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Morphological, anatomical and pharmacological studies in *Clitoria ternatea* L.

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ABSTRACT

Clitoria. ternatea L. *i.e.* 'Shankhpushpi' (Shankha) snail shell shaped flowers, categorized under Ayurvedic Medhya Rasayana drugs that claim as brain tonic and have memory and intelligence enhancing properties. The leaf mucilage contains anhydro-galacatan, anhydro pentosan and methyl pentosan and an alkaloid. The anthocyanins and delphinidin glucoside present in blue flowered variety. Pharmacologically it is reported for improved cognitive abilities, learning and memory, neuronal degenerative disorders; anticonvulsant activities; antimicrobial and insecticidal; antipyretic, analgesic, anti-inflammatory; antioxidant, hepatoprotective, antidiabetic, and platelet aggregation inhibitory activities. The detailed pharmacognostic studies of *C. ternatea* L. have not been reported so far. Therefore, an attempt has been made to standardize the macro-morphological and phytochemical studies of the plant *C. ternatea* L.. rheumatism, syphilis.

Keywords: *Clitoria. ternatea* L., Shankhpushpi, Pharmacology, Phytochemistry, Anatomy, Morphology

INTRODUCTION

Clitoria ternatea L. belongs to Family Fabaceae, commonly known as 'Butterfly pea' a perennial twining herb, found throughout India in tropical areas. Traditionally it is recommended for the treatment of snakebite, scorpion sting, chronic bronchitis, indigestion, constipation, fever, arthritis, eye ailments, sore throat, skin diseases, eye and ear-diseases in India (Mukherjee *et al*, 2008); mental problems, epilepsy, insanity, for muscular strength and complexion tonics (Anonymous, 1976); as a remedy for hemicranias and in swollen joints (Morris, 1999) beside this it is a good source of forage legumes in India (Gomez and Kalamani (2003). Ethno botanically it is used in various urinary troubles like infection, burning sensation in urinary tract, lack of urination, frequent urination (Singh *et al*, 2010) and also reported for purification after surgical removal of tumor (Das et al 2003). *C. ternatea* L. *i.e.* 'Shankhpushpi' consists of conch or 'Shankha' shaped flowers, categorized under Ayurvedic Medhya Rasayana drugs that claim as brain tonic and have memory and intelligence enhancing properties.

The blue-flowered variety is generally mixed with white-flowered one. Various groups of phytochemicals have been reported from white variety of *C. ternatea* L. like phenolics- kaempferol, quercetin, myricetin and their glycosides (Sharma, 2001). The fatty acid content of *C. ternatea* L. includes palmitic, stearic, oleic, linoleic, and linolenic acids (Vianni & Souto1971). Lactones aparajitin and clitorin were also reported from leaves (Gupta, 1959).

The leaf mucilage contains anhydro-galacatan, anhydro pentosan and methyl pentosan and an alkaloid (Sinha, 1960). The anthocyanins and delphinidin glucoside present in blue flowered variety. Pharmacologically it is reported for improved cognitive abilities, learning and memory, neuronal degenerative disorders (Kazuma et al 2003); nootropic and anticonvulsant activities (Jain et al 2003); antimicrobial and insecticidal; antipyretic, analgesic, anti-inflammatory (Kelemu, *et al* 2004); antioxidant, hepatoprotective, antidiabetic, (Devi, 2003) and platelet aggregation inhibitory (Zingare et al 2013) activities. The detailed pharmacognostic studies of *C. ternatea* L. blue have not been reported so far. Therefore, an attempt has been made to standardize the macro-morphological and phytochemistry of the plant *C. ternatea* L.

MATERIAL METHODS

Collection and authentication of plant material:
The selected blue and white variety of *Clitoria ternatea* L. were collected from Lucknow, India in April 2012, their herbarium specimens were prepared as per standard herbarium procedure (Honda *et al*, 1991) and deposited in the herbarium of CSIR-National Botanical Research Institute, Lucknow wide voucher specimen number LWG-002 and LWG-32 respectively.

