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MEDICO-BOTANICAL STUDY OF *CLITORIA TERNATEA* LINN.VAR. *TERNATEA*

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ABSTRACT:
The aim of the present work to study the Pharmacognostic properties of *Clitoria ternatea* Linn. The study includes of collection, identification, pharmacognostic and phytochemical evaluation of different parts of the plant. The preliminary phytochemical test on alcoholic and water extracts indicates the presence of alkaloids, glycosides, flavanoids, saponins, carbohydrates, phenols, lignins and terpenoids. Present intensive studies on morphology, histochemistry, studies of vegetative organs would be very revealing and significant in the evaluation of these drug plants.

Keywords: Pharmacognosy, Phytochemical, flavanoids, saponins, glycosides, lignins.

INTRODUCTION:

Clitoria ternatea Linn. var. *ternatea* is a twining herb traditionally used as *Shanka pushpi*, an Ayurvedic medicine used to promote neurological health. It shows promise in animal models for its memory enhancing effects, and has a wide spectrum of neurological benefits (Sivaranjan, 1994). In traditional Ayurvedic medicine, it is ascribed various qualities including memory enhancing, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing, and sedative properties (*Mukherjee, et al., 2008*). In traditional Chinese medicine, due to its appearance similar to the female reproductive organ, and consistent with the Western concept of the doctrine of signatures, the plant has been ascribed properties affecting this organ (*Fantz, 1991*) there are no experimental studies to show that this how this herb standardize. Thus, this study was designed to find the standard parameters of the drug.

Morphology and Medicinal Uses
Classification:
Kingdom : Plantae
Division : Angiosperms
Class : Eudicots
Order : Fabales
Family : Fabaceae
Genus : *Clitoria*
Species: *C. ternatea* Linn.var. *ternatea*

C. ternatea is a vigorous, strongly persistent, herbaceous perennial legume; stems fine twining, sparsely pubescent, suberect at base, 0.5-3 m long. Leaves pinnate with 5 or 9 leaflets; petioles 1.5-3 cm long; stipules persistent, narrowly triangular, 1-6 mm long, subulate, prominently 3-nerved; rachis 1-7 cm long; leaflets elliptic, ovate or nearly orbicular, 1.5-5 cm long, 0.3-3 cm wide, with apex acute or rounded, often notched, and base cuneate or rounded, both surfaces sparsely appressed pubescent. Flowers large, axillary, solitary, single or paired; colour ranges from light blue to dark blue; pedicles 4-9 mm long. Bracteoles persistent, broadly ovate or rounded, 4-12 mm long. Calyx 1.7-2.2 cm long with a few fine hairs; tube campanulate, 0.8-1.2 cm long; lobes triangular or oblong, 0.7-1 cm long, acute or acuminate. Standard obovate, funnel-shaped, 2-5.5 cm long, 2-4 cm wide, notched or rounded at apex, blue with a pale yellow base. Pods linear-oblong, flattened, 4-13 cm long, 0.8-0.2 cm wide, with margins thickened and style persistent, sparsely pubescent when mature, pale brown, dehiscent when dry. Seeds 8-10/pod, oblong, somewhat flattened, 4.5-7 mm long, 3-4 mm wide, yellowish brown to almost black, shiny, often mottled, minutely pitted. (Jones, *et al.*, 2000, Yadav and Sardesai, 2002).

Medicinal Properties and Uses:

In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant tranquilizing and sedative agent (Aulakh, *et al.*, 1998).

In animal tests the methanolic extract of *Clitoria tenatea* roots demonstrated neutropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. The active constituents include tannins, resins, starch, taraxerol and taraxerone (Kiranmai, *et al.*, 2001).

