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ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL HERBAL EXTRACTS ON CLINICALLY IMPORTANT BACTERIAL PATHOGENS

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ABSTRACT

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The antibacterial activity of methanol extract of *Abutilon indicum*, *Hygrophila spinosa* and *Mimosa pudica* were studied by agar well diffusion method *in vitro*. The effect of antibacterial potential was examined against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Salmonella typhi* and *Salmonella paratyphi*. The methanol extract of these medicinal plants have showed consistently significant inhibitory activity on different bacterial pathogens tested. Furthermore, the Minimum Inhibitory Concentration (MIC) studies carried out by broth dilution assay and found the MIC ranged between 0.2 to 0.9mg/ml. Overall the methanol extracts was found to be more effective. The results of the extracts were compared with the standard antibiotics Kanamycin.

INTRODUCTION: Plants are traditionally used in the treatment of bacterial and fungal infections for its wide range of bioactive molecules. Photochemicals are applied as natural anti pathogenic which can be derived from leaves, stems, barks and flowers of plants¹. The traditional plant medicine is getting back with modern science all over the globe. The extracts from medicinal plants are used in the treatment of different diseases of humans, plants and animals².

Approximately 80% of the world's population still relies on traditional plant medicines for the treatment of common illness^{3,4}. The bacterial strains developed its genetic ability to various pharmacological antibiotics⁵. The synthesized drugs associated with adverse effects which lead to immunosuppression and allergic reactions⁶. The formulation of appropriate and efficient antimicrobial drugs to the patient is ultimate goal in this decade.

Plants are the traditional helpers having alkaloids, flavonoids, saponins, tannins, protein and amino acids as its chemical constituents⁷.

It has been estimated that 3,000 to 3,500 species of higher plants seen in India⁸. India and China are richest in herbs among other countries in Asia. Use of medicinal plant in health care system is in practice since ancient time in India. India gets identity as botanical garden of the world by the abundance of herbs.

Abutilon indicum (indian mallow) is a small shrub in the Malvaceae family and often used as a Siddha medicine. The plant is considered for antibacterial, antihelmintic, carminative and diuretic treatments. It is used locally for colds, high fever, mumps, and tuberculosis⁹. The phytochemicals presence of this plant is luteolin, chrysoeriol, quercetin, triacontanoic acid, ursenol,

methylstigmastrol, glucopyronoside etc¹⁰. *Hygrophila spinosa* T Ander, belonging to the family Acanthaceae, is a promising medicinal plant with great economic potential. The medicinal value of *H. spinosa* has been appreciated in the ancient medical literature. The plant contains terpenoids, alkaloids, and flavonoids and is traditionally known as an aphrodisiac, renal tonic, and for its health-promoting properties⁹. *Mimosa pudica* also called Sensitive Plant is a perennial herb often grown for its curiosity value. The leaves of the plant are used in the treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations and burning. Leaf contains an alkaloid mimosine. Root contains tannin, ash, calcium oxalate crystals and mimosine¹¹.

Making antibacterial drug therapy effective, safe and affordable has been focus of interest during recent years. There have been reports on antimicrobial activity of different herbal extracts. Considering the above aspects, an attempt has been made to carry out the screening for preliminary antibacterial activity of different plants used in Indian folk medicine.

MATERIALS AND METHODS:

Sample Collection: For the present study, alternative medicinal plants such as *Abutilon indicum*, *Hygrophila spinosa* and *Mimosa pudica* were selected. Sufficient quantities of these plants were collected in and around the regions of Chidambaram, Tamil Nadu, during June month of 2011. Specimens were cleaned for removing adhering soil/dust in the field by shaking and quick rinsing with tap water. Plants were placed in paper bag and transferred to the laboratory. Any remaining particles of soil were removed by use of pressurized airflow and by the use of a paintbrush and in some cases, by quick rinsing with distilled water.

