

**PHYTOCHEMICAL, ANTIMICROBIAL ANALYSIS AND ANTIOXIDANT ACTIVITY
OF CLITORIA TERNATEA LEAVES (APRAJITA)**Tisha Agarwal¹, Yuvraj Singh Gaurav², Amit Kumar³, Srishti Priya⁴, D. Venkat Vijaya^{*5}^{1,2,3,4}Student, Netaji Subhas University, Department of Pharmacy, Pokhari, Jamshedpur, Jharkhand, 831012.^{*5}Assistant Professor, Netaji Subhas University, Department of Pharmacy, Pokhari, Jamshedpur, Jharkhand, 831012.***Corresponding Author: D. Venkat Vijaya**Assistant Professor, Netaji Subhas University, Department of Pharmacy, Pokhari, Jamshedpur, Jharkhand, 831012. DOI: <https://doi.org/10.5281/zenodo.21701655>**How to cite this Article:** Tisha Agarwal¹, Yuvraj Singh Gaurav², Amit Kumar³, Srishti Priya⁴, D. Venkat Vijaya^{*5}. (2026). Phytochemical, Antimicrobial Analysis and Antioxidant Activity of Clitoria Ternatea Leaves (Aprajita). European Journal of Pharmaceutical and Medical Research, 13(8), 218–226.

This work is licensed under Creative Commons Attribution 4.0 International license.



Article Received on 20/06/2026

Article Revised on 10/07/2026

Article Published on 01/08/2026

ABSTRACT

This study is about a beautiful blue flowering plant called *Clitoria ternatea*, commonly known as 'Aprajita' in India and globally as the butterfly pea, blue pea, or Asian pigeonwings belonging to the family "Fabaceae". This plant has been used in traditional ayurvedic medicine as a memory enhancer, antistress, anticonvulsant and sedative agent. Its extracts possess a wide range of pharmacological activities including antimicrobial, antioxidant, antipyretic, anti-inflammatory and antidiabetic properties. This study aims to confirm the antimicrobial and antioxidant properties included in plant leaves with studies using phytochemical analysis. The phytochemical analysis of *C. ternatea* plants leaves, flowers and seeds contain powerful natural chemicals including alkaloids, flavonoids, tannins, saponins and phenols. These compounds had strong antimicrobial action against common disease that caused by pathogens and infectious agents. *Clitoria ternatea* is not just a beautiful flower-it is a scientifically promising medicinal plant with real potential to contribute to future healthcare, especially in the field of infection treatment and oxidative stress related diseases.

KEYWORDS: *Clitoria ternatea*, antimicrobial, antioxidant, Ayurveda, phytochemicals, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* *Streptococcus*.

INTRODUCTION

Herbal medicine's renewed interest has prompted a surge in scientific research worldwide. Investigation into traditional plant species. Among them is the perennial *Clitoria ternatea*. The Fabaceae family, which includes the herbaceous plants, is distinguished by its unique characteristics. Extensive ethnopharmacological history.^[1] Commonly referred to as. Known as the "Butterfly Pea," Shankhpushpi, or Asian Pigeonwings, the plant originates from tropical gardens. In Asia, it has a pan-tropical distribution, with thriving populations in Africa, America and Australia.^[2] While the A striking and vivid blue flower is renowned and extensively employed as a natural food colorant and antioxidant.^[3] Furthermore, the plant components, particularly the leaves, exhibit an exclusive and robust quality. Profile of phytochemicals that need thorough scientific analysis. Taxonomically, *C. ternatea* is identified by its twining habit, pinnate leaves and deep blue coloration. (Or white axillary flowers. The plant is a component of traditional

