International Journal of Research and Review
Vol. 9; Issue: 5; May 2022
Website: www.ijrrjournal.com
Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Pharmacognostical Screening of Blue Flowered Variety of *Clitoria ternatea* Linn. - A Phytocentric Overview

M. P. V. Vikram Singh[1], Vinay Kumar Prajapati[2]

[1]Department of Botany, Shri Jai Narain Mishra P.G. College, Lucknow, UP-India-226001
[2]Department of Botany, M.B.P. Govt. P. G. College, Ashiyana, Lucknow, UP-India-226012

Corresponding Author: M. P. V. Vikram Singh

DOI: https://doi.org/10.52403/ijrr.20220525

ABSTRACT

Pharmacognostic screening is the first and foremost step to determine identity and to assess the quality and purity of the crude drug. The genus *"Clitoria"* includes about 48 species under the family "Fabaceae". *Clitoria ternatea* Linn. isa traditional Ayurvedic medicinal plant commonly known as "*Aparajita*", "Butterfly pea" and also "*Shankhapushpi*", is found all over India. *Clitoria ternatea* Linn. have reported to possess a number of pharmacological activities such as nootropic, anxiolytic, anticonvulsant, sedative, antipyretic, anti-inflammatory, analgesic, anthelmintic and antidote to animal stings. The present study provided the scientific data for the proper identification and establishment of standards for the *Clitoria ternatea* Linn. The pharmacognostic screening were focused on macroscopic, qualitative and quantitative microscopy, powder behavior, physico-chemical parameters, quantitative estimation of primary and secondary metabolites and also the TLC profile. The plant extracts were subjected to phytochemical analysis for screening of medical constituents. Establishing standard is an essential step to identify the quality and purity of the drug. The study establishes the pharmacognostical standards of the crude drug and helps to differentiate the plant sample from the adulterants.

Keywords: *Clitoria ternatea* Linn., Fabaceae, Pharmacognosy, Qualitative analysis, Quantitative analysis, Phytochemical screening, TLC profile.

INTRODUCTION

It is estimated that more than 80% of the world's population rely solely on indigenous systems of medicines, which are mainly plant based. The world health organization (WHO) estimate some 20,000 sps. of higher plants are used medicinally throughout the world. Our country India and its neighbourhood has inherited a very rich ligancy of natural resources, particularly with respect of medicinal flora. *Clitoria ternatea* Linn. (Fabaceae) known as "*Aparajita*" and "Butterfly pea", is widely used as substitute of "*Shankhapushpi*". It consists of "conch" or "*shankh*" shaped flowers, categorized under Ayurvedic *Medhya Rasayna* drug that claim as brain tonic and have memory and intelligence enhancing properties. It is vigorous, strongly, persistent, herbaceous, perennial, climber, conspicuous, blue flowers legume. The plant is native to south-east Asia and distributed in tropical Asia including India. Almost all parts of the plant are reported to have medicinal properties widely used in the Ayurvedic system of medicine.

Traditionally, it is recommended for the treatment of fever, rheumatism, syphilis, skin diseases, arthritis, eye and ear diseases, snake bite, scorpion sting, chronic bronchitis, indigestion, constipation in India (Mukherjee et al., 2008, Meena et al., 2010), epilepsy, mental problems, insanity for muscular strength (Anonymous, 1976), as a remedy for

hemicranias and in swollen joints (Morris et al., 1999, Anonymous, 2001). Ethnobotanically, it is used in various urinary troubles like infection, burning sensation in urinary track, lack of urination, frequent urination (Singh et al., 2010) and also reported for purification after surgical removal of tumor (Das et al., 2003). Pharmacologically, it is reported for improved cognitive abilities, memory and learning, neuronal degenerative disorders (Rai et al., 2000), nootropic and anticonvulsant activities (Rai et al., 2005), antimicrobial and insecticidal (Yadava et al., 2003), anti-inflammatory, antipyretic, analgesic (Parimaladevi et al., 2004), antidiabetic, antioxidant, hepatoprotective (Devi et al., 2003) and platelet aggregation inhibitory (Zingare et al., 2013) activities, anthelmintic (Salhan et al. 2011). The plant contains several secondary metabolites such as Kaempferol and its glucoside- clitorin, taraxerol and a lactone aparajitin (Barik et al., 2007). The anthocyanin and delphinidin glucoside present in blue flowered variety (Sinha et al., 1960), Seeds contain sistosterol, hexacosanal and anthoxanthin (Yoganarasimhan, 2000).

It is extremely important to determine the qualitative and quantitative parameters of the medicinal plant by undertaking extensive study of the plant. Knowledge about taxonomic attribute of plant species, plant parts and property of medicinal plants which in turn depends upon the prevalence of primary and secondary metabolites. Most of the previous studies of the species in rare aspects is not sufficient and found lacking. To the best of our knowledge, there are few pharmacognostical studies on *Clitoria ternatea Linn.* has been reported in the literature. Therefore, in the present investigation, an attempt has been made to standardize the plant through macro-microscopical, physico and phytochemical parameters.

