

**Anxiolytic Activity of Extract of Flowers of *Clitoria Ternatea* Linn****Prashant Kumar Dhakad<sup>1</sup>, Rajveer Singh<sup>2</sup>, Siddhi Kumari Yadav<sup>3</sup>, Priya Singh<sup>4</sup>**<sup>1</sup>Assistant Professor, School of Pharmaceutical Science, Jaipur National University, Jaipur, India<sup>2</sup>Assistant Professor, School of Pharmaceutical Science, Jaipur National University, Jaipur, India<sup>3</sup>M. Pharm (Pharmacology), School of Pharmaceutical Science, Jaipur National University, Jaipur, India<sup>4</sup>M. Pharm (Pharmacology), School of Pharmaceutical Science, Jaipur National University, Jaipur, India

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**Abstract**

*Clitoria ternatea* (CT) or commonly known as butterfly pea originates from the Fabaceae family. Parts of the plant such as its leaves, flowers, and roots are believed to possess sought-after medicinal values such as analgesic, antipyretic and anti-inflammatory properties. The plant also possesses a number of advantages such as antioxidant, antidiabetic, antimicrobial, anthelmintic, hepaprotective, antiasthmatic and anxiolytic properties that are beneficial and useful in reducing health disorders. This plant has a long use in traditional Ayurvedic medicine for several diseases and the scientific studies have reconfirmed those with modern relevance. It has high calcium concentration, which is a significant source of calcium brewed as an herbal drink.

**Keywords:** *Clitoria Ternatea*; Aparajita; Butterfly Pea, Anxiolytic Activity, Anxiety.

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**Introduction:**

*Clitoria ternatea* (butterfly pea) is a perennial and herbaceous plant species from the Fabaceae family. It has for a long time been used as a forage and cover crop and as part of traditional medicine[5]. Recently, *C. ternatea* has drawn immense attention because of its potential applications both in modern agriculture and medicine. Plants as a source of medicine, has been used throughout history for treatment of mood disorders (anxiety,

depression, sleeplessness, and physiologically related conditions). Today there are over 10 medicinal plants that are used commercially as regulated Natural Health Products (NHPs), or EU phytomedicines to treat mood disorders related to anxiety. These medicinal plants have been well studied pharmacologically and phytochemically and there is extensive animal behavior and clinical research supporting their efficacy[1,2].

*Clitoria ternatea* is well known tropical perennial climber herb with slender downy stem, found throughout the tropical region of India, growing wild and also in gardens, bearing white or blue flowers[2]. It is commonly known as Aparajita and Koyal in Hindi and Butterfly pea in English. The extracts of CT have been used as an ingredient in "Medhya Rasayana" a rejuvenating herbal formulation for treatment of various neurological disorders and to strengthen intellectual ability. Various traditional uses of CT are shown. The root part of CT has been used for its laxative, purgative, diuretic, inflammation, indigestion, constipation, fever, arthritis, eye ailments, sore throat and anthelmintic. Kirtikar and Basu also reported the usefulness of CT for treatment of severe bronchitis, asthma and fever. CT is also being used by the local tribes to cause abortion, to cure abdominal swelling, sore throat, mucous disorder and fever. The root juice of CT is given with cold milk to reduce phlegm in chronic bronchitis[8,9].

### Phytochemical Constituents[13,15]

The major phyto-constituents found in the plant are the pentacyclic triterpenoids such as taraxerol and taraxerone Ethanol extract of *Clitoria ternatea* shows presence of terpenoid, flavonoid, tannin and steroid which may act as antioxidant. The major phytoconstituents found in *Clitoria ternatea* are the pentacyclic triterpenoids such as taraxerol and taraxerone. Phytochemical screening of the roots shows the presence of ternatins, alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, resins, starch, taraxerol and taraxerone.

