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Research article

EFFICACY OF EXTRACTS OF SIX MEDICINAL PLANTS OF INDIA AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT: The sensitivity of the pathogenic multi-drug resistant bacteria (*Aeromonas hydrophila, Bacillus licheniformis, Bacillus mycoides, Bacillus niacini, Bacillus subtilis, Escherichia coli, Geobacillus thermodenitrificans, Klebsiella pneumoniae, Paenibacillus koreensis, Paenibacillus larvae larvae, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas flourescens, Pseudomonas putida* and *Staphylocccus aureus*) was tested against aqueous, acetone and ethanol extracts of mature leaves of *Mimosa pudica* Linn. (Mimosaceae) and *Moringa oleifera* Lam. (Moringaceae), stems of *Michelia champaca* Linn. (Magnoliaceae) and *Musa paradisiaca* Linn.(Musaceae), roots of *Momordica charantia* Linn. (Cucurbitaceae) and *Murraya koenigii* Linn. (Rutaceae) by agar well diffusion method. Gatifloxacin was the most effective antibiotic against all the reference bacteria. Though all the extracts were found effective, the ethanol extract showed maximum inhibition against the test microorganisms followed by acetone and aqueous extract. *Bacillus niacini* is the most resistant bacteria and *Klebsiella pneumoniae* is the most sensitive bacteria against all the extracts used. MIC values of each bacterium were also determined.

Keywords: Antibacterial activity, medicinal plant, pathogenic bacteria, India

INTRODUCTION

The medicinal plants in the world are genuinely useful for primary health care. Plants have been the traditional source of raw materials for medicines. A rich heritage of knowledge on preventive and curative medicines was available in ancient scholastic work included in the Atharva veda, Charaka and Sushruta Samhitas. An estimate suggests that about 13,000 plant species worldwide are known to have use as drugs. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials (Akueshi *et al.* 2002; Anwar *et al.* 2000; Balakrishnan *et al.* 2006; Bhattacharjee *et al.* 2005, 2006; Borges *et al.* 2004; Chatterjee *et al.* 2007; Das *et al.* 1999; Doughari *et al.* 2007; Oumadevi *et al.* 2007). The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair *et al.* 2005).

The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto *et al.* 1995). The present article deals with the examination of six plants namely *Michelia champaca* Linn., *Momordica charantia* Linn., *Murraya koenigii* Linn., *Moringa oleifera* Lam., *Musa paradisiaca* Linn. and *Mimosa pudica* Linn. for their antibacterial activities against as many as fifteen pathogenic bacteria.

MATERIALS AND METHODS

The leaves of *Mimosa pudica* Linn. (Mimosaceae) and *Moringa oleifera* Lam. (Moringaceae), stems of *Michelia champaca* Linn. (Magnoliaceae) and *Musa paradisiaca* Linn.(Musaceae), roots of *Momordica charantia* Linn. (Cucurbitaceae) and *Murraya koenigii* Linn. (Rutaceae) were initially rinsed with distilled water and air-dried. Hot aqueous, acetone and ethanolic extracts of different plant parts were obtained from air-dried plant materials (Bauer *et al.* 1966).