Macro-microscopical studies:
The macroscopic study of two varieties of *C. ternatea* L. was described with the help of Flora (Jain & Rao, 1977). Plant materials were dried at 40 ± 2°C for 4-5 days in a hot air oven. The samples were stored at 25 ± 2°C in airtight containers and grounded to form fine powder when required and filtered through sieve of 345 micron pore size. Qualitative were done by hand cut sectioning in transverse planes best sections were picked out for mounting after the staining and dehydration were completed, quantitative microscopy for stomatal number, stomatal index and palisade ratio were done by slide preparation after clearing with chloral hydrate solution. Observation and photography was made under the microscope (Olympus CX3) and compatible software (Magnus Pro image analysis software) in different magnification (10X, 40X). Fluorescence analysis (in Ultraviolet fluorescent analysis cabinet, Sonar) and powder studies were done according to the standard methods (Kokate, 2006).

Physicochemical and phytochemical studies:
Total ash, acid-insoluble ash, alcohol soluble extractive, water soluble extractive and residual moisture content were calculated as per pharmacopeial methods (Evans, 2003). The preliminary phytochemical screening for the presence of steroid, triterpenoids, flavonoids, alkaloids, carbohydtrate, glycosides, tannins and saponins etc. was done according to Evans (Evans, 2003) and estimation major phytoconstituents according to following described methods.

Estimation of total sugar:
Total amount of sugar present in the drug was calculated based on the Montgomery method Anonymous (2000) using a spectrophotometer (Thermo electronic, Double Beam UVvis Spectrophotometer). 10% homogenate of the plant tissue in 80% ethanol was prepared and centrifuged at 2000 rpm for 15 minutes. The supernatant obtained was made upto known volume (10 ml or depending expected conc. of sugar). 0.1 ml aliquote was taken and 0.1 ml of 80% phenol and 5 ml conc. H_2SO_4 were added. Cooled and then the absorbance at 490 nm were noted. D-Glucose was taken as positive control. Standard curve was made by plotting a graph between optical density (OD) and concentrations of different dilutions (0.01, 0.02, 0.03, 0.04 and 0.05mg/ml) of standard D-Glucose. The percentage of sugar was calculated using formula. % sugar = Con. At UV x Ext value x 100/1000.

Estimation of total starch:
Total amount of starch present in the drug was calculated based on the Montgomery method Anonymous (2000) using the spectrophotometer (Thermo electronic, Double Beam UVvis Spectrophotometer). 10% homogenate of the plant tissue in 80% ethanol was prepared and centrifuged at 2000 rpm for 15 minutes. Added 4 ml of distilled water to the residue heated on a water bath for 15 minutes and macerated with the help of glass rod. To each of the samples, added 3 ml 52% perchloric acid and centrifuged at 2000 rpm for 15 minutes. The

supernatant thus obtained was made up to known volume (generally up to 10 ml). 0.1 ml aliquot was taken, 0.1 ml of 80% phenol and 5 ml conc. H_2SO_4 were added to it. Cooled and then noted the absorbance at 490 nm. The percentage of starch was calculated using formula. % sugar = Con. At UV x Ext value x 100/1000.

Estimation of total phenolic content (TPC): The amount of total phenolics present in the drug was calculated according to the Bray and Thrope (Montgomery, 1957)]. A stock solution of 1 mg/ml methanolic plant extract was prepared. 0.5 ml stock solution was taken in the test tube and added 10ml distil water and 1.5 ml folin reagent, kept for 5 minutes then added 4 ml 20% Na_2CO_3 made the volume up to 25 ml with distil water, and kept for 30 minute. The OD (optical density) was taken at 765 nm using the spectrophotometer (Thermo electronic, Double Beam UVvis Spectrophotometer). Gallic acid of different dilutions (0.2, 0.4, 0.6, 0.8 µg/ml) was used as standard. TPC was calculated in percent by the following formula: TPC = conc. In 1 ml x Ext. value x 100/1000.

