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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF *CLITORIA TERNATEA* FLOWERS

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Abstract:
The genus " Clitoria" includes about 48 species under the family "Fabaceae". Clitoria ternatea is a widely distributed floral garden plant in India.Literature search revealed numerous pharmacological actions, including nootropic, anxiolytic, anticonvulsant, sedative, antipyretic, anti-inflammatory, and analgesic effects, have been observed in C.ternatea. For the treatment of snake bites, the roots, stems, and flowers are suggested. The flower's powder microscopy reveals pitted vascular cells, elongated rectangular epidermal cells with thin walls, fiber, and two different kinds of trichomes: capitate glandular trichomes with an apical point and simple unicellular trichomes. According to the preliminary phytochemical analysis of the ethanolic extract, alkaloids, glycosides, tannins, proteins, amino acids, flavanoids, saponins, carbohydrates, phenols, lignins, anthocyanins, and terpenoids are present.

Key Words: *Clitoria ternatea, Pharmacognosy, Microscopy, Phytochemistry, Chemical constituents.*

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INTRODUCTION:

The Genus *Clitoria*

Since the morphology of these plants' flowers matches the shape of human female genitalia, the genus *Clitoria* is a direct translation of the Ternate native term telang, which means "Clitoris". A Polish naturalist named Jakob Breyne first mentioned the genus in 1678. He named it Flos clitoridis ternatensibus, which translates to "Ternatean flower of clitoris." Breyne's description includes a picture of the plant. The Ternate location, which is part of the northern Maluku Islands, is the location from which botanist Carl Linnaeus obtained his specimens, and this is where the species name comes from. [1,2]

***Clitoria ternatea* Linn**

It is usually referred to as Butterfly pea belonging to the family Fabaceae[3,4,5]and sub-family Papilionaceae, originates from tropical Asia and is a perennial herbaceous plant with elliptic, obtuse leaves and known as leguminous twiner. It was thereafter widely transported throughout South and Central America, the East and West Indies, China, and India, where it became naturally occurring.[4] *Clitoria ternatea* also known as Asian pigeon wings, blue bellvine, blue pea, cordanfan pea and darwin pea. It blooms alone or in pairs from summer to fall and grows as a vine or creeper that does well in damp, neutral soil. The plant *C.ternatea* is traditionally used for food colouring, stress, infertility and gonorrhoea.

SYNONYMS OF *CLITORIA TERNATEA* LINN[3,6,7,14]

- *Clitoria albiflora* Mattei
- *Clitoria bracteata* Poir
- *Clitoria principissae*

VERNACULAR NAMES OF *CLITORIA TERNATEA* LINN[6,10,14]

- Sanskrit : Ashphota, Aparajitha, Saukarnika, Ardrakarni, Girikarnika, Supuspi
- Hindi : Aparajitha
- Telugu : Neela-ghentana, Dintana, and Gilarnika, Sankhupuvvu
- Kannada : Billisaiuga, Satugadagida
- Marathi : Gokurna
- Tamil : Kuruvilai, Kakkanam, Kakatan, Kavachi
- Malayalam : Shankapuspam, Aral, Malai-amukki
- English : Butterfly pea, Blue-pea vine, Pigeon wings

SCIENTIFIC CLASSIFICATION OF *CLITORIA TERNATEA* LINN[2,6,7,8,14]

Kingdom	: Plantae
Division	: Angiosperms
Phylum	: Tracheophyta
Class	: Eudicots
Order	: Fabales
Family	: Fabaceae
Genus	: *Clitoria*
Species	: *ternatea*
Species authority	: Linn

Fig 1: Plant of *Clitoria ternatea*

BOTANICAL DESCRIPTION OF *CLITORIA TERNATEA*[4,5,6,7,8,9]