medicine in Ayurveda and other systems .Referred to as the Medhya Rasayana', it is a rejuvenating energy of intellect and memory.^[4] The roots, seeds, In the past, leaves have been used to treat various ailments, including many different types of illnesses. From indigestion and constipation to skin diseases and eye infections, there are also other issues. Recent. The use of pharmacological studies has reinforced numerous conventional. assertions. Anti-inflammatory, anticonvulsant, anxiolytic, and anti-depressant activities. The root's potential as a medicinal plant is more extensively researched than the leaf". and flower. Clitoides, which are bioavailable cyclic peptides found in the leaves, along with certain glycosides of kaempferol and quercetin, which may have a significant impact on blood pressure regulation.^[5] Antimicrobial properties. The emergence of multidrug-resistant (MDR) bacterial strains is a noteworthy phenomenon. New antimicrobial agents must be discovered to address the global health crisis.^[6] Plant-derived. Multi-target mechanisms of action and intricate structures make

phytochemicals a valuable class of compounds. A potential avenue for the development of drug.



Figure 1: *Clitoria parviflora* flower and foliage.^[7]

Methods of Extraction

A). Cold Maceration

The simple process of maceration involves cold extraction from the plant material, followed by a prolonged soak in the materials. Solvent at ambient temperature. Adequate for thermolabile elements and as a starting point for extraction. To eliminate pigments before using them under hot water.^[8,9]

Protocol

1. In a wide-mouth glass conical bottle, weigh 50g of leaf powder.
2. 500mL of ethanol (95%) is mixed with approximately 500 milliliters of menstrual fluid.
3. Use a cotton plug to seal the flask and leave it stand at 25°C with some time. Shaking for 72 hours.
4. Sort the meat by using Whatman No. 1. Filter paper; collect the filtrate.
5. Macerate the mixture with a new solvent twice to achieve complete extraction.
6. All the filtrates are mixed and then pumped into the 40°C rotary evaporator.
7. Store the semi-solid extract in an airtight container at 4°C.

Protocol

1. Measure 23gm of coarsely powdered leaf material and blend it with a pre-cut herbicide. Cellulose thimble.



Figure 2: weighing of CT powder.

2. Place 150 mL of petroleum ether (60-80°C) in the distillation flask and use as the solvent.

B). Aqueous Extraction (Decoction/Infusion)

Aqueous extraction imitates the traditional preparation methods (kadha /decoction) used in ritual. Ayurveda. Glycosides and tannins, among other polar compounds, are highly effective solvents in water. Mucilage and some alkaloids.^[10]

Protocol

1. For 30 minutes, dissolve 50g of leaf powder in 500 mL of distilled water (decoction). Set aside.
2. To cool down, pass through muslin cloth and then fill the freezer with Whatman No.
3. Dry aqueous extract powder can be obtained by freezing the filtrate or using lyophilization. Stay attentive while taking a water bath at 60°C.
4. Containers kept at -20°C should be airtight to prevent Contamination by bacteria.

C). Soxhlet Extraction (Hot Continuous Extraction)

The process of Soxhlet extraction involves continuous refluxing of solvents. The complete extraction of bioactive compounds is achieved through the drug bed.^[11,12] It's particularly. Efficient for sparingly soluble constituents.^[13] Equipment: Soxhlet machine (extraction flask, thimble, Condenser, heating mantle)



Figure 3: Measuring of Petroleum ether.

3. Install the Soxhlet apparatus and heat until its temperature is appropriate; cook for 18-24 hours. Hours (approximately 15-20 siphon cycles).



Figure 4: Extraction of clitoria ternatea.

4. Following that, extract the material and browse through Whatman No.1.5. Using the 40°C Rotary vacuum evaporator, extract solvent and concentrate it until it is dry.



Figure 5: Clitoria ternatea extract.

6. The process is repeated with chloroform, ethanol (95%), and Water.

PHYTOCHEMICAL ANALYTICAL METHODS QUANTITATIVE ANALYSIS

Spectrophotometric methods such as the Folin-Ciocalteu method were used to determine TPC. The extract was Oxidized with the reagent of Folin-Ciocalteu and neutralized by sodium carbonate. The absorbance of. At 765 nm, the blue complex that resulted was measured. The outcome was indicated as Gallic Acid .Equivalents (mg GAE/g extract).^[14]

Total Flavonoid Content (TFC)