Recently, several biologically active peptides called cliotides have been isolated from the heat stable fraction of *C. terrnatea* extract. Cliotides belong to the cyclotides family and activities studies show that cliotides display potent antimicrobial activity against *E. coli, K. pneumonia, P. aeruginosa* and cytotoxicity against Hela cells. These peptides have potential to be lead compound for the development of novel antimicrobial and anti-cancer agents. (Ali Esmail Al-Snafi, 2016)

In traditional medicinal: Owing to its similarity to a human body part, this plant has been described properties affecting the same (a phenomenon also found in connection with the mandrake among other plants). It was used traditionally in an attempt to treat sexual aliments like infertility and gonorrhea, to control menstrual discharge and also as an aphrodisiac. This practice aligns with an ancient belief recorded in the Doctrine of Signatures. (Ali Esmail Al-Snafi, 2016)

MATERIAL AND METHODS

Collection and identification of plant:

The fresh healthy, mature, plants were collected from Dhule away from main city to unpolluted area. The plant materials were identified using the flora of Dhule district and Nandurbar district (Patil, 2003) and Flora of Kolhapur District (Yadav and Sardesai, 2002) at Post-graduate Department of Botany, S. S. V. P. Sansthas, L. K. Dr. P. R. Ghogrey Science College, Deopur, Dhule-424005(M.S) India.

The leaves, stem and roots were washed and used for the present study. The macroscopic observations of the mature plants, leaves, inflorescences and other parts were noted down. For microscopic studies, some plant material preserved in 70% alcohol. Leaf epidermal studies were carried out on fresh specimens. Peels were removed mechanically using some chemicals. They were stained in 1% safranin mounted in glycerin and made semi-permanent by ringing with DPX solution. Stomatal index (SI), stomatal frequency, vein-islet, vein termination number and palisade ratio were calculated as defined by Salisbury, (Salisbury, 1927; 1932).

viz.,

$$SI = \frac{S}{E + S} \times 100$$

Where 'S' = number of stomata per unit area and 'E' = number of epidermal cells in the same area. Stomatal index (SI), stomatal frequency, vein-islet, vein termination number and palisade ratio have been calculated out of an average of 10 readings. Palisade ratios (PR) was calculated as the average of palisade cells (P) beneath each epidermal cell (E). Vein islet number is defined as the number of vein islets per sq. mm of the leaf surface midway between the midrib and the margins.

Transections of leaf, stem and root were taken by free hand. Fresh and preserved materials were used. Sections were stained in safranin (1 %), light green (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of leaf, petiole, stem and root sections were taken by using DIGI- EYE High resolution Cameras affixed to microscope Olymphus CH 20*I.*

For study of vessels, fragments of plant organs, especially stem at nodal region and root, were macerated using nitric acid (10%) and potassium dichromate (10%) solution in equal proportions. The vessel elements were stained with aqueous saffranin (1%), dehydrated and mounted in DPX. Some vessel members were also examined in glycerine. The line and cellular sketches of the figures were drawn using a Camera Lucida. The range of length and diameter of vessel elements was determined by the measurement of 20 – 25 vessel elements.

The fresh, healthy plants were rooted out and washed with water. They were separated as root, stem and leaves and shade dried. The dried plant

materials were pulverized into fine powder using a grinder (mixer). About 1 kg of powdered material was prepared. After that powder were kept into air tight bags. Physiochemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values as petroleum ether-soluble extractives, etahnol-soluble extractives, methanol-soluble extractive, and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia (Annonymous, 1966; 1985).

RESULTS AND DISCUSSIONS

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Thomas, *et al.*, 2009; Usha Kuamari *et al.*, 2009). Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. The result of this study as follows:

Microscopic Characters:

T.S. of leaflets of *C. ternatea* Linn.var. *ternatea* (Plate 2:E)

In transverse section leaf shows the dorsiventral structure. The epidermis distinguishes into upper and lower epidermis. The epidermis unilayered on both side with thick cuticle, cuticle strianted hence appearing lobed in cut section at the midrib. All along the veins prismatic crystals of calcium oxalate are present. The mesophyll is differentiated into palisade and spongy tissue. A vascular bundle is present in the cortex of midrib region. Stele is haplostele. Xylem elements surrounded by phloem.

Stomata: Stomata found on both surfaces and its type is anomocytic and anisocytic with wavy cell walls. (Plate 1:B,C).

