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

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ABSTRACT ■ The bactericidal activities of the aqueous, ethanol and acetone extracts of the leaves of two plants used as popular medicine in India were studied. The dried leaf extracts of *Moringa oleifera* Lam., and *Mimosa pudica* Linn. were tested in vitro by the disk diffusion method against four bacterial strains, namely, *Staphylococcus aureus* (MTCC 2940), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 739), and *Pseudomonas aeruginosa* (MTCC 2453). The organic and aqueous extracts of *Moringa oleifera* are comparatively more effective than *Mimosa pudica*. Aqueous extracts of the leaves of all the plants appeared to have less antibacterial activity than the ethanol and acetone extracts. The present study calls for further investigation to isolate and characterize the chemicals attributing antimicrobial property to the plants *M. oleifera* and *M. pudica.*

Key words: Bactericidal activity, disk diffusion method, *Moringa oleifera*, *Mimosa pudica*.

INTRODUCTION

Different national and international pharmaceutical companies are utilizing plant-based formulations in treatment of various diseases and disorders around the world (Gur et al., 2006; Bhattacharjee et al., 2006; Chatterjee et al., 2007; Shyur and Yang, 2008; Baquero et al., 2009; Bhattacharjee et al., 2010; Chatterjee et al., 2011).

Plants have been, and still are, a rich source of many natural products in major part of India and other countries, most of which have been extensively used for traditional human health care systems. The vast majority of people in the world takes care of themselves and uses healing plants that have been used for hundreds of generations. (Cordell, 1995; Farnsworth and Soejarto, 1991; Shengji, 2002; Taylor et al., 2001). India is a country of vast biodiversity and traditional knowledge of using herbal medicines to cure many ailments. It has nearly 20,000 species of plants of medicinal and economic importance. Indian System of Medicine, which includes Ayurveda and Siddha systems of medicine, depends on the medicinal herbs for the treatment of various ailments. Estimate of 250,000 flowering plants in the world (Heywood, 1993), more than 8000 species are weeds (Holm et al., 1979).

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The study of medicinally important weeds has not been realized as fully as other traditional communities elsewhere such as wild plants in forest ecosystems which often exclude weed species (Lawrence, 1959; Rosakutty et al., 1999; Prasad et al., 1996; Subramaniam, 1999).

In view of the rapid loss of diversity of plants, natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, documentation of medicinally important weeds is an urgent matter. Secondly, search for new medicines with low cost, more potential and without adverse side effect is needed to solve the major health problems (Green et al., 2005).

In view of the above, the present study has been designed to determine the extracts (aqueous extract, acetone and ethanol) of *Moringa oleifera* Lam. (sajina - Bengali), and *Mimosa pudica* Linn. (lajjabati -Bengali) for potential antibacterial activity against two gram positive bacteria i.e. *Staphylococcus aureus* (*S. aureus*) MTCC 2940 and *Bacillus subtilis* (*B. subtilis*) MTCC 441 and two gram negative bacteria i.e. *Escherichia coli* (*E. coli*) MTCC 739 and *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 2453. The observed inhibition zones were measured (in mm) and compared against standard antibiotics chloramphenicol, cotrimoxazole, gatifloxacin and gentamycin. The results of the present study are expected to highlight the prospective use of the plants in *M. oleifera* and *M. pudica* regulating microbes harmful for human and domestic animals.

2. Materials and methods

2.1. Plant materials collection

The plant materials used in this study consisted of mature leaves of *Moringa oleifera* Lam., and *Mimosa pudica* Linn., collected from outskirts of Vidyasagar University Campus (22°25'48"N, 87°17'44"E), W.B., India during spring season (mid-March to mid-April). The leaves were initially rinsed with distilled water and dried on paper towels in the laboratory at (37±1°C) for 24 hours.

2.2. Preparation and preservation of plant extract

2.2.1. Preparation of aqueous extract

Each of the two samples, which consisted of mature leaves of *Moringa oleifera* Lam., and *Mimosa pudica* Linn. was weighed out (50 g) and soaked separately in 500 mL of cold water contained in conical flasks fitted with rubber corks and left undisturbed for 24 h. They were then filtered off using sterile filter papers (Whattman No. 1) into clean conical flasks and subjected to water bath evaporation, where the aqueous water solvents were evaporated at boiling temperature of 100°C. The standard extracts thus obtained were then stored at 4°C in a refrigerator until further use.