The TFC was measured by the colorimetric method of Aluminum Chloride. The extract was mixed. When the absorbance of AlCl₃ solution was measured at 415 nm. Results were expressed as Quercetin Equivalents (mg

QE/g extract).^[15]

Chromatographic Analysis (HPLC)

High-Performance Liquid Chromatography (HPLC) was employed to achieve the desired results. The detection and quantification of particular biological markers. On what date was the split arranged. A C18.Using the reverse-phase column and its gradient mobile phase, Acetonitrile and 0.1% Phosphoric Acid.^[16]

Qualitative Phytochemical Screening

The crude extracts obtained from different Solvents were subjected to standard chemical tests to detect the presence of various phytoconstituents.^[17]

TABLE – 1

Phytochemical	Test Name	Procedure	Observation
Tannins	Lead acetate test	Sample +10% lead acetate solution	A bulky white ppt forms (1- 2mins)
Steroid Test	Salkowski's Test	Dissolve the extract(sample) in chloroform + an equal volume of conc sulphuric acid.	A reddish brown or cherry red color.
Phenols Test	Ferric Chloride Test	Sample + few drops of 5% ferric chloride.	A bluish black or brownish green
Saponins	Foam Test	Extract + Water (Shake vigorously)	Persistent foam honeycomb
Flavonoids Test	Anthocyanin Test	Sample + few drop of 2M sodium hydroxide/ammonia.	Changes towards blue, green or yellowish
Sulphur Powder Test	Powder Test	Test solution +small amt. of Sulphur powder.	Sulphur sinks at the bottom.

STUDY OF ANTIMICROBIAL EFFECT

Principle

Plant extracts are effective antimicrobial agents because they disrupt the activity of bacterial cells. Disrupting enzyme production, walls, or DNA replication are all forms of damage. Phenolics and tannins bind to Terpenoids can interfere with the lipid bilayer of cell membranes, while adhesin molecules and transport proteins are involved.

Experimentation

Test Organisms

STAPH-positive bacteria include *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633). The bacteria *Pseudomonas aeruginosa* (ATCC 27853) is classified as gram-negative *Escherichia coli* (ATCC25922).

• Fungi: *Candida albicans* (ATCC10231).

METHODS OF ANTIMICROBIAL ANALYSIS

Agar Well Diffusion Method Principle

Antimicrobial compounds are diffused from the well into the agar of the plant extract. Medium containing microorganisms.

If the extract inhibits microbial growth, a zone known as "clear space" will occur. Around the well lies the Zone of Inhibition. The magnitude of antimicrobial activity is indicated by its size in this zone.^[18]

PROCEDURE

i). Preparation of Culture Media

Autoclaving is employed to purify the preparation of Nutrient agar. The medium is poured into sterile petri dishes and allowed to solidify.

ii). Inoculation of Microorganisms

To evenly distribute test microorganisms (such as bacteria or fungi) on the appropriate-sized agar plate, place them in an airtight container. Swab.

iii). Formation of Wells

iv). Addition of Extract

Wells are filled with different concentrations of *Clitoria ternatea* leaf extract.

v). Controls

Ampicillin is a standard antibiotic that can be used as 'positive control'. Negative control: Solvent used for extraction.

vi). Incubation

The plates are incubated at 37°C for 24 hours to prepare the bacterial cultures.

vii). Observation

The zone of inhibition around the well is measured in millimeters through the use of a ruler or similar device Caliper.

Minimum Inhibitory Concentration (MIC)

Principle

MIC is the minimum concentration of plant extract that can inhibit visible microorganism growth. Identifies the effectiveness of antimicrobial compounds.'

Procedure

i). The preparation involves placing a sample in nutrient broth.

Each tube contains varying amounts of *Clitoria ternatea* extract.

ii). In each tube, a small amount of microbial culture is put in.

iii). The process of incubating tubes at 37°C takes 24 hours.

Turbidity (cloudiness) is observed in the tubes.^[19]

Calculation

The antimicrobial activity of *Clitoria ternatea* can be determined by the plate shown:

Sample Zone of Inhibition (mm)

A 24 mm.

