimination of detrimental humours from the body necessitates the use of *Mundij Sawda'* (concoctive for black bile) and *Mushil-i Sawda'* (purgative for black bile), which form the foundation of cancer treatment (7,8,9). The management of the cancer was done by the single as well as compound formulations. The *Aftimoon* (*Cuscuta reflexa* Roxb.), *Turbud* (*Operculina turpethum* (L.) Silva Manso), *Bisfayej* (*Polypodium vulgare* L.), *Sadabahar*

*** Corresponding Author:**
Mohammad Zakir
Department of Ilmul Advia, National Research Institute of Unani Medicine for Skin Disorders,
Hyderabad, India.
Email Id: urzakir@rediffmail.com

(*Catharanthus roseus* (L.) G. Don), and *Kutki* (*Picrorhiza kurroa* Royle ex Benth.) has been described in literature and their antioxidant and anticancer activities has been confirmed in scientific studies (10).

Contemporary cancer therapies encompass chemotherapy and radiation, both of which entail considerable side effects. Most chemotherapy agents target rapidly proliferating cells, impacting the bone marrow, gastrointestinal system, and hair follicles. These medications induce myelosuppression, nausea, vomiting, gastrointestinal complications, mucositis, alopecia, reproductive diseases such as sterility and infertility, and infusion reactions. Moreover, there is an increased susceptibility to infections due to impaired immune function (immunosuppression) (11).

An established Unani formulation called IA comprises ten components, several of which exhibit scientifically corroborated antioxidant and anticancer attributes was selected for this study. This formulation is beneficial for the treatment of *Sawdawi Amrad* (melancholic illnesses) and acts as a *Mushil Sawda* (black bile purgative) and *Mushil Balgham* (phlegm purgative) (table-1). This formulation is described in Unani classical pharmacopoeia '*Ilaj-ul-Amraz'* and '*Al Qarabadeen*' and its antioxidant and anticancer potential was evaluated by In vitro model (12,13).

Table 1: Ingredients of the IA

S. No.	Name	Botanical Name	Part used	Quantity
1	Post halela Kabuli	*Terminalia chebula* Retz.	Pericarp of mature fruit	35 gm
2	Post halela zard	*Terminalia chebula* Retz.	Pericarp of semi mature fruits	35 gm
3	Amla	*Phyllanthus emblica* L.	Fruits	35 gm
4	Sana Makki	*Senna alexandrina* Mill	Leaves	17.5 gm
5	Turbud safaid	*Operculina turpethum* (L.) Silva Manso	Root	17.5 gm
6	Aftimoon	*Cuscuta reflexa* Roxb.	Whole plant	17.5 gm
7	Sheetraj Hindi	*Plumbago zeylanica* L.	Barks	10.5 gm
8	Anisoon	Pimpinella anisum L.	Seeds	7 gm
9	Namak Hindi	Black salt	Crystals	7 gm
10	Bisfayej	*Polypodium vulgare* L.	Rhizomes	3.5 gm
11	Asal khalis	*Honey*	Honey	560 gm

Material and methodology

Preparation of test formulation (IA)

The crude drugs were authenticated by the botanist at the SMPU, NRIUMSD Hyderabad, and voucher specimen number for *Halela zard, halela Kabuli, amla, sana Makki, turbud, sheetraj Hindi, anisoon, bisfayej,* and *aftimoon* were assigned as SMPU/CRI-Hyd15136, 15137, 15138, 15139, 15140, 15141, 15142, 15143, and 15144, respectively. The IA was prepared in the GMP certified pharmacy of the Institute, by following the guidelines outlined in the *Al Qarabadeen* and *Ilaj-ul-Amraz* books. The AQ, HE, and ME extracts were prepared by combining 10 gm powder of IA with 100 ml of the corresponding solvent. The mixture was stirred at a speed of 50 rpm at a temp of 37°C, subsequently, filtered, dried in rotatory vacuum evaporator and stored in a desiccator.

Standardization of IA

Organoleptic parameters

Organoleptic parameters like texture, colour, odour, taste was evaluated to differentiate them from other plants (14,15,16).

Microscopic evaluation

All powders were stained separately with phloroglucinol 1% and conc. HCl for identification of lignified cells and tissues, H_2SO_4 to detect calcium oxalate crystals, iodine solution was used to find starch granules, Sudan red-G for cuticular cell walls, and Sudan red G in acetic acid and ethanol for the presence of essential oils, fats and resins and observed under microscope (WHO, 2011) (14).

Physico-chemical evaluation

The parameters included measurements of weight loss upon drying, pH of 1% and 10% aqueous suspensions, aqueous extract, alcoholic extract, and chloroform extract (14,15,16,17).

