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

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Abstract—
Introduction and Objectives:
Infections caused by gram negative bacteria are important causes of morbidity and mortality and have a major impact on public health. Extracts of plants and herbs like *Clitoreaternatea (C.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* (ATCC25922), clinical strains of *Klebsiella pneumoniae*, *Escherichia coli* and *Candida albicans*.
Method:
The agar well diffusion method was done using Muller Hinton agar and Sabouraud's dextrose agar. The culture medium was inoculated with the microorganism separately grown in Peptone water. A 4 mm diameter wells punched into the agar was filled with 20µl of aqueous and alcoholic rootextracts *C. ternatea* extracts in various concentrations (100 µg /mL - 25µg/mL). The plates were then incubated at 37˚C for 18 h. The antimicrobial activity was evaluated by measuring the diameter of zone of inhibition. Positive controls were ampicillin10 µg/disc and ketoconazole 15 µg/disc.
Results:
The aqueous root extract of *C. ternatea* with the concentration of 100 µg /ml showed zone of inhibition against *Escherichia coli* (ATCC 27853) 18mm, *Pseudomonas aeruginosa* (ATCC 27853) 14mm, multidrug resistant strain of *Klebsiella pneumoniae* 15 mm.
Alcoholic extract of *C.ternatea* with the concentration of 100 µg /ml showed zone of inhibition of 35 mm against *Escherichia coli* (ATCC 27853), *Pseudomonas aeruginosa* (ATCC 27853) 22 mm, multidrug resistant strain of *Klebsiella pneumoniae* 28 mm. *Candida albicans*was resistant to both aqueous and alcoholic extract of *C.ternatea* root.
Conclusions:
Alcoholic extract of *C.ternatea* is a better antibacterial agent than aqueous root extractagainst 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.

I. INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction[1].A large and increasing number of patients in the world use medicinal plants and herbs for health purpose. Therefore, scientific scrutiny of their therapeutic potential, biological properties, and safety will be useful in making wise decisions about their use[2, 3]. India throughout its long history has accumulated a rich body of experiential facts of the use of medicinal plants for the treatment of various diseases[4]. Many efforts have been made to ascertain new antimicrobial compounds from plants. Recently several workers have reported antibacterial activities medicinal plants[5], [6]. Researchers are focusing their attention more and more to herbal medicine pursue for new direction to develop superior drugs against microbial infections[7]. *Clitoriaternatea 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 *Clitoriaternatea* 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 *ClitoriaternateaL(C. ternatea*)[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.

II. MATERIALS AND METHODS

Plant collection:
C. ternatea roots collected from 2–3yearold plants, identified and confirmed as *C. ternate* in the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, were used for extraction.

Aqueous root extraction[11]
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.

Ethanol Extract preparation[12]

The shade dried Clitoriaternatea roots were grinded to powder. A known amount ofpowdered material was added to ethanol in the ratio of 1:16. Extract was prepared using Soxhlet apparatus. The extraction was done for 48 hours duration. The crude extracts thus obtained were filtered using Watmen 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.

Antimicrobial activity[13]

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

The antibacterial and antifungal activity were 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 Muller-Hinton Broth (MHB) and *Candida albicans* was transferred to Sabouraud's Dextrose Broth (SDB) and incubated overnight. 0.5 McFarland's turbidity standard was used to adjust the concentration of growth of microorganisms to10^5CFU/mL. Drugs used as positive control were ampicillin 10µg and ketaconazole 15µg. DMSO was used as a negative control.

Determination of Antibacterial Activity: Muller-Hinton Agar (MHA) measuring 20 mL each was poured into petri dishes. The bacterial culture was spread over the surface of the MHA plate. 4mm diameter wells were punched into the agar and filled with 20µl solution of test compounds in various concentrations (100 µg /mL, 50 µg /mL, 25µg/mL 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 eachpetridishes. Culture of the *Candida albicans* was spread over the surface of the SDA plate. Wells were punched into the agar plate measuring 4mm in diameter and filled with 20µl solution of test compounds in various concentrations (100 µg /mL, 50 µg /mL, 25 µg/mL 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.

Results:

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

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

Table 1 :
ZONES OF INHIBITION OF AQUEOUS EXTRACT OF *C. TERNATEA*AGAINST VARIOUS ORGANISMS

Organism	Zone of inhibition (mm)			
	Extract 100 mg/ml	Extract 50 mg/ml	Extract 25 mg/ml	Extract 12.5 mg/ml
E. coli (ATCC 27853)	18	13	10	-
Pseudomonas aeruginosa (ATCC 27853)	14	11	10	-
MDR Klebsiella pneumoniae	15	10	8	-
Candidaalbicans	-	-	-	-

Table 2 :
ZONES OF INHIBITION OF ALCOHOLIC EXTRACT OF *C. TERNATEA* AGAINST VARIOUS ORGANISMS

Organism	Zone of inhibition (mm)			
	Extract 100 mg/ml	Extract 50 mg/ml	Extract 25 mg/ml	Extract 12.5 mg/ml
E. coli (ATCC 27853)	35	30	22	10
Pseudomonas aeruginosa (ATCC 27853)	22	15	10	8
MDR Klebsiella pneumoniae	28	17	9	-
Candidaalbicans	-	-	-	-

III. DISCUSSION

Studies have shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria[14]. Haripriya et al observed that petroleum ether extracts of *S. involvens* showed higher antibacterial activity against *E. coli* and *Pseudomonas*[15]. Selvamaleeswaran P et.al in their study showed Ethyl acetate, Ethanol, Acetone and petroleum ether extracts had maximum zone of activity against *A. formicans, A. hydrophilia, B. subtilis, E. coli, K. pneumonia, P. aeruginosa* and *S. agalactiae*.In our present study ethanol extract of *C. ternatea* had better antibacterial activity than aqueous extract of *C. ternatea* (p value < 0.05). This *C. ternatea* was also effective against multidrug resistant strains of *Klebsiella pneumoniae*. Our study was in

concordance with the study conducted by Haripriyaet. al and Selvamaleeswaran Pet al.

CONCLUSION

Presentstudy suggests that *C. ternatea*was effective against multidrug resistant microorganism especially *K. pneumoniae*. Further research is required to identify the active compounds responsible for the plants biological activity which can be used in drug development program for safe health care services.

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