B 25 mm.

C 23 mm.

D 24 mm.

Interpretation

Maximum activity: Sample B (25 mm)

Moderate performance: A and D (24 mm) specimens.

Minimum activity: Sample C (23 mm) size

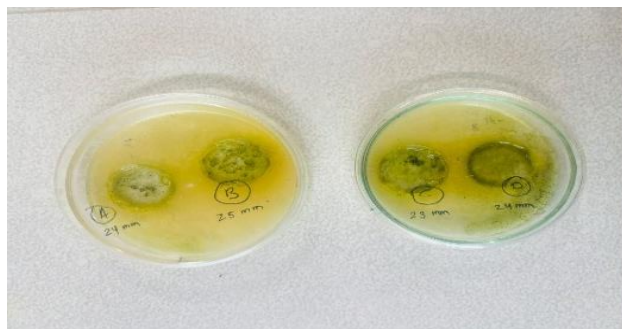


Figure 6: zones of inhibition.

a).24mm b).25mm c).23mm d).24mm

Broth Dilution Method

Principle

The concentration of plant extract needed to inhibit microbial growth is determined by this approach. Growth in a liquid medium. Unless there is a deficiency in nutrients, microorganisms can thrive in nutrient broth antimicrobial compounds.^[20]

Procedure

- Stack several test tubes with the intention of producing nutrient broth.
- Use different amounts of *Clitoria ternatea* leaf extract

in each tube.

iii). Infest each tube with a small quantity of microbes.

iv). Keep the tubes in a temperature-controlled incubator for 24 hours at 37°C.

v). Seek out a cloudiness that forms in the broth.'

Observation

Clear broth: Microbial growth inhibited.

Cloudy broth: Microbial growth present.

The Minimum Inhibitory, which is the concentration that inhibits growth at a minimum level.

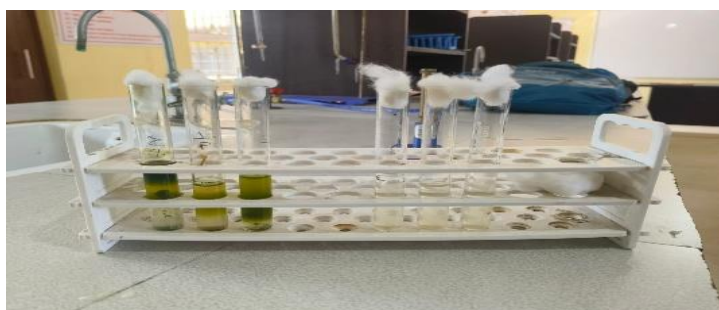


Figure 7: observation of bacterial growth.

Minimum Bactericidal Concentration

Principle

MBC determine the lowest concentration of plant extract that completely kills microorganism, not just inhibits them.

Procedure

- MIC test tubes are used to extract samples without any growth detected.
- Fresh nutrient plates are utilized to serve the samples.
- For 24 hours, plates are incubated at 37°C.
- The presence of bacteria is detected on the plate.

Interpretation

The absence of colonies is due to the bactericidal nature of extract.

There are colonies: Extraction only delayed the growth.

The MBC value is the smallest quantity that can eradicate bacteria.

Time-Kill Assay^[21]

Principle

This method gauges the duration at which the plant extract eliminates microorganisms.

Procedure

- Create a culture organism in the presence of nutritious broth.
- Alternatively, use plant extract at specific concentrations.
- Samples at varying intervals (0, 2, 4, 6, and 24 hours).
- Plating is done on nutrient-plated agar.
- Plates are incubated and colonies accumulated.

Result

The reduction of colonies is indicative of the effectiveness of antimicrobial activity.

Agar Dilution Method

Principle

By adding the antimicrobial extract to agar medium and cultivating microorganisms, we achieve sterilization. In relation to the extract, on the surface to ascertain their growth potential.

Procedure^[22]

- i). The addition of molten agar results in different amounts of plant extract.'
- ii) The agar is then placed into sterile plates and allowed to solidify.
- iii) The agar surface is observed to contain microorganisms.
- iv). Incubation of the plates takes place for one day at 37°C.

