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Pharmacognostical investigation of *Clitoria ternatea* L. leaves

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Abstract

Clitoria ternatea L. (Family: Fabaceae) is also commonly known as Aparajita or Shankapushpi. The folklore claims the use of whole plant for curing various ailments. The leaves are used for hepatic problems, otalgia and eruptions. Pharmacognosy is an indispensable aid in standardization of herbal drugs. For the present investigation, pharmacognostical evaluation of *C. ternatea* L. leaf is carried out for quality standards. The study involves the following parameters like macroscopic, microscopic, histochemical analysis, powder microscopy, preliminary phytochemical screening, and physicochemical analysis. The keystone characteristic of microscopic studies under light and scanning electron microscopy revealed the presence of sclerenchyma ring around the vascular bundle in the midrib region and; two types of non-glandular bicellular trichomes, first type is smooth walled with curved apex, and second type is warty walled with blunt apex. The presence of wax crystalloids on the surface of the leaflets was clearly observed in ESEM. Powder study goes concurrent with microscopy. The physicochemical studies revealed i.e. total ash (8.15 %), water soluble ash (6.58 %), acid insoluble ash (1.88 %) and sulphated ash (9.05%); water soluble extractive value (14.92%) and alcohol soluble extractive values of (9.66%) which are comparatively higher to other solvents. The preliminary phytochemical and histochemical studies showed the presence of alkaloids, saponins, anthraquinone glycosides, terpenoids, flavonoids etc. In the current studies, pharmacopeial standards are laid down for the leaves of *C. ternatea* L.

Keywords

Aparajita, *Clitoria ternatea*, leaves, Pharmacognosy, Shankapushpi

Introduction

Clitoria ternatea L., commonly known as Aparajita/ Shankapushpi/ Gokarna is a climber (1). It belongs to family Fabaceae. The whole plant is used in treating dysentery, asthma in ascites, snakebites and abdominal enlargement by the aboriginals (2). The root is used as diuretic, in fever, inflammation, brain tonic etc. The seeds are recommended as nerve tonic and as laxative (3). The leaves are used in otalgia, hepatopathy and eruptions (4 - 6). The present investigation deals with macroscopic, microscopic evaluation of the leaf, including physicochemical and phytochemical analysis. (Fig. 1. A, B).

