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ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF SELECTED SOUTH INDIAN MEDICINAL PLANTS AGAINST SELECTED BACTERIAL PATHOGENS

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Abstract:
Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents; hence resistance tool and newly produced drugs is on the rise. The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. Thus the present study was undertaken to investigate the antibacterial activity of 3 medicinal plants against E.coli, P.aeruginosa, S.aureus, S.pneumonia The antibacterial activity of methanol, Extract of Mirabilis jalapa L., Acalypha lanceolata Wild and Curculigo orchioides Gaertn. (leaves was used), by Disc diffusion and Minimal Inhibitory Concentration (MIC) method. Our studies concluded that crude extracts of the selected plants especially the methanol extracts exhibited significant activity against bacterial pathogens. It can be concluded that these plants can be used to discover natural products that may serve as lead for the development of new pharmaceuticals addressing the major therapeutic needs to help new researchers and Pharmacologist for future studies.

Key words: *bacterial pathogens Medicinal plants, Antibacterial activity, Minimal Inhibitory Concentration (MIC) method.*

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INTRODUCTION:

Microbial infections are still a threat to public health despite the progress in synthesis chemistry and the discovery o f new antimicrobial agents from natural sources [1]. In the course of our study for a better valorization and in order to find some potential activities of *Scoparia Mirabilis jalapa L.*, *Acalypha lanceolata Wild* and *Curculigo orchioides Gaertn*. was selected.

Plant produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4, 00, 000 plant species on Earth only a small number has been systematically investigated for their antimicrobial activities. Additionally, there is a rich local ethnobotanical knowledge and bibliography describing the species most frequently used by population to cure various diseases [2]**.**However there has been seldom effective collaboration between the traditional and western medical therapeutics, largely due to the perception that the use of traditional and herbal medicines has no scientific basis. According to World Health Organization (WHO, 2000), medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy **[**4].

Tribal people of India have been using several types of plants as medicine since the ancient time which has not been studied extensively. Although screening of Indian medicinal plants has revealed varying degrees of antimicrobial activity against pathogenic and opportunistic microorganisms [3] but there is still a lack of experimental scientific studies confirming the possible antimicrobial properties of a great number of these remedies. Hence the present study was conducted to evaluate the antibacterial potential of fifteen plants used in folk medicine by tribals of chitteri region against bacterial pathogens.

The production, processing, and marketing of agricultural goods are central to food security and economic growth. Products derived from ethonobotanical [5] and animals' earthworms [6]. According to World Health Organization the goal of health for all can't be achieved without herbal medicines, while the demand for herbal medicine is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicine [7]. Plants have been noteworthy accountability in on condition that the human race with remedies. At present phototherapy is a documented complementary and substitute medicine (CAM) therapeutic modality [8].

Thus the present studies were focused Antibacterial activity of different solvent extracts of selected south Indian medicinal plants against selected bacterial pathogens.

MATERIALS AND METHODS:

Collection and processing of plants

Many south Indian tribal communities are still using plants extensively in their materia medica. Several hundred medicinal plant species from the Indian sub continent have been identified and their useges are documented in the ethnobotanical literature (Indian Medicinal plants and Home Remedies*;* Thirugnam *et al*,2010). This literature provides the information regarding the useage of medicinal plants by the tribals in India as anti bacterial and anti fungal agents, and for the treatment of wounds, cuts, sores, colds, cough and diarrhoea. For the present study, the medicinal plants were selected by the help of tribal people who are the resident of Chitheri mallai (Hills) in Dharmapuri Dt,Tamilnadu,India.

Collection of the plant speciemns were carried out during the growing and flowering season (March and April, 2009 to2012) from different places in Chitheri Malai of Dharmapuri districts of Tamil Nadu, India. The plants were identified and classified upto family (Matthew,1983) for each plant taxonomic classification was detaily given in the thesis of the current study. Bulk plant specimens were acquired based on ethno-directed collection procedures and air-dried in the shade. The dried materials were ground with domestic mixture to fine powder and stored until further use. At the time of collection, two pressed voucher herbarium specimens were prepared per species, of which one specimen was deposited as a reference in the Herbarium of PG Department of Zoology J. K. K Nataraja college of Arts and science, Komorapalayam, Tamil Nadu, Whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction Method

Samples of each of the selected plant species were extracted with three different solvents in a successive manner, in order to produce crude extracts containing a wide range of active compounds. About 20g of coarse plant material was placed in a dry 500 ml glass jar and 100 ml of acetone was added and allowed to macerate overnight. The next day, the

mixture was vigorously stirred for 10 min and allowed to settle for 5 min. The supernatant liquid was filtered through a Whatman No. 1 filter paper to remove any solid plant materials. The residual plant material was extracted two more times using 100 ml acetone. Finally, the three filtrates were combined and the solvent was evaporated under reduced pressure at 40°C to yield the acetone crude extract. The residual plant material was then sequentially extracted with chloroform and methanol using the same procedure described for acetone, giving chloroform and methanol extracts respectively. The final extracts were collected in a sterilized borosil glass vial, weighed and then stored in the desiccator until further use

Antimicrobial Assay

In vitro antimicrobial evaluation of the acetone, chloroform and methanol crude extracts were carried out against seven bacterial strains, two Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermids*) and five Gram-negative bacterias (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* and *Pseudomonas aureginosa).* Each bacterial strain was obtained from the Department of Medical Microbiology, Rajah Muthiah Medical College, Chidambaram, Tamil Nadu, India.