2.2.2. Preparation of ethanol and acetone extracts

After drying, the plant materials were ground separately in a grinding machine (MX-110 PN, Japan) in the laboratory. Exposure to sunlight was avoided to prevent the loss of active components. The ethanol and acetone extraction fluid (500 ml) was mixed with 50 g each of powdered plant materials. The mixtures were then kept for 24 h in tightly sealed vessels at room temperature, protected from sunlight, and stirred thoroughly several times a day with sterile glass rods. The mixtures thus obtained were filtered through Whatman No. 1 filter papers and the residues adjusted to the required concentration (50 ml of ethanol and 50 ml of acetone for the residue of 50 g of powdered plant material) with the extraction fluid for further extraction. This was repeated three-times, and a clear colorless supernatant extraction liquid was finally obtained. The extracted liquids were subjected to rotary evaporation in order to remove the

ethanol and acetone. The semisolid extracts produced were kept at -80°C (REVCO model No. ULT 790-3-V 32) in a freezer overnight and then subjected to freeze-drying for 24 h at -60°C at 200 ml vacuum. Then the extracts were stored in an airtight container at 4°C in the refrigerator until further use. All the dried extracts were exposed to UV rays (200-400 nm) for 24 h and checked frequently for sterility by streaking on nutrient agar plates (Chessbrough, 2000).

2.3. Antibacterial assay

2.3.1. Disc diffusion method

Antibiogram was done by disc diffusion method (NCCLS M2-A5, 1993; Bauer et al., 1966) using plant extracts and commonly used antibiotics. The test quantity of specific extracts, as shown in Tables 1 and 2, were dissolved in either distilled water or dimethylsulphoxide (DMSO), depending upon the solubility of the extracts. The dissolution of the organic extracts (ethanol and acetone) was aided by 1% (v/v) DMSO and that of the aqueous extracts with water, which did not effect the growth of microorganisms, in accordance with our control experiments. The surfaces of media were inoculated with bacteria from a broth culture. High-potency bio-discs (Himedia) were placed on the agar. After 18 h of incubation at a specific temperature [(30±1) °C for *B. subtilis* and 37°C for *S. aureus*, *E. coli*, and *P. aeruginosa*], the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter.

2.3.2. Dilution method for minimum inhibitory concentration

Of the two plants tested, the ones that showed antibacterial activity against some of the selected pathogens were selected for further tests to calculate their minimum inhibitory concentration (MIC) by dilution method. These tests were performed in sterile 96-well microplates and macroplates. The microdilution was performed in 96-well microtiter plates with U-shaped wells, while the macrodilution technique was as described by the National Committee for Clinical Laboratory Standards was followed (NCCLS, 1993; Paiva et al., 2003). In brief, the cultures were diluted in Müeller-Hinton broth at a density adjusted to 0.5 McFarland turbidity. The final inoculum was 5×10^5 CFU/ml of bacterial colony. Controls with 0.5 ml of culture medium only or others with plant extracts were used in the tests. The wells were filled with 100 ml of sterile H_2O, and 100 ml of the plant extracts were added to the wells by serial two-fold dilution from the suspension of plant extract stock solution. Each well was inoculated with 100 ml of 0.5 McFarland standard bacterial suspensions so that each well received 5×10^5 CFU/ml. The plates were covered, placed in plastic bags, and incubated at 37°C for 24 h. The experiments were conducted thrice. In this study, the MIC was the lowest concentration of plant extracts that exhibited no growth of the organisms in the wells by visual reading.

2.3.3. Test microorganisms

Gram positive bacteria *S. aureus* MTCC 2940 and *B. subtilis* MTCC 441, and Gram-negative bacteria *E. coli* MTCC 739 and *P. aeruginosa* MTCC 2453 were used in this study. All the tested strains are reference strains and were collected from the Microbiology Laboratory of Burdwan Medical College. The bacterial cultures were maintained in nutrient broth (Himedia, M002) at 37°C and maintained on nutrient agar (Himedia, MM012) slants at 4°C.

2.3.4. Statistical analysis

Since the readings of control (distilled water) 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.

3. Results

The antibacterial activities of the leaf extract of *Moringa oleifera* Lam., and *Mimosa pudica* Linn. in different solvents (aqueous, ethanol and acetone) against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus* are shown in Table 1. In Table 1, all the bacterial strains were found to be effective against all the extracts with varying inhibition diameters. The ethanol extracts of the plants were comparatively more effective than their aqueous counterparts against the bacterial strains except *P. aeruginosa*. Thus, from Table 1, it is evident that the organic extracts are comparatively more effective than the aqueous extracts. The organic and aqueous extracts of *Moringa oleifera* are comparatively more effective than *Mimosa pudica*. Antibiogram of the commonly used antibiotics is shown in Table 2, and the MIC value of the tested plant extracts against the tested microorganisms is shown in Table 3.