Materials and Methods

Procurement of Materials

For the present investigation, the mature leaves of *C. ternatea* L. were col-

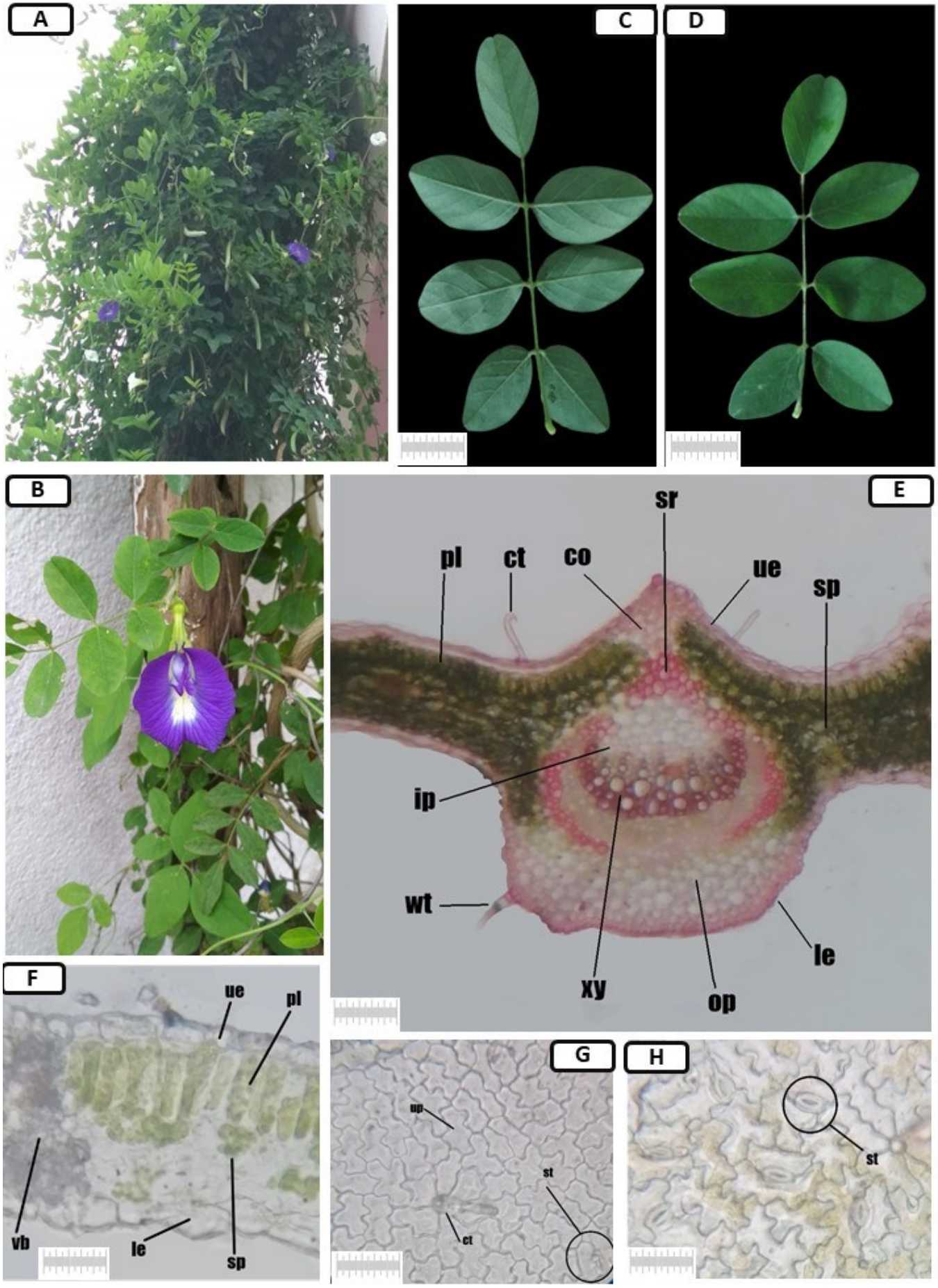

Fig. 1. **A & B**: Habit of plant; **C**: Lower Surface of lamina; **D**: Upper Surface of lamina; **E**: T.S. of leaflet passing through lamina and midrib x 100M; **F**: T.S. of leaflet passing through lamina x 450; **G**: Upper epidermis showing curved trichome x 450; **H**: Lower epidermis showing stomata x 450.

lected from Tungareshwar, part of Sanjay Gandhi National Park, Mumbai. The plant specimen was authenticated from Botanical Survey of India, Pune Regional Centre. The fresh leaflets were used for macroscopic and microscopic

studies. The remaining leaflets were preserved in FAA. (formaldehyde: acetic acid: alcohol) solution. The leaflets were separated and dried in shade. The dried leaflets were made into moderately coarse powder for further analysis and stored in airtight container (7, 8).

Pharmacognostic study

The fresh leaves were studied for its macroscopical and organoleptic parameters. The fresh hand cut sections were prepared for microscopic analysis (9). The cell contents were measured using stage and occular micrometer. The leaf parameters like stomatal type, stomatal index, trichome density, palisade ratio and vein islet numbers, were performed as per the standard procedure (10, 11). The sections were also treated with different reagents, to find the cellular contents (12, 13).

A few dried and fresh leaf samples were sent to Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai for SEM studies. The sections were taken according to the standard method of SEM and were analysed in ESEM mode with magnification from 25X up to 20000X.

The dried leaflet powder was treated with aqueous solution of chloral hydrate and mounted on the slide infused in 50% glycerine for microscopic studies (14). Fluorescence analysis were performed on the dry powder using various reagents and observed under Ultraviolet (UV) and ordinary light (15, 16). Various physicochemical parameters such as ash values, extractive values and moisture contents were established using the powdered drug (17). The powder was extracted with water, alcohol and methanol. These extracts were tested for their respective phytoconstituents using the standard procedure (18, 19).

Results

Macroscopy

The leaves are alternate, imparipinnately compound but each leaflet is opposite in phyllotaxy. The length from the base of the petiole to apex of the leaflet is 10.4 - 12.50 cms. The apical leaflet's length from the base of the leaflet to apex is 4.30 - 5.00 cms and breadth is 2.60 - 3.4 cms. The length of the lower leaflet sizes from 4.20 - 4.80 cms and breadth is 2.30 - 2.50 cms. The leaves are with petioles; each leaflet has petiolule with stipules at the base. The lamina is ovate in shape; base is symmetrical, with entire margin and mucronate apex. Each leaflet shows unicostate reticulate venation. The surface of lamina is smooth with hairy texture. The upper surface of the leaflet is dark green in colour while the lower surface is light green in colour. The odour is characteristic with bitter taste (Fig. 1. C, D).