MATERIAL AND METHODS

In the present investigation detailed pharmacognostical studies of plant genera *Clitoria ternatea* Linn. (Fabaceae), were undertaken and following methods were followed for investigation.

Collection of genuine plants material

Specimens of plant genera *Clitoria ternatea* Linn. were collected from their natural habitat. The plant was identified with the help of floras and by matching them with the type specimens deposited in the institute's herbarium. The herbarium specimens were made and deposited to national herbarium of National Botanical Research Institute, Lucknow, India.

Botanical name - *Clitoria ternatea* Linn.
Family - Fabaceae
Vernacular name - Aparajita
Place of collection - Banthra farm of NBRI Banthra, Lucknow
Voucher No. - 262526
Part used - Whole plant

Processing of plant material for study

The plant materials were properly dried in shade at 40°C and powdered. The fresh material was preserved in FAA solution (formaldehyde: acetic acid: alcohol: water in a ratio of 10:5:50:35) for microscopic studies.

Studies of organoleptic characters

This study includes surface markings, texture, fracture, internal appearance, cut surface, odour and taste of the crude drug.

Microscopic methods for herbal raw material

Microscopic evaluation deals with identification of the various characters of tissues, cells and cell contents by microscopic methods by preparing specimens of crude material. Microscopic studies vary, depending on the part used like, leaf, stem, root, bark, flower, and fruit and also on the nature of the material i.e. entire, cut or powdered.

A. Disintegration of hard and woody tissues

Cut the material into small pieces and transfers few pieces to test tube containing 4ml of dil. HNO_3 and heat to boiling. Add powdered potassium chlorate warm it gently and allow to react. Tissue starts to disintegration, when completely bleached. Apply pressure with glass rod for complete disintegration of the tissues. Allow the material to settle down, decant the liquid and wash the bottled material repeatedly with waters until the acidity is removed.

B. Preparation of sections

For microscopically studies, the sections were cut by the razor/ blade or through microtome and double staining were performed in safranin and hematoxylin. The sections of 13-18 μm thickness were taken from the plant genera. The permanent slides (T.S. /T.L.S.) were prepared by using dehydration method.

C. Leaf surface preparation

For the surface study and quantitative microscopy, boil pieces of leaves in a test tube with chloral hydrate for several minutes until completely clarified and then examine them in chloral hydrate solution after clarification, leaf pieces are placed on a microscopic slide and then divided into two parts with the help of scalpel or needle and carefully turn one part.

D. Quantitative microscopy

Draw a square with the help of microscope, stage micrometer scale and camera Lucida. Place transparent leaf fragments of about 5x5 mm in size on a microscope slide and prepare the mount, with 1 drop of safranin and 1 drop of glycerin.

(a) Stomatal number / density: Is the number of stomata present per mm^2.

(b) Stomatal Index: Is the percentage which the number of stomata forms to the total number of epidermal cell, each stoma being counted as one cell. Stomata Index can be calculated by using the following equation.

S.I. = S/E+S x 100

Where's

S.I. = Stomata Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area.

(c) Vein-islet number:Is the number of vein–islets per sq. mm of the leaf surface mid-way between the midrib and the margin.

(d) Vein-termination number:Is the number of vein let termination per sq. mm of the leaf surface midway between midrib and margin.

(e) Determination of palisade ratio:Is the average number of palisade cell beneath each epidermal cell. Count the palisade cells under the four epidermal cells where a cell is intersected. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4.

Maceration

To observe the shape, size and structure of isolated thick walled elements, small pieces of material are placed in a test tube and boil with 40% HNO_3 for 15-45 minutes. Wash thoroughly with water, place the material on the microscopic slide and then macerate with the help of a needle then add 1 drop of glycerol and 1 drop of safranin, cover with a cover slip.

Powder Studies

Different characters of powdered drugs like organoleptic characters viz. color, odour, fineness, degree of uniformity of the particles and sensation of smoothness were recorded. For examining characters of the powder, take sufficient amount of powder in chloral hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time. Fluorescence test of powder

(under UV light and visible light) were performed according to the method described by Chase and Pratt (1949) and Kokoski et.al. (1958).

Physico-chemical parameters for the standardization of crude drugs

The physicochemical analysis often plays an important role in herbal drug standardization. These tests are simple and quick to perform and give valuable information about the nature and purity of a crude drug. The values given in the results are replicate of six samples. The tests which are normally performed include:

A. Determination of foreign matter

Drug should be entirely free from visible sign of contamination by moulds or insects and other animal contamination. No abnormal odour, discoloration, slime or sign of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from harmful foreign mater or residue. Morphological examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable.

Procedure:100-500g of the drug sample to be examined weighed it and spread out in a thin layer. Detect the foreign matter by inspection with the unaided eye or by the use of a lens (6 xs). Separate the other material weight it and calculated the percentage present. The amount of foreign matter shall not be more than the percentage prescribed in the pharmacopoeia (2%).

B. Determination of moisture content (loss on drying)

Determination of the amount of volatile matter in the drug is measure of loss on drying for substances.