Habitat	Open in mesic forest or shrub land
Habit	Perennial, twinning climber (2-3m height)
Flowering class	Dicot
Root	Tap root system with numerous slender lateral roots
Stem	Aerial, weak stem and a twinning fine stem, 0.5-3m long
Trichomes	Multicellular, with two basal cells smaller than the terminal cells are present
Leaf	Pinnate compound leaves that are obovate and entire with emarginate tips. Leaves imparipinnate with 2-4 pairs of leaflets (3- 5cm long), elliptic to lanceolate shortly pubescent underneath. The epidermis on both leaf surfaces consists of single layer cell protected by a thick cuticle and with trichome out growths. A layer of palisade cells, lignified xylem and paracytic stomata lie underneath the upper epidermis
Inflorescence	Axillary & solitary
Calyx	Tubular, consisting of 5 sepals which are fused about two thirds of their length
Corolla	Showy, consists of 5 free sepals, with one large &rounded banner, two wrinkled wings which are often half the length of the banner
Androecium	Stamens 10, diadelphous (9+1), attached to each filament is a pollen bearing white anther, which consists of four lobes
Gynoecium	Ovary superior, stipitate and monocarpellary, unilocular ovary bearing 10 ovules, long & thick style with a bent tip and incurved with feathery stigma
Pods	Narrow, linear, oblong, 6-13m long,0.7-1.2mm wide shortly pubescent, beaked and flattened with pointy tips, and they contain 10 seeds with length range of 5-7cm and are edible when tender
Fruit	Legume
Flowers	Pentamerous zygomorphic pea-shaped flowers, bracteate, bracteolate, deep blue to blue mauve, very short pedicellate and 4-5cm long.
Seed	non-endospermous, oval shaped & kidney shaped, having a olive blackish or yellowish-brown colour ,often mottled, 4.5-7mm long and width from 3-6mm

Fig2:Leaf

Fig3:Flower

Fig 4 : Seed

Fig 5: Pods

Fig 6: Root

Fig 7: Stem

Microscopical studies of *Clitoria ternatea* flower

POWDER MICROSCOPY

Powdered material consists of pitted vessel cells, elongated rectangular epidermal cells with thin walls, fibre, and two types of trichomes: Simple unicellular trichomes and Capitate glandular trichome with an apical tip.

Fig no: 8 Capitate glandular trichome

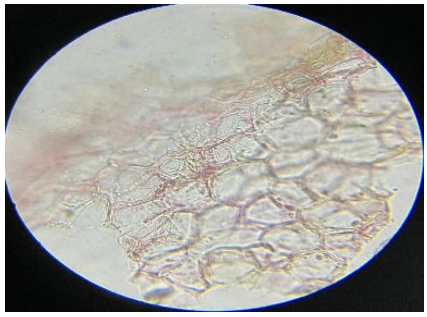

Fig no: 9 Straight anticlinal walls

Fig no: 10 Pollen grains

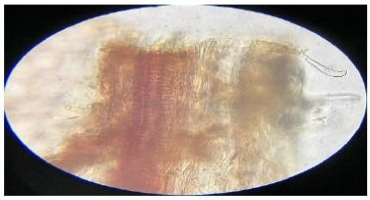

Fig no: 11 Thin walled and elongated parenchyma rectangular epidermal cells

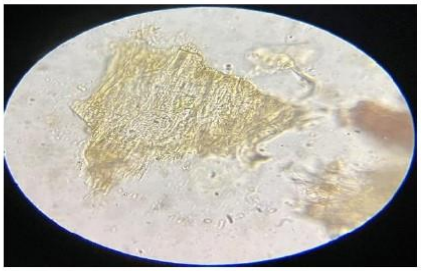

Fig no: 12 Sinuous anticlinal walls

Fig no: 13 Pitted vessel

Fig no: 14 Simple unicellular trichome

CHEMICAL CONSTITUENTS [4,9,14]

Pentacyclic triterpenoids, namely taraxerol and taraxerone, are the main phytoconstituents of *Clitoria ternatea*. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, , taraxerol, taraxerone, flavonoids, saponins, tannins, carbohydrates, proteins, resins and starch. Four kaempferol glycosides I,II,III and IV were isolated from the leaves. Kaempferol-3-glucoside (I), kaempferol-3-rutinoside (II), kaempferol-3-neohesperidoside (III) and kaempferol-3-o-rhamnosyl glucoside (IV) named as Clitorin. Nucleoprotein, delphinidin-3,3,5-triglucoside, essential amino acids, anthoxanthin glucoside, p-hydroxycinnamic acid polypeptide, 6% ash, and a poisonous alkaloid are all found in seeds. According Yoganarasimhan seeds contain g-sitosterol, b-sitosterol, hexacosanol & anthocyanin glucoside.

