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# PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CLITORIA TERNATEA* LEAVES AGAINST *STAPHYLOCOCCUS AUREUS*

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## ABSTRACT

Plants have played a major role in the production of biological compounds for the formation of drugs. Their role may either become a base for the development of medicine, a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases. Secondary metabolites are responsible for medicinal activity of plants. *Clitoria ternatea* commonly known as butterfly pea has diuretic and laxative effect. It also possess anti-inflammatory and anti-pyretic properties. Hence in the present study phytochemical and antimicrobial activity was carried out to confirm the presence of various phytochemical like saponins, terpenoids, steroids, tannins and to determine the minimum inhibitory concentration of extracts. The results suggest that the phytochemical properties for curing various ailments and possess potential antioxidant and leads to the isolation of new and novel compounds. The methanolic extract inhibited the growth of Gram positive bacteria *Staphylococcus aureus*.In reference to the role of plants in ethnomedicines and in traditional system the presence of phyotchemical consitutents in them have been studied.

**KEYWORDS:Phytochemical screening, Medicinal plants, *Staphylococcus aureus*, Secondary metabolites.**

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# INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The bioactive substances in plants are their secondary metabolites. Plants exhibit protective mechanism against pathogens as both evolved at same period and survives in same niche. Therefore, it is reasonable to expect a variety of plant-compounds to have specific as well as a general activity. Medicinal plants, with their wide variety of chemical constituents, offer a promising source of new antimicrobial principle. Plants are good source of antimicrobial substance this shows the way to identify new compounds for pharmaceutical uses. India is one of the richest with vast resource medicinal and aromatic plants. As people are conscious about the synthetic drug usage because of its side effects, the need of traditional herbal remedies is more demanding and is a viable research initiative for new pharmaceuticals. In traditional herbal remedies plant extracts is used to treat diseases and the present investigation is yet another search for specific medicinal property in plants. Plants are the richest source of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [4]. The recent return to the natural medicine throughout the world has emphasized the importance of gathering information about medicinal plant species traditionally used by the indigenous communities. Phytochemical screening is important in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best use of available natural wealth. Many medicinal plants have been chemically investigated for this purpose. The various solvent extracts of *Riccinus communis* leaves against five species of bacteria 9 have found positive antibacterial effect [13] have also reported antibacterial activity of *Punica granatum*. There is now growing evidence that indicates a strong relationship between ethnic knowledge and sustainable use of biodiversity.*Clitoria ternatea* commonly known as Aparajita in India is a flowering plant. *Clitoria ternatea* L. (Family: Fabaceae) a perennial twing herb, stems are terete, more or less pubescent. It is used to cure sever bronchitis, asthma. Plant has reported number of pharmacological activities such as anticonvulsant, sedative, antipyretic, anti-inflammatory and analgesic. It has diuretic and laxative effects. It also has anthelminitic and anti-ulcer properties. According to Ayurveda literature, this plant has the property of healing all types of wounds. From many years especially in India where they are widely distributed, they have been shown to possess antibacterial, antifungal properties. The leaves and flowers have the cooling effects. Phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other. In view of the necessity to search the suitable antibacterial drugs from plants, the present investigation was proposed to be investigated against *Staphylococcus aureus, a* causative agent of skin diseases. The present study carried out on the plant samples revealed the presence of medicinally active constituents.

# MATERIALS AND METHODS

***Preparation of plant extracts:***

Leaves were collected from mature plants and were air- dried and powdered. The dry leaf powder was extracted by reflexed in 100 mL methanol, ethyl acetate and water respectively for 24 h, using a Soxhlet apparatus. The extracts were filtered using What man filter paper, No. 1. The filtrates were then evaporated using rotatory evaporator and dried at 55°C. Dried extract was stored at 20°C in labeled, sterile screw-capped bottles for further

phytochemical analysis and antimicrobial activity.

***Phytochemical tests for active compounds***

The tests were done to find the presence of the active chemical constituents such as alkaloids, terpenoids and steroids, flavonoids, tannin etc. by the following procedure

***Test for carbohydrates***

Molisch's test: One drop of concentrated sulphuric acid was added to the extracts, and then three drops of 1% α-napthol in 80% ethanol were added to the mixture. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates.

Detection of proteins and amino acids

***a) Xanthoproteic Test:***

The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

***b) Ninhydrin Test:***

To the extracts, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Terpenoid and steroid

To five milligrams of each plant extracts 0.5 ml of acetic anhydride and 0.5 ml of chloroform was added. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids [16].