Procedure: 10 gram of drug were kept in oven at 100°c for 3h and made it moisture free, weighted till constant weight was attained and calculated the percentage of moisture by the following formula-

$$\text{Moisture percentage} = \frac{Pw - Fw}{W} \times 100$$

Where's,
Fw = Final constant weight of the sample
Pw = Pre weight of sample
W = Total weight of sample

C. Ash Value

Ash value is determined to estimate the total amount of the inorganic salts present in the drug. This includes total ash, and acid insoluble ash.

(a) The total ash: Method is designed to measure the total amount of material remaining after ignition. This includes both "Physiological ash" which is derived from the plant tissue itself, and "Non Physiological ash" which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Procedure: Place 2 gm of ground air-dried material was accurately in a previously ignited and tarred crucible. Spread the material as an even layer and ignite it by gradually increasing the temperature not exceeding 450°C, until it become white, indicating the absence of carbon. Cool in desiccators and weigh. If carbon free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2ml of water. Dry on a plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, then weight without delay. Calculate the content of total ash of air-dried material.

$$\text{Total ash percentage} = \frac{Pw - Fw}{W} \times 100$$

Where's,
Pw = Pre weight of crucible
Fw = Final weight of crucible
W = Total weight of powdered plant material

(b) Acid insoluble ash:

Procedure: Boil the ash obtained as total ash with 25 ml of dilute hydrochloric acid in the crucible, cover with a watch glass and boil gently for 5 minutes. Rinsed the watch glass

with 5ml of hot water and add this liquid in the crucible. Collect the insoluble matter on an ash-less filter paper and wash with hot water until the filtrate neutral. Transfer the ash-less filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, and then weight with delay. Calculate the content of acid-insoluble ash of air-dried material.

Acid insoluble ash percentage =

$$\frac{FWb - FWa}{W} \times 100$$

Where's,

FWa = Final weight of crucible with acid insoluble ash

FWb = Final weight of crucible with total ash

W = Total weight of powdered plant material

D. Extractive values

It is the amount of soluble constituents (active or otherwise) extracted with solvents like alcohol, water, methanol, hexane and other solvents from a given amount of medicinal plant material. These are used to determine the amount of the matter, which is soluble in the solvents used; it includes alcohol soluble extractive, water soluble extractive, and hexane soluble extractive etc.

(a) Determination of alcohol soluble extractive: Procedure– Macerate 5 g of the coarsely powdered air- dried drug with 100 ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hour and loss of solvent. Take 25 ml of the filtrate in a tarred flat-bottomed shallow dish, evaporate and dry at 105°C to constant weight. Calculate the percentage of alcohol soluble extractive with reference to the air-dried drug (Anonymous, 1966).

(b) Determination of water soluble extractive: Procedure - Macerate 5 g of the coarsely powdered air-dried drug with 100ml of chloroform water (0.1%) in a closed flask for twenty-four hour, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precaution against loss of solvent. Take 25 ml of the filtrate in a tare-bottomed shallow dish, evaporate and dry at 105°C to constant weight. Calculated the percentage of water–soluble extractive with reference to the air-dried drug (Anonymous, 1966).

(c) Determination of successive soxhlet extractive values: Procedure- Extract 5g of the air dried coarsely powder drug exhaustively with hexane, chloroform, acetone, alcohol and water in a successive order. Collect the hexane, chloroform, acetone, alcohol and water soluble extractives obtained separately, concentrate and dry. Calculated the percentage of each extractive with reference to the air dried drug.

E. Sugar estimation (Montgomery 1957)– Total amount of sugar present in the drug

Procedure: Prepare 10 percent homogenate of the plant tissue in 80 percent ethanol. Centrifuge at 2000 rpm for 50 minutes. The supernatant obtained is made up to known volume (generally up to 10 ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot and add 0.1 ml of 80 percent phenol and 5 ml conc. H_2SO_4. Cool and then read the absorbance at 490 nm. Calculate the percentage according to the absorbance.

Total amount of sugar percentage =

$$\frac{3.1 \times \text{Absorbance}}{\text{Sample amount}}$$

Phytochemical screening / tests (Qualitative analysis)

Determination of various class of primary (carbohydrates, lipids, proteins, etc.) as well as secondary (alkaloids, glycosides, saponins, flavonoids, terpenoids, tannins etc.) metabolites was estimated. General screening of the alcoholic, aqueous and other extracts of the plant material is used for quantitative determination of the group of organic compound present in them. The preliminary phytochemical studies are used for testing the different chemical groups present in plant extracts. 10% (w/v) solution of extract is taken unless otherwise

mentioned in the respective individual test. General screening of the extracts of the plant material is used for qualitative determination of the groups of organic compound present in them.

A. Alkaloids- *Dragendorff's test:* Dissolve few mg of alcoholic or aq. extract of the drug in 5 ml of distilled water, add 2 M hydrochloric acid until an acidic reaction occur, then add 1 ml of Dragendorff's reagent, an orange or orange - red ppt. produced immediately indicate the presence of alkaloid.