Observation

They report either growth or no growing.
Growth at that concentration lacks antimicrobial activity.



Figure 8: Presence of bacterial growth 23mm and Absence of bacterial 24mm spot in agar surface.

ANTIOXIDANT ACTIVITY

A significant contributor to oxidative stress is the overproduction of reactive oxygen species (ROS) and free radicals, Aging, cellular degradation, cancer and cardiovascular disease are all fundamental pathologies. Plant-Enhanced by hydrogen atom transfer (HAT) or single electron transport, these antioxidants are derived and have been shown to reduce oxidative damage. (SET) mechanisms.

Its properties are directly related to the fact that *C. ternatea* is one of the most potent antioxidant reservoirs. its Ternatin and flavonol concentrations.^[23]

MATERIAL REQUIRED**TABLE – 2**

Category	Items
Plant Material	Dried <i>Clitoria ternatea</i> flowers
Instruments	Absorption (Ultraviolet - visible) Spectrophotometer, Analytical balance, Centrifuge, Thermostatic Water bath, Vortex mixer
Chemicals	H ₂ O ₂ (30%), Phosphate buffer saline (PBS, pH 7.4), Ascorbic acid (standard), Ethanol (80%)
Glassware	Test tubes, Volumetric flasks, Cuvettes, Micropipettes

PROCEDURE

- i). Prepare phosphate buffer and adjust to (pH 7.4).
- ii). Prepare 40. The solution of Mm hydrogen peroxide in phosphate buffer (pH 7.4) is.
- iii). Leaf Extract stock solution can be prepared in 10ml by adding a small amount of water to the solution.
- iv). Calculate the concentration in 0.2 mg/ml, 0.4 mg/5 ml, 0.6 mg/3 ml and 0.8 mg/8 ml or 1 mg/15 ml. Set these values.?
- v). Control, Blank, Sample tubes and Standard tubes are all label test tubes.
- vi). Prepare Reaction Mixture.

• Control

Take one scoop of H₂O₂ and place it in a dish, fill it with 3.4 mL of phosphate buffer.
(No plant extract added)

• Sample

0.6mL H₂O₂. Solution and Extract solution prepared at

the concentration of 0.2 mg/ml.

Add the Phosphate Buffer to increase the volume.'

• Standard

0.6ml H₂O₂. Solution, Standard solution and Utilizing phosphate buffer increases the total volume.

• Blank

3.4. Wait 10 minutes at room temperature before incubating all test tubes.

vii). UV-Visible Analysis.

Switch on the UV-Visible spectrophotometer.
Allow the instrument to rest for 10-15 minutes before using.

Identify the desired wavelength and adjust it to approximately 200 nanometers (H₂O₂ displays an absorbance of about 110 microns).

Since it is not possible to measure the UV range, choose a quartz cuvette instead. Efficiently employing plastic or glass cuvettes for precise measurements.

• **Calibrate with blank**

Fill cuvette with blank solution.

Place in spectrophotometer.

Mark the blank/zero value as 0.000 to reduce absorbance. What's the significance?

• **Measure control absorbance**

Use a control solution to clean the cuvette.".

Use tissue to clean the area surrounding a cuvette.

Insert into instrument.

Record absorbance (A_0 1.673nm).

• **Measure sample absorbance**

Separate extracts from *Clitoria ternatea*.

The absorbance of a sample is recorded as follows: 0.2ml equals 0.561, 0.497 and 0.588., 1ml=0.978nm)

• **Measure standard absorbance**

Take standard (ascorbic acid) solution.

Measure absorbance and record values.

Wash cuvette to prevent contamination of the cuvette, wash it with distilled water in between samples.