***Test for saponins***

To test the presence of saponins frothing test was done.To the extract water was added and on warming frothing showed the presence of saponins.

***Test for tannins***

Approximately 5g of each portion of the extract was stirred with 10ml of distilled water on a magnetic stirrer, filtered, and ferric chloride reagent added to the filtrate. A blue black, green, or blue-green precipitate indicates for the presence of tannins.

***Flavonoid***

Four milligram of each plant extracts solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 - 6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and Orange color for flavones [16].

***Detection of alkaloids***

Extract was dissolved in dilute Hydrochloric acid and filtered.

***a) Mayer's Test:***

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

***b) Dragendroff's Test:***

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

***c) Wagner's Test:***

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**Table 1**
***Preliminary Phytochemical Screening of Clitoria ternatea leaves***

| Chemical Tests | Aqueous extract | Ethyl acetate extract | Methanol extract |
|---|---|---|---|
| Test for carbohydrate | – | + | – |
| Test for alkaloids | | | |
| a) Mayer's test | – | + | + |
| b)Dragendorff's test | – | + | + |
| c) Wagner's test | + | + | + |
| Test for flavonoids | + | + | + |
| Test for Amino acids | + | – | – |
| Test for Proteins | + | – | – |
| Test for Saponins | + | + | + |
| Test for Steroids | – | + | – |
| Test for Tannins | + | – | + |

### *Antimicrobial activity of Clitoria ternatea*

The antimicrobial activity of *Clitoria ternatea* leaves extract of water, ethyl acetate and methanol was tested by disc diffusion bioassay on bacterial isolate *Staphylococcus aureus*. The extract was tested for their Minimum Inhibitory Concentrations using the MIC agar dilution method .The Minimum Inhibitory Concentration (MIC) values of the extract are shown in table 3 which shows dose dependent inhibition of bacterial growth.

**Table 2**
***Antimicrobial activity of Clitoria ternatea***

| | Mean diameter inhibition zone(mm) | | | |
|---|---|---|---|---|
| | Aqueous | Ethyl acetate | Methanol | Ampicilline |
| *Staphylococcus aureus* | 5 | 10 | 12 | 20 |

**Table 3**
***Minimum Inhibitory Concentration (MIC) of Methanolic extract of Clitoria ternatea***

| | Diameter of zone of inhibition (mm)<br>Methanol extract concentration (mg/ml) | | |
|---|---|---|---|
| *Staphylococcus aureus* | 250 | 500 | 1000 |
| | 7.23 ± 0.2 | 11.12 ± 0.6 | 13.87 ± 1.2 |

## RESULTS AND DISCUSSION

Many plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. Herbal medicinal products are virtually known to contain phytochemicals. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents

Recent studies have showed that plants with medicinal values with antibiotic resistant bacteria can be used in drug discovery which need of an hour Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening. The distribution of different phytochemical constituents in extracts of leaves of *Clitoria ternatea* were evaluated qualitatively and presented in table1. The results of the qualitative phytochemical study revealed that the methanol extracts of leaf of *Clitoria ternatea* showed the presence of alkaloid, flavonoid, steroid, tannin, terpenoid. The methanolic

extract of *Clitoria ternatea* showed antimicrobial activity against gram positive bacteria *Staphylococcus aureus*. The maximum zone of inhibition (12 mm) was observed in methanol extract while the minimum zone of inhibition (05 mm) was observed in water extract. The MIC, extract exhibiting antimicrobial activity showed variation in the three fractions.

## CONCLUSION

Preliminary phytochemical analysis revealed the presence of alkaloids, tannins and saponins, flavonoids, steroids, etc. were present in the plant. The result of the phytochemical analysis revealed the presence of medicinally active constituents. Many of the phytochemical analysis showed positive results which showed the presence of their active compounds. Preliminary phytochemical screening was helpful in prediction of nature of drugs and also useful for the detection of different polarity solvent. The results of phytochemical and antimicrobial activity suggest that *Clitoria ternatea* can be used for curing various ailments as it possess potential antioxidant properties and leads to the isolation of new and novel compounds. Therefore, it could be helpful to extract out particular constituents by solvent.*Clitoria ternatea* methanolic extract have considerable promise to be used as antimicrobial agents. It can be concluded that the methanolic extract of *Clitoria ternatea* shows significant activity against *Staphylococcus aureus*. It suggests that the plants can be used as antioxidant, anti-inflammatory, antidiabetic, anticancer agents in the future.

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