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AN *IN-VITRO* STUDY ON ANTIBACTERIAL ACTIVITY OF *MIMOSA PUDICA*, *CYNODONDACTYLON* AND *IMPATIENS BALSAMINA*

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ABSTRACT

Medicinal plants are nature's gift to human to make disease free healthy life. Infectious diseases continue to represent a significant challenge to human medicine. The present investigation evaluates antibacterial activity of *Mimosa pudica*, *Cynodondactylon* and *Impatiens balsamina*. They were tested against gram negative bacteria such as *Escherichia coli*,*Pseudomonas aeruginosa* and gram positive bacteria such as *Bacillus subtilis* using agar well diffusion method using acetone and aqueous solvent extracts. The susceptibility of the microbes to the extract of the plants was compared with standard antibiotics tetracyline and azithromycin (30mcg/ml) as a positive control. The results revealed that alcoholic extracts of *Mimosa pudica* and acetone extracts of *Impatiens balsamina* showed very high antibacterial activity (16-19mm) against three pathogens at low concentration 10µl, while *Cynodondactylon* plant extract exhibited moderate zone of inhibition (10-12mm) at the same concentration. The acetone and alcohol extracts of three medicinal showed more effective zone of inhibition against both gram negative and gram positive bacteria. The present findings of the study suggest these three medicinal plant extracts can play a promising roleas antibacterial agents in against pathogenic bacteria.

Key words: *Mimosa pudica, Cynodondactylon,Impatiens balsamina*and antimicrobial susceptibility.

INTRODUCTION

Infectious diseases are the leading cause of death world-wide. For a long period of time plants have been valuable sources of natural products for maintaining human health, especially in the last decade with more intensive studies for natural therapies [1-4]. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased abnormally [5-9]. Literature reports and ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry [10].

The use of plants and plant products as medicine can be traced as far back as the beginning of human civilization of medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicine, pharmaceutical intermediates and lead compound in synthetic drugs [11]. The plant based natural constituent can be derived from any part of the plant like bark, leaves, roots fruit and seeds, etc., Accordingly to world health organization more than 80% of the world's population relies on traditional medicines for their primary health care needs. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have enormous therapeutic advantages [12].

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming important cause morbidity in immune-compromised patients in developing countries [13]. The increasing prevalence of multi-drug resistant strains of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. For long time, plants have been an important source of natural products for human health. Although the active constituents may occur in lower concentrations, plant extracts may be a better source of antimicrobial compounds than synthetic drugs. Development of bacterial resistance to synergistic drug combinations, such as those found in plants, may be slower than for single drug therapies [14].

The increasingly high numbers of bacteria that are developing resistance for classical inhibits drive much of the current interest on natural antimicrobial molecules in hope that may provide useful leads into anti-infective drug candidates. About 74% of all plant derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate directly with their traditional uses on numerous occasions, the folkloric records

of many different cultures have provided information of plants with useful medicinal properties. A renewed interest has occurred in the last decade to search for phytochemicals of native and naturalized plants pharmaceutical are nutritional purposes; with the recognition that plant derived products have great potential as sources of pharmaceuticals [15].

According to the medicinal point of view and global environmental perspective, herb is on measurable wealth of nature. It plays a significant role ameliorating the disease resistant ability and combating various unfavorable metabolic activities within the living system. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. The increasingly high numbers of bacteria that are developing resistance to classical antibiotics drive much of the current interest on natural antimicrobial molecules in hope that they may provide useful leads into anti infection drugs [16].

The objective of the study was to evaluate antibacterial activity of *Mimosa pudica, Imaptiensbalsamia*and*Cynodondactylon* and against the human pathogens such as *E. coli, Bacillus subtilis* and *Pseudomonas aeuruginosa.*

MATERIAL AND METHODS

Collection of Plant Materials

The plants leaves of *Mimosa pudica, Imaptiensbalsamia*and*Cynodondactylon* were collected from Karnatak Science College, Dharwad campus. The plant leaves were plucked and washed under running tap water and oven dried at 60°C for 6 hours and finally powdered. The voucher specimens are deposited and maintained in department of botany, Karnatak Science College, Dharwad.

PHOTOS OF MEDICINAL PLANTS

Mimosa pudicaCyanodondactylon

Impatiens balsamina

Collection of Bacterial Culture

The test organisms like *Escherichia coli, Pseudomonas aeuroginosa andBacillus subtilis* were procured from MTCC, Chandigarh, India.

Chemicals, Media and Antibiotics used

All chemicals, culture media (Muller Hinton Agar, Nutrient Agar and Nutrient Broth), antibiotics (Tetracycline and Azithromycin) and organic solvent Alcohol and Aqueous solvents were procured from Hi-Media PVT. Ltd. Mumbai.

Preparation of inoculum

Suspension of organism was prepared as per McFarland standards (NCCLS- National committee for clinical Laboratory standards), 24hrs old cultures were used for the preparation of bacterial suspension. A suspension of organism was made in a sterile isotonic solution of sodium chloride and turbidity was adjusted. It was obtained by adjusting optical density (650 nm) equal to 0.5 ml of 1.175% Barium chloride in 100 ml of 1% sulphuric acid.