The extracts (2,000 µg/ml) were stored as a stock solution in a refrigerator at 4°C until testing for antibacterial properties on fifteen bacterial strains: *Aeromonas hydrophila* MTCC 646, *Klebsiella pneumoniae* MTCC 432, *Bacillus licheniformis* MTCC 429, *Escherichia coli* MTCC 739, *Proteus vulgaris* MTCC 1771, *Pseudomonas aeruginosa* MTCC 2453, *Pseudomonas flourescens* MTCC 103, *Pseudomonas putida* MTCC 1654, *Staphylocccus aureus* MTCC 2940, *Bacillus mycoides* MTCC 8340, *Bacillus subtilis* MTCC 8322, *Bacillus niacini* MTCC 8323, *Geobacillus thermodenitrificans* MTCC 8341, *Paenibacillus koreensis* MTCC 8342, *Paenibacillus larvae larvae* MTCC 8343 (obtained from IMTECH Chandigarh, India). The bacteria were grown in nutrient broth (Hi media, M002) at 37°C and maintained in nutrient agar slants at 4°C. During the first experiment, the synthetic antibiotic sensitivity test discs (Span Diagnostics Limited, Surat, India) were used to determine antibiotic sensitivity profile/appearance of bacteria by the disc diffusion method (Chessbrough, 2000; NCCLS, 1993). Antibiotic sensitivity was tested in Müeller-Hinton agar plates. The second experiment consisted of antibacterial assay with the plant extracts using the agar well diffusion method (Perez *et al.* 1990). Wells (diameter, 6 mm) were punched in the agar (present in the 90-mm-diameter Petri plates) and filled with 30 µL of 2,000 µg/mL extracts. DMSO and distilled water were used as control. Antibacterial activities were evaluated by measuring the inhibition zone diameters. The third experiment consisted of determination of minimum inhibitory concentration (MIC) by dilution method (NCCLS, 1993; Paiva *et al.* 2003). Since the readings of control (distilled water and DMSO) experiments in the *in vitro* antibacterial studies of those plants were zero, the data were analyzed by simple arithmetic means of the different extracts and the standard errors were compared with the control.

RESULTS AND DISCUSSION

Antibiogram of the commonly used antibiotics is shown in Table 1. All the bacteria were sensitive to the new generation antibiotics except *B. subtilis* because of its complex growth requirements, definitive NCCLS cut off values for antibiotics sucesptibility and resistance have not been established. All the values given are the mean of the three sets of observations and for the sake of convenience it has been rounded off. Gatifloxacin was the most effective antibiotic against all the reference bacteria.

The antibacterial activities of all the extracts against all the test bacteria are shown in Table 2. Leaf extract seems to have greater antibacterial activity followed by stem and root respectively. *Bacillus niacini* is the most resistant bacteria and *Klebsiella pneumoniae* is the most sensitive bacteria against all the extracts used. The organic extracts of *Moringa oleifera* are comparatively more effective followed by *Mimosa pudica, Musa paradisiaca, Michelia champaca, Momordica charantia* and *Murraya koenigii.* The MIC value of the tested plant extracts having great potentiality (ethanol extract) against the tested microorganisms is shown in Table 3.

The antibacterial compounds extracted from these plants might inhibit bacteria by a different mechanism to that of currently used antibiotics and have therapeutic values as antibacterial agents.

All types of extracts of all the plants showed varied antibacterial efficacies against all the reference pathogenic bacteria causing human and animal diseases. The ethanol extracts showed best result followed by acetone and aqueous extracts. Aqueous extracts showed less activity than acetone and ethanol extracts possibly because of the presence of similar active substances in aqueous extracts, in low concentrations or active substances were soluble in organic solvents and, therefore, not present in aqueous extracts as also suggested by de Boer *et al.* 2005. The antibacterial action of the extracts is more pronounced on Gram - positive than on Gram-negative bacteria, and these findings corroborate to the observations of previous screenings (Nair *et al.* 2005; Rabe and Staden, 1997) of medicinal plants for antibacterial activity. The activity against both the types of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins. Although this study investigated the *in vitro* antimicrobial activity, the results substantiate the ethnobotanical use of the 6 studied plant species for the treatment of various bacteria related diseases. The results of this study support the use of these plants for human and animal disease therapy and reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances.

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Table 1. Susceptibility of bacterial strains to antibiotics

Bacterial strains	Antibiotics (in µg/ml)			
	Chloramphenicol (30)	Cotrimoxazole (25)	Gatifloxacin (10)	Gentamycin (10)
	Diameter of the inhibitory zones (in mm)			
Aeromonas hydrophila	23	25	26	18
Bacillus licheniformis	28	30	32	22
Bacillus mycoides	22	14	37	19
Bacillus niacini	0	0	32	22
Bacillus subtilis	30	38	39	21
Escherichia coli	0	27	37	22
Geobacillus thrmodenitrificans	34	7	28	20
Klebsiella pneumoniae	0	0	36	24
Paenibacillus koreensis	35	15	38	18
Paenibacillus larvae larvae	30	38	39	21
Proteus vulgaris	16	30	29	18
Pseudomonas aeruginosa	30	38	39	21
Pseudomonas fluorescens	28	6	11	0
Pseudomonas putida	0	6	19	10
Staphlococcus aureus	25	25	29	23