Solvent Extraction: Leaf of the plant materials taken for this study was shade dried individually at room temperature and then powdered by using electric blender then, sieved the powder individually by using a nylon sieve in order to remove plant fibers. The large particles were again ground with the electric blender and sieved through a fine cloth (mesh size < 50 µm) to obtain the products with uniform particle size. 100 g of shade-dried powder was filled in the thimble and extracted successively with methanol by using Soxhlet

apparatus for 48hrs. The extract was collected in bottles and it is kept in vacuum drier for 3 days. The extract was concentrated to one-fifth of the original volume and stored at 4°C for further use.

Microorganisms and Culture Media: Microbial cultures such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *salmonella typhi* and *Salmonella paratyphi* were obtained from Raja Muthiah Medical College Hospital, Annamalai University, Chidambaram. Bacterial strains were maintained on nutrient agar slants (Hi-media).

Inoculum preparation: Bacterial cultures were subcultured in liquid medium (Nutrient broth) and incubated at 37°C for 8 hours and further used for the antibacterial assay (10⁵-10⁶ CFU/ml). The cell density was standardized spectrophotometrically (A600nm). These suspensions were prepared immediately before the test was carried out.

Assay for Anti-Pathogenic Activity: Assay for the anti-pathogenic activity of plant extracts were done by agar-well diffusion method using Muller Hinton Agar¹². 0.1ml of test organism was taken from the stock (broth) and swabbed on the agar medium. The methanol extract of above mentioned plants were dissolved in dimethyl sulphoxide (250mg/10ml). Different concentrations of extracts (25µl, 50µl, 100µl) were added into the wells. The diameter of the zone of inhibition (mm) around the well was measured after incubation at 24-28°C after 48hours. Kanamycin was used as positive control and DMSO as Negative control.

MICs were determined by broth dilution method. Duplicates of serial dilutions of broth with crude extract of *A. indicum*, *H. spinosa* and *M. pudica* were made. The MICs were determined against 1 x 10⁶ cells of each culture, as the lowest concentration of extract that reduced the growth of these microbes.

RESULTS AND DISCUSSION: The results showed unique characters of the plants in inhibiting bacterial growth. The anti pathogenic activity was interpreted after 24 hrs of incubation in different concentration. All the extracts showed good antibacterial activity against the tested pathogens with the methanol extract.

A. indicum demonstrating the highest activity (25 mm zone diameter of inhibition against *K. pneumoniae*, followed by the *M. pudica* extracts (23 mm zone diameter of inhibition) against *S. aureus*, while the *H. spinosa* extracts gives 22mm zone of inhibition in *P. vulgaris* at 100 µl (Table 1). The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumoniae* is the most important member of the

Klebsiella genus of Enterobacteriaceae and it is emerging as an important cause of neonatal nosocomial infection¹³. *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients. Infection caused by *Salmonella typhimurium* is a serious public health problem in developing countries and represents a constant concern for the food industry¹⁴.

TABLE 1: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF DIFFERENT PLANT EXTRACTS ON BACTERIAL PATHOGENS.

Pathogens	Zone of inhibition(mm)									Kanamycin (standard)
	<i>Abutilon indicum</i>			<i>Hygrophila spinosa</i>			<i>Mimosa pudica</i>			
	25µl	50 µl	100 µl	25µl	50 µl	100 µl	25µl	50 µl	100 µl	
<i>Escherichia coli</i>	5	7	15	4	7	15	6	10	14	33
<i>Pseudomonas aeruginosa</i>	5	9	15	7	13	17	8	12	16	34
<i>Staphylococcus aureus</i>	7	10	18	6	11	19	9	16	23	29
<i>Bacillus cereus</i>	4	8	12	4	7	10	5	9	12	30
<i>Proteus vulgaris</i>	6	13	16	8	16	22	6	11	14	28
<i>Enterococcus faecalis</i>	6	11	15	6	10	13	4	7	12	32
<i>Klebsiella pneumoniae</i>	9	15	25	8	12	18	7	12	16	35
<i>Vibrio cholera</i>	7	14	16	5	8	16	4	9	16	31
<i>Salmonella typhi</i>	8	12	16	5	9	14	5	9	18	35
<i>Salmonella paratyphi</i>	7	14	20	8	13	17	6	10	15	29