The pesticide Residue

The pesticide residue of the test drug was carried out by using standard methods of vegetable drugs by using Gas Chromatography- Mass Spectrometry (GC-MS). The test for the pesticide was done by M/s Bureau Veritas India Testing Services Pvt. Ltd., Sanath Nagar, Hyderabad, Telangana 500018, India

HPTLC analysis and phytochemicals screening

The 5 g test drug was weighed and transferred to a conical flask containing 100 ml of chloroform, and it was placed in an orbital shaker for 6 hours to ensure complete extraction. The drug sample extract was filtered and concentrated to 5 ml, followed by the execution of high-performance thin layer chromatography.

HPTLC was performed on 10 cm × 10 cm Pre-coated Aluminium Sheets of Silica Gel 60 F^{254} (Merck). Sample solution of about 7µl was applied as 10 mm width bands using fully automatic TLC applicator system of the CAMAG ATS 4. A linear ascending development with Toluene: Ethyl Acetate (8:2) as mobile phase was carried out in a twin trough glass chamber previously saturated with mobile phase vapour for 20 minutes at room temperature (25 ± 2°C). The development of solvent distance was 80 mm. After development plates were air-dried. TLC plate was scanned by CAMAG TLC scanner 4 at 254 and 366 nm. Then the TLC plate derivatized with 1% Vanillin-sulfuric acid and heated on the TLC plate heater at 105^0C for 5 minutes and then photographed under CAMAG TLC visualizer 2 and then scanned by CAMAG TLC scanner 4 under the UV visible chamber at 560 nm for detection of spots (14).

Qualitative analysis of phytoconstituent of the extracts

The AQ, ME and chloroform extracts of IA were subjected to test the presence of therapeutically active chemicals like alkaloid, glycoside, carbohydrate, flavonoids, saponins, tannins, protein, and phenols by using standard colour reactions tests (17,18).

Antioxidant and anticancer activity of IA.

The AQ, HE and ME extract of IA, obtained by continuous extraction and dried in lyophilizer was used to test antioxidant and anticancer activity of IA. The dried extract was dissolved in dimethyl sulfoxide (DMSO) to prepare 10 mg/ml stock solution (19).

Antioxidant assay

The DPPH assay was performed to evaluate the antioxidant capability. A 100 µL aliquot of 0.1 mM DPPH in methanol was combined with 50 µL of MA extracts in a micro test plate. The plate was positioned in a dark setting and incubated for 30 minutes to facilitate the intended response. The optical density of the plate was subsequently measured using a TECAN multimode reader at a wavelength of 517 nm. Ascorbic acid served as the control for the experiment (20).

The free radical scavenging activity (% inhibition) was calculate using following formula-

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

Cytotoxicity Assay

Estrogen receptor (ER)-positive MCF-7 cells were obtained from the NCCS in Pune, India. MCF-7 breast cancer cells were cultivated in T-25 and T-75 flasks, utilizing Dulbecco's Modified Eagle's Medium augmented with 10% fetal bovine serum, 1% 200 mM L-glutamine, and 0.5% antibiotic-antimycotic solution. The cultures were preserved in a CO_2 incubator at conditions of 95% air, 100% relative humidity, 5% CO_2, and a temperature of 37°C. Cultures were passaged every five days, and the culture media was replenished three times weekly. The Trypan Blue method was employed to enumerate viable cells using a hemacytometer (21).

MTT Assay

In a 96-well flat-bottom plate, 200 µl of medium was added to each well, and 5000 MCF-7 cells were seeded per well. The cells were permitted to adhere overnight, after which different quantities of AQ, HE, and ME extracts (0.1, 1, 10, and 100 µg/ml) of the IA were introduced to the corresponding wells. Following a 24-hour incubation at 95% air, 100% relative humidity, and 5% CO^2 at 37°C, 100 µl of MTT reagent (2.5 mg MTT in 10 ml phosphate-buffered saline) was added to each well. Following an extra 4 hours of incubation, 100 µl of DMSO solution was administered to each well to solubilize the MTT crystals. Optical density was measured with a TECAN multimode reader at a wavelength of 590 nm with paclitaxel as control. Each concentration was replicated in triplicate, and data was collected through three measurements. The untreated cells served as the control for viability assessment, reflecting 100% vitality. The outcomes are presented as percentages reflecting the degree of feasibility. The efficacy of cell growth inhibition for each extract will be represented by its IC_{50} value (22).