### Traditional Uses[1,9]

*Clitoria ternatea* is known to as a very bioactive plant and used in various diseases as folklore medicine. The roots are being used as diuretic and seeds as cathartic. In the traditional system of medicine particularly in Ayurveda, the roots, seeds and leaves of CT

have long been widely used as a brain tonic and is believed to promote memory and intelligence. Juice of leaves is mixed with salt and applied around ears in headache and swelling of adjacent gland to relieve pain. Juice is used as an anti-dote against snake bite. *Clitoria ternatea* seed powder mixed with pepper was given in constipation. Leaves are used as poultices for swollen joints. Seeds are used as mildly laxative, purgative and anthelmintic. For hiccups, the seeds are burned for fume inhalation, also for asthma. Also used for throat, eye infections, skin diseases. Root ash is used for facial care. Root powder is used for jaundice. Root juice applied in the nose for migraine. For painful boils mix the root juice with vinegar and apply to the boils.

### Pharmacological Profile

- 1. Anxiolytic activity:** Oral treatment with alcoholic extract of *Clitoria ternatea* at a dose of 460 mg/kg significantly prolonged the time taken to traverse the maze as produced by chlorpromazine in rat demonstrated significant effect on anxiety. The animals treated with *Clitoria ternatea* (100mg/kg) showed a significant increase in the inflexion ratio and discrimination index which provides evidence for the species nootropic activity[4,10].
- 2. Anti inflammatory and analgesic activity:** the anti inflammatory, analgesic studies of petroleum ether extract (60-80c) from the flowers of *Clitoria ternatea* showed that it exhibited significant antiinflammatory activity at both the dose level (200 and 400 mg/kg body weight) ( $P < 0.01$ )[6,12]. The methanol extract of *Clitoria ternatea* showed a significant antipyretic activity. *Clitoria ternatea* roots methanol extract when given by oral route to rats is found to inhibit both the rat paw oedema caused by carrageenin and vascular permeability induced by acetic acid in rats.

3. **Anti-microbial activities:** The methanolic extracts of the leaves and root of *Clitoria ternatea* are tested for their antibacterial activity against different pathogenic drug resistant Gram-positive and Gram-negative clinical isolates[12]. The leaf is found to possess powerful antibacterial activity against *Escherichia coli* and *Vibrio cholera*, known for causing dysentery, and *Staphylococcus aureus*, causative agent of fever. The leaf extract showed stronger antibacterial activity than root extract. Both extracts are shown to be bactericidal in their mode of action.
4. **Antipyretic effect:** The ethanolic extract of aerial parts of CT at the doses of 230 mg/kg and 460 mg/kg, p.o. caused marked reduction in normal body temperature as recorded between 30 to 120 minute which was almost similar to chlorpromazine. In another study, methanolic extract of CT root showed significant antipyretic effect in yeast induced fever. The ethanolic and acetone extracts of CT leaves at the dose of 400 mg/kg also showed the antipyretic effect in rats.
5. **Diuretic activity:** The powdered form of dried whole root and ethanol extract are evaluated for diuretic activity and only single I.V. dose of extract produce moderate increase in urinary excretion of Na, K and decrease in Cl but no change in urine volume. Also, an appreciable effect is seen on oral dosing.
6. **Antioxidant:** Extracts of *Clitoria ternatea* (butterfly pea) flowers are used in Thailand as a component of cosmetics and the chemical composition of the flowers. It is also suggested that they may have antioxidant activity[6,7]. The aqueous extracts of *Clitoria ternatea* were shown to have stronger antioxidant activity than ethanol extracts.

7. **Effect on miscellaneous diseases:** It has also been reported that the extract from the white-flowered plant can cure goiter. The juice of the root of white flowered variety is blown up the nostrils as a remedy for hemicranias.

#### **Anxiolytic activity of extract of flowers of *Clitoria ternatealin***

##### **Sample collection**

For sample collection, the plant has to be collected from the nursery first.

##### **Extraction**

Soxhlet extraction is the process of transferring the partially soluble components of a solid of the liquid phase using a Soxhlet extractor. The solid is placed in a filter paper thimble which is then placed into the main chamber of the Soxhlet extractor. This method is used for the extraction of flowers. Ethanol is used as the solvent. Ethanol is taken in the quantity of 500ml. The process of extraction is done by reflux condensation method using Soxhlet apparatus at less than 70 degree for 16 hours. Then the extract is obtained from the syphon tube after which the extract is kept in china dish and evaporated until a dry product is obtained. The extract is obtained.

