RESEARCH PAPER

Phytochemical Analysis And Evaluation Of Antimicrobial Activity Of Ethanolic Flower Extract Of Clitoria Ternatea.

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ABSTRACT
The Clitoria ternatea plant, commonly known as Butterfly Pea, is a valuable source of bioactive compounds with numerous therapeutic benefits. This study aimed to investigate the phytochemical profile and antimicrobial potential of the ethanolic flower extract of Clitoria ternatea. The extraction process was conducted using ethanol, followed by phytochemical screening and analysis using Liquid Chromatography-Mass Spectrometry (LC-MS). The identified phytoconstituents include flavonoids, anthocyanins, alkaloids, and phenolic compounds, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. The antimicrobial activity was assessed against various bacterial and fungal strains using the cup plate method, demonstrating significant inhibition at concentrations of 15, 30, and 45 mg/ml. The study highlights the potential of Clitoria ternatea as a source of natural antimicrobial agents and its therapeutic relevance in treating infectious diseases. Further research is necessary to explore the specific compounds responsible for these effects and their mechanisms of action..

Keywords: Clitoria ternatea, Ethanolic Extract, Phytochemical Analysis, LC-MS, Antimicrobial Activity, Flavonoids, Anthocyanins, Alkaloids, Antioxidant Properties, Antibacterial, Fungal Inhibition, Medicinal Plants, Natural Products
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INTRODUCTION

Natural products, including those derived from plants, have played a crucial role in the development of pharmaceuticals for centuries. These products, often referred to as secondary metabolites, are non-essential for the survival of the organism but serve vital functions in plant defense and reproduction. Over the years, the use of plant-derived compounds has gained significant attention due to their therapeutic potentials, including antimicrobial, anti-inflammatory, and antioxidant properties. Medicinal plants, particularly those with a long history of use in traditional systems of medicine, continue to be a valuable source for discovering novel bioactive compounds that could lead to the development of new therapeutic agents.

Clitoria ternatea, commonly known as Butterfly Pea or Shankpushpi, belongs to the Fabaceae family and has been widely used in Ayurvedic and traditional medicine for its various therapeutic benefits. The plant has a rich history of use as a memory enhancer, anxiolytic, antidepressant, anticonvulsant, and sedative agent. It is also known for its neuroprotective, cardiovascular, and antimicrobial effects. *Clitoria ternatea* contains a wide range of bioactive compounds, including flavonoids, anthocyanins, alkaloids, saponins, and glycosides, which contribute to its diverse pharmacological activities.

Recent studies have focused on the chemical composition of *Clitoria ternatea* and its potential applications in modern medicine. Among its many uses, the antimicrobial activity of *Clitoria ternatea* has gained particular attention. Several studies have demonstrated its effectiveness against various bacterial and fungal pathogens, suggesting its potential as a natural alternative to synthetic antibiotics. The ethanolic extract of *Clitoria ternatea* flowers, in particular, has shown promising results in inhibiting the growth of both Gram-positive and Gram-negative bacteria, as well as fungi.

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Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful analytical technique that is widely used for the identification and quantification of bioactive compounds in plant extracts. This technique offers high sensitivity and precision, enabling researchers to profile complex mixtures of plant constituents. In this study, LC-MS was employed to identify the phytochemical constituents present in the ethanolic flower extract of *Clitoria ternatea*, and the antimicrobial activity was evaluated against selected bacterial and fungal strains.

The aim of this study is to conduct a comprehensive phytochemical analysis of the ethanolic flower extract of *Clitoria ternatea* using LC-MS and evaluate its antimicrobial potential. This will not only contribute to the understanding of its chemical composition but also provide insights into its therapeutic potential, especially in the context of combating microbial infections.

MATERIALS AND METHODS

Materials:

The materials used in this study included chemicals, reagents, microbial strains, and laboratory instruments. The chemicals used for extraction and analysis were ethanol (HPLC grade), acetone, formic acid (AR grade), and HPLC-grade water (Milli-Q or equivalent). Streptomycin was used as a positive control for antimicrobial assays. The microbial strains selected for the antimicrobial activity testing included *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), and the fungal strain *Candida albicans*.