% Inhibition of extract against cell line was measured by formula given bellow:

$$\% \text{ Viability} = \frac{\text{absorbance of test sample}}{\text{absorbance of control (untreated cells)}} \times 100$$

$$\% \text{ Cell inhibition} = 100 - \% \text{ Cell viability}$$

Statistical analysis

The statistical calculation was performed using Graph Pad Prism 5.0, with ANOVA conducted through Open Epi software. The values of $p \leq 0.05$ were regarded as statistically significant.

Results

Physicochemical analysis

The results of organoleptic parameters, physicochemical analysis along with extractive values are given in the table-2.

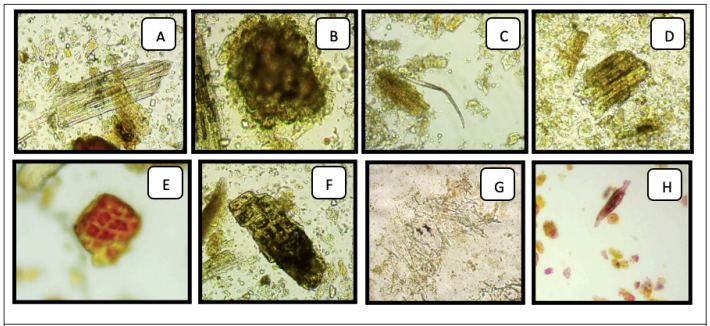
Table 2: Physicochemical analysis of IA

S. No.	Parameters	Values
1	Appearance	Semisolid
2	Colour	Blackish brown
3	Odour	Pleasant sweet
4	Taste	Sweet and astringent
5	Texture	Slightly rough
6	Alcohol soluble extractive (% w/w)	47.1442 ± 0.04 %
7	Water soluble extractive (% w/w)	48.2973 ± 0.22 %
8	Chloroform soluble extractive (% w/w)	4.1585 ± 0.07 %
9	pH of 1% aqueous solution	4.0166 ± 0.03
10	pH of 5% aqueous solution	4.35 ± 06
11	Loss in weight on drying at 105°C	3.6633 ± 0.11%
12	Total ash value (% w/w)	8.7424 ± 0.02 %
13	Acid insoluble Ash (% w/w)	1.3616 ± 0.04%
14	Bulk density g/ml	0.6366 ± 0.0032

Microscopic evaluation

The microscopic characters of IA powder are shown in Figure 1. It consists of pitted vessel, endosperm, trichomes, spiral vessels, epidermal cells, calcium oxalate crystals, palisade parenchyma, and sclereids (figure 1). These microscopic characters may be used as the fingerprinting and will help in the identification and quality control of the formulation in future.

Figure 1. Microscopy of the powder form of IA



A (pitted vessel), B (endosperm), C (trichomes), D (spiral vessels), E (epidermal cells), F (calcium oxalate crystals), G (palisade parenchyma), H (sclereids).

Qualitative phytochemical analysis

The results of the colour reaction for the presence of secondary metabolites are depicted in Table-3. The results reveal the

presence of therapeutically active secondary metabolites like alkaloids, glycoside, carbohydrate, flavonoids, saponins, tannins, protein, phenols and terpenoids etc (table 3). The pesticide residues were estimated and formulation have not shown presence of any pesticide (table-4).

HPTLC of IA

The HPTLC analysis performed under UV light at 254 nm indicates the presence of six notable peaks at Rf values of 0.006, 0.075, 0.129, 0.589, 0.657, and 0.728. Under UV light at 366nm, eight prominent peaks are observed with Rf values of 0.006, 0.108, 0.385, 0.594, 0.647, 0.715, 0.776, and 0.868. Moreover, upon exposure to the visible spectrum following derivatization with 1% Vanillin-sulfuric acid, the HPTLC profile reveals six peak values with Rf values of 0.010, 0.074, 0.124, 0.333, 0.486, and 0.733. This thorough HPTLC analysis employing diverse wavelengths and derivatization methods offers an in-depth understanding of the formulation's composition and attributes (Figure 2, 3).

