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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF ROOT EXTRACT OF *CLITORIA TERNATEA*

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ABSTRACT

Objective: Infections caused by Gram-negative bacteria are important causes of morbidity and mortality. Extracts of plants and herbs such as *Clitorea ternatea* are used as diuretic. This work attempts to find out antimicrobial activity of aqueous and alcoholic extract of *C. ternatea* roots against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), clinical strains of *Klebsiella pneumoniae*, and *Candida albicans*.

Methods: The agar well-diffusion method was done using Mueller Hinton agar and Sabouraud's dextrose agar. The microorganism grown in peptone water was inoculated into culture medium. 4 mm diameter well punched into the agar was filled with 20 µl of aqueous and alcoholic root extracts *C. ternatea* extracts in various concentrations (100-25 µg/ml). The plates were incubated and antimicrobial activity was evaluated.

Results: Aqueous root extract of *C. ternatea* with the concentration of 100 µg/ml showed zone of inhibition against *E. coli* (ATCC 25922) 18 mm, *P. aeruginosa* (ATCC 27853) 14 mm, multidrug resistant strain of *K. pneumoniae* 15 mm. Alcoholic extract of *C. ternatea* with the concentration of 100 µg/ml showed zone of inhibition of 35 mm against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) 22 mm, and multidrug resistant strain of *K. pneumoniae* 28 mm. *C. albicans*was resistant to both extract of *C. ternatea* root.

Conclusions: Alcoholic extract of *C. ternatea* is a better antibacterial agent against multidrug resistant *Klebsiella* species and other Gram-negative pathogens. Further, studies are required to identify active substances from the alcoholic extracts of *C. ternatea* for treating infections.

Keywords: *Clitorea ternatea,* Antimicrobial sensitivity, Multidrug resistant.

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INTRODUCTION

Health-related quality of human life has been benefited since the introduction of antibiotics [1]. Medicinal plants and herbs are used for treatment of infections. Hence, their therapeutic potential, biological properties, and safety have to be useful to take decisions of their use [2,3]. History of India reveals the usage of medicinal plants to treat various diseases [4]. Lots of efforts are made to ascertain new antimicrobial compounds from plants. Antibacterial activities in medicinal plants have been reported [5,6]. In the need to develop superior drugs toward microbial infections, researchers are focusing their attention more toward herbal medicine [7]. *Clitoria ternatea L.* (butterfly pea in English) belongs to the family Fabaceae and subfamily Papilionaceae is an herbaceous perennial legume valued for its forage and medicinal importance. Extracts of *C. ternatea* has been used since time immemorial to treat mental disorders since it has property of being a good nervine tonic [8]. There are reports on callus induction and antimicrobial activity of seed and callus extracts of *C. ternatea* L. [9,10].

The objective of our study is to find out the antimicrobial activity of aqueous and alcoholic extract of *C. ternatea* roots against Gram-negative bacteria and fungi.

METHODS

Plant collection

C. ternatea roots collected from 2 to 3 years old plants, identified and confirmed as C. ternate by the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, were used for extraction.

Aqueous root extraction

Fresh roots of *C. ternatea* were collected, cleaned, cut into small pieces, and dried sunshade. It was then hand powdered. Dry powder was weighed and mixed with distilled water at 1:10 ratio and boiled over a low flame for half an hour, cooled, and decanted. Residue was mixed with distilled water (1:10) and boiled for 30 minutes, cooled, and decanted. The above procedure was repeated twice. The clear supernatant obtained each time was decanted and then centrifuged (3000 rpm for 5 minutes) and the supernatant was evaporated on low flame, to get a thick paste like extract, which was later dried in an incubator at 37°C and the dry powder so obtained was stored in a dessicator [11].

Ethanol extract preparation

The shade dried *C. ternatea* roots were grinded to powder. A known amount of powdered material was added to ethanol in the ratio of 1:16. Extract was prepared using Soxhlet apparatus. The extraction was done for 48 hrs duration. The crude extracts thus obtained were filtered using Whatmen filter paper No. 1 and the solvents were evaporated to dry using water bath at 40°C. The dry extract was labeled and stored in the desiccator [12].

Antimicrobial activity

Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), clinical isolate (multidrug resistant strain) of *Klebsiella pneumoniae,* and *Candida albicans*was used.

The antibacterial and antifungal activity was determined using the agar well-diffusion method.

The above mentioned bacterial and fungal strains were revived by plating on nutrient agar and Sabouraud's dextrose agar (SDA), respectively. Isolated colonies were selected after overnight incubation at 37°C. Identification of the organisms was done by standard procedure. Isolated bacterial colonies were then transferred to sterile Mueller-Hinton broth, and *C. albicans* was transferred to Sabouraud's dextrose broth and incubated overnight. 0.5 McFarland's turbidity standard

was used to adjust the concentration of growth of microorganisms to 10^5 CFU/ml. Drugs used as positive control were ampicillin 10 µg and ketaconazole 15 µg [13].

Determination of antibacterial activity

Mueller-Hinton Agar (MHA) measuring 20 ml each was poured into petri dishes. The bacterial culture was spread over the surface of the MHA plate. 4 mm diameter wells were punched into the agar and filled with 20 µl solution of test compounds in various concentrations (100, 50, 25, and 12.5 µg/ml). The inoculated plates were then kept in the incubator for 18 hrs at 37°C. Tests were done in triplicates and the average of the three was considered for the study.

