

Antimicrobial Activity and Phytochemical Evaluation of *Clitoria Ternatea*

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Abstract: The aim of the present study was to investigate the antibacterial properties and phytochemical evaluation of *Clitoria ternatea*. The organic solvent (Ethanol, Methanol, Hexane) and water extracts from the whole plant of *Clitoria ternatea* (Fabaceae) were tested against, *Salmonella typhimurium*, *Proteus vulgaris*, *Shigella dysenteriae* and a fungal pathogen *Candida albicans* by agar disc diffusion method. The results showed prominent antibacterial activity against the tested microbial pathogens. Of all those, methanol extract was found to give a strong antimicrobial effect when compared to the other extracts (Ethanol, Hexane and water). Phytochemicals like tannins, flavonoids, alkaloids, steroids and terpenoids are found. The anthraquinones and saponins were found to be absent in plant material observed.

Keywords: *Clitoria ternatea*, Agar Disc Diffusion Method, Phytochemicals, Antimicrobial Properties

1. Introduction

Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way.. While 30 to 50% of current pharmaceuticals are derived from plants, only few of them are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which have been found in vitro to have antimicrobial properties. *Clitoria ternatea* (Family-Liguminoceae OR Papillioneceae), a perennial herbaceous twiner, stems terete, more or less pubescent. Leaves imparipinnate, petioles 2.1-2.6 cm long; stipules 4 mm long, linear, acute. Leaflets 5-7, sub coriaceous, 2.5-5 by 2-3.2 cm, elliptic-oblong, obtuse, stipules filiform. Flowers axillary, solitary, standard bright or blue or sometimes white, with an orange centre, seed- 6-10, yellowish brown, smooth. Two types- white variety and blue flowered variety; widely distributed throughout India, used as an ornamental plant. In Southeast Asia the flowers are used to colour food. In Malay cooking, an aqueous extract is used to colour glutinous rice. In Kelantan it is used to colour white rice for *Nasi kerabu*. In Thailand, a syrupy blue drink is made called *nam dok anchan*, it is sometimes consumed with a drop of sweet lime juice to increase acidity and turn the juice into pink-purple. In Burmese and Thai cuisine the flowers are also dipped in batter and fried. In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent.^[1] In traditional Chinese medicine, owing to its similarity to the

female reproductive organ, this plant has been ascribed properties affecting the same. It was used traditionally in an attempt to treat sexual ailments, like infertility and gonorrhea, to control menstrual discharge, and also as an aphrodisiac. This practice aligns with an ancient belief recorded in the Doctrine of Signatures.^[2] Various parts of *C. ternatea* have been reported to have tranquilizing property, anti-inflammatory, analgesic activity and antipyretic activities. Recently, several biologically active peptides called cliotides have been isolated from the heat-stable fraction of *Clitoria ternatea* extract. Cliotides belong to the cyclotides family^[4] and activities studies show that cliotides display potent antimicrobial activity against *E. coli*, *K. pneumonia*, *P. aeruginosa*. These peptides may have potential to be developed as antimicrobial and anti-cancer agents.^[5] Recently, several biologically active peptides called cliotides have been isolated from the heat-stable fraction of *Clitoria ternatea* extract. Cliotides belong to the cyclotides family^[4] and activities studies show that cliotides display potent antimicrobial activity against *E. coli*, *K. pneumonia*, *P. Aeruginosa*. These peptides may have potential to be developed as antimicrobial and anti-cancer agents.^[5] In animal tests the methanolic extracts of *C. ternatea* demonstrated anxiolytic, antidepressant, anticonvulsant and antistress activity^[3]. The active constituents include tannins, resins, starch, taraxerol, and taraxerone^[7]. This work attempts to find out the antimicrobial properties of *Clitoria ternatea*, against select list of microbes and extraction, isolation and characterization of compounds that give these properties to these plants.



Figure 1: Clitoria ternatea Blue and White varieties

2. Materials and Methods

Clitoria ternatea plants were collected from various places in and around the areas of Kurnool. Whole Plants of both the species were collected from mature plants and identified by comparing with herbarium specimens. The plants were air-dried and powdered. The dry powder was extracted by refluxed in 100 mL methanol for 24 h, using a Soxhlet apparatus (Khan *et al.*, 1988). The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator and dried at 55°C. Ethanol, methanol, hexane and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20°C in labeled, sterile capped bottles. Stock cultures of microbes are maintained at a temperature of 4 degrees centigrade, active cultures are prepared by growing in tubes of Muller-Hinton (MHB) / Potato dextrose agar (PDA) for bacteria and Sabouraud dextrose broth (SDB) for fungi.

2.1 Microorganisms

The bacterial colonies were isolated from hospital samples at Kurnool, their pure cultures were maintained in nutrient agar and stored at 4°C. Three gram negative bacterial species were grown, namely *Salmonella typhimurium*, *Proteus vulgaris*, *Shigella dysenteriae* and the fungus *Candida albicans*.