Table 3: Chemical constituents of IA

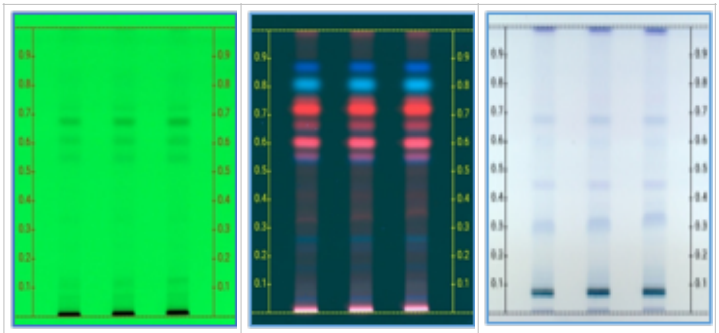
S. No.	Constituents	Results		
		Ethanolic extract	Aqueous extract	Chloroform extract
1	Alkaloids	+++	+++	++
2	Glycosides	++	-	+
3	Carbohydrate	+++	+	-
4	Flavonoids	+++	++	-
5	Saponins	++	-	+
6	Steroids	-	-	-
7	Tannin	++	+++	++
8	Proteins	+	+	-
9	Phenols	++	+	-
10	Terpenoids	+	+	++

Table 4: Pesticide residue analysis for IA

S. No.	Test Parameters in mg/kg	Test Result	LOQ	S. No.	Test Parameters in mg/kg	Test Result	LOQ
1	Alachlor	BLQ	0.01	26	Endosulfan sulphate	BLQ	0.01
2	Aldrin, dieldrin	BLQ	0.01	27	Endrin	BLQ	0.01
3	Dieldrin	BLQ	0.01	28	Ethion	BLQ	0.01
4	Azinophos methyl	BLQ	0.01	29	Fenitrothion	BLQ	0.01
5	Bromopropylate	BLQ	0.01	30	Fenvalerate	BLQ	0.01
6	Chlordane cis	BLQ	0.01	31	Heptachlor	BLQ	0.01
7	Chlordane trans	BLQ	0.01	32	Heptachlor epoxide	BLQ	0.01
8	Chlorfenvinphos	BLQ	0.01	33	Hexa-chlorobenzene	BLQ	0.01
9	Chlorpyrifos	BLQ	0.01	34	Alpha-HCH	BLQ	0.01
10	Chlorpyrifos methyl	BLQ	0.01	35	Beta-HCH	BLQ	0.01
11	Cypermethrin I	BLQ	0.01	36	Delta-HCH	BLQ	0.01
12	Cypermethrin II	BLQ	0.01	37	Methidathion	BLQ	0.01
13	Cypermethrin III	BLQ	0.01	38	Parathion	BLQ	0.01
14	Cypermethrin IV	BLQ	0.01	39	Methyl parathion	BLQ	0.01
15	O,P-DDT	BLQ	0.01	40	Permethrin I	BLQ	0.01
16	P,P-DDT	BLQ	0.01	41	Permethrin II	BLQ	0.01
17	O,P-DDE	BLQ	0.01	42	Phosalone	BLQ	0.01
18	P,P-DDE	BLQ	0.01	43	Piperonyl butoxide	BLQ	0.01
19	P,P-DDD	BLQ	0.01	44	Pirimiphos-methyl	BLQ	0.01
20	Deltamethrin	BLQ	0.01	45	Pyrethrins	BLQ	0.01
21	Diazinon	BLQ	0.01	46	Quintozone	BLQ	0.01
22	Dichlorvos	BLQ	0.01	47	Pentachloroaniline	BLQ	0.01
23	Dithiocarbamates	BLQ	0.01	48	Pentachlorophenyl methyl sulfide	BLQ	0.01
24	Endosulfan alpha	BLQ	0.01	49	Malathion (malathon and malaoxon)	BLQ	0.01
25	Endosulfan beta	BLQ	0.01	50	Lindane (gamma isomers of hexachlorocyclohexane)	BLQ	0.01

*BLQ – below the limit of quantification; LOQ – limit of quantification

Figure 2: HPTLC finger print of IA in chloroform extract at 254 nm, 366 nm and visible light



UV at 254 nm	UV at 366nm	Visible light (after derivatization)

Figure 3: Densitometry chromatogram of IA in chloroform extract at 254 nm, 366 nm and visible light

UV at 254 nm	UV at 366nm	Visible light (after derivatization)

DPPH radical scavenging activity

The antioxidant activity of all extracts exhibited dose-dependent findings, rising with greater concentrations. The results indicated the maximum activity in AQ extract (IC_{50} value 61 µg/ml), followed by ME extract (IC_{50} value 71 µg/ml), and finally HE extract (IC_{50} value 75 µg/ml), in comparison to Ascorbic acid (IC_{50} value 18 µg/ml) (table-5, table-6 and figure-4).