Laboratory instruments used for this study included the Soxhlet extraction apparatus, nutrient agar medium for microbial culture, and a LC-MS system consisting of a Waters Alliance e-2695 HPLC system coupled with a SCIEX QTRAP 5500 mass spectrometer with an electrospray ionization interface. Other instruments such as Vernier calipers, a microscope, and a refrigerator set at 5°C were also utilized. The LC-MS conditions were set with a Waters X-Terra RP-18 column (150mm × 4.6mm, 3.5 µm), and the analysis was performed with the following mass spectrometer parameters: ion spray voltage of 5500 V, collision energy of 14 V, source temperature of 550°C, and drying gas temperature between 120–250°C.

Collection and Preparation of Plant Material:

The flowers of *Clitoria ternatea* were collected from the local area of Gudlavalleru, Andhra Pradesh. After collection, the flowers were shade-dried for 7 to 14 days to remove moisture. The dried flowers were then weighed (approximately 100 g) and ground into a fine powder using a mechanical grinder to facilitate the extraction process.

Fig No. 1: *Clitoria Ternatea* Flower

Extraction of Ethanolic Flower Extract:

The powdered *Clitoria ternatea* flowers were subjected to Soxhlet extraction using ethanol as the solvent. The extraction process was conducted over 72 hours to ensure thorough extraction of the bioactive compounds present in the flowers. Once the extraction was complete, the ethanol was removed under reduced pressure using a rotary evaporator, and the remaining concentrated extract was further dried on a water bath to obtain a solid residue.

Preliminary Phytochemical Screening:

The ethanolic flower extract of *Clitoria ternatea* was subjected to preliminary phytochemical screening using standard qualitative tests. These tests were conducted to identify the presence of various bioactive compounds, including carbohydrates, alkaloids, flavonoids, saponins, glycosides, tannins, phenolic compounds, and terpenoids. The presence of these compounds was determined by using the following tests:

Phytochemical Analysis by LC-MS:

The ethanolic flower extract was analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS) to identify

and profile its bioactive compounds. The LC-MS analysis was conducted using a Waters Alliance e-2695 HPLC system coupled with a SCIEX QTRAP 5500 mass spectrometer. The chromatographic conditions involved using a Waters X-Terra RP-18 column (150mm × 4.6mm, 3.5 µm), with acetonitrile and buffer (30:70 ratio) as the mobile phase. The flow rate was set to 1.0 mL/min, and the injection volume was 10 µL. The mass spectrometer was operated in positive electrospray ionization mode, with the following parameters: ion spray voltage at 5500 V, collision energy at 14 V, source temperature of 550°C, and drying gas temperature ranging from 120–250°C.

Antimicrobial Activity Testing:

The antimicrobial activity of the ethanolic flower extract of *Clitoria ternatea* was evaluated against selected bacterial strains and fungal strains using the cup plate method. The bacterial strains tested included *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, while *Candida albicans* was used as the fungal strain.

For the antimicrobial testing, bacterial suspensions were prepared by sub-culturing the strains overnight in nutrient agar medium at 35°C. The fungal strain was similarly cultured. The microbial suspensions were spread onto nutrient agar plates, and wells were created in the agar. Different concentrations (15, 30, and 45 mg/mL) of the ethanolic flower extract were introduced into the wells. Streptomycin (50 mg/mL) was used as a positive control. The plates were incubated at 35°C for 24 hours, after which the zones of inhibition were measured using Vernier calipers.

Determination of Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration (MIC) of the ethanolic flower extract was determined using the cup plate method. Serial dilutions of the extract were prepared to concentrations of 15, 30, and 45 mg/mL. The MIC was defined as the lowest concentration at which no visible microbial growth was observed after 24 hours of incubation at 35°C.

RESULTS AND DISCUSSION:

Extraction Yield and Phytochemical Screening:

The extraction of *Clitoria ternatea* flowers using ethanol as a solvent yielded a solid extract after evaporation of the solvent. The crude extract was subjected to preliminary phytochemical screening, which revealed the presence of several bioactive compounds. Carbohydrates, alkaloids, flavonoids, glycosides, phenolic compounds, and terpenoids were detected, while saponins, tannins, and proteins were found to be absent. The positive results for alkaloids, flavonoids, and glycosides suggest that *Clitoria ternatea* flowers possess compounds that are widely known for their therapeutic effects, including antioxidant, anti-inflammatory, and antimicrobial properties

The phytochemical composition of the extract aligns with previous studies, which have reported the presence of flavonoids, alkaloids, and glycosides in *Clitoria ternatea*, contributing to its wide range of pharmacological activities . These findings confirm that the ethanolic extract of *Clitoria ternatea* contains a rich diversity of bioactive compounds, which may contribute to its medicinal properties.