T.S. of petiole of *C. ternatea* Linn.var. *ternatea* (Plate 2:F)

The transverse section of petiole shows a triangular outline, upper two angles extending into a multicellular lob like structure. Epidermis thick cuticle, unilayered small cells compactly arranged which is followed by parenchymatous 5-8 layered, cortex with scattered chlorenchymatus cell. In the center cortex is present. Phloem consists of sieve tubes, campanian cells and phloem parenchyma also xylem consists of vessels and xylem pavenchyma.

T.S. of Stem of *C. ternatea* Linn.var. *ternatea* (Plate 2:G)

The transverse section of the stem shows a circular outline. Epidermis has small cells compactly arranged with 6-8 angles extending into a multicellular lobe like structure. following the epidermis is a thick cortex 6-10 layered which is parencymatous with scattered chlorenchymatus cell. Cortex in wing 2-5 layers of compact Parenchyma. Stele consisting of band of xylem disrupted at poles. Polar xylem is lax, phloem is present in the form of continues band on both side of xylem band. The central pith is present.

T.S. of Root of *C. ternatea* Linn.var. *ternatea* (Plate 2:H)

The transverse section of petiole shows on circular outline shows outermost phloem composed of 12-25 rows of thin walled longitudinally elongated cells of which are compressed and few exfoliating. Phellogen is single layered and phelloderm is two to three layered, some cells contain calcium oxalate. Cortex is composed of 8-12 layers of thin walled trangentially elongated cells or polygonal mostly. Secondary growth found in root. Stele consisting of Contineuous ring,

xylem is surrounded by phloem zone. Phloem appears as conical strands separating by narrow medullary rays. All the ray cells are fully packed with starch grains and some contains calcium oxylates too. Vessels are embedded with thick-walled xylem sclerenchyma. The central pith is present.

Quantitative Microscopy of Leaves: (Table 1)

Quantitative microscopy of leaves *C. ternatea* has illustrated into the Table-1 which contains stomatal index and stomatal numbers of plants. Stomatal number ranges from 15-28. Stomatal index ranges from 25-36.

Qualitative Phytochemical Analysis of *C. ternatea* Linn.var. *ternatea*: (Table 2)

Qualitative phytochemical analysis was done to diagnose the presence or absence of various chemical constituents such as carbohydrates, reducing sugars, pentose sugars, hexose sugars, galactose, proteins, amino acids, glycosides, saponins, flavonoids, alkaloids and tannin and phenolic compounds.

Results shows that in *C. ternatea*, there is presence of carbohydrates, hexose sugars, proteins, amino acids, saponis are present in appreciable amount. Reducing sugars, pentose sugars, flavonoids, alkaloids and tannin and phenolic compounds are present in moderate amount. There is complete absence of anthroquinone glycosides.

Histochemical Tests (Leaf) of *C. ternatea* Linn.var. *ternatea* (Table 3)

For the histochemical studies free hand sections of the organs to be studied, were taken and treated with the respective reagents to localize componants, viz. Starch, proteins, tannins, saponins, Fat and alkaloids in the tissues.

The present pharmacognostic study of *Clitoria ternatea* provides useful information on its correct identity. Leaves are small, petiolated. The stem is rounded.

The epidermis cells are cubical to oval. The stomata are reported on the both surface, lower surface and upper surface of the leaf only. The shape of stomata is generally circular, oval or elliptical in outline. The stomata are anomocytic and paracytic. The stomatal index is reported as 29.67. The lower epidermis is followed by one to two layers of collenchymatous hypodermis followed by parenchymatous cortex. The transverse section of the stem shows a circular outline. Epidermis has small compactly arranged cells. Phyllotaxy is alternate. In center pith is present.

The transverse section of the root shows a circular outline. Epidermis has small cells compactly arranged. The cortex consist of tangentially elongated cells. Secondary growth is more. The preliminary phytochemicals studies carried out so far reveal the presence of various phytochemicals like protein, alkaloids, starch, fats, saponins and tannins.

CONCLUSION:

The present investigation pertains detail pharmacognostical studies of *Clitoria ternatea*. This is the most popular and widely used drug plants in Ayurvedic system of medicines. The present study intends to find out their efficacy towards medicinal importance, medicinal potentiality and taxonomic identification.