Disc diffusion method

The Plant extracts were screened for antimicrobial activity using the agar well diffusion method. Bacterial strains were inoculated into 10 mL of sterile nutrient broth, and incubated at 37 ℃ for 8 hours. The aliquots (0.5 ml) were spread evenly onto the surface of the sterile Mueller-Hinton agar plates using a sterile cotton swab and incubate for 30 minutes at 37 ºC. Sterile paper discs (6 mm diameter) containing 30 mg of acetone, chloroform and methanol crude extracts were screened for antibacterial activity. Simultaneously paper discs dipped with pure acetone, chloroform and methanol were used as controls. The Petri plates were then pre-incubated for 3 h at 5°C to permit maximum diffusion of the extracts into the media and incubated at 37 ºC for 24-48 hours. After incubation period, the diameter of the inhibition zone (mm) was measured. The formation of clear inhibition zone of ≥7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract. Gentamicin (30µg/ml) for *E. coli, S. aureus and P. aeruginosa*, Ofloxacin (10µg/ml) for *P.vulgaris, K. pneumoniae* and Chloremphenicol (30µg/ml) for *P. mirabilis* and *S. epidermids* were used as reference standards.

Determination of minimal inhibitory concentration (MIC)

A modified agar microdilution method of Lorian (1996) was used to determine the MIC of acetone, chloroform and methanol extracts of plants which produced inhibition zones. One microliter of an overnight culture of each bacterial strain was applied onto MHA supplemented with the plant extracts 250 µg/ml concentration, 125µg/ml (for fractions tested) and 31.25µg/ml. The microtiter plates were incubated at 35°C for 18 h. Observations were performed in triplicates and results were expressed as the lowest concentration of plant extracts that produced a complete suppression of colony growth. Minimal bactericidal concentration using agar dilution method in petri dishes with millipore filter (Lorian, 1996) was performed with the extracts that gave significant MIC values against each bacterial strain.

RESULTS AND DISCUSSION:

In the present study, 3 plant species belonging to various families were analysed for their antibacterial activities of 3 medicinal plants against *E.coli, P.aeruginosa, .aureus, S.pneumonia* The antibacterial activity of methanol, Extract of *Mirabilis jalapa L., Acalypha lanceolata Wild* and *Curculigo orchioides Gaertn*. (Leaves was used), by Disc diffusion and Minimal Inhibitory Concentration (MIC) method (Table 1-4).

In general, among the tested bacterial strains, *E.coli, P.aeruginosa, S.aureus, S.pneumonia* The antibacterial activity of methanol, Extract of *Mirabilis jalapa L., Acalypha lanceolata Wild* and *Curculigo orchioides Gaertn* were found to be more sensitive to many of the test agents than other microbes. The antibacterial activity was more pronounced on the Gram-positive bacteria than the Gram-negative bacteria(Table 1- 4). The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram-negative organisms which are more complex than the Gram-positive ones act as a diffusional barrier and making them less susceptible to the antimicrobial agents than are Gram-positive bacteria.In spite of this permeability differences, however, some of the extracts have still exerted some

degree of inhibition against Gram-negative organisms as well.

Infectious diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment [9].

Table-1 Antibacterial activity (zone of inhibition, mm) of different solvent extracts of *Mirabilis jalapa* tested against selected bacterial species

Solvents tested	Gram positive bacteria		Gram negative bacteria				
	Staphylococcus aureus	*Staphylococcus epidermids*	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Hexane	15c	11b	8c	13cd	11c	10d	15c
Diethyl ether	19b	12b	11c	12d	10c	9d	19c
Dichloro methane	17bc	16b	18b	15c	14bc	8d	17c
Ethyl acetate	19b	10bc	15bc	18c	17b	15c	12cd
Methanol	23b	20b	21b	24b	21b	22b	24b
Control	33a	36a	29a	33a	35a	28a	25a

Values represent zone of inhibition (in mm). Values with different alphabet in the column are statistically significant at p<0.005, LSD(4.2) - Tukey's test performed on the basis of mANOVA. *Gentamycine (30µg/ml; for *E. coli, S. aureus and P. aeruginosa (*Hailu Tadeg et. *al.,* 2005*)*), Ofloxacin (10µg/ml for *P.vulgaris, K. pneumoniae*; Karman *et al*., 2002) and Chloremphenicol (30µg/ml for *P. mirabilis* and *S. epidermids*; Nancy *et al*., 2000) were used as reference standards.