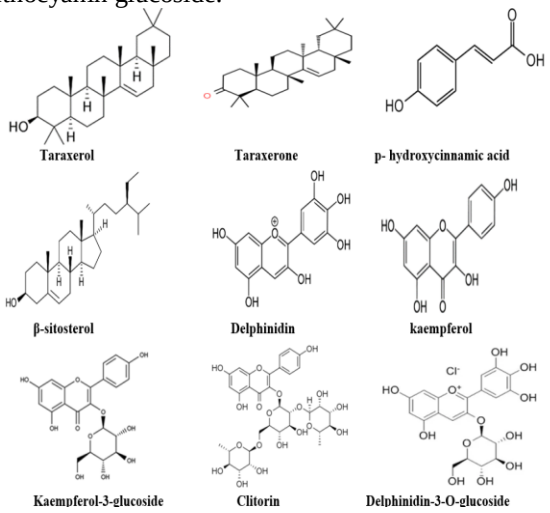

Fig 12: Chemical constituents of *Clitoria ternatea*

PHYTOCHEMICAL STUDIES [16]

The whole plant or organism serves as an active laboratory for the production of natural products from primary metabolites. Primary metabolites are the products of vital metabolic pathways such as respiratory chain, TCA cycle etc. Secondary metabolites are varieties of simple to sophisticated bizarre molecules. They are fascinating chemical molecules, very useful and of great importance in nature, as well as highly diversified in structures, properties, uses, chemistry etc.

Extraction

The process of separating active principles from powdered crude drugs by using suitable solvents is called extraction. The choice of solvents depends upon the characteristics of the secondary metabolites like polarity, pH, and thermal stability. Successive solvent extraction is suitable to extract the constituents of different polarity. It involves the extraction of the same plant material with solvents of different polarity ranging from non-polar to polar.

Successive Soxhlet Extraction

Soxhlet extraction is also known as hot continuous percolation. Here the plant material is continuously flushed with fresh solvent which is obtained by evaporation and subsequent condensation of the solvent containing extracted materials. In successive soxhlation the plant material is extracted with solvents of increasing polarity and finally macerated with chloroform water.

Soxhlet Apparatus

A Soxhlet extraction is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material.

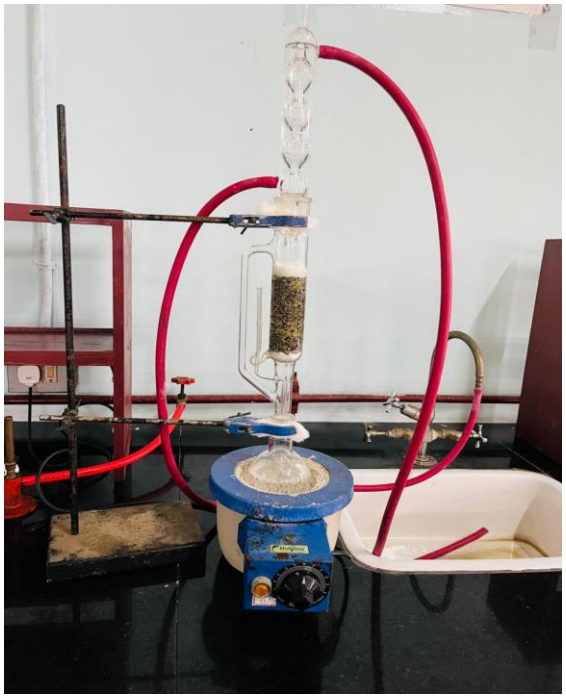

Fig 13: Soxhlet Apparatus

Operation

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly

fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically means of a rotary evaporator. the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded.

EXTRACTION:

S.No	Extract	Colour	Nature	Percentage yield
1.	Ethanol	Green	Semisolid	92%

PRELIMINARY PHYTOCHEMICAL SCREENING[16,17,18,19,20,21,22]

Preliminary phytochemical screening was done to identify different constituents like carbohydrates, proteins, lipids, flavonoid, tannins, glycosides, alkaloids, essential oils etc. Extracts of ***Clitoria ternatea*** **Linn** leaves were subjected to preliminary phytochemical screening.