2.2 Antimicrobial assay

Sensitivity tests were performed by disc diffusion with standard antibiotics, following Kirby-Bauer method (Bauer *et al.*, 1966). The assessment of antimicrobial activity was done based on measurements of the diameter of inhibition zones (NCCLS, 1998). Of the four extracts, methanolic extract has given interesting results and the aqueous extract showed no killing.

2.3 Phytochemical screening

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

- Braemer's test for Tannins
- Liebermann-burchardt test for Steroids
- Liebermann-burchardt test and Salkowski test for Terpenoids
- Dragendroff's reagent test for Alkaloids
- Shinoda test for Flavanoids

- KOH test FOR Anthraquinones
- Keller-Kiliani test for Cardiac glycosides
- Frothing test for saponins

2.4 Antimicrobial disc diffusion assay

Antibacterial and antifungal activities of the four plant extracts were investigated by the disc diffusion method [6]. The MHA plates, containing an inoculum size of 10⁶ colony-forming units (CFU)/mL of bacteria or 2x10⁵ CFU/mL yeast cells on SDA were spread on the solid plates with a glass rod. Then discs (4.0-mm diam.) impregnated with 20 µL of each extract at a concentration of 100.0mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding solvent control alone in the centre, and antibiotic discs (6.0-mm diam.) of (20 µg/mL, Streptomycin sulphate for bacteria) and Nystatin (20 µg/mL, for fungal) were also used as a positive control. All of the plates were incubated at 37°C for 18 hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the discs were measured after 18 hours of incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones on the agar surface around the discs.

3. Results and Discussion

The aqueous and hexane extracts of plant has shown negligible antimicrobial activity on tested pathogens, whereas the methanolic extract of plant has shown maximum inhibition on *Shigella dysenteriae* (14±0.9) and minimum on *Candida albicans*. Ethanolic extract of plant has shown maximum inhibition on *Shigella dysenteriae* (13.06) and minimum on *Proteus vulgaris* (10±0.8). Of all the extracts methanolic extracts have shown maximum inhibition.

The results of the phytochemical screening to test the presence of tannin, anthraquinone, alkaloid, saponin, phlobatannin, flavonoid, cardiac glycosides, volatile oils, terpenoids and steroids in the methanolic extracts from various parts of *C. ternatea* are shown in Table I. The preliminary phytochemical screening study revealed that the leaf of *C. ternatea* contains intermediate amounts of tannin, cardiac glycosides and steroids and small amounts of alkaloids. There were no secondary metabolites noted in the stem. Both the flowers of *C. ternatea* contain phlobatannin, flavonoid, terpenoid in moderate amounts. The roots contain small amount of flavonoid, volatile oil and terpenoids. Anthraquinone and saponins were found to be absent in the entire plant.

Treating Gram-negative bacterial infections can be difficult because of several unique features of these bacteria. For example, the unique nature of their cell wall makes them resistant to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by carbapenems. However, even these drugs have become ineffective against some bacteria, leaving researchers to go for natural resources, which are

medicinal plants. New drugs to combat Gram-negative bacterial infections are needed. In addition, researchers are unraveling the molecular mechanisms of drug resistance in Gram-negative bacteria to identify novel strategies to combat these pathogens. This paper helps in formulating natural principles to combat drug resistance of certain gram negative bacteria.

Table 1: Antimicrobial activity of *Clitoria ternatea*

Solvent extracts	μL	Zone of inhibition in mm (Microbes)			
		<i>Salmonella typhimurium</i>	<i>Shigella dysenteriae</i>	<i>Proteus vulgaris</i>	<i>Candida albicans</i>
Aqueous	20	-N-	-N-	-N-	-N-
Methanol	20	12.7 \pm 1.1	14 \pm 0.9	12 \pm 0.8	11 \pm 0.5
Ethanol	20	11 \pm 0.9	13.06	10 \pm 0.8	12 \pm 0.9
Hexane	20	-N-	-N-	-N-	-N-
Streptomycin sulphate	20	32 \pm 0.7	26 \pm 0.8	22 \pm 0.5	-
Nystatin	20	-	-	-	18 \pm 1.7

-N- No Action

Table 2: Phytochemical Screening of Secondary Metabolites from *Clitoria ternatea*

S.No	Secondary metabolites	Name of the test	Leaf	Stem	Flower	Root
1.	Tannins	Braemer's test	2+	--	--	--
2.	Flavonoids	Shinoda test	--	--	2+	+
3.	Anthraquinone	KOH test	--	--	--	--
4.	Saponins	Frothing test	--	--	--	--
5.	Cardiac glycosides	Keller-Kiliani test	2+	--	--	--
6.	Alkaloid	Dragendorff test	+	--	2+	2+
7.	Steroids	Lieberman Burchardt test	--	--	--	+
		Steroids test	2+	--	--	--
		LiebermannBurchardt test	--	--	2+	2+
8.	Terpenoids	Salkowski test	--	--	2+	2+

'2+' Moderate, '+' Small amounts, '-' Absent

4. Conclusion

In conclusion, all the extracts investigated possessed activity against at least one strain of bacteria and/or fungi. Further studies aimed at the isolation and identification of active substances from the methanol extracts of *C. ternatea* could also evolve compounds with effective natural medicinal values for the cure of microbial diseases.

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