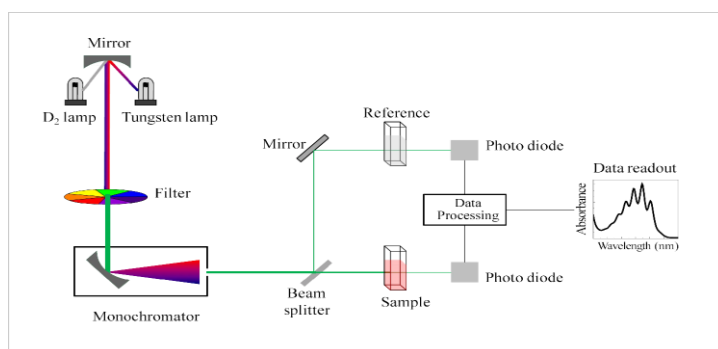


Figure 9: Schematic representation of a UV-Visible spectrophotometer.^[24]

CALCULATION

$$\text{Scavenging Activity} = \frac{\text{Absorbance control} - \text{Absorbance Sample}}{\text{Absorbance Control}}$$

Control = Absorbance of H₂O₂ Without Extract

Sample = Absorbance of H₂O₂ With Extract

$$\begin{aligned} &\text{For Sample 0.2ml} \\ &= \frac{1.673 - 0.563}{1.673} \times 100 \end{aligned}$$

$$= 66.35\%$$

$$\begin{aligned} &\text{For Sample 0.4ml} \\ &= \frac{1.673 - 0.679}{1.673} \times 100 \\ &= 59.41\% \end{aligned}$$

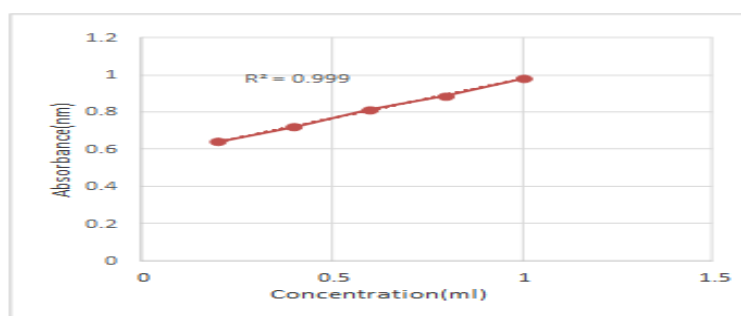
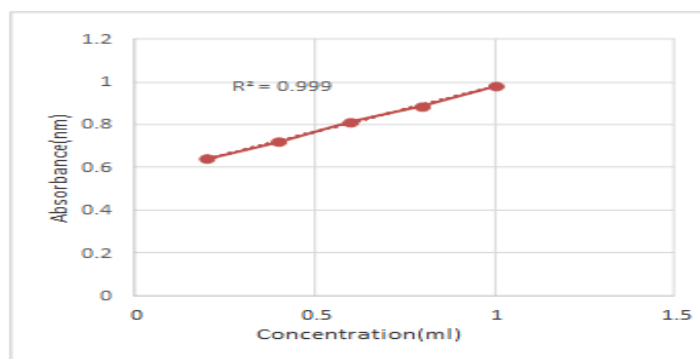
$$\begin{aligned} &\text{For Sample 0.8ml} \\ &= \frac{1.673 - 0.885}{1.673} \times 100 \\ &= 47.10\% \end{aligned}$$

$$\begin{aligned} &\text{For Sample 0.6ml} \\ &= \frac{1.673 - 0.711}{1.673} \times 100 \\ &= 57.50\% \end{aligned}$$

$$\begin{aligned}
 &\text{For Sample 1.0ml} \\
 &= \frac{1.673-0.978}{1.673} \times 100 \\
 &= 41.54\%
 \end{aligned}$$

OBSERVATION**TABLE - 3**

Concentration (mL)	Absorbance(nm)	%Scavenging
0.2	0.563	66.35
0.4	0.679	59.41
0.6	0.711	57.50
0.8	0.885	47.10
1	0.978	41.54
H2O2(Std)	1.673	

GRAPH**GRAPH 1: STANDARD CALIBRATION CURVE FOR HYDROGEN PEROXIDE (H2O2) SCAVENGING ASSAY.****Result of Hydrogen peroxide scavenging Assay.****RESULT**

The H₂O₂ scavenging activity of lowest concentration (0.2mg/ml) shows best results i.e. has higher antioxidant property and can be used for further method development.