B. Carbohydrates- *Anthrone test:* To 2 ml of anthrone solution, add 0.5 ml of aq. extract of the drug. A green or blue color indicates the presence of carbohydrates.

C. Flavonoids: Schinoda test: In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids, a pink, reddish pink or brown color is produced.

D. Triterpenoids: *Liebermann -Burchard's test:* Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid through the side. A violet color colored ring formed indicating the presence of triterpenoids.

E. Proteins- *Biuret's test:* To 1ml of hot aq. extract of the drug add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3%w/v copper sulphate solution. A redor violet color is obtained.

F. Resins: Dissolve the extract in acetone and pour the solution into distilled water. Turbidity indicates the presence of resins.

G. Saponins: In a test tube containing about 5 ml of an aqueous of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mnts. Honeycomb like forth formed indicates saponins.

H. Steroids: *Liebermann-Burchard's test:* Add 2 ml of acetic anhydride solution to 1 ml petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish color is developed which turns to blue.

I. Tannins: To 1-2 ml of extract of the drug add a few drops of 5% $FeCl_3$ solution. A green color indicates the presence of Gallo tannins while brown color indicates tannins.

J. Starch: Dissolve 0.015 g of iodine and 0.075 g of potassium iodide in 5 ml of distilled water and add 2-3 ml of an extract of drug. A blue color is product.

Chromatographic Analysis

A. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is frequently used for the rapid and positive analysis of herbal medicines. The time required for the demonstration of most of the characteristic constituents by TLC is very short and in addition to qualitative detection, the TLC also provides semi- quantitative information on the chief constituents of the plant drug and thus enables an assessment of drug quality. It is a open bed technique in two phases a stationary phase acting through adsorption and a mobile phase in the form of a liquid. Identification can be effected by adsorption of spots of identical Rf. value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi- quantitative estimation.

TLC is used for the separation of simple mixtures where speed, low cost, simplicity are required. It provides a chromatographic drug fingerprints. It is therefore suitable for monitoring the identity

and purify of drug. In TLC the various steps involved are.

1. Application of sample
2. Chromatographic development
3. Detection of spots
4. Quantification
5. Documentation

1. Application of sample- A known quantity of sample is dissolved in a known volume of solvent and the sample applied on percolated TLC plate in the form of a spot or a band.

2. Chromatographic development (separation) - Development of the chromatographic is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chamber or twin through chamber is commonly used for TLC development.

3. Detection of spots- For detection of spot UV light is generally preferred.

4. Quantification and documentation- Densitometry is *in situ* instrumental measurement of visible UV absorbance, fluorescence quenching directly. The scanner convents the spot/band on the layer into a chromatogram consisting of peaks similar in appearance of HPLC.

The portion of the scanned peaks on the recorder chart is related to Rf. value of the spots on the layer and the peaks light or area is related to the concentration of the substance on the spot.

B. High performance thin layer chromatography (HPTLC)

HPTLC is an advanced versatile chromatographic technique for quantitative analyses with high sample throughout and is complementary to HPLC/GLC. It provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs. In HPTLC the various steps involved are

1. Application of sample.
2. Chromatographic development
3. Detection of spots
4. Quantification
5. Documentation

Applications of sample– An automatic applicator (Linomat) is used for sample application.

A known quantify of sample is dissolved in a known volume of solvent and the sample on percolated TLC plate either in the form of a spot or a band. However a band form is preferred because:

- Larger quantities of sample can be handled for application.
- Better separation because of rectangular area in which compounds are present on the plate.
- Response of densitometry in better due to variable concentration of substances in a spot.

1. Chromatographic development (separation): Development of the chromatogram is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

2. Detection of spots: For densitometry scanning, detection under UV light is generally preferred. But post chromatographic derivatisation reactions are essentially required for detection when individual compounds does not respond to UV light or do not have intense fluorescence.

3. Quantification and Documentation: Densitometry is *in situ* instrumental measurement of visible, UV absorbance, fluorescence quenching directly. The scanner converts the spot/band on the layer into a chromatogram consisting of speaks similar in appearance to HPLC. The portion of the scanned peaks on the recorder chart is related to Rf values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

RESULT

Taxonomy: Following is the detailed descriptive taxonomy of the plant *Clitoria ternatea* Linn.

Botanical name – *Clitoria ternatea* Linn.

Family– Fabaceae

Vernacular names–

Hindi	–	Aparajita
Sanskrit	–	Girikar
Assamese	–	Aparajita
Bengali	–	Aparajita
English	–	Clitoria
Gujrati	–	Gokarni
Kannada	–	GiriKarnika
Malayalam	–	Shankhapushapam
Marathi	–	Gokarna
Oriya	–	Aparajita
Punjabi	–	Koyal
Tamil	–	Kakkanam
Telugu	–	Dintena

Identification:

Plants with seeds, ovules enclosed within the ovary Angiosperms

Venation reticulate, flowers pentamerous Dicotyledanae

Petals free Polypetalae

Thalamus cup-Shaped, ovary inferior Calyciflorae

Alternate, stipulate leaves, carpals one or more Rosales

Flower zygomorphic, gynoecium usually one, corolla papilionaceous with descending imbricate aestivation,

Ovary monocarpellary Fabaceae

Petals very unequal, stamens diadelpho *Clitoria*

Petals very unequal, stamens monoadelphous *ternatea*

Habit: A perennial climbers.