Morphological and anatomical studies of different organs have been studied in detail. Number of leaves at the node, their shape, size, colour, texture, venation including the leaf surface data, inflorescence, bracts, colour of flower, trichomes, petiole, stem and root structure are the characters which help to differentiate these species. Phytochemical tests for primary screening of active ingredients have been carried out for Glycosides, Alkaloids, Tannin and Phernolic compound, Steroid, Saponin glycoside and redusing Sugars. In the present study most of the positive results are found in Chloroform extract. Present intensive studies on morphology, histochemistry, studies of vegetative organs would be very revealing and significant in the evaluation of these drug plants.

Further phytochemical studies are necessary for appropriate and safe conclusion.

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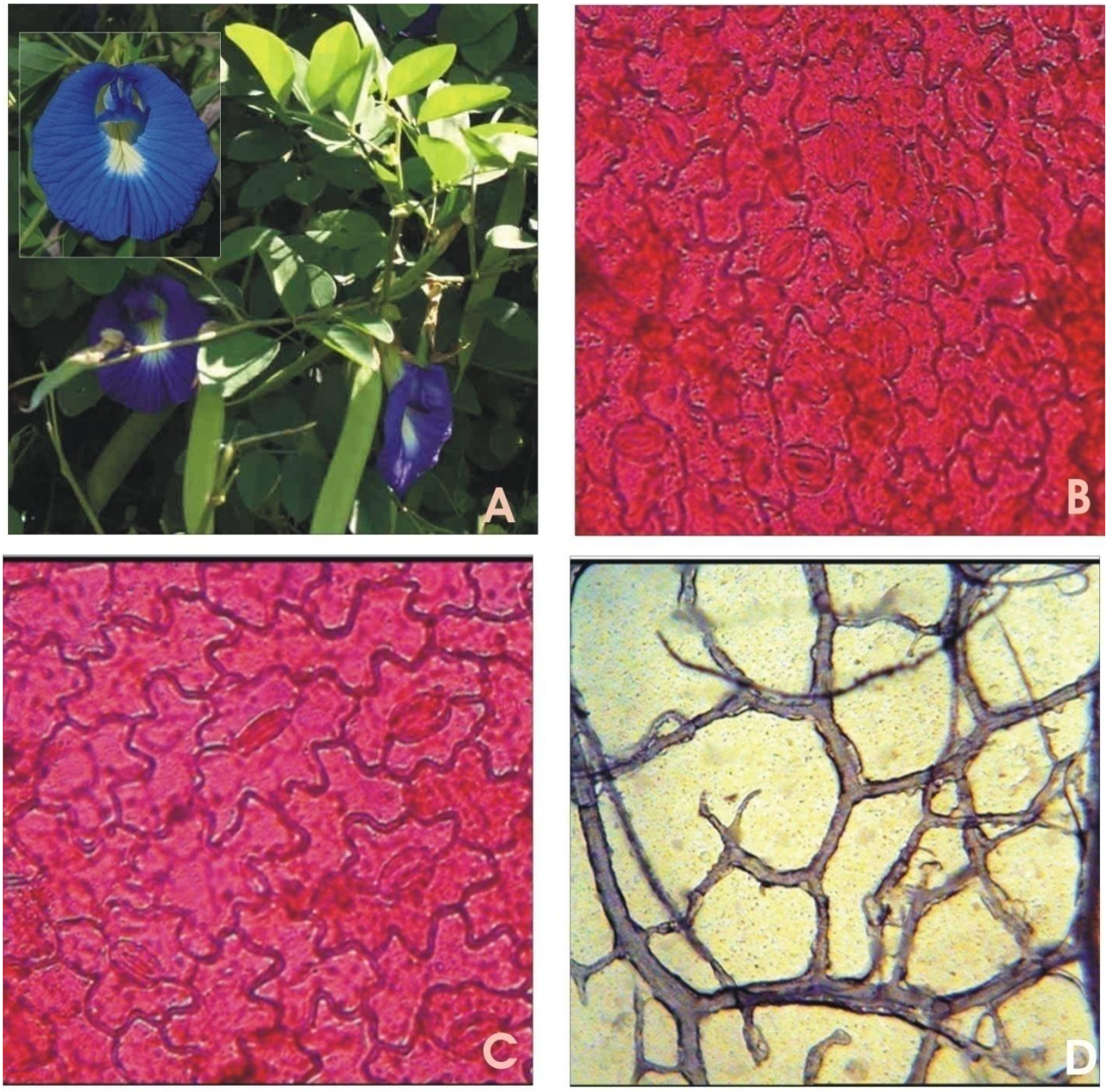