Table 2. Antibacterial activities of specific concentrations (30 mg/disc) of aqueous (AqE), ethanol (EE) and acetone (AE) extracts of six medicinal plants compared to control (distilled water and DMSO) and standard antibiotics (Ampicillin– 10 µg / disc)

Bacterial strains	Name of the plants																	
	Michelia champaca			*Momordica charantia*			*Murraya koenigii*			*Moringa oleifera*			*Musa paradisiaca*			*Mimosa pudica*		
	AE	AqE	EE	AE	AqE	EE	AE	AqE	EE	AE	AqE	EE	AE	AqE	EE	AE	AqE	EE
A. hydrophila	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	1	0	1
B. licheniformis	15	14	18	11	9	15	10	8	12	25	23	26	19	15	20	22	21	23
B. mycoides	0	0	1	0	0	1	0	0	0	3	2	5	1	0	2	2	1	4
B. niacini	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
B. subtilis	2	0	3	2	1	3	1	0	1	6	5	8	4	3	5	4	3	6
E. coli	7	6	10	8	5	9	7	5	8	12	11	14	10	9	11	10	9	12
G.thermodenitrificans	0	0	0	0	0	0	0	0	0	2	1	3	0	0	1	2	0	2
K. pneumoniae	16	15	19	13	11	16	11	9	13	26	25	28	19	17	22	23	20	24
Pa. koreensis	3	1	5	3	2	4	2	1	3	9	7	10	5	4	7	6	5	8
Pa. larvae larvae	5	3	6	5	3	5	3	2	4	8	7	10	6	5	7	7	6	9
Pr.vulgaris	1	0	2	1	0	1	0	0	0	5	3	7	2	1	3	3	2	5
Ps. aeruginosa	13	12	17	10	8	14	9	7	11	23	21	23	15	14	18	18	17	20
Ps.flourescens	11	10	15	10	7	13	8	7	10	19	18	20	14	12	17	17	15	19
Ps. putida	9	8	12	9	6	11	8	6	9	14	13	15	12	11	13	13	11	14
S. aureus	6	5	9	6	4	7	5	4	6	10	8	12	9	8	10	10	8	11

The values of control (distilled water and DMSO) and standard antibiotics (Ampicillin– 10 µg / disc) = 0

Table 3. . Minimum inhibitory concentration (MIC) of ethanol extract by dilution method

Bacterial Strains	*Michelia champaca*									*Momordica charantia*									*Murraya koenigii*								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
A	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
B	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
C	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-
D	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
E	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-
F	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
G	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-
H	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
I	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-
J	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-
K	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	-	-	-
L	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
M	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
N	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
O	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-

Bacterial Strains	*Moringa oleifera*									*Musa paradisiaca*									*Mimosa pudica*								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
A	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
B	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
C	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-
D	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-
E	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
F	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
G	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
H	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
I	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
J	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
K	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
L	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
M	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
N	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
O	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-

+ = Growth, - = No growth.
Concentration of the extracts are 0,5,10,15,20,25,,30,35,40 mg/ml are denoted as 1 ,2,3,4,5,6,7,8,9 respectively.
A= *Aeromonas hydrophila,* B= *Bacillus licheniformis,* C= *Bacillus mycoides,* D= *Bacillus niacini,* E= *Bacillus subtilis,* F= *Escherichia coli,* G= *Geobacillus thermodenitrificans,* H= *Klebsiella pneumoniae,* I= *Paenibacillus koreensis, J= Paenibacillus larvae larvae,* K= *Proteus vulgaris* ,L= *Pseudomonas aeruginosa,* M= *Pseudomonas flourescens,* N= *Pseudomonas putida,* O= *Staphylococcus aureus*

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