Habitat: A common garden plant, also occurs among hedges, all over the tropical region from Himalayas to Ceylon.

Root: Stout tap root with a few tortuous branches, cylindrical 1-5 mm in thickness, a few places show cracks due to presence of lenticles, color light brown, fracture fibrous, taste bitter.

Stem: Climbing, branched, cylindrical, herbaceous, hallow.

Leaf: Leaves 2-3 inch long, shortly petiolate, broadly lanceolate, leaflets1-3inch long, ovate obtuse, subcoriaceous, stipules minute linear.

Flower: Flowers showy, blue, bracteate, bracteolate, pedicellate, zygomorphic, papilionaceous. Petals unequal, style bearded below the stigma.

Fruit: Pods flattish 2.5 inch long, sparingly hairy.

Seed: Compressed smooth, seeds 6-10, black.

Flowering and Fruiting: July-March.

Fig.-1: Plant of *Clitoria ternatea* Linn.

Microscopy of the Crude Drug

Root: T.S. of root shows 10-15 layers of rectangular thin-walled cells filled with starch grains, a few cells contain calcium oxalate in this region, single or groups of 2-8 lignified cuticle fibers, distributed in the lower half of the cortex, secondary phloem consists of usual elements, phloem fibers 2-8 in groups, a few solitary fibers which are long, thin-walled with narrow lumen and pointed tips, secondary xylem consists of usual elements, vessels pitted with oblong, bordered pits and have short conical tall at one end, mostly occur 2-3 in groups, xylem fibers similar to those of irregular in shape and pitted walls, medullary rays, 1-5 cells wide, oblong and pitted. Starch grains are common in secondary cortex, phloem and xylem parenchyma.

Stem: Shows single layered epidermis covered with cuticle, cortex 8 to 10 cells wide, consisting of oval to circular, thin-walled, parenchymatous cells, groups of

pericycle fibers, phloem consists of usual elements, xylem consists of usual elements pith wide, consisting of thin-walled round to oval parenchymatous cells.

Leaf: Shows dorsiventral structure, both upper and lower epidermis consists of single-layered cells, covered externally with thick cuticle, some epidermal cells of both surfaces elongated outwards forming uni to tri-cellular warty hairs, basal cells smaller and apical cells longer, palisade single layered, spongy parenchyma 4 or 5 layered with crystals of calcium oxalate, stomata paracytic, present on both surfaces.

Physical constants of leaves:

S.No.	Parameters	Results
1	Stomatal Number (Upper surface)	300-343 per sq.mm
2	Stomatal Number (Lower surface)	212-247 per sq. mm
3	Stomatal Index (Upper surface)	31-42 persq.mm
4	Stomatal Index (Lower surface)	58-68 per sq.mm
5	Vein-islet Number	22-24 persq.mm
6	Vein-termination Number	34-37 per sq. mm
7	Palisade ratio	3-4

Power studies

A. **Organoleptic characters of leaves:** Following are the organoleptic characters of leaves of *C. ternatea.*

S.No.	Characters	Observations		
		When fresh	After drying	Powder
1	Colour	Dark green	Olive green	Yellowish green
2	Odour	Characteristic	Characteristic	Characteristic
3	Texture	Soft	Fibrous	Coarse powder
4	Taste	Bitter	Bitter	Bitter spicy

B. **Organoleptic characters:** Following are the organoleptic characters of whole plant powdered drug.

Color – Yellowish-brown
Taste – spicy
Odour – Slight characteristic

C. **Microscopic study:** On microscopic study of C. ternatea whole plant powder shows groups of spongy parenchyma, palisade cells, fibers, xylem vessels with spiral thickenings, fragments of hairs with warty cuticle, wary thin-walled, epidermal cells with paracytic stomata in surface view.

D. **Fluorescence analysis:** The behavior of the powdered drug with different chemicals reagents has been shown in the table.

S. No.	Treatment	Day light	UV-254nm	UV-336nm
1	Powder (P)as such	Yellowish green	Yellow	Pale white
2	P + NaOH in water	Green	Yellowish green	Brown
3	P + 1N NaOH in methanol	Green	Yellowish green	Brown
4	P + 50% KOH	Green	Yellowish green	Brown
5	P + 1 N HCl	Yellowish green	Pale yellow	Black
6	P + 50% H_2SO_4	Yellowish green	Pale yellow	Light Black
7	P +50% HNO_3	Light red	Yellow	Dark black
8	P + Conc. HNO_3	Red	Yellow	Dark black
9	P + Acetic acid	Yellowish black	Whitish yellow	Whitish brown
10	P + Conc. H_2SO_4	Black	Blackish yellow	Black
11	P + Iodine water	Yellow	Light yellow	Whitish black

Physico-chemical studies: The different physico-chemical values obtained are recorded for identity, purity and strength.