Plate 1

***Clitoria ternatea* Linn.var. *ternatea*: A**-Morphology of Plant; **B**-Stomata present on upper epidermis of leaf; **C**-Stomata present on Lower epidermis of leaf; **D**-Vein Islets and terminations

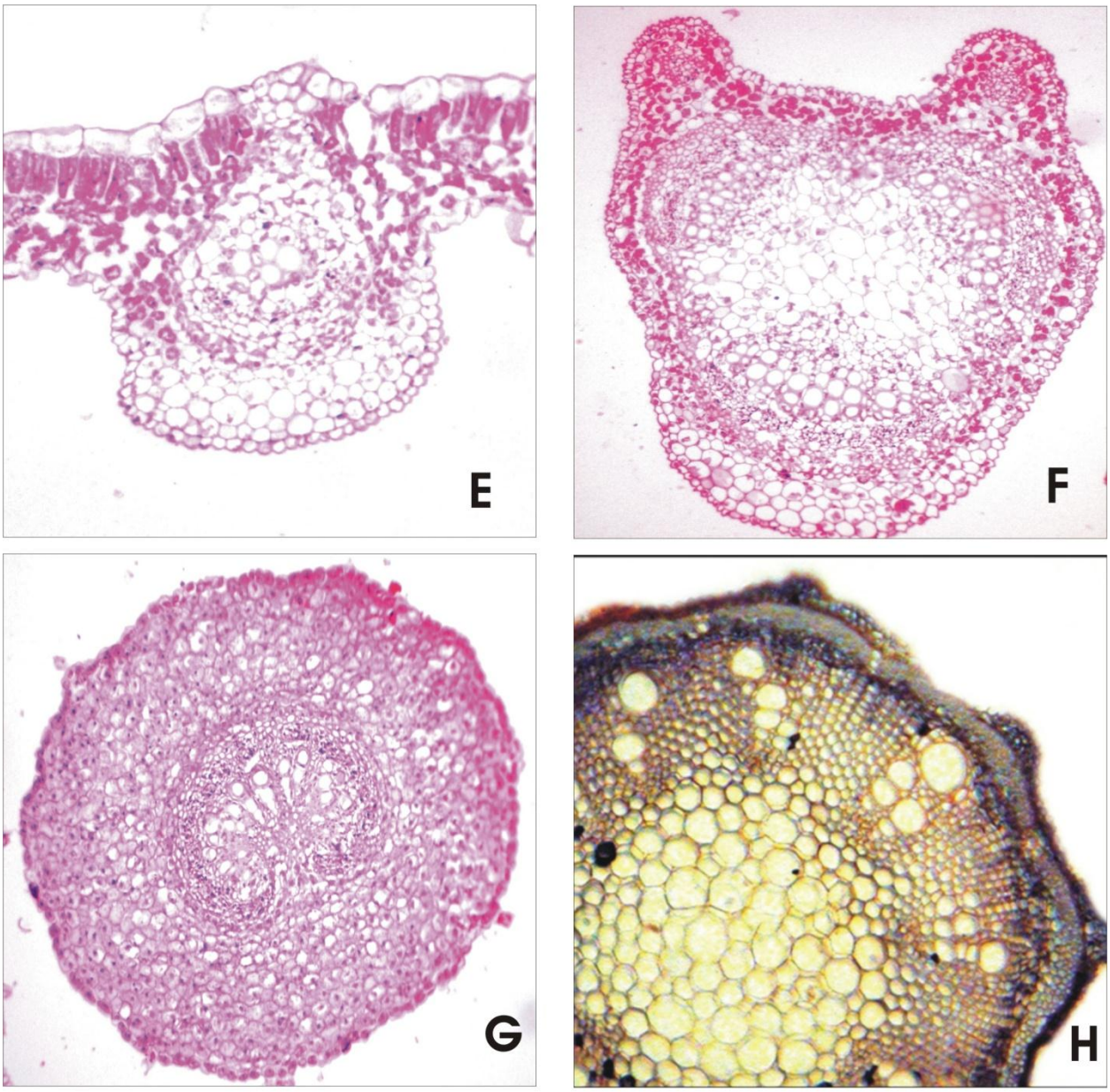

Plate 2

***Clitoria ternatea* Linn.var. *ternatea* Anatomy of Plant** : **E-** T.S. of Leaflet; **F**-T.S. of Petiole; **G**-T.S. of Stem; **H**-T.S. of Root

Table -1 Quantitative Microscopy of leaves:

Sr. No.	Parameter	Range	Mean
1.	Stomatal Index	25-36	29.67
2.	Stomatal Number	15-28	19.66

Table -2 Quanlitative Phytochemical of *C. ternatea* Linn:

Sr. No.	Test	Stem	Leaf
		Extract in Methanol	Extract in Methanol
1.	Protein	+++	++
2.	Glycosides	-	-
3.	Alkaloids	++	+

4.	Starch	+	+
5.	Fat	++	+
6.	Saponins	++	+
7.	Tannin	++	+

Table 3 : Histochemical Tests (Leaf) of *C. ternatea* Linn.

Sr. No.	Reagent	Constituents	Colour	Histological zone	Degree of intensity
1.	T.S. + Iodine + Potassium iodide solution	Starch	Blue Green	Mesophylls	++
2.	T.S. + Potassium Ferrocyanide + Glacial acetic acid + FeCl3.	Proteins	Blue Yellow	Mesophylls M. cortex	++
3.	T.S. + $FeCl3 + Na_2CO_3$	Tannins	Blue	Merophylls, M. Cortex	++
4.	T.S. + $Ba(OH)_2 + K_2Cr_2O_7 + CaCl_2$	Saponins	Yellow	Mesophylls	+++