Microscopy

T.S of leaflet passing through lamina

The upper epidermis is covered with thick-walled cuticle. The epidermis is single layered with tangentially elongated cells measuring 9.60 - 19.20 µm in length and 7.20 - 12.00 µm in breadth. The epidermal cells show's 2 types of non-glandular trichomes which are bicellular and uniserriate. In one type of trichome, the apical cell is curved with smooth wall. The curved trichomes are smaller measuring 26.40 - 48.00 µm in length and 7.20 - 9.60 µm in breadth. In second type of trichome, the wall is warty with apical blunt end, measuring 96.00 - 216.00 µm in length and 9.60 - 14.40 µm in breadth. The trichomes are present at regular intervals along with stomata. The mesophyll is differentiated into palisade and spongy tissues. It consists of single layered elongated palisade cells measuring 26.40 - 31.20 µm in length and 7.20 - 9.60 µm in breadth. It is filled up with chloroplast, below the palisade layer; there is a presence of 2 - 3 layered spongy tissues, measuring 9.60 - 12.00 µm in width. The spongy tissues are chlorenchymatous with intercellular space. The mesophyll is interrupted with poorly developed vascular bundles. The lower epidermis is made up of wavy, single layered, tangentially elongated cells measuring 9.60 - 19.20 µm in length and 7.20 - 12 µm in breadth. It is interrupted with 2 types of non-glandular, bicellular trichomes, which are uniserriate and similar to that of upper epidermis. On the lower epidermis, stomata and trichomes are comparatively more than upper epidermis (Fig. 1. E, F).

T.S leaflet passing through midrib

The upper epidermis is single layered with thick cuticle. The cells are round or slightly elongated measuring 4.80 - 9.60 µm in length and 4.80 - 9.60 µm in breadth. Trichomes are present more in midrib region. No stomata are reported on the epidermis of midrib. The upper epidermis is followed by 5 layered collenchyma cells, measuring 12 - 24 µm in width. The collenchyma is continued by ring of sclerenchyma, which is 2-4 layered, measuring 7.20 - 12 µm in width. In the younger leaves, the sclerenchyma is present in the form of arch and at maturity 2 endings of the curved arch joins together to form a ring of sclerenchyma around the vascular bundles. In mature leaflets, the central patch of parenchymatous cells measures 14.40 - 28.80 µm in width. In immature leaves, this patch is absent. Inner to the sclerenchyma ring, there is presence of vascular bundle, which consists of xylem and phloem. The protoxylem faces the upper epidermis and metaxylem towards lower epidermis. Outer to sclerenchyma just above the lower epidermis, 6-8 layered parenchyma cells measuring 16.80 - 48 µm in width is present. The parenchyma cells shows the presence of prismatic calcium oxalate crystals. The lower epidermis is single layered in thickness, wavy and tangentially elongated. The midrib region shows more number of trichomes than lamina (Fig. 1. E).

Scanning Electron Microscopy

The SEM of leaflets showed similar cells as that of light microscope. The detailed surface examination of cuticle layer has revealed the existence of wax crystalloids. In comparison with lower epidermis, upper epidermis has sharp form of crystalloid. The cells of collenchyma shows angular thickening with the deposition of cellulose. In the mature collenchyma, the thickening is increased and the lumen becomes smaller. At maturity, sclerenchyma shows the shrunken lumen because of the lignin deposition in the inner wall. The thickening are clearly visible under the high power magnified images. The ultra-magnified view shows the presence of xylem with annular and pitted vessels. The xylem cells also show the slight deposition of the lignin layer (Fig. 2. I – O).

Leaf constants

The leaflet shows two types of stomata i.e anisocytic and paracytic measuring 12.00 - 14.40 µm in length and 7.20 - 9.60 µm in breadth. The stomatal index of upper epidermis is 4.76 % while on lower epidermis it is 22.2 %. The trichome density of upper epidermis is 8 - 11 and that of lower epidermis is 35 - 42. The palisade ratio of leaflet is 7. The vein islet termination number ranges between 10 - 13.