Table 1. Antibacterial activity of specific concentration (30 mg/disc) of aqueous (AqE), ethanol (EE) and acetone (AE) extracts of two medicinal plants compared to control (distilled water and DMSO) and negative control Nystatin by disc diffusion method.

Bacterial Strains	Moringa oleifera (Inhibition Zone Diameter in mm) in mm)			Mimosa pudica (Inhibition Zone Diameter		
	AE	AqE	EE	AE	AqE	EE
B. subtilis	6.0 ± 0.29	5.0 ± 0.17	4.0 ± 0.23	4.0 ± 0.05	3.3 ± 0.58	16.00 ± 0.23
E. Coli	12.3 ± 29	11.0 ± 0.47	14.6 ± 0.47	10.0 ± 0.23	9.00 ± 0.23	12.00 ± 0.17
P. aerugiosa	23.6 ± 0.05	21.0 ± 0.17	13.0 ± 0.17	18.00 ± 0.58	17.60 ± 0.05	10.60 ± 0.58
S.aureus	10.3 ± 0.23	8.0 ± 0.58	22.0 ± 0.58	10.60 ± 0.29	8.60 ± 0.47	27.00 ± 0.05
Distilled water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
* DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nystain	–	–	–	–	–	–

*DMSO: dimethylsulphoxide.

-, no inhibition zone.

Table 2. Susceptibility of four reference bacterial strains to some antibiotics in nutrient agar.

	Diameter of the inhibitory zones (mm)			
Antibiotics (µg/ ml)	*P. aeruginosa*	*S. aureus*	*E. coli*	*B. subtilis*
Chloramphenicol (30)	29.3	25.7	0	30
Cotrimoxazole (25)	36.0	25.0	27.0	38.3
Gatifloxacin (10)	38.30	29.30	37.0	39.7
Gentamycin (10)	24.60	23.00	22.30	21.00

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

	Moringa oleifera									*Mimosa pudica*								
Bacterial Strains	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
B. subtilis	+	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
E. coli	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
P. aeruginosa	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
S. aureus	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-

+, =Growth, -, = no growth

4. Discussions

The results indicate that the crude extracts of all plants studied showed antibacterial activities toward the Gram-positive bacteria (*S. aureus* and *B. subtilis*). These results are consistent with previous reports on related plants with respect to Gram-positive bacteria (Cowan, 1999; Chatterjee et al., 2007). The resistance of Gram-negative bacteria (*E. coli* and *P. aeruginosa*) to plant extracts was expected as, in general, this class of bacteria is comparatively more resistant than the Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan & AbuHasan, 1998). Infections caused by these bacteria, especially those with multidrug resistances, are among the most difficult ones to treat with conventional antibiotics (CDC NNIS System, 1999). Therefore, it seems likely that the antibacterial compounds extracted from these plants might inhibit bacteria by a different mechanism than that of currently used antibiotics and have therapeutic values as antibacterial agents.

The MIC of the crude extracts of individual plant varies against different test strains. The relationship between zone of inhibition and MIC value may or may not be related. The crude extracts have mixture of phytoconstituents, which may influence the diffusion power of the active constituents. Several workers have made similar observations by using essential oils or complex mixture from higher plants (Ahmad and Aqil, 2007; Arjuna et al., 2011; Tewari et al., 2011; Idogun and Kasia, 2011). Therefore direct relationship of zone of inhibition size with MIC value is expected with pure compounds not with crude extracts. On the other hand, these test strains may have different levels of intrinsic tolerance to antimicrobials and thus the MIC values differ from isolate to isolate.

It appears that the microorganisms were not as sensitive to the aqueous extracts compared to the methanol extracts as determined by diffusion. The reasons for this could be that all of the identified components from plants active against microorganisms, aromatic, or saturated organic compounds are most often obtained through initial methanol extraction (Cowan, 1999).

In conclusion, it is suggested that these plants may be used to discover natural bioactive products that might lead to the development

of new drugs. Such screening of various natural organic compounds and identification of active agents must be considered as a fruitful approach in the search for new pharmaceuticals.

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