1. **Detection of alkaloids:**
   a. **Mayer's test:** 2ml of the extract was treated with 2ml of Mayer's reagent.
   b. **Dragendorff's test:** 2ml of the extract was treated with 2ml of Dragendorff's reagent.
   c. **Hager's test:** 2ml of the extract was treated with 1-2ml of Hager's reagent.
   d. **Wagner's reagent:** 2ml of the filtrate was treated with 1-2ml of Wagner's reagent.
   e. **Tannic acid test:** 2ml extract was treated with 2ml of Tannic acid solution.
2. **Detection of carbohydrates:**
   a. **Molisch's test:** 1ml of the test solution was mixed with 2ml Molisch reagent, shaken the mixture and added 1ml of concentrated sulphuric acid along the sides of the test tube.
   b. **Benedict's test:** Mixed 2ml of the Benedict's reagent with 2ml of the test solution. Boiled in a water bath.
   c. **Fehling's test:** Boiled 1ml of the test solution with 1ml Fehling's solution A and 1ml of Fehling's solution B on a water bath.
   d. **Barfoed's test:** Mixed 2ml of Barfoed's reagent with 1ml of the test solution and boiled in a water bath.
   e. **Iodine test:** Mixed 0.5ml of Iodine solution with 1ml of test solution.
   f. **Seliwanoff's test:** Boil 2ml of Seliwanoff's reagent with 1ml of test solution.
3. **Detection of proteins and amino acids:**
   a. **Biuret test:** About 2ml of the extract was mixed with 2ml of Biuret reagent.
   b. **Millon's test:** 2ml of the extract was mixed with 2ml Millon's reagent and boiled.
   c. **Xanthoprotein test:** 2ml of the extract was treated with 1ml of conc. Nitric acid and Sulphuric acid. Cooled the solution and made alkaline with 10% NaOH.
   d. **Ninhydrin test:** Boiled 2ml of the extract with 1ml of 5% ninhydrin solution in a water bath for 5 minutes.
4. **Detection of glycosides:**
   a. **Borntrager's test:** To a little quantity of sample solution added Sulphuric acid and carbon tetrachloride. Separated the organic layer and shaken with dilute ammonia.
   b. **Modified Borntrager's test (modified anthraquinone test for C-glycosides):** To little quantity of sample solution added ferric chloride solution, hydrochloric acid and carbon tetrachloride. Separated the organic layer and shaken with dilute ammonia.
   c. **Legal test:** Mixed 1ml of the test solution with 2ml of Pyridine and sodium nitroprusside.
   d. **Baljet test:** Mixed 2-3g of sample in 2ml sodium picrate solution.
5. **Detection of tannins and phenolic compounds**
   a. **Ferric chloride test:** Mixed 2ml of the test solution with few ml of 5% ferric chloride solution.
   b. Lead acetate test: Mixed 2ml test solution with 1ml of lead acetate solution.
   c. Dilute iodine test: Mixed 2ml of the test solution with dilute iodine solution.
   d. Potassium dichromate test: Mixed 2ml of the test solution with potassium dichromate solution.
   e. Dilute Nitric acid test: Mixed 2ml of the test solution with dilute nitric acid.
   f. Bromine water test: Mixed 2ml of the test solution with bromine water.
6. **Detection of flavonoids:**
   a. **Shinoda test:** To 2 ml of the sample solution added magnesium powder or zinc powder and few drops of concentrated hydrochloric acid or sulphuric acid.

b. **Sulphuric acid test:** Added few drops of concentrated sulphuric acid to few ml of sample solution.
c. **Lead acetate test:** Mixed 2 ml of the test solution with lead acetate solution.
d. **Alkali test:** Treated the test solution with increasing amount of sodium hydroxide.

7. **Detection of fixed oils and fats:**
   a. Filter paper test: Pressed the powder between filter paper
8. **Detection of steroids and triterpenoids:**
   a. **Libermann test:** Mixed 2 ml of test solution with 2 ml of acetic anhydride and boiled. Then added 0.5 ml of concentrated sulphuric acid.
   b. **Libermann-Buchard test:** Mixed 2 ml of the test extract with 1 ml of Chloroform and 1 ml acetic anhydride. Then added 1 drop of concentrated sulphuric acid.
   c. **Salkowski test:** Dissolved 1-2 mg of sample in 1 ml of chloroform and added 1 ml of concentrated sulphuric acid.
9. **Detection of saponins:**
   a. **Foam test:** Shaken few mg of extract with 20 ml of distilled water.
10. **Detection of mucilage:**
    a. **Ruthenium red test:** Treated the powder with ruthenium red.
    b. **Swelling test:** Dissolved the powder in water.
11. **Detection of anthocyanins**
    a. **HCl test :** 2 ml plant extract with 2ml 2 N HCl with addition of few ml ammonia
12. **Detection of terpenoids :**2ml chloroform + 5mL plant extract, (evaporated on water bath) + 3mL conc. H2SO4 (boiled on water bath)