RESULTS & DISCUSSION

Macromorphology:

In *Clitoria* petals are blue in colour. Stem: 20-45 cm, slender, terate, downy, splintery fibrous, surface smooth, internode 6-13 cm, taste bitter; Leaf: leaflets 4, opposite imperipinnate, ovate or oblong, obtuse, subcoriaceous 2-5 cm in length and 1-2 cm in width, apex macronate, surface hairy; Stipules: minute, linear; Flowers: solitary, axillary, bracteoles large, obtuse**;** Calyx: 1-1.5 cm, teeth lanceolate nearly as long as the tube; Corrolla: 3-5 cm long; Pod: 6-8 cm, flat, sparingly hairy, 6-10 seeded (Plate.1).

Micromorphology & Anatomy:

T.S. of Stem:

The T.S. of Stem of *clitoria ternatea* shows upper most layer is epidermis. The epidermis is composed of barrel shaped thick walled cells. The epidermis is covered by thin cuticle. The epidermal cells were measured about 1-2 x3-4 µ. The epidermis is followed by cortex. Cortex is of 4-5 layers. The cortical cell was measured about 4-54 x 5-6 lµ. The centrally steles is present at the centre of t.s of *Clitoria* stem. The stele is composed of phloem parenchyma & xylem elements. The phloem cells are measured about 3-4-x 4-5 µm &xylem elements measured about 1.2-1.3 x 1.3-1.4µ

T.S. of Leaf:

The T.S. of leaf of *clitoria ternatea* shows two layers epidermis i.e. - upper & lower epidermis. The upper epidermis is composed of compactly arranged barrel shaped cells. Epidermal cells are measured about 3-4-x 4.5-5µm. The upper epidermis is followed by single layer of palisade cells of chlorophylls cells The palisade cells were measured about 2.5-x10-13µm The palisade cells were followed by spongy parenchyma. The spongy parenchyma measured about 3.5-4x4.5-5µm. The spongy parenchyma was rich in chlorophyll.

The spongy parenchymatous is followed by bundle heath cells. The bundle sheath cells are of xylem elements the bundle sheath cells followed by vascular strand. The vascular strand composed of phloem parenchyma & xylem elements. The phloem parenchyma is measuring about 1-2x1.5-2.5µwere xylem element is measured about 3.5-4x4.5-5µ. The lower most is lower epidemic which is of thick walled compactly arranged cells. Lower epidermal cells were measured about 2.0-2.5x2.5-3.0.

T.S. Root:

The upper epidermis is composed of thick wall cells. The cortex is reduced with parenchymatous cells Xylem element in vascular tissue is interrupted to phloem parenchyma the phloem parenchyma were measured about 2.5- 3x3-3.5µ while xylem element measured about 4-5x4.5-5.5µ

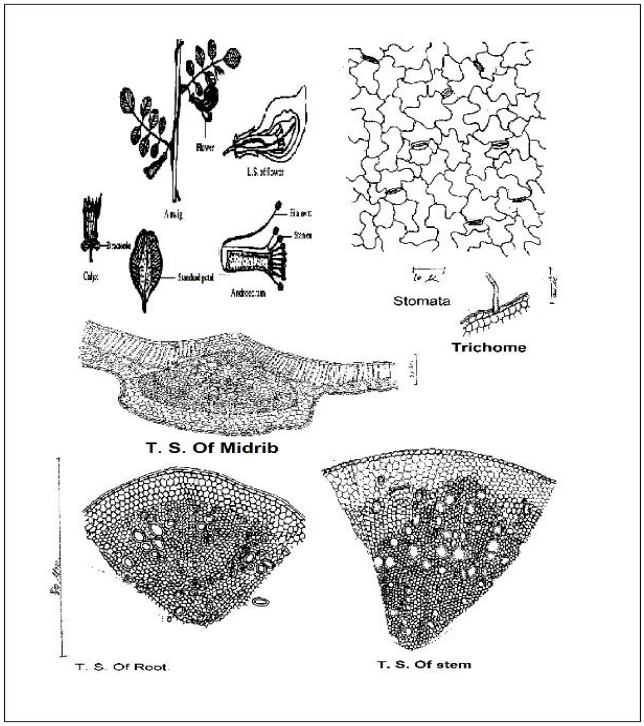


Plate.1, 1, Morphology, 2. Stomata, 3. Trichomes, 4. T. S. of Midrib, 5. T. S. of Root, 6. T.S. of Stem

Trichome: The unicellular unicostate type of trichomes is present in *clitoria ternatea* which is measured about 20-25 µin length

Stomata: *Clitoria ternatea* L. shows the anisocytic type of stomata (cruciferous) Which is measured about 5-7x4-5.