S. No.	Parameters	Range (in percent)	Mean (in percent)
1	Foreign matter	0.20-0.3000.25	
2	Moisture content	11.37-13.02	12.19
3	Total ash	06.19-07.04	07.11
4	Acid in soluble ash	01.23-01.42	01.32
5	Hexane soluble extract	2.50-3.00	02.75
6	Alcohol soluble extract	7.02-8.63	07.87
7	Water soluble extract	8.92-10.98	09.91
8	Sugar	0.176-0.317	00.2562

Phytochemical studies: The preliminary phytochemical screening of whole plant drug are recorded for different chemical groups present in different extractives are as fallows.

S. No.	Phytochemicals	Water	Alcohol	Chloroform	Acetone	Hexane
1	Alkaloids	+		+		
2	Carbohydrate		+			
3	Flavonoids					
4	Triterpenoids			+	+	
5	Protein			+		
6	Resin			+		
7	Saponins	+		+		
8	Steroids			+		
9	Tannins	+				
10	Starch					

TLC Assay and HPTLC Analytical studies

Test solution– Extract 5gm of powdered drug in soxhlet apparatus with methanol. Filtrate and concentrate the methanolic extract. Take 10 mg of the residue and dissolve in 1 ml of methanol and use the same for TLC and HPTLC analysis of the drug.

Solvent system– Toluene: Ethyl acetate: Formic acid (8:2:05).

Procedure– Apply 10ml of the test solution on precoated silica gel 60f254 TLC plate (E. Merck) of uniform thickness of 0.2mm. Develop the plate in solvent system at distance of 8cm.

Visualization and Evaluation– Visualize the plate under UV light at 366nm (Fig.-3,4) shows one fluorescence zones at Rf 0.24 which are not identical and corresponding to substituent's like C. pluricaulis and E. alsinoides etc., conforms in the variation of chemical contents.

Discussion: Clitoria ternatea Linn. Known as "butterfly pea" is a perennial climber with slender downy stem and beautiful blue flowers, found throughout the tropical region of the country from Himalayas to Ceylon. It is a versatile medicinal plant used singly or in combination with other medicinal plant for treating a variety of ailments. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents which is starts with right identification of plant material. Taxonomical, morphological, microscopical, physico-chemical and phytochemical analysis are major pharmacognostical screening parameters used for pharmacognostic study of a medicinal plant (Woisky et al.,1998). Microscopical method of valuing medicinal plants is based on the examination of mounts of the thin sections of them under a compound microscope. Every plant possesses a characteristic histology in respect to its organs and diagnostic features of these are ascertained through the study of the tissue and their arrangement, cell walls and cell contents, when properly mounted in stains, reagent or mounting media. The examination of powder showed the colour developed due to respective compound for which fluorescence analysis also an important parameter for quality control point of view, because some phytochemicals showed fluorescence in different UV range after reacting with different reagents (Woisky et al.,1998). The physico-chemical parameters are helpful in judging the purity and quality of the drug (Bigoniya et al.,2012). The percentage of active principals in the plant is determined only in the dry condition. Hence, the moisture lost percentage is very important to decide about the condition of crude drug. The moisture should be kept minimum to prevent the drug from various kinds of decomposition (Mythii et al.,2012). The percentage of ash was 07.11% and acid insoluble ash was 01.32%. The total ash and the acid insoluble ash indicate the presence of any foreign matter, inorganic composition and purity of drug. Extensive value of the crude drug also useful parameter for the evaluation and standardization and gives the idea of nature of the chemical components soluble in particular solvent. These parameters are useful in determining authenticity and purity of drug and also these values are important quantitative standards (Anonymous, 2000, Evans, 2003).

The phytochemical tests are useful for the detection of various chemical constituents help in detection and identifying new sources of therapeutical and industrial importance (Salhan et al., 2011). The preliminary phytochemical screening showed presence of primary and secondary metabolites like alkaloids, tannins, glycosides, carbohydrate, resins, steroids, saponins, and flavonoids etc. The secondary metabolites have infacuated different pharmacological effects and liable for various pharmacological activities of Clitoria ternatea Linn. The characteristic bands showed in TLC profiling (Fig.-3) are significant point of quality standards. HPTLC analysis (Fig.-4) is more characteristic to the plant extract, play an important role in detection of various chemical constituents. However, presence of different secondary metabolites indicates its more therapeutic values. Further pharmacological investigations are required for therapeutic activities of Clitoria ternatea Linn.

CONCLUSION

It is concluded that *Clitoria ternatea* Linn.is a plant with a variety of ethnic medicinal uses. Quality of herbal drug in term of chemical constituents and their efficacy necessitates the need of quality control studies of raw drug materials using pharmacognostical standardization. World health organization (WHO) has also created awareness towards validation of plant-based drug to maintain the quality, safety and efficacy. The macro-microscopic characterization is an important parameter for proper authentication of crude drug even in powdered form. However, the physico-chemical values are useful to ascertain the identity, purity and strength of the *Clitoria ternatea* Linn. The parameter which are reported here can be considered as enough to identify and decided the authenticity of the more medicinally valuable plant of *Clitoria ternatea* Linn. in herbal industries. This is valuable information for preparation of drugs in pharmaceutical industry and stress the need for more intensive research in this medicinal plant since the compounds play a great role in healthcare.