Preparation of Antibiotic Solutions

Stock solution of broad spectrum antibiotics tetracycline and azithromycin were prepared (30 mcg/ml) in sterile distilled water.

Preparation of aqueous plant extract

10gms of fine powder of plants leaves were macerated separately with 100 ml of sterile distilled water using pestle and mortar .The macerate was first filtered through muslin cloth and then centrifuged at 8000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120^0C for 30 min. The extract was preserved aseptically in a brown bottle at 4^0Cuntil further use.

Preparation of Solvent extract

About10gm fine powder of plant leaves were macerated with 100 ml of alcohol in a conical flask at wrist action rotary shaker for three days at 220 rpm. The whole extract was filtered with the help of Whatman No.4 filter paper .The collected solvent was then is evaporated to dryness with rotary vacuum evaporator at 40^0C to afford a thick residue. The thick residue was then converted in dried powder in a desecrator. They are stored for 4^0 C in air tight bottles for further use. 1gm of each solvent residue was dissolved in 10 ml of alcohol and aqueous solvents were used in test extracts for antimicrobial assay.

Antimicrobial Assay

The antimicrobial assay of five medicinal plants extracts against humans pathogens were determined by agar well diffusion method on Muller Hinton Agar as per NCCLS method. The Alcohol and aqueous solvents were used for antimicrobial assay. The sterile Muller Hinton Agar media plates were prepared. To make wells a sterilized cork borer (6mm) was used. The bacterial inoculums (100 µL,10^6 CFU/mL) were spread on the solid agar plates with sterile swab moisture with the bacterial suspension. The agar plates were allowed to dry for 15 min. The different concentrations (10, 40, 70 and 100 µL) of test sample wereadded into the well. The antibiotics tetracycline and azithromycin (30mcg/ml) were also added into the wells for control. The plates were labeled and incubated overnight at 37^0C.. The zone of Inhibition was measured and the results were recorded.

RESULTS AND DISCUSSION

The results of antibacterial acitivity of three Indian medicinal plants *Mimosa pudica, Cynodondactylon* and *Impatiens balsmina*were tested against two gram negative bacteria such as *E. coli,Pseudomonas* aeruginosa andgram positive *Bacillus subtilis* were illustrated in table.1 and figure 1-3.

It was observed that all the three plants showed antibacterial activity in alcohol and aqueous extracts. Alcoholic extracts of *M. pudica*and*balsamina* showed most effective zone of inhibits against human pathogens as *E. coli, Pseudomonas aeruginosa and* Bacillus*subtilis* at least. The alcoholic extracts of showed moderate zone of inhibition (10-12mm) against three pathogens. The aqueous extracts of *M. pudica* and *I. balsamina* showed better activity (40-16mm) against three pathogens. The aqueous extract of *C. dactylon*showed less activity (10-12mm) against the three pathogens. Among the three organisms the gram negative bacteria (*E. coli* and *P. aeruginosa)* showed more inhibitory effect than gram positive bacteria (*B.*

subtilis). The *E. coli* higher zone of inhibition than *P. aeruginosa*against *Mimosa pudica*and *Impatiens balsamina*than the *C. dactylon*in alcoholic extracts.

Table.1 Zone of inhibition (mm) of medicinal plant extract against human pathogens.

Test organism	Conc^{n}	*Mimosa pudica,*		*Cynodond actylon*		*Impatiens balsmina*		Control	
		Al	Aq	Al	Aq	Al	Aq	Tet	Azt
E. coli	10µl	19	16	10	12	19	14	30	28
	40µl	17	14	-	11	16	12		
	70µl	12	12	-	10	17	11		
	100µl	11	10	-	-	12	10		
Pseudomonas aeruginosa	10µl	19	16	10	-	14	12	25	23
	40µl	17	14	-	-	12	11		
	70µl	14	13	-	-	11	10		
	100µl	12	10	-	-	10	10		
Bacillus subtilis	10µl	18	14	12	10	17	14	24	26
	40µl	16	13	11		15	13		
	70µl	14	12	10		13	12		
	100µl	12	11			11	10		

N.B. Al-Alcohol, Aq- Aqueous, Tet – Tetracycline, Azt – Azithromycin

The alcoholic plant extracts showed higher zone of inhibition against tested bacteria than the aqueous plant extracts of three plants. The zone of inhibition was increased with decreased concentration of the three plant extracts in alcohol and aqueous solvents. The *C. dactylon*did not show any antibacterial activity against gram negative bacteria. *Pseudomonas aeruginosa*was resistant to all the extracts except aqueous extracts of *Cynodondactylon*. The difference in the observed zone inhibition activity of the various extracts may be due to varying degrees of solubility of the active constituents in the solvents used. It has been documented that different solvents have diverse solubility capacities for different phyto-constituents [17]. Out of all the bacteria tested the gram negative bacteria were more resistant to the plant extract than the gram positive bacteria. The susceptibility of the bacteria to the

extracts of the plants was compared with standard antibiotics, tetracycline and azithromycin as well as a positive control.