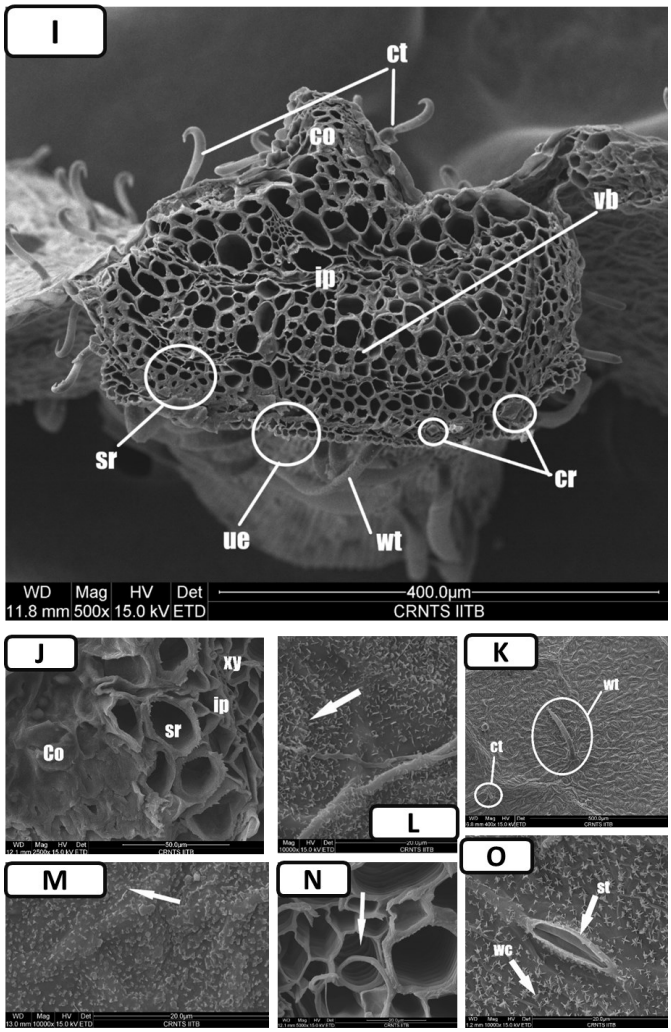

Fig. 2. Scanning Electron Microscopy images- **I & J**: Transverse section of leaf passing through midrib region, **KJ**: Section showing different types of trichomes (Upper epidermis), **L**: Magnified view of wax crystalloids (Upper epidermis), **M**: Magnified view of wax crystalloids (lower epidermis), **N**: Magnified section showing annular xylem rings. **O**: Surface view of cuticle covering stomata (Upper epidermis).

Histochemical analysis

The section of the fresh leaves were taken and stained with different reagents. The results are mention in (Table 1).

Powder study

The colour of the course leaf powder is green, with characteristic odour and bitter taste. Diagnostic microscopic features of the powder includes epidermal cells 16.80 - 24.00 µm in length, 9.60 - 16.80 µm in breadth, palisade cells 31.20 - 36.00 µm in length and 9.60 - 12.00 µm in width, spongy tissues is 7.20 - 12.00 µm in width, calcium oxalate crystal is 7.20 - 9.60 µm in length and 12.00 - 4.40 µm in width, sclerenchyma 7.20 - 12.00 µm in width, parenchyma 16.80 - 21.60 µm in length and 21.60 - 26.40 µm in width, xylem 7.20 - 12.00 µm in width and stomata 14.40 - 19.20 µm in length and 9.60 - 12.00 µm in width. Two types of trichomes are noticed trichomes which is bicellular, nonglandular pointed and with curved apex and smooth surface measuring 28.80 - 43.20 µm in length and 4.80 - 9.60 µm in width and second bicellular with the blunt and straight apex with; warty surface measuring 19.20 - 48.00 µm in length and 9.60 - 12.00 µm in width (Fig. 3. P -V).

Table 1. Histochemical analysis of *Clitoria ternatea* L. leaflets.

Sr. No.	Plant constituent Tests	Observations
1	Test for Starch	+
2	Test for Lipids	-
3	Test for Proteins	+
4	Test for Tannins	+
5	Test for Alkaloids	++
6	Test for Saponins	+++
7	Test for Glucosides	+++
8	Test for Mucilage	+++
9	Test for Calcium oxalate crystals	++

Keys: "+++" High concentration, "++" Moderate concentration, "+" Less concentration, and "-" Absent.