Physicochemical and phytochemical studies

Phytochemistry: The results of physicochemical parameters viz. water extractive; alcoholic extractive, total ash, acid insoluble ash, residual moisture content, total sugar, starch; table 1. The results of preliminary phytochemical screening are presented in Table 1.

Table 1: Biochemical analysis of metabolites in *Clitoria ternatea*.

Sr. No.	Test	Plant Part		
		Root	stem	leaf
1	Starch	+	+	+
2	Protein	+	+	+
3	Fat	+	+	+
4	Tannin	_	+	+
5	Saponin	+	+	+
6	Glycoside	+	+	+
7	alkaloids	+	+	+

Table 2. Ash content in *Clitoria ternatea* L.

Sr. No	Part of the plant	Total ash	Water insoluble ash	Water soluble ash	Acid soluble ash	Acid insoluble
1	Root	13.4%	12%	1.4%	11.2%	2.2%
2	Stem	18.6%	16%	2.6%	15.9%	2.7%
3	Leaf	21.3%	19.1%	2.2%	19.3%	02%

Table 3. Moisture and sugars content in *Clitoria terneta* L.

Sr. No	Part of the plant	Moisture	Total sugar	Reducing sugar	Non reducing sugar
1	Root	6.34%	0.83%	0.53%	0.30%
2	Stem	7.1%	0.94%	0.63%	0.31%
3	Leaf	7.9%	1.1%	0.83%	0.27%

Table 4. Biochemical analysis in *Clitoria terneta*

Sr. No	Plant Part	Amino Acid (%)	Crude protein (%)	P (%)	Ca (%)	K (%)	N (%)	Total Alkaloides (%)
1	Root	0.5	18.1	0.79	1.7	0.171	0.9	07
2	Stem	0.5	20.9	2.21	2.1	0.72	1.2	09
3	Leaf	0.9	17.2	2.32	3.3	0.211	4.1	11.3

Qualitative analysis:

Histochemistry: *Clitoria ternatea* (L): Root gave the negative test for tannin but other metabolites like starch, fat, protein saponin lipid are scattered cells of cortex. Stem shows their presence in the cortical and pith parenchyma the fresh leaf section shows the localization of starch, protein lipid fat, glycosides alkaloids and tannin are present in scattered cells of the mesophyll tissue.

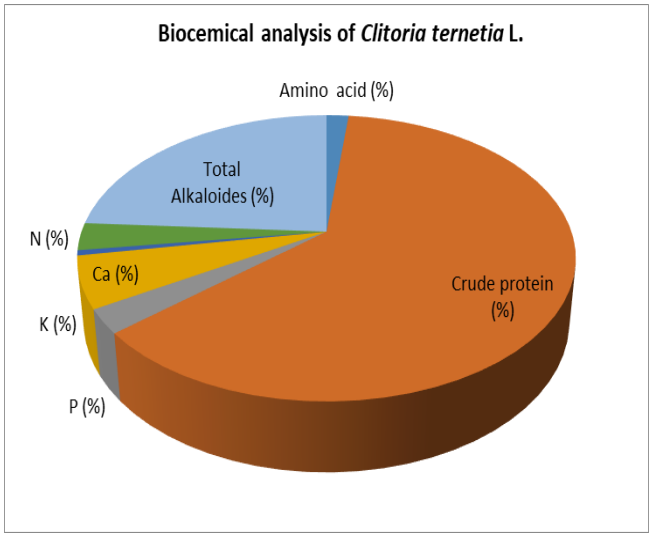
Physical evaluation:

Ash values: Total amount of ash in the root was 13.4 %, water soluble was found to be 1.4% water insoluble ash was12%, acid soluble ash was 11.2% acid insoluble ash

was found to be 2.2%. Total amount of ash in the stem was 18.6 %, water soluble was found to be 2.6% water insoluble ash was16%, acid soluble ash was 15.9% acid insoluble ash was found to be 2.7%. Total amount of ash in the leaf was 21.3 %, water soluble was found to be 2.2% water insoluble ash was19.1%, acid soluble ash was 19.3% acid insoluble ash was found to be 02%.