Table: 5 Antioxidant activity of IA by DPPH assay

Concentrations (µg)	AQ extract	HE extract	ME extract	Ascorbic acid
0.1	7	2	5	28
1	11	20	11	44
10	21	21	30	59
100	75	60	65	79

Table 6: IC_{50} of different extracts of IA using DPPH assay

Parameter	AQ extract	ME extract	HE extract	Ascorbic acid
IC_{50} Value (µg/ml)	61	71	75	18

MTT assay

The IC_{50} value of paclitaxel was 21 µg/mL, while the IC_{50} values of the ME, HE, and AQ extracts of IA were 64 µg/mL, 82 µg/mL, and 83 µg/mL, respectively. The results indicated that the activity was dose-dependent and increased as the dose was increased. (table-7, table-8 & Figure-5).

Figure: 4: Antioxidant activity of IA

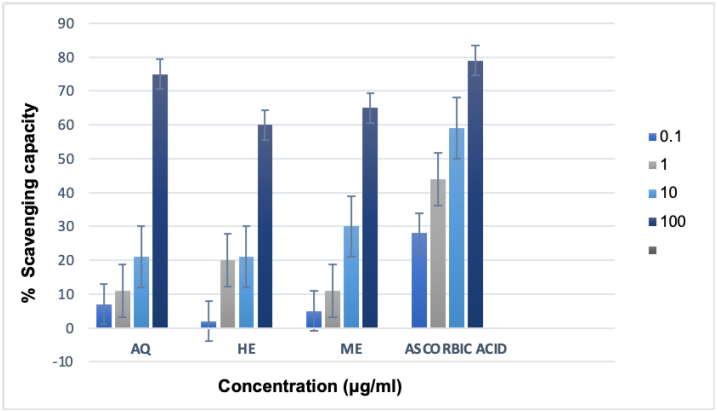


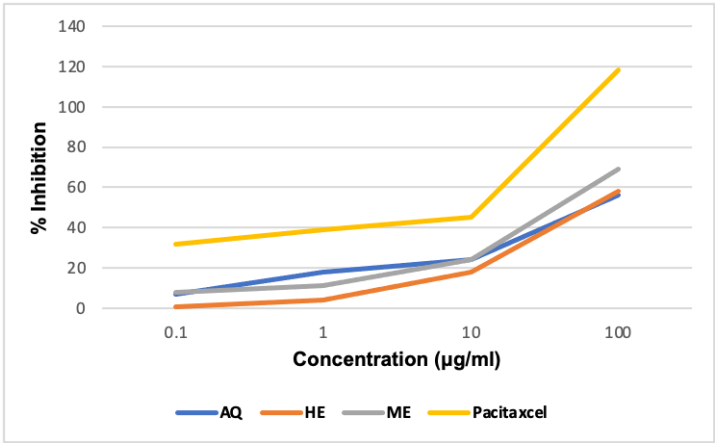
Table 7: Anticancer activity of different extracts of IA

Concentrations (µg/ml)	AQ extract	HE extract	ME extract	Paclitaxel
0.1	7	0.9	8	32
1	18	4	11	39
10	24	18	24	45
100	56	58	69	118

Table 8: IC_{50} Value of different extracts of IA

Parameter	ME extract	HE extract	AQ extract	Paclitaxel
IC_{50} Value (µg/ml)	64	82	83	21

Figure: 5: Anticancer activity of IA by MTT assay



Discussion

The study aimed to investigate the anticancer and antioxidant properties of the Unani formulation, IA, to validate its claimed anticancer effects as documented in Unani literature. We performed phytochemical screening of extracts to identify the active secondary metabolites in this formulation and evaluated specific physicochemical standardization parameters. Although Unani physicians have historically employed IA for cancer treatment, there is a lack of scientific evidence to substantiate these claims. This research represents a preliminary effort to validate the Unani tradition by examining its anticancer properties through contemporary scientific methods. This initial investigation is expected to establish a foundation for subsequent

clinical research regarding the potential therapeutic applications of this formulation.

In the ABTS assay, *T. chebula* demonstrated the highest efficacy in scavenging free radicals. The evaluation of superoxide-free radical scavenging activity through the NBT assay has shown notable effectiveness, with free radical inhibition percentages ranging from 80 to 100%. Standardized extracts from T. chebula demonstrated significant levels of antioxidant-related components and displayed a strong superoxide anion scavenging activity, with IC_{50} values ranging from 0.06 to 0.13 mg/mL (23). The methanolic extracts derived from *T chebula* tubers exhibit anti-inflammatory and antioxidant properties, along with cytotoxic effects, inducing apoptosis in the oral cancer cell line at a concentration of 30 µg/mL (24).