Physicochemical evaluation

The physicochemical values such as moisture content, ash values (total ash, water soluble, acid insoluble ash and sulphated ash) and extractive values (water, alcohol, ace-

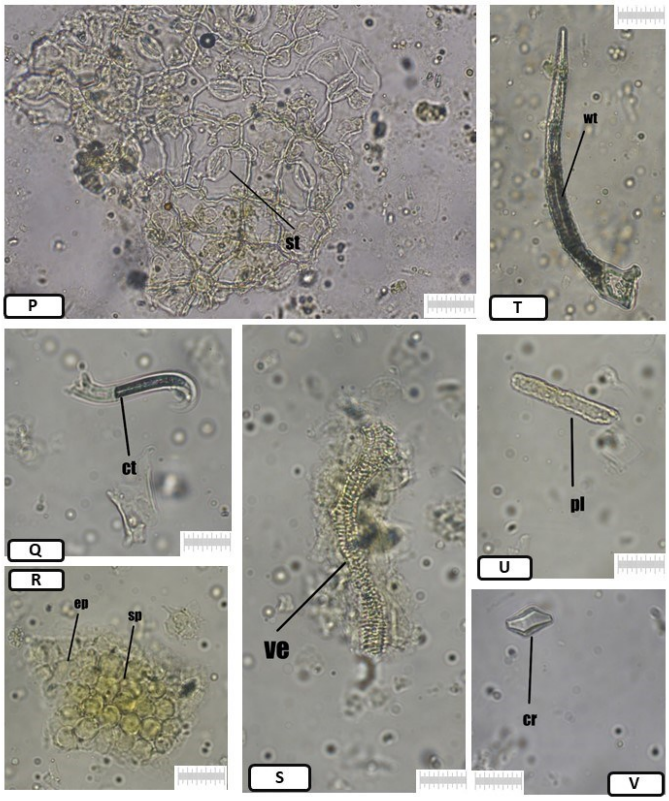

Fig. 3. Powder study showing **- P**: stomata x 450, **Q**: trichome with apical cells curved and smooth wall x 450, **R**: palisade cells upper view x 450, **S**: annular vessels x 450, **T**: trichome with apical cells with blunt end and warty wall x 100, **U**: palisade cell x 450, **V**; prismatic calcium oxalate crystal.
Abbreviations: **co**: collenchyma cells, **cr**: calcium oxlate crystals, **ct**: curved trichomes, **ip**: inner parenchyma, **le**: lower epidermis, **op**: outer parenchyma, **ph**: phloem, **pl**: palisade cells, **sp**: spongy parenchyma, **sr**: sclerenchyma ring, **st**: stomata, **ue**: upper epidermis, **vb**: vascular bundles, **ve**: vessels, **wc**: wax crystalloids, **wt**: warty trichomes, **xy**: xylem.

tic acid, butanol, chloroform, methanol, benzene, ethyl acetate, acetone soluble extractives) were established for the powdered drug. Results are given in (Table 2.).

Fluorescence analysis

The dried powder was treated with different regents and exposed to UV light (short and long). The observations are recorded in (Table 3).

Table 2. Physicochemical evaluation of *Clitoria ternatea* L. leaflets.

	Parameters	Observations	
	Moisture content %	3.63	
	Ash Values		
i.	Total ash % w/w	8.15	± 0.13
ii.	Water soluble ash % w/w	6.58	± 0.10
iii.	Acid insoluble ash % w/w	1.88	± 0.08
iv	Sulphated ash % w/w	9.05	± 0.21
	Extractive Values		
i	Water soluble extractive	14.92	± 0.73
ii	Alcohol soluble extractive	9.66	± 0.35
iii	Acetic acid soluble extractive	7.28	± 0.55
iv	Butanol soluble extractive	2.37	± 0.68
v	Chloroform soluble extractive	1.24	± 0.27
vi	Methanol soluble extractive	4.48	± 0.51
vii	Benzene soluble extractive	0.85	± 0.30
viii	Ethyl acetate soluble extractive	2.38	± 0.69
ix	Acetone soluble extractive	0.44	± 0.18

Preliminary phytochemical analysis

The qualitative phytochemical analysis of powder drug revealed the presence of various primary and secondary metabolites. The results are displayed in (Table 4.).