##### **Phytochemical estimation of extract**

**Phytochemical screening:** Phytochemical testing is done for the promising extract of all the four types of extracts as it has shown the interesting activity.

- 1) Braemer's test for Tannins.
- 2) Liebermann-burchardt test for Steroids.
- 3) Liebermann-burchardt test and Salkowski test for Terpinoids.
- 4) Dragendroff's reagent test for Alkaloids.
- 5) Shinoda test for Flavanoids
- 6) KOH test for Anthraquinones
- 7) Keller-Kiliani test for Cardiac glycosides
- 8) Frothing test for Saponins

##### **Test for Tannin- Braemer's test:**

Add 2 ml of water to 1 ml of extract, boil and then filter. Add few drops of 5% ferric chloride to the filtrate. A dark green, blue or brown color indicated the presence of tannin.

#### **Test for Steroids- Liebermann-Burchard test:**

Extract (1ml) is treated with chloroform, acetic anhydride and 2 drops of H<sub>2</sub>SO<sub>4</sub> is added and observed for the formation of dark green colour.

#### **Test for Sterols:**

##### **Liebermann-Burchard test:**

Extract (1ml) is treated with chloroform, acetic anhydride and 2 drops of H<sub>2</sub>SO<sub>4</sub> is added and observed for the formation of dark pink or red colour.

##### **Dragendorff's reagent:**

Is a color reagent to detect alkaloids in a test sample. Alkaloids, if present in the solution of sample, will react with Dragendorff's reagent and produce an orange or orange red colour.

##### **Test for Terpenoids:**

Salkowski test give a positive result and confirms the presence of Terpenoids.

##### **Salkowski test:**

The extract is mixed with 2ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> (3ml) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive result for the presence of terpenoids.

##### **Test for alkaloids:**

Few quantities is stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml is taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent are added; occurrence of orange-red precipitate is taken as positive. To the second 1 ml, Mayer's reagent is added and appearance of buff-coloured precipitate

will be an indication for the presence of alkaloids.

##### **Shinoda's test for flavonoids:**

About 0.5 of each portion is dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips is then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

##### **Keller-killiani Test for Cardiac glycosides:**

Have an 80% alcohol extract equivalent to ten grams of plant material. Evaporate until dryness over a water bath. Defat extraction by trituration with hexane to remove as much of the color pigments as possible. Discard the hexane solution. Warm the defatted residue over a water bath to remove the residual hexane solvent. Add three ml of FeCl<sub>3</sub>. Stir to mix well and transfer to a test tube. Hold the test tube in a slant position, and carefully add 1 ml of conc. Sulfuric Acid that allows the acid to roll inside the walls of the test tube. Allow the mixture to stand for a while. Determine any change of color in the junction (+) result is presence of reddish brown color which may gradually become bluish or purplish color indicate the presence of two deoxy sugars.

##### **Test for Saponins:**

Foam test- 1ml solution of extract is diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins. 1ml extract is treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins.

##### **Anxiolytic activity**

Anxiolytic activity is investigated using well established model, i.e., elevated plus maze (EPM) model. To check the anxiolytic activity of *Clitoria ternatea*, the experimental animals are divided into 3 groups of control, standard and test. The control group animals receive

only vehicle (2 % Tween 80, p.o.); the standard group animals receive diazepam as standard drug (2 mg/kg, p.o.) for comparison and test group animals receive the test substances (*Clitoria ternatea*) (50, 100 and 200 mg/kg, p.o.). The results are expressed as mean $\pm$ SD. The inter group variation is measured by one way analysis of variance (ANOVA) followed by Tukey's test.

The EPM model is commonly used in investigations because it is cost effective, easy to operate, less time consuming, and require no preliminary training to the mice and do not cause much discomfort to the animals while handling. The model is principally based on the phobia due to height.

### Conclusion

*Clitoria ternatea* is not only a wild herb but is also a medicinal plant. It has so many traditional usages as well a number of medicinal usages. Even, it is useful in treatment of some incurable diseases such as cancer, neurological disorder, nephrological disorder, hyperglycemia, urinary disorder, goiter, respiratory disorders etc. Its anxiolytic activity is reported prominently in various studies and may prove to be beneficial in management of anxiety.

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