Many ingredients of test formulation have shown antioxidant and anticancer activities when tested in different studies. The DPPH assay, hydroxy radical scavenging assay, superoxide radical scavenging assay, nitric oxide assay, and peroxynitrite scavenging assay of the methanolic extract of *E. officinalis* fruit (25), along with the DPPH assay of ethanolic fruit flesh, seed coat, and seed extracts (26), demonstrated notable antioxidant activities, including DPPH scavenging activity, hydroxy radical scavenging activity, superoxide radical scavenging activity, FRAP assay, and inhibition of lipid peroxidation assay in the water extract (27). It has also demonstrated cytoprotective properties against H2O2-induced damage in PC12 cells (28). The aqueous and ethanolic extract of *E. officinalis* shown dose-dependent antiproliferative activity against HeLa, HT29, HCT116, MCF7, and Jurkat cells (29).

The flavonoids obtained from *C. angustifolia* extracts demonstrated significant anticancer activity against MCF-7 (IC_{50}, 4.0 µg/µL), HeLa (IC_{50}, 5.45 µg/µL), and Hep2 (IC_{50}, 7.28 µg/µL). Significant antioxidant activity was observed, with an IC_{50} of 2.41 µg/mL against the DPPH radical (30). A study examined the anticancer effects of stem extracts of *O. turpethum* in a female rat model with breast tumors induced by 7,12-dimethylbenzanthracene (DMBA) and evaluated their antioxidant efficacy. Ethanolic extracts of stem bark were administered orally at a dosage of 100 mg/kg, with DMBA used as an inducer at a dosage of 20 mg over a duration of 45 days. The findings indicated a notable reduction in lipid peroxidation and increased antioxidant levels, alongside a decrease in breast tumour weight (31).

The *C. reflexa* stem extract demonstrated a dose-dependent antioxidant effect, with IC_{50} values of 87.38 µg/mL for DPPH, 318.34 µg/mL for reactive nitrogen oxide, 359.96 µg/mL for FRAP, 526.12 µg/mL for hydroxy free radicals, 698.45 µg/mL for ABTS, and 892.71 µg/mL for superoxide anion. Additionally, it exhibited cytotoxic activity against A549 cells with an IC_{50} of 436.80 µg/mL. Analysis of morphological characteristics in treated cells has revealed indicators of apoptotic properties. Treatment with *C. reflexa* stem extract increased ROS production and mitochondrial depolarization in A549 cells, suggesting that this therapy exhibits significant apoptotic properties (32). The DPPH assay revealed IC_{50} values of 72.33, 84.71, and 79.69 for the aqueous, ethanol, and methanol extracts of *P. anisum*, respectively, indicating that the aqueous extract exhibits the highest antioxidant activity (33). A study confirmed the anticancer effects of the ethanolic extract of *P. vulgare* on a human melanoma cell line. The findings indicated that a concentration of 0.123 mg/ml of herb extract significantly enhanced apoptosis in melanoma cells (34).

The findings of this investigation indicate that the AQ extract exhibited the most significant free radical scavenging activity among the three extracts, with an IC_{50} value of 61 µg/ml. IA at a dosage of 61 µg/ml was capable of inhibiting 50% of the free radicals in the experiment. The ME extract had somewhat reduced activity with an IC_{50} value of 71 µg/ml, but the HE extract displayed the lowest activity among the three, with an IC_{50} value of 75 µg/ml. at conclusion, these results indicate that the AQ extract is especially proficient at scavenging free radicals, surpassing both the ME and HE extracts in this particular experiment.

The research revealed that distinct extracts of IA exhibited differing degrees of cytotoxicity on MCF-7 cells, with ME extract (IC_{50}: 64 µg/ml), HE extract (IC_{50}: 82 µg/ml), and AQ extract (IC_{50}: 83 µg/ml). All extracts of IA had concentration-dependent cytotoxic effects, with the ME extract showing the most significant reduction in cell viability. The findings of this investigation indicate that formulation IA had a significant cytotoxic impact in comparison to the control medication paclitaxel. This strongly indicates its potential efficacy on the MCF-7 cell line.

Conclusion

This study identified bioactive substances including alkaloids, glycosides, flavonoids, terpenoids, phenols, tannins, and saponins in IA. The IA, had antioxidant and cytotoxic effects on MCF-7 cell lines, equivalent to conventional agents such as ascorbic acid and paclitaxel, respectively. This study corroborates Unani assertions that IA may be effective in treating breast cancer. The synergistic impact of the herb in IA may combat breast cancer cells and diminish malignancy. The Unani perspective posits that natural remedies are safe, easily assimilated, and metabolizable without harmful secondary metabolites due to their similarity to the human body. Nonetheless, additional preclinical and clinical research are required to substantiate its anticancer efficacy in humans. This study may act as a foundational probe for subsequent research in animal models and clinical trials.

Competing Interests

The authors have declared that no competing interest exists.