Acknowledgement: The authors are thankful to Dr. A.K.S. Rawat, Principal Scientist & Head and Dr. S.K. Srivastava, Senior Scientist, Pharmacognosy & Ethnopharmacology Division (CSIR-NBRI), Lucknow for providing laboratory facility, for the research work and first author is also thankful to Council of Science and Technology, Lucknow, U.P. for financial support (CST/SERPD/D-488).

Conflict of Interest: None
Source of Funding: None

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Abbreviations-

%	-	Percentage	dr. wt.	-	Dry weight
°C	-	Degree centigrade	g	-	Gram
aq.	-	Aqueous	h	-	Hours
cm	-	Centimeter	l	-	Liter
conc.	-	Concentrated	ml	-	Milliliter
mm	-	Millimeter	COL	-	Collenchyma
ppt	-	Precipitate	CU	-	Cuticle
s	-	Second	EP	-	Epidermis
sq. mm	-	Square millimeter	LE	-	Lower epidermis
TLC	-	Thin Layer Chromatography	MR	-	Medullary rays
µm	-	Micrometer	PH	-	Phloem
UV	-	Ultraviolet	PI	-	Pith
v	-	Volume	PP	-	Palisade parenchyma

v/v	-	Volume per volume	RZ	-	Rizome
w	-	Weight	SC	-	Scleroids
w/v	-	Weight per volume	SG	-	Starch grain
dil.	-	Dilute	SP	-	Spongy parenchyma
sps.	-	Species	TR	-	Trichrome
CC	-	Cork cambium	UE	-	Upper epidermis
CHL	-	Chlorenchyma	VB	-	Vascular bundle
CK	-	Cork	XY	-	Xylem