Table -2. Antibacterial activity - Minimum Inhibitory Concentration (MIC) value of different solvent extracts of *Mirabilis jalapa* tested against selected bacterial species

Bacteria tested	**Control***	**Hexane extract**		**Ethyl acetate extract**		**Methanol extract**	
		MIC	**C_{max}**	**MIC**	**C_{max}**	**MIC**	**C_{max}**
Staphylococcus aures	1.0	12.5b	147	12.5a	135	6.25a	127
Staphylococcus epidermids	8	12.5b	187	12.5a	155	6.25a	143
Escherichia coli	8	25a	137	6.25b	147	6.25a	117
Klebsilla pneumoniae	0.5	12.5b	187	12.5a	108	6.25a	145
Proteus mirabilis	8	6.25c	224	12.5a	142	3.125b	132
Proteus vulgaris	10	12.5b	207	12.5a	211	6.25a	211
Pseudomonas aeruginosa	10	6.25	90	12.5a	100	3.125	87

Values are represent in µg/ml . Values with different alphabet in the column are statistically significant at p<0.005, LSD (4.75) - Tukey's test performed on the basis of mANOVA. *Gentamycine (for *E. coli, S. aureus and P. aeruginosa*), Ofloxacin (for *P.vulgaris, K. pneumoniae*) and Chloremphenicol (for *P. mirabilis* and *S. epidermids*) were used as control.

Table-3. Antibacterial activity - Minimum Inhibitory Concentration (MIC) value of different solvent extracts of *Curculigo orchioides* tested against selected bacterial species

Bacteria tested	Control*	Hexane extract		Ethyl acetate extract		Methanol extract	
		MIC	C_{max}	MIC	C_{max}	MIC	C_{max}
Staphylococcus aures	1.0	12.5b	147	12.5b	126	6.25b	117
Staphylococcus epidermids	8	25a	187	25a	162	12.5a	123
Escherichia coli	8	25a	122	12.5b	152	6.25b	115
Klebsilla pneumoniae	0.5	12.5b	173	25a	124	12.5a	119
Proteus mirabilis	8	6.25c	224	12.5b	133	3.125b	120
Proteus vulgaris	10	12.5b	210	6.25c	225	3.125b	204
Pseudomonas aeruginosa	10	12.5b	90	12.5b	100	6.25	90

Values are represent in µg/ml . Values with different alphabet in the column are statistically significant at p<0.005, LSD (5.25) - Tukey's test performed on the basis of mANOVA. *Gentamycine (for *E. coli, S. aureus and P. aeruginosa*), Ofloxacin (for *P.vulgaris, K. pneumoniae*) and Chloremphenicol (for *P. mirabilis* and *S. epidermids*) were used as control.

Table 4. Antibacterial activity (zone of inhibition, mm) of different solvent extracts of *Curculigo orchioides* tested against selected bacterial species

Solvents tested	Gram positive bacteria		Gram negative bacteria				
	Staphylococcus aureus	*Staphylococcus epidermids*	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Hexane	10c	14c	10d	13cd	8e	16c	12d
Diethyl ether	8cd	15c	16c	14c	6e	13d	16c
Dichloro methane	7d	13cd	11cd	10e	11d	17c	8e
Ethyl acetate	11c	16c	15c	16c	15c	16c	13d
Methanol	18b	19b	23b	27b	26b	27b	23b
Control	33a	36a	29a	33a	35a	28a	25a

Values represent zone of inhibition (in mm). Values with different alphabet in the column are statistically significant at p<0.005, LSD (3.0)) - Tukey's test performed on the basis of mANOVA. *Gentamycine (30µg/ml; for *E. coli, S. aureus and P. aeruginosa (*Hailu Tadeg et. *al.*, 2005*)*), Ofloxacin (10µg/ml for *P.vulgaris, K. pneumoniae*; Karman *et al*., 2002) and Chloremphenicol (30µg/ml for *P. mirabilis* and *S. epidermids*; Nancy *et al*., 2000) were used as reference standards.

CONCLUSION:

From the study we conclude that most of the plants showed antimicrobial activity against one or more pathogens isolates. Broad spectrum activity was observed in *E.coli, P.aeruginosa, S.aureus, S.pneumonia* The antibacterial activity of methanol, Extract of *Mirabilis jalapa L., Acalypha lanceolata Wild* and *Curculigo orchioides Gaertn.*. The antibacterial potential of these plants against causing pathogens have been reported earlier and needs extensive investigation to understand their antibacterial principles which may allow the scientific community to recommend their use as accessible alternative to synthetic antibiotics.

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