Sources of funding

This study was supported by internal funding from the CCRUM, Ministry of Ayush, Government of India.

Acknowledgments

The authors are thankful to Director General, CCRUM, New Delhi for providing the necessary facilities and infrastructure for carrying out the research work at NRIUMSD, Hyderabad.

Author contributions

Mohammad Zakir: Conceptualization, Methodology, Supervision, Writing - Original Draft; **Salman Suhail**: Conceptualization, Investigation, Writing- Reviewing and Editing; **Tasleem Ahmad:** Methodology, Formal analysis **K. Lahari:** Investigation**; Uzma Viquar**: Reviewing and Editing; **Sidama Gopal:** Investigation for quality control, **Younis Iftikhar Munshi:** Resources and supervision.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-249. https://doi.org/10.3322/caac.21660
2. Maseehi AAIQ. Kitabul Umda fil Jarahat, (Urdu translation), Vol-I & II, CCRUM, Ministry of Health and Family Welfare, Govt of India, New Delhi 1995:169-75.
3. Ibn Rushd AW. Kitabul Kulliyat. 1st ed. (Urdu translation), CCRUM, Ministry of Health and Family Welfare, Govt of India, New Delhi, 1980. p.89-90.
4. Sina I. Al Qanoon Fit Tib, Vol-4: Idara Kitabus Shifa, New Delhi; 2010. p. 1278-80.
5. Hubal I. Kitabul Mukhtarat Fil Tibb, (Urdu translation) Vol-IV, CCRUM, Ministry of Health and Family Welfare, Govt of India, New Delhi, 2005. p.182-83.
6. Razi AB. Kitab al-Mansuri (Urdu translation), CCRUM, Ministry of AYUSH, Government of India, New Delhi, 1991. p. 266-67.
7. Arzani HA. Mizan Al-Tib. Urdu translation by Hkm. Kabiruddin, Idara Kitabus Shifa, New Delhi, 2002; pp-22, 34, 39, 246.
8. Qamri MH. Ghina Muna (Urdu translation), CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 2008, pp.508-10.
9. Zuhr I. Kitabul Taiseer Fil Madawat (Urdu translation), 1st ed., CCRUM, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 1986. pp. 217.
10. Mannan MN, Zakir M, Ahmad I, Ahmad T, Kazmi MH. A Simplified Review on Aftimoon (Cuscuta Reflexa Roxb.), A Parasitic Vine with Special Reference to Unani Medicine, New Horiz., 2021;18:113-127.
11. Amjad MT, Chidharla A, Kasi A. Cancer Chemotherapy. 2023 Feb 27. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 33232037.
12. Kabiruddin M. Al-Qarabadeen, CCRUM, Ministry of AYUSH, Govt. of India, New Delhi, 2006, pp. 1095-96
13. Khan HMS. Ilaj-ul-Amraj, CCRUM, Ministry of AYUSH, Govt. of India, New Delhi, 2005, pp.7462.
14. WHO, Quality control methods for medicinal plant materials-1998. WHO Library Cataloguing-in-Publication Data, Malta, 2011.
15. QSIMP, Quality standards of Indian Medicinal Plants, Indian council of medical research, New Delhi, 2003.Vol-I, 205-11
16. UPI, The Unani Pharmacopoeia of India. 1st Ed. New Delhi: Ministry of Health & Family Welfare Dept. of AYUSH, Govt. of India; 2008, part-I, vol-V, pp- 27-28.
17. Lohar DR. Quality Control Manual for Ayurvedic Siddha & Unani Medicine. PLIM, Dept. of Ayush, Min. of Health and Family Welfare, Govt. of India, Ghaziabad. 2008 pp.51-53.
18. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. Int. J. Adv. Res. Chem. Sci. 2015 Apr;2(4)25-32.
19. Kumar A, Nirmal P, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, Sneha K, Oz F. Major phytochemicals Recent advances in health benefits and extraction method. Molecules. 2023;28(2):887. https://doi.org/10.3390/molecules28020887
20. More GK, Makola RT. In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by *Solanum sisymbriifolium* extracts. 2020, Sci. Rep. 16;**(10):**6493, https://doi.org/10.1038/s41598-020-63491-w
21. Rai V, Pai VR, Kedilaya P. A preliminary evaluation of anticancer and antioxidant potential of two traditional medicinal plants from Lamiaceae-Pogostemon heyneanus and Plectranthus amboinicus. J. Appl. Pharm. Sci. 2016; 6(8):73-78 https://doi.org/10.7324/JAPS.2016.60811