CONCLUSION

The focus of the project was on examining the phytochemical components, antimicrobial activity and other relevant elements, Antioxidant potential of *Clitoria ternatea*.

The initial evaluation of phytochemical samples demonstrated the presence or absence thereof. Flavonoids, alkaloids and tannins as well as glycosides

(particularly glutarallic acids), saponins and phenolic compounds are all significant bioactive compounds.

Terpenoids and compounds may be involved in the medicinal properties of it.

We analyzed the antimicrobial potential of *Clitoria ternatea* extract by diffusion into agar and then administering broth dilution Methods. Select microorganisms were selectively targeted by the agar well diffusion assay and the extract was effective against them. The observation of zones of inhibition indicates its antimicrobial capabilities. Moreover, The broth dilution method further. MIC was used to confirm the effectiveness of antimicrobials, proving that the plant

extract can inhibit microbial growth at specific concentrations.

UV spectroscopy was employed to gauge antioxidant activity, which exposed large quantities of free radicals. Scavenging ability of the extract. Phenolic and other compounds are known to possess antioxidant properties. Deficiency of cells against cellular damage through flavonoid compound.

Conflict of interest statement The authors declare no conflict of interest.

Consent for publication Not applicable.

Funding None.

ACKNOWLEDGEMENT All the authors are thankful to the Principal and Head of the Department, Pharmacy, Netaji Subhas Institution of Pharmacy, Pokhari, Jamshedpur, 831012, for their encouragement of this work.

REFERENCE

1. Indian Medicinal Plants Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International Book Distributors; 2006.
2. The Wealth of India The Wealth of India. Raw Materials Series: *Clitoria ternatea*. New Delhi: CSIR; 2003.
3. Journal of Ethnopharmacology Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic medicine *Clitoria ternatea*—From traditional use to scientific assessment. *J Ethnopharmacol*, 2008; 120(3): 291–301.
4. Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic medicine *Clitoria ternatea*—From traditional use to scientific assessment. *J Ethnopharmacol*. 2008 Dec 8; 120(3): 291–301.
5. Nguyen GKT, Zhang S, Nguyen NT, Nguyen PQ, Chiu LL, Hardjohaju W, et al. Discovery and characterization of novel cyclotides from *Clitoria ternatea*. *J Biol Chem*. 2011 Jul 8; 286(27): 24275–87.
6. Anand SP, Jeyachandran R. Antibacterial activity of *Clitoria ternatea* L. a high value medicinal plant. *Int J Pharma Bio Sci*. 2011; 2(3): 147–53.
7. Vecteezy. *Clitoria ternatea* commonly known as butterfly pea flower [Internet]. Available from: <https://www.vecteezy.com/>
8. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 26th ed. Pune: Nirali Prakashan; 2023.
9. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998.
10. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2023.
11. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 26th ed. Pune: Nirali Prakashan; 2023.
12. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998.
13. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2023.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965; 16(3): 144–58.
15. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*. 2002; 10(3): 178–82.
16. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2011). Introduction to Modern Liquid Chromatography. John Wiley & Sons.
17. Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer Science & Business Media.
18. Balouiri M, Sadiki M, Ibensouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016; 6(2): 71–79. doi: 10.1016/j.jpha.2015.11.005.
19. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimum inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*. 2008; 3(2): 163–175
20. Balouiri M, Sadiki M, Ibensouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016; 6(2): 71–79.
21. Pankey GA, Sabath LD. Clinical relevance of bactericidal mechanisms of antimicrobial agents. *Clin Infect Dis*. 2004; 38(6): 864–870.
22. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimum inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*. 2008; 3(2): 163–175.
23. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 5th ed. Oxford: Oxford University Press; 2015.
24. ResearchGate: Schematic diagram of a double beam UV-Vis spectrophotometer (Attributed to Samira Hosseini et al., 2022)