Table 1: Preliminary Phytochemical Screening of Ethanolic Flower Extract of *Clitoria ternatea*

S. No.	Phytoconstituent	Test(s) Conducted	Result
1	Carbohydrates	Molisch's test, Benedict's test	Positive
2	Alkaloids	Mayer’s test, Hager’s test	Positive
3	Flavonoids	Shinoda test, Lead acetate test	Positive
4	Saponins	Foam test	Negative
5	Glycosides	Specific reactions	Positive
6	Tannins	FeCl3 test	Negative
7	Phenolic Compounds	Potassium dichromate, Iodine solution	Positive
8	Terpenoids	Specific tests	Positive

Phytochemical Analysis by LC-MS:

The *Clitoria ternatea* ethanolic flower extract was subjected to LC-MS analysis to identify and profile its bioactive compounds. The LC-MS spectrum revealed several compounds with distinct mass-to-charge ratios (m/z). The major compounds identified include acetylated delphinidin 3-glucoside, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, cyanidin-3-O-glucoside, and delphinidin-3-O-glucoside. These compounds are known for their antioxidant, anti-inflammatory, and anticancer properties .

Among the identified compounds, anthocyanins like delphinidin and cyanidin, which are known for their strong antioxidant activity, were present in significant quantities. Additionally, flavonoid glycosides such as quercetin and

kaempferol have been reported to exhibit anti-inflammatory, anticancer, and cardiovascular protective effects . The identification of these compounds confirms the potential therapeutic relevance of *Clitoria ternatea* in various disease conditions.