22. Bahuguna A, Khan I, Bajpai VK, Kang SC. MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh J. Pharmacol. 2017 Apr 8;12(2)115-8. https://doi.org/10.3329/bjp.v12i2.30892
23. Limsuwan S, Awaeloh N, Na-Phatthalung P, Kaewmanee T, Chusri S. Exploring Antioxidant Properties of Standardized Extracts from Medicinal Plants Approved by the Thai FDA for Dietary Supplementation. Nutrients. 2025 Mar 4;17(5):898. https://doi.org/10.3390/nu17050898
24. Kumar DS, Alex A, Neha B, Kalpana R, and Vimal S. Studies on In Vitro Antimicrobial, Anticancer, and Anti-oxidative Inflammatory Response of Methanolic Tuber Extracts Derived from Terminalia chebula. Cureus, 2024;16(7): e63930. https://doi.org/10.7759/cureus.63930
25. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of Terminalia chebula, Terminalia belerica and Emblica officinalis. BMC Complement. Altern. Med. 2010;10(1):1-15. https://doi.org/10.1186/1472-6882-10-20
26. Mondal R, Polash SA, Saha T, Islam Z, Sikder MM, Alam N, Hossain MS, Sarker SR. Investigation of the Phytoconstituents and Bioactivity of Various Parts of Wild Type and Cultivated *Phyllanthus emblica* L. Adv. Biosci. Biotechnol. 2017;8(7):211-227. https://doi.org/10.4236/abb.2017.87016
27. Li Y, Chen J, Cao L, Li L, Wang F, Liao Z, Chen J, Wu S, Zhang L. Characterization of a novel polysaccharide isolated from Phyllanthus emblica L. and analysis of its antioxidant activities. J. Food Sci. Technol. 2018;55:2758-2764, https://doi.org/10.1007/s13197-018-3199-6
28. Zhang Y, Zhao L, Guo X, Li C, Li H, Lou H, Ren D. Chemical constituents from Phyllanthus emblica and the cytoprotective effects on H 2 O 2-induced PC12 cell injuries. Arch. Pharm. Res. 2016;39:1202-1211, https://doi.org/10.1007/s12272-014-0433-2
29. Kumnerdkhonkaen P, Saenglee S, Asgar MA, Senawong G, Khongsukwiwat K, Senawong T. Antiproliferative activities and phenolic acid content of water and ethanolic extracts of the powdered formula of Houttuynia cordata Thunb. fermented broth and Phyllanthus emblica Linn. fruit. BMC Complement. Altern. Med. 2018 Apr 11;18(1):130. https://doi.org/10.1186/s12906-018-2185-x
30. Ahmed SI, Hayat MQ, Tahir M, Mansoor Q, Ismail M, Keck K, Bates RB. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of Cassia angustifolia Vahl. BMC Complement Altern Med. 2016 Nov 11;16(1):460. https://doi.org/10.1186/s12906-016-1443-z.
31. Anbuselvam C, Vijayavel K, Balasubramanian MP. Protective effect of Operculina turpethum against 7,12-dimethyl benz(a)anthracene induced oxidative stress with reference to breast cancer in experimental rats. Chem. Biol. Interact. 2007 Jul 20;168(3):229-36. https://doi.org/10.1016/j.cbi.2007.04.007.
32. Elasbali AM, Al-Soud WA, Mousa Elayyan AE, Alhassan HH, Danciu C, Elfaki EM, Alharethi SH, Alharbi B, Alanazi HH, Mohtadi ME, Patel M, Adnan M. Antioxidative and ROS-dependent apoptotic effects of *Cuscuta reflexa* Roxb. stem against human lung cancer: network pharmacology

and *in vitro* experimental validation. J. Biomol. Struct. Dyn. 2024;42(21):11651-11676, https://doi.org/10.1080/07391102.2023.2263889

33. Boubker A, El Ouardi A, El Kamli T, El Hamidi A, Kaicer M, Kichou F, Ameur N, Errafii K, Ben Aakame R, Sifou A. Phytochemical Analysis, Antioxidant and Antibacterial Activities, Minerals Element Profiling, and Identification of Bioactive Compounds by UPLC-HRMS Orbitrap in Four Aromatic and Medicinal Plants. Molecules. 2025; 30(6):1279. https://doi.org/10.3390/molecules30061279
34. Tabeshpour J, Shakiban D, Qobadi A, Aghazadeh E, Yousefsani BS. Cytotoxic Effects of Ethanolic Extract of Polypodium Vulgare on Human Malignant Melanoma Cell Line. Asian Pac. J. Cancer. Prev. 2023 Jan 1;24(1):275-281. https://doi.org/10.31557/APJCP.2023.24.1.275

*****