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

Research Article

Kalyani Sutarkar[1*], Chakradhar Panherkar[1], Prerna Bankar[1], Jayashree Dalal[2], Akash Kapse[1]

1. Agnihotri Institute of Pharmacy, Wardha (MS), India-442001.
2. Agnihotri College of Pharmacy, Wardha (MS), India-442001.

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).

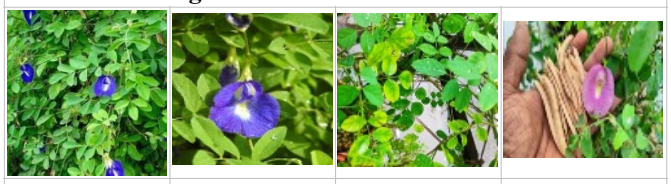
Clitoria ternatea, commonly referred to as Butterfly pea, is a perennial leguminous vine that belongs to the Fabaceae family and the Papilionaceae subfamily. The Clitoria genus includes 60 species, primarily found in tropical regions, with a few species occurring in temperate zones. The species most 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

*** Corresponding Author:**
Kalyani Sutarkar
Agnihotri Institute of Pharmacy, Sindhi Meghe Ramnagar, Wardha (MS), India-442001
Email Id: kalyanisutarkar11@gmail.com

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.

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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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