Quantitative analysis:

1. **Total sugar in root:** Total sugar content in root 0.83%, reducing sugar is found in 0.53% and non reducing sugar is 0.30 %. Total sugar content in stem0.94 %, reducing sugar is found in 0.63% and non reducing sugar is 0.31%. Total sugar content in leaf 1.1%, reducing sugar is found in 0.83% and non reducing sugar is 0.27%.
2. **Moisture contents:** Moisture content in root 6.34%, stem 7.1% and leaf is found in 7.9%. The values were found in increase in number root < stem< leaf.
3. **Total alkaloids :** Total alkaloids in root is found in 2.1%, in stem 10.18% and leaf 07% is found
4. **Nitrogen:** Amount of nitrogen in root 0.9%, stem 1.2 % and in leaf 4.1% is found
5. **Potassium:** Amount of potassium in root is 0.171%, stem 0.72% and in leaf 0.211% is found.
6. **Calcium:** Amount of calcium in root 1.7%, stem 2.1% and in leaf 3.3 % is found.
7. **Phosphorus:** Amount of Phosphorus in root 0.79%, stem 2.4% and in leaf 2.32% is found.
8. **Crude protein:** Amount of Crude protein in root 18.1%, stem 20.9% and in leaf 17.2% is found.
9. **Total free amino acid:** Amount of Total free amino acid in root 0.5%, stem 0.5% and in leaf 0.9% is found



DISCUSSION

Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents which is starts with wrong identification of plant material (Woisky & Salatino, 1998). There are several evidences of unraveling this problem by pharmacognostic studies of medicinal plants even present time (Chanda, 2014). Morphological, microscopical, phytochemical and physicochemical analyses are major pharmcognostical parameters for above incongruity. 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 possess 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 microscopical features of *C. ternatea* L. (Table 1) clearly showed anatomical structures like more starch grains in transverse section of root; broad patches of pericyclic fibers, more vessels with broad lumen and broad pith region in the stem of white variety (Figure 2 and 3). Further, the quantitative leaf microscopy showed slight variation in stomatal number, somatal index and palisade ratio (Table 1). 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. *C. ternatea* L. showed some significant presence of biochemicals in biochemical analysis (Table 2). Further, the preliminary phytochemical screening showed presence of steroid, flavonoids, alkaloid, carbohydrate, glycosides, tannins and saponins in *C. ternatea* L. better results for flavonoid, alkaloid, carbohydtare and glycosides while blue variety for saponins and tannins (Table 3 and 4). These reports validated the traditional claim of *C. ternatea* L. However, presence of high flavonoid, phenolics, sugar and starch content in the white variety indicated its more therapeutic values. Further pharmacological investigations are required for therapeutic activities of *C. ternatea* L.

CONCLUSION

Every plant possesses characteristic chemical, histological and anatomical peculiarities in respect to its ecological conditions. The microscopical features of *C. ternatea* L. clearly showed anatomical structures like

more starch grains in transverse section of root; broad patches of pericyclic fibers, more vessels with broad lumen and broad pith region in the stem of white variety. Further, the quantitative leaf microscopy showed slight variation in stomatal number, somatal index and palisade ratio. *C. ternatea* L. showed some significant presence of biochemicals in biochemical analysis. Further, the preliminary phytochemical screening showed presence of steroid, flavonoids, alkaloid, carbohydrate, glycosides, tannins and saponins in *C. ternatea* L. better results for flavonoid, alkaloid, carbohydtare and glycosides while blue variety for saponins and tannins. These reports validated the traditional medicinal properties claim of *C. ternatea* L. for various ailments. However, presence of high flavonoid, phenolics, sugar and starch content in the white variety promisingly indicated more therapeutic values. Further pharmacological investigations are required for therapeutic activities of *C. ternatea* L. as these studies are primary studies reveals its importance. There are greater chances of increase in the present knowledge about *Clitoria ternatea* L. if analyzed with modern techniques.