Determination of antifungal activity

20 ml of SDA was poured into each petridishes. Culture of the *C. albicans* was spread over the surface of the SDA plate. Wells were punched into the agar plate measuring 4 mm in diameter and filled with 20 µl solution of test compounds in various concentrations (100, 50, 25, and 12.5 µg/ml). The plates were then kept in the incubator for 18 hrs at 37°C. Tests were done in triplicates and the average of the three was considered for the study.

Statistical analysis

Data are summarized as mean ± standard deviation. All comparison between solvent and concentration was done using two way ANOVA and $p<0.005$ was considered statistically significant.

RESULTS

The root of *C. ternatea* (aqueous extract) with the concentration of 100 µg/ml showed zone of inhibition against *E. coli* (ATCC 25922) 18 mm, *P. aeruginosa* (ATCC 27853) 14 mm, and multidrug resistant strain of *K. pneumoniae* 15 mm (Table 1).

Root of *C. ternatea* (alcoholic extract) with the concentration of 100 µg/ml showed zone of inhibition of 35 mm against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) 22 mm, multidrug resistant strain of *K. pneumoniae* 28 mm. *C. albicans* was resistant to both aqueous and alcoholic extract of *C. ternatea* root (Table 2).

There is a decrease in zone of inhibition with decrease in concentration which is statistically significant ($p<0.001$). Similarly, when we compare the zone of inhibition between the aqueous and alcoholic extracts there is statistically significant difference in the mean zone of inhibition ($p<0.001$) (Table 3).

Comparison of zone of inhibition between the aqueous and alcoholic extract for each concentration depicted a statistically significant difference in the means for the concentration of 100, 50, and 25 µg/ml ($p<0.001$) for *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and MDR *K. pneumoniae* and for the concentration of 12.5 µg/ml the aqueous extract was resistant to all the organism used in the test.

DISCUSSION

Medicinal plants are rich sources of antimicrobial agents which are used medicinally in different countries and are a source of many potent drugs used for traditional medicine. Medicinal plants exhibit antimicrobial activity by different mechanisms. This can be achieved by inhibition of cell wall synthesis, interference with the permeability of cell membrane, cause membrane disruption, modifying cellular constituents, and cell

Table 1: Zones of inhibition of aqueous extract of *C. ternatea* against various organisms

Organism	Zone of inhibition (mm)			
	Extract 100 µg/ml	Extract 50 µg/ml	Extract 25 µg/ml	Extract 12.5 µg/ml
E. coli (ATCC 25922)	18	13	10	-
P. aeruginosa (ATCC 25922)	14	11	10	-
MDR *K. pneumoniae*	15	10	8	-
C. albicans	-	-	-	-

C. ternatea: Clitorea ternatea, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, C. albicans: Candida albicans, P. aeruginosa: Pseudomonas aeruginosa, MDR: Multidrug resistant

Table 2: Zones of inhibition of alcoholic extract of *C. ternatea* against various organisms

Organism	Zone of inhibition (mm)			
	Extract 100 µg/ml	Extract 50 µg/ml	Extract 25 µg/ml	Extract 12.5 µg/ml
E. coli (ATCC 27853)	35	30	22	10
P. aeruginosa (ATCC 25922)	22	15	10	8
MDR *K. pneumoniae*	28	17	9	-
C. albicans	-	-	-	-

C. ternatea: Clitorea ternatea, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, C. albicans: Candida albicans, P. aeruginosa: Pseudomonas aeruginosa, MDR: Multidrug resistant

Table 3: Descriptive statistics - Dependent variable

Concentration	Mean±SD					
	E. coli (ATCC 25922)		*P. aeruginosa* (ATCC 27853)		MDR *K. pneumoniae*	
	**Aqueous	Alcoholic	**Aqueous	Alcoholic	**Aqueous	Alcoholic
100 µg/ml*	18.000	34.667	13.667	22.000	9.667	27.667
50 µg/ml*	12.667	30.000	10.667	15.000	9.667	16.667
25 µg/ml*	10.000	21.667	9.667	10.000	8.333	8.667
12.5 µg/ml	-	9.667	-	7.667	-	-

*p<0.001 for comparison between the concentration 100, 50 and 25 µg/ml; **p<0.001 for comparisons between the aqueous extract with the alcoholic extract. SD: Standard deviation, *C. ternatea: Clitorea ternatea, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, C. albicans: Candida albicans, P. aeruginosa: Pseudomonas aeruginosa,* MDR: Multidrug resistant

damage or cell mutation [14]. Most of the solvents such as ethanol, hexane, and methanol when used for plant extract showed inhibitory effect on Gram-positive and Gram-negative bacteria [15].

Haripriya *et al.* [16] observed that petroleum ether extracts of *Selaginella involvens* showed higher antibacterial activity against *E. coli* and Pseudomonas. Ponnusamy *et al.* [4] in their study showed ethyl acetate, ethanol, acetone, and petroleum ether extracts had maximum zone of activity against *Aeromonas formicans, Aeromonas hydrophila, Bacillus subtilis, E. coli, K. pneumonia, P. aeruginosa,* and *Streptococcus agalactiae*. In our present study of *C. ternatea* (ethanol extract) had better antibacterial activity than *C. ternatea* (aqueous extract) $p<0.001$. The *C. ternatea* (ethanol extract) was also effective against multidrug resistant strains of *K. pneumoniae*. Our study was in concordance with the study conducted by Haripriya *et al.* [4] and Ponnusamy *et al.* [16].

CONCLUSION

Present study suggests that *C. ternatea* was effective against multidrug resistant microorganism, especially *K. pneumoniae*. There is a good scope for the development of natural drugs. Further, research is required in drug development program to identify the active compounds which is responsible for the plants biological activity.

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