A. indicum has been used as a remedy for jaundice, piles, ulcer and leprosy in the Siddha System. A chemical compound, β-sitosterol is an active agent reported in *A. indicum*. The plant medicine is range from antibiotic to antitumor which gets more attention in these days. *H. spinosa* have been being used for as rejuvenators, immunomodulators and tonic¹⁵ Singh and Handa¹⁶ have reported that methanolic extracts of the seeds of the plant show hepatoprotective activity against paracetamol and thioacetamide intoxication in rats. Some potent anti-proliferative and

apoptotic effective compounds like alkaloid have been reported in *M. pudica*. The present study reported good antibiotic activity against the collected clinical pathogens. The MIC of the methanol extracts ranged from 0.2 to 0.9 mg/ml (Table2), with the *A. indicum* and *H. spinosa* extracts demonstrating the lowest values (MIC 0.2 mg/ml) against *E. coli* and *K. pneumoniae* respectively. Low MIC values were also an indication of high efficacy of herbal extracts against pathogens.

TABLE 2: MIC OF EXTRACTS OF DIFFERENT HERBAL LEAVES

Pathogens	MIC(mg/ml)		
	<i>Abutilon indicum</i>	<i>Hygrophila spinosa</i>	<i>Mimosa pudica</i>
<i>Escherichia coli</i>	0.2	0.7	0.5
<i>Pseudomonas aeruginosa</i>	0.5	0.3	0.3
<i>Staphylococcus aureus</i>	0.3	0.6	0.6
<i>Bacillus cereus</i>	0.7	0.8	0.4
<i>Proteus vulgaris</i>	0.4	0.5	0.7
<i>Enterococcus faecalis</i>	0.9	0.9	0.5
<i>Klebsiella pneumoniae</i>	0.3	0.2	0.4
<i>Vibrio cholerae</i>	0.8	0.4	0.6
<i>Salmonella typhi</i>	0.6	0.7	0.8
<i>Salmonella paratyphi</i>	0.7	0.5	0.9

Plant products provide unlimited opportunities for new drugs because of the unmatched availability of chemical diversity¹⁷. The methanol extracts of leaves of *Abutilon indicum* exhibited antimicrobial activity on

10 pathogens. *Hydrophilia spinosa* and *Mimosa pudica* also exhibits antimicrobial activity against 10 pathogens. There are several reports about the antimicrobial activity of methanol extracts prepared

from plants^{18, 19}. Although strategies have been proposed an attempt to control the spread²⁰, the search for new methods to treat infections stimulates the investigation of natural compounds as an alternative method to treat this infection.

The methanolic extracts of three ayurvedic antimicrobial herbals such as *A. indicum*, *H. spinosa* and *M. pudica* have the capacity to suppress the bacterial activity. The study by Bonjar²¹ observed that methanolic extracts of *Abutilon indicum*, *Hygrophila spinosa* showed maximum inhibitory effect against *E. coli* (20 and 21mm) respectively.

In recent years there has been a drastic development in the fields of science and technology extensively, due to certain side effects and drawbacks. Some countries have made it obligatory to switch over to natural products for many goals. Thus, like in other countries in the world, in India also the plants known by people are identified and used in the treatment of various diseases as traditional medicine. The present study showed that these three plants can be used in treatment against the infection disease.

CONCLUSION: In general in the folk medicine the plant latex was found to play a role in curing the wounds caused by the thorns of plants. This therapy using plant leaf extracts was familiar in many parts of India especially in Tamil Nadu. The antimicrobial property of the plant was due to the presence of some bioactive compounds. These three plants are used to treat the many diseases as shown in the study. The demonstration of activity against both gram-negative and gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. So, further research is needed to carry out for drug formation from these plants.

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