5.	T.S. + Sudan III/IV	Fats	Pink	Mesophylls M. cortex	+++
6.	T.S. + Mayer's reagent	Alkaloids	Golden Yellow	Mesophylls M. cortex	++

Table 4 : Histochemical Tests (Stem) of *C. ternatea* Linn.

Sr. No.	Reagent	Constituents	Colour	Histological zone	Degree of intensity
1.	T.S. + Iodine + Potassium iodide solution	Starch	Blue	Mesophylls	++
2.	T.S. + Potassium Ferrocyanide + Glacial acetic acid + $FeCl_3$.	Proteins	Blue Yellow	Mesophylls M. cortex	+++
3.	T.S. + $FeCl_3 + Na_2CO_3$	Tannins	Blue	Merophylls	++
4.	T.S. + $Ba(OH)_2 + K_2Cr_2O_7 + CaCl_2$	Saponins	Yellow	Mesophylls M. cortex	+++
5.	T.S. + Sudan III/IV	Fats	Red Pink	Mesophylls M. cortex	+++
6.	T.S. + Wagner's reagent	Alkaloids	Golden Yellow	M. cortex Mesophylls	+++

Table 5 : Histochemical Tests (Petiole) *Clitoria ternatea* Linn.

Sr. No.	Reagent	Constituents	Colour	Histological zone	Degree of intensity
1.	T.S. + Potassium Ferrocyanide + Glacial acetic acid + $FeCl_3$.	Proteins	Blue Yellow	Mesophylls M. cortex	+++
2.	T.S. + $FeCl_3 + Na_2CO_3$	Tannins	Blue	Merophylls	++
3.	T.S. + $Ba(OH_2) + K_2Cr_2O_7 + CaCl_2$	Saponins	Yellow	M. cortex	+
4.	T.S. + Sudan III/IV	Fats	Pink	Mesophylls M. cortex	+++
5.	T.S. + Wagner's reagent	Alkaloids	Golden Yellow or Brown	Mesophylls M. cortex	++