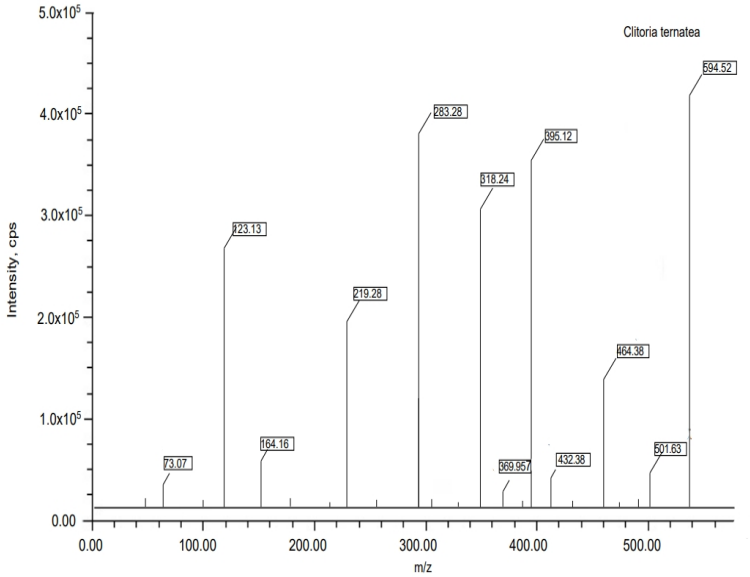


Fig No. 2: LC- MS Spectral peak for Phytoconstituent Analysis

Table 3: Peak Analysis

Peak No.	m/Z	Mol. F	Nomenclature	Phytoconstituent Identified	Class
1	73.07	C3H5O2	3-O-[(2S)-2-acetoxy-3,4-dihydroxyphenyl]-5,7-dihydroxy-2-phenylchromenylium-3-glucoside	Acetylated delphinidin 3-glucoside or acetyl delphinidin.	Anthocyanin
2	123.13	C7H7O2	Salicylaldehyde (2-Hydroxybenzaldehyde)	Hydroxyphenyl derivatives	Flavonoids
3	164.16	C9H8O3	(E)-3-Phenylprop-2-enoic acid	Hydroxycinnamic acid or P-Coumaric acid	Phenyl Propanoid
4	219.28	C13H17NO2	N-Methyl-1H-indole-3-ethanamine	N-methyltryptamine	Alkaloid
5	283.28	C16H13NO4	Kaempferol-3-O-glucoside	Kaempferol	Flavonoid Glycoside
6	318.24	C15H10O8	Quercetin-3,7-O-diglucoside	Quercetin	Flavonoid Glycoside
7	369.95	C15H11O6	Cyanidin-3-O-glucoside	Cyanidin	Anthocyanin Glycoside
8	395.12	C15H15O7	Delphinidin-3-O-glucoside	Delphinidin	Flavonoid
9	432.38	C21H20O10	Isorhamnetin-3-O-rutinoside	None	Flavonoid Glycoside
10	464.38	C21H20O12	Quercetin-3-O-glucoside	Isoquercitrin	Flavonoid Glycoside
11	501.63	C21H21O10	Delphinidin-3-O-rutinoside	Anthocyanin Glycoside	Anthocyanins
12	594.52	C27H30O15	Quercetin-3-O-robinobioside	Quercetin	Flavonoid Glycoside

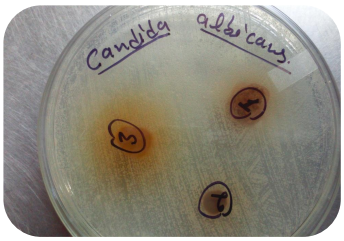
Antimicrobial Activity:

The antimicrobial activity of the ethanolic flower extract of *Clitoria ternatea* was evaluated against selected bacterial and fungal strains using the cup plate method. The extract

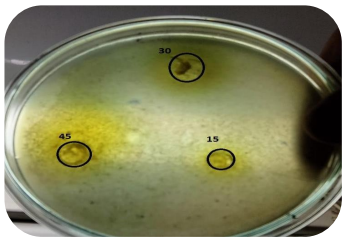
exhibited significant antimicrobial activity against all tested strains, with varying degrees of inhibition. The zone of inhibition ranged from 0.9 mm to 1.6 mm at 15 mg/ml, 0.2 mm to 1.3 mm at 30 mg/ml, and 0.3 mm to 1.5 mm at 45 mg/ml.

The extract showed the highest activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*, with inhibition zones reaching 1.6 mm and 1.4 mm, respectively, at 45 mg/ml. The lowest activity was observed against *Candida albicans*, with a maximum inhibition zone of 0.9 mm at the highest concentration. These results suggest that *Clitoria ternatea* has a strong antimicrobial potential, particularly against Gram-positive bacteria, and to a lesser extent, against fungi .

The observed antimicrobial activity can be attributed to the presence of bioactive compounds identified in the LC-MS analysis. Flavonoids and anthocyanins, known for their antimicrobial properties, could be responsible for inhibiting microbial growth. Previous studies have reported similar antimicrobial effects of *Clitoria ternatea* extracts, highlighting its potential as a natural antimicrobial agent .



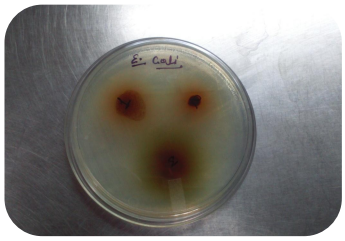
Zone of Inhibition of Clitoria ternatea extract against Candida Albicans



Zone of Inhibition of Clitoria ternatea extract against Bacillus Substilis

Zone of Inhibition of Clitoria ternatea extract against Staphylococcus aureus

Zone of Inhibition of Clitoria ternatea extract against Pseudomonas aeruginosa



Zone of Inhibition of Clitoria ternatea extract against E. Coli

Fig No. 3: Zone of Inhibition of *Clitoria ternatea* Extract

Table 4: Zone of Inhibition of Ethanol Flower Extract of *Clitoria ternatea* against Selected Gram-Positive and Gram-Negative Bacteria and Fungi

S. No.	Extract	Strain	Zone of Inhibition (mm) Standard (50 mg/ml)	Concentration (mg/ml) Control
1	Ethanol Flower Extract of *Clitoria ternatea*	*Pseudomonas aeruginosa*	1.4 ± 0.1 mm	0
2		*E. coli*	1.5 ± 0.2 mm	0
3		*Staphylococcus aureus*	1.2 ± 0.1 mm	0
4		*Bacillus subtilis*	1.6 ± 0.1 mm	0
5		*Candida albicans*	0.9 ± 0.2 mm	0

Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration (MIC) values were determined for the ethanolic flower extract of *Clitoria ternatea*. The MIC for *Pseudomonas aeruginosa* was found to be 30 mg/ml, while the MIC for *E. coli* and *Staphylococcus aureus* was 45 mg/ml. The extract showed the lowest MIC value for *Bacillus subtilis* at 30 mg/ml, indicating a stronger antimicrobial effect against this Gram-positive bacterium. The MIC values for the fungal strain

Candida albicans were less conclusive, as the inhibition observed was lower compared to the bacterial strains.
The results suggest that *Clitoria ternatea* has a higher efficacy against bacterial strains, especially Gram-positive bacteria, compared to fungal strains. This aligns with previous research indicating the antimicrobial potential of *Clitoria ternatea* against a broad spectrum of pathogens.

DISCUSSION:
The results of this study confirm that the ethanolic flower extract of *Clitoria ternatea* is rich in bioactive compounds, including flavonoids, anthocyanins, alkaloids, and glycosides. These compounds contribute to the plant's significant therapeutic properties, including antimicrobial activity. The LC-MS analysis provided a detailed profile of the bioactive compounds present in the extract, which supports its potential use in pharmaceutical applications for treating infections caused by bacteria and fungi.
The antimicrobial activity observed in this study further substantiates the ethnomedicinal use of *Clitoria ternatea* in treating infections. The significant antimicrobial effects against Gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, highlight its potential as a natural alternative to synthetic antibiotics. However, the moderate activity observed against fungi, particularly *Candida albicans*, suggests that further studies are needed to explore its effectiveness against fungal pathogens.
In conclusion, the ethanolic flower extract of *Clitoria ternatea* holds promise as a source of natural antimicrobial agents. The phytochemical composition, coupled with its antimicrobial activity, underscores the therapeutic potential of *Clitoria ternatea* in the treatment of microbial infections. Further research, including in vivo studies, is required to explore its full therapeutic potential and to isolate specific compounds responsible for its antimicrobial effects.

SUMMARY:
The present study aimed to investigate the phytochemical composition and antimicrobial activity of the ethanolic flower extract of *Clitoria ternatea*. The flowers were collected, dried, and subjected to extraction with ethanol, followed by preliminary phytochemical screening. The extract was found to contain various bioactive compounds, including alkaloids, flavonoids, glycosides, phenolic compounds, and terpenoids, which are known for their therapeutic properties.
Liquid Chromatography-Mass Spectrometry (LC-MS) analysis identified several key phytochemicals, including acetylated delphinidin 3-glucoside, kaempferol-3-O-glucoside, quercetin-3-O-glucoside, and cyanidin-3-O-glucoside. These compounds are well known for their antioxidant, anti-inflammatory, and antimicrobial activities, contributing to the medicinal value of *Clitoria ternatea*.
The antimicrobial activity of the ethanolic flower extract was tested against several bacterial strains (*Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*) and the fungal strain *Candida albicans* using the cup plate method. The extract demonstrated significant antimicrobial activity, with the highest inhibition observed against *Bacillus subtilis* and *Pseudomonas aeruginosa*. The Minimum Inhibitory Concentration (MIC) for the bacterial strains was determined, and the extract showed a strong antibacterial effect, particularly against Gram-positive bacteria.

CONCLUSION:
The findings of this study confirm that *Clitoria ternatea* flowers are a rich source of bioactive compounds, which contribute to its wide range of pharmacological activities. The ethanolic extract of the flowers exhibited significant antimicrobial properties, making it a potential candidate for the development of natural antimicrobial agents. The identification of key compounds through LC-MS analysis further underscores the therapeutic potential of this plant, particularly in the treatment of bacterial infections.
Given its promising antimicrobial activity, *Clitoria ternatea* could serve as a valuable addition to the arsenal of natural remedies for managing infectious diseases. However, further in vivo studies and isolation of specific bioactive compounds are necessary to fully understand the mechanisms behind its antimicrobial effects and to explore its full therapeutic potential. Additionally, the use of *Clitoria ternatea* in combination with other antimicrobial agents may enhance its effectiveness and provide a broader spectrum of activity.

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