SI.no	Qualitative tests	Result
1.	**Detection of alkaloids**	
	Mayer's test	+
	Dragendorff's test	+
	Hager's test	+
	Wagner's test	+
	Tannic acid test	+
2.	**Detection of carbohydrates**	
	Molisch's test	+
	Benedict's test	+
	Fehling's test	+
	Barfoed's test	+
	Iodine test	–
	Seliwanoff's test	–
3.	**Detection of proteins and amino acids**	
	Biuret test	–
	Millon's test	+
	Xanthoprotein test	+
	Ninhydrin test	+
4.	**Detection of glycosides**	
	Borntrager's test	–
	Modified Borntrager's test	–
	Legal test	+
	Baljet test	+
5.	**Detection of tannins and phenolic compounds**	
	Ferric chloride test	+

	Lead acetate test	+
	Dilute iodine test	–
	Potassium dichromate test	–
	Dilute Nitric acid test	–
	Bromine water test	–
6.	**Detection of flavonoids**	
	Shinoda test	–
	Sulphuric acid test	–
	Lead acetate test	+
	Alkali test	+
7.	**Detection of fixed oils and fats**	
	Filter paper test	–
8.	**Detection of steroids and triterpenoids**	
	Libermann test	+
	Libermann-Buchard test	+
	Salkowski test	+
9.	**Detection of saponins**	
	Foam test	+
10.	**Detection of mucilage**	
	Ruthenium red test	–
	Swelling test	–
11.	**Detection of anthocyanins**	
	Hcl test	+
12.	**Detection of terpenoids**	+

Traditional uses of *Clitoria ternatea*[7,8,11]

Clitoria ternatea is known to as a very bioactive plant and used in various diseases as folklore medicines. All part of the plant is used as medicine. The roots being used as diuretic and seeds as cathartic. Seeds are mildly laxative, purgative and antihelmintic. Root was used in the treatment of ascetics, enlargement of the abdominal viscera, sore throat and skin diseases. They were also administered with honey and ghee as a general tonic to children for improving mental faculties, muscular strength and complexion tonics. Seeds and leaves were widely used as a brain tonic and to promote memory and intelligence. Juice and flowers were used as an antidote for snake bite. Seeds were used in the swollen joints, crushed seeds are taken with cold or boiled water for urinary problems. Root ash is used for facial care. Root powder is used for jaundice. Root juice applied in the nose for migraine. In Ayurvedic medicine, it has been used as a memory enhancer, antistress, anti-inflammatory, anti-cancer, anxiolytic, anti-depressant, anticonvulsant, tranquilizing and sedative agents. It is also used in neurological disorders.

CONCLUSION

Clitoria ternatea, known as Butterfly pea, belongs to the family **Fabaceae**, holds immense medicinal potential and has been utilized since ancient times for various therapeutic purposes. Powder microscopy of the flower shows pitted vessel cells, elongated rectangular epidermal cells with thin walls, fibre, and two types of trichomes: Simple unicellular trichomes and Capitate glandular trichome with an apical tip. The preliminary phytochemical test on ethanolic extract indicates the presence of alkaloids, glycosides,tannins, proteins and amino acids, flavanoids, saponins, carbohydrates, phenols, lignins, anthocyanins and terpenoids.. Its various extracts possess pharmacological activities such as, anxiolytic, anti-convulsant, sedative, anti-pyretic, anti-inflammatory, anti-diabetic, anti-oxidative, anti-stress, immunomodulatory, larvicidal, proteolytic,

antihelmintic, diuretic, anti-microbial and memory enhancing.. Additionally, its antioxidant, neuroprotective, and anthelmintic activities have been well-documented.

Concluding remarks would likely suggest avenues for future research. This may include further exploration of its mechanisms of action, clinical trials to validate traditional uses, phytochemical analysis for identifying active compounds, and cultivation techniques for sustainable harvesting.

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