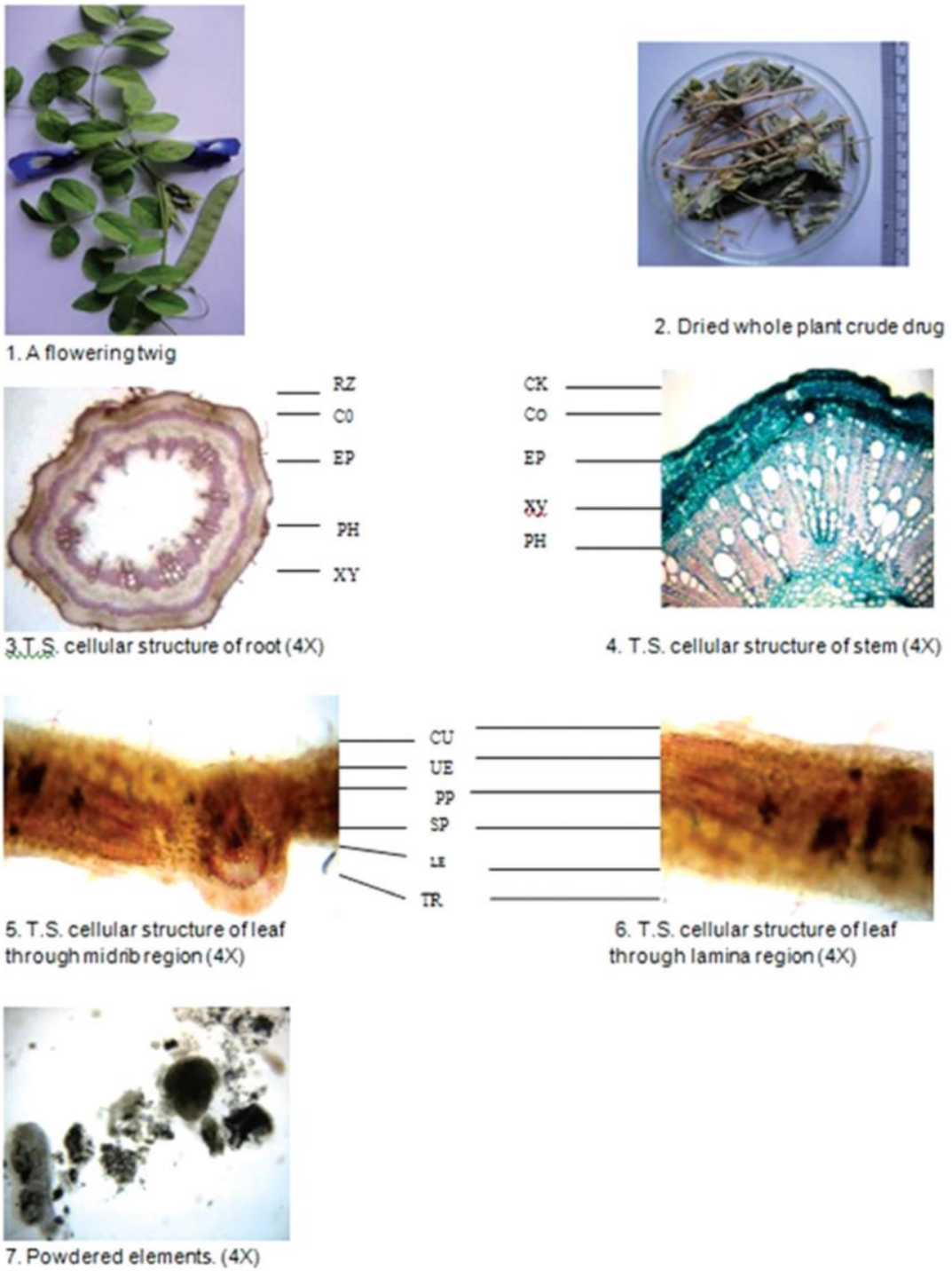

Fig.-2: Macroscopic and Microscopic characters of *Clitoria ternatea* Linn.

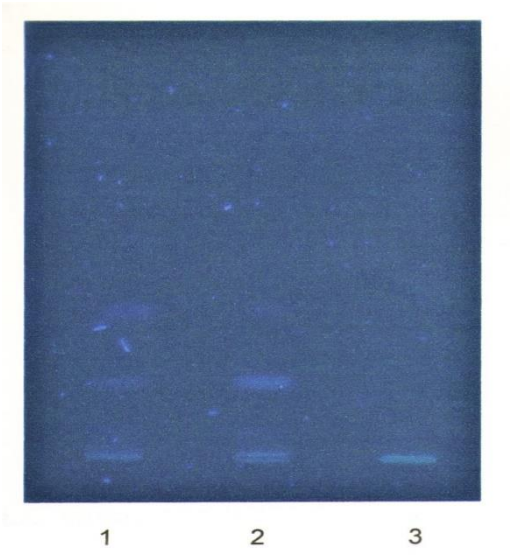

Fig.-3: TLC profile of whole plant extract of *Convolvulus pluricaulis* (1), *Evolvulus alsinoides* (2), and *Clitoria ternatea* (3).

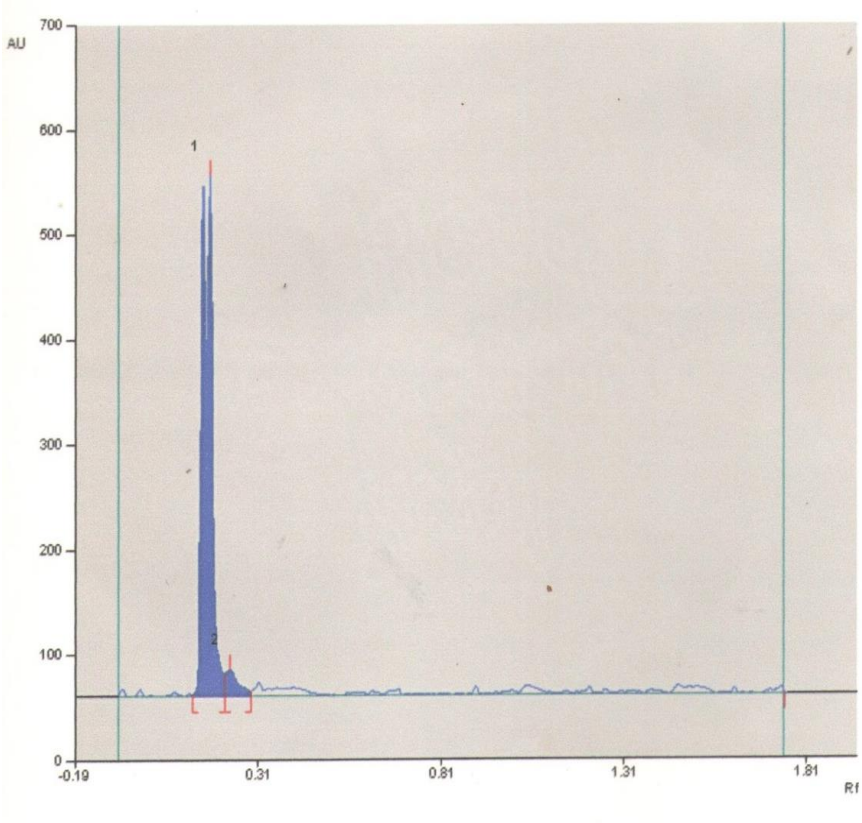

Fig.-4: HPTLC Chromatogram (densitometric scan at 366 nm) of *Clitoria ternatea* Linn. whole plant extract.

How to cite this article: M. P. V. Vikram Singh, Vinay Kumar Prajapati. Pharmacognostical screening of blue flowered variety of *Clitoria ternatea* Linn.- a phytocentric overview. *International Journal of Research and Review*. 2022; 9(5): 168-181. DOI: *https://doi.org/10.52403/ijrr.20220525*

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