Table 3. Fluorescence analysis of *Clitoria ternatea* L. leaflets

S. N	Test	Wavelength		
		Visible	254 nm	365 nm
1	Nitrocellulose in amyl acetate + dried plant powder. Allow it to dry and then observe.	Moderate Green	Yellowish Green	Light Orange
2	Methanolic sodium hydroxide + dried plant powder. Observe the colour.	Light green	Light Green	Light yellow
3	Nitrocellulose in amyl acetate + Methanolic sodium hydroxide + dried plant powder. Observe colour.	Moderate Green	Yellowish Green	Light Brown
4	Dil. Hydrochloric acid + dried plant powder. Observe colour.	Light Green	Light Green	Light Green
5	Nitrocellulose in amyl acetate + dil. Hydrochloric acid + dried plant powder. Observe colour.	Moderate Green	Yellowish Green	Light Orange
6	Aqueous Sodium hydroxide + dried plant powder. Observe colour.	Yellowish green	Light Green	Moderate Green
7	Dil. Nitric acid + dried plant powder. Observe colour.	Light green	Light Green	Light yellow
8	Dil. Sulphuric acid + dried plant powder. Observe colour.	Light green	Light Green	Light yellow

Discussion

Clitoria ternatea L., is used as herbal remedy in curing various ailments since ages. The leaves are known for its hepatoprotective activity (20). In order to formulate a standard for the leaflets of the said plant pharmacognostical analysis is of utmost important. Though the plant has already been studied for standardization by (21, 22), there is lot of lacunae. The macroscopy states the presence of 4 leaflets were as the current studies proves the presence of 5 - 7 leaflets (21). The apex of the leaflets were said to be emarginate, while in the current study the leaflets found were of mucronate apex. In microscopy as well as powder study, previous workers have not mentioned the measurements of the cells, which is very well explained in the present work. In microscopy, the researcher has stated the presence of simple and glandular trichomes while in present investigation glandular trichome is absent. The presence of collenchyma cells below the upper and lower epidermis of lamina was not found in the recent investigation. Addition to this, parenchyma (bundle sheath cells) were reported, but it actually has sclerenchymatous ring around the vascular bundles. In the current investigation ESEM studies have also been carried out, which has shown substantial amount of evidence to identify the said plant drug. The stomatal index of lower epidermis was very less in present studies which was 4.76% while in former studies it was 12.25 - 14.45 which was not promising because very less stomata was observed in lower epidermis. The Vein termination number was missing in previous research papers. Anatomical characters like 2 types of trichomes, calcium oxalate crystals, layers of palisade and spongy tissues are significant in identification of fragmented leaflets. Powder drug can be authenticated

Table 4. Preliminary Phytochemical Screening of *Clitoria ternatea* L. leaflets

Tests	Extracts		
	Water	Alcohol	Methanol
Test for starch	++	+	+
Test for carbohydrates	++	+	+
Test for mucilage	++	+	+
Test for proteins	+	+	+
Test for aleurone grains	-	-	-
Test for amino acids	++	-	-
Test for fats and oil	-	-	-
Test for tannins and phenolic compounds	++	+	+
Test for steroids	++	+	+
Test for flavonoids	+	+	+
Test for cardiac glycoside	-	-	+
Tests for anthraquinone glycosides	+++	+	+
Tests for cyanogenic glycoside	-	-	-
Test for coumarin glycosides	+	+	+
Test for saponin glycosides	++	-	-
Tests for alkaloids	+++	+	+
Test for terpenoids	++	++	++

Keys: "+++" High concentration, "++" Moderate concentration, "+" Less concentration, and "-" Absent.

based on paracytic and anisocytic stomata as well as non-glandular trichomes. The physicochemical and fluorescence analysis will be useful parameters in detecting the adulteration of this drug. The histochemical and qualitative phytochemical assay goes concurrent with each other and is a useful data in interpreting the various phytoconstituents present in the plant part. The phytochemical screening was carried out previously using methanolic extract only (23, 24). In the current studies in supplement to ash values, the sulphated ash was also included. In addition to the methanolic extracts, water and alcoholic extracts are also put forth in the current study to know the various bioactive components present in the crude drug. Different solvent systems have been tried for analysis of extractive values, which gave significant results. The quantitative phytochemical and pharmacological investigation for the said plant part is in progress.

Conclusion

For the current studies *C. ternatea* L., is investigated for its leaf drug. The standardization of the leaf drugs is of great significant value in authentication of the plant drug in whole as well as in powdered form. Thus, these Pharmacopeial standards are of utmost important for the entry of the said plant part in the main stream of herbal medicine.

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Authors contributions

SR carried out the Pharmacognostical experiments, analysed it and drafted the manuscript. BG participated in the designing, analysis of the experiment and finalization of manuscript. Both the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: **:** Authors do not have any conflict of interests to declare.

Ethical issues: None.

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