REFERENCES

Anonymous (1976) Medicinal Plants of India. Indian Council of Medical Research, New Delhi 260-261.

Anonymous (2000) The Ayurvedic pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare, New Delhi, India.

Chanda S (2014) Importance of pharmacognostic study of medicinal plants: *An overview. J Pharmacog Phytochem* 2: 69-73.

Das S, Dash S, Padhy SN (2003) Etheno-medicinal information from Orissa state, India, *A Review. J Hum Ecol* 14: 165-227.

Devi BP Boominathan R, Mandal SC (2003) Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. *Fitoterapia* 74: 345-349. https://doi.org/10.1016/s0367-326x(03)00057-1

Evans WC (2003) Trease and Evans Pharmacognosy. (15thedn) Saunders, London: 545-547.

Gomez SM, Kalamani A (2003) Butterfly Pea (*Clitoria ternatea*): A Nutritive Multipurpose Forage Legume for the Tropics - An Overview. *Pakistan Journal of Nutrition* 2: 374-379. https://doi.org/10.3923/pjn.2003.374.379

Gupta RK (1959) Chemical examination of the leaves of *Clitoria ternatea* Linn. *J Ind Chem Soc* 36: 243-246.

Honda T, Saito N, Kusano T, Ishisone H, Funayama N, et al. (1991) Isolation of anthocyanins (Ternatin A A2, B B2, D and D2) from *Clitoria ternatea* cv. (double blue) having blood platelet aggregation-inhibiting and vascular smooth muscle relaxing activities. *Kokai Koho, Japan*, 7.

Jain NN Ohal CC, Shroff SK, Bhutada RH, Somani RS, et al. (2003) *Clitoria ternatea* and the CNS. *Pharmacol Biochem Behav* 75: 529-536. https://doi.org/10.1016/s0091-3057(03)00130-8

Jain SK, Rao RR (1977) A hand Book of field and herbarium methods. Today and Tomarrow Printers and Publications, New Delhi.

Kazuma K Noda N, Suzuki M (2003) Flavonoid composition related to petal color in different lines of *Clitoria ternatea.* Phytochemistry 64: 1133-1139. https://doi.org/10.1016/s0031-9422(03)00504-1

Kelemu S Cardona C, Segura G (2004) Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea*, a tropical forage legume. *Plant Physiol Biochem* 42: 867-873.

Kokate CK (2006) Practical Pharmacognosy. (1stedn) Vallabh Prakashan, New Delhi.

Montgomery R (1957) Determination glycogen. *Arch Biochem Biophys* 67: 378- 386.

Morris JB (1999). Legume Genetic Resources with Novel Value Added Industrial and Pharmaceutical Use. ASHS Press, USA. 1-6.

Mukherjee PK, Kumar V, Kumar NS, Heinrich M (2008) The Ayurvedic medicine *Clitoria ternatea*--from traditional use to scientific assessment. *J Ethnopharmacol* 120: 291-301. https://doi.org/10.1016/j.jep.2008.09.009

Sharma PV (2001) Caraka Samhita. (7thedn), Chaukhambha Orientalia, Varanasi.

Singh PK, Kumar V, Tiwari RK, Sharma A, Rao Ch V, et al. (2010). Medico- Ethnobotany of 'Chatara' Block of District Sonebhadra, Uttar Pradesh, India. *Adv Biol Res* 4: 65-80.

Sinha A (1960). Mucilage from the leaves of *Clitoria ternatea*. *Proceedings of the Institution of Chemists India.* 32: 228-231.

Vianni R Souto SM (1971) Oil content and fatty acidcomposition of seeds of *Clitoria ternatea* L. Arquivos, Universidade *Federal Rural do Rio de Janeiro* 1: 47-50.

Woisky R, Salatino A (1998). Analysis of propolis: some parameters and procedures for chemical quality control. *J Apic Res* 37: 99-105. https://doi.org/10.1080/00218839.1998.11100961

Zingare ML, Zingare PL, Dubey AK, Ansari MA (2013) *Clitoria ternatea* (Aparajita): A Review of the Antioxidant, Antidiabetic and Hepatoprotective potentials. IJPBS 3: 203-213.