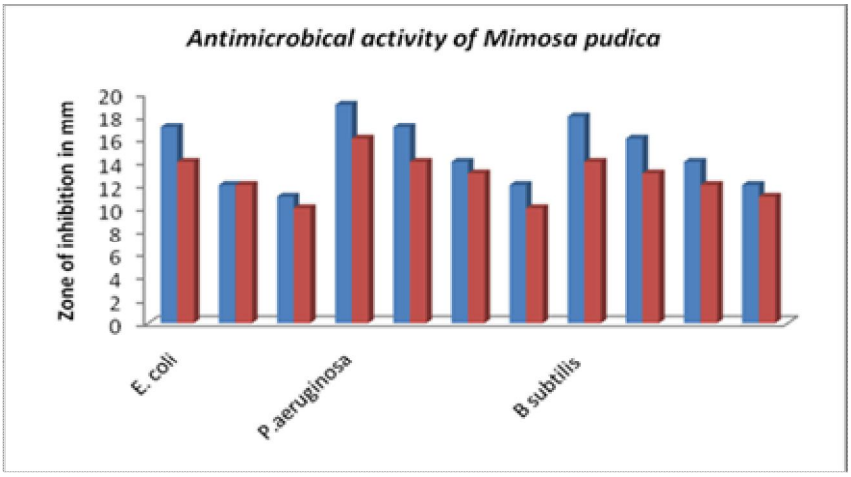

Fig 1. Antimicrobial activity of*Mimosa pudica*

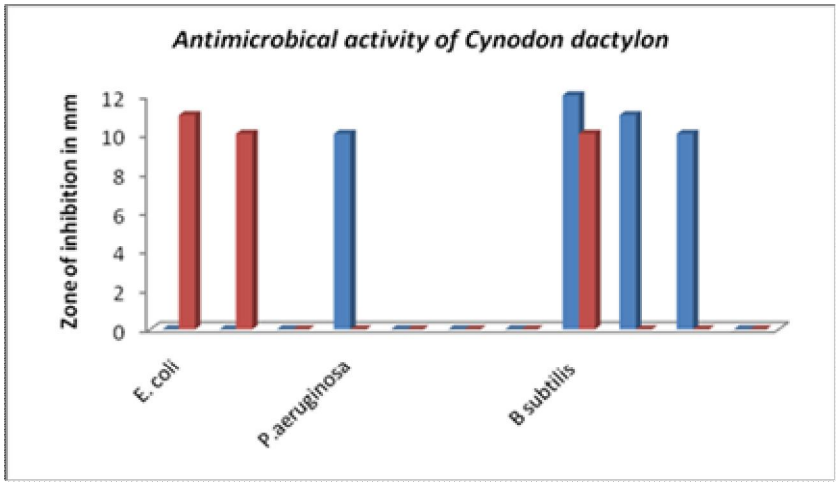

Fig 2. Antimicrobial activity of*Cynodon dactylon*

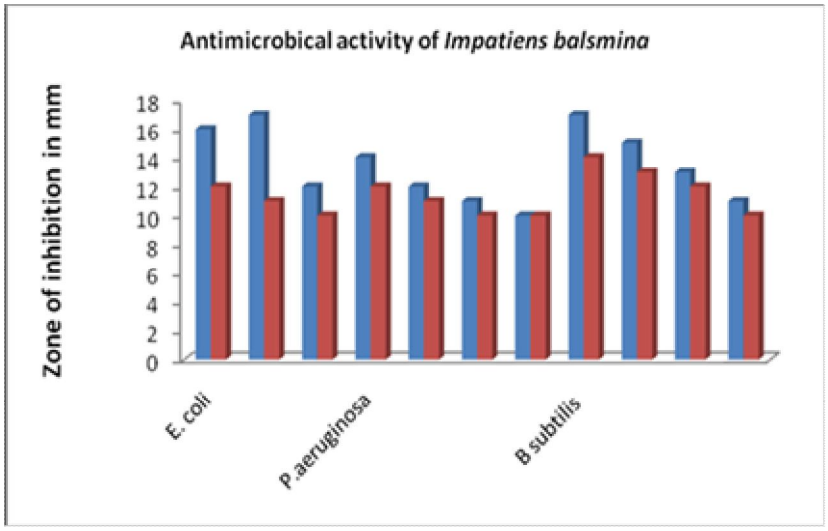

Fig 3. Antimicrobial activity of *Impatiens balsmina*

CONCLUSION

From the study it is concluded that the antibacterial activity and its active components of three medicinal plants extracts would be helpful in treating various diseases caused by the three pathogens (*E. coli, P. aeruginosa* and *B. subtilis*). It also revealed that alcoholic extracts have broad spectrum of activity against both gram negative and gram positive bacteria. Further investigation of their activity against a wider range of bacteria, identification and purification of its active chemical constituents and toxicological investigations of the plant extracts will be carried out with a view to developing novel drugs for human consumption.

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