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Full Length Research Paper

Antibacterial activity of seed extracts of *Argemone mexicana* L. on some pathogenic bacterial strains

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Antibacterial activity of seed extracts of *Argemone mexicana* L. (Papaveraceae) was evaluated against some pathogenic bacterial strains. Chloroform extract of seeds exhibited varying level of antibacterial activity, with minimum inhibitory concentrations (MIC) of 2.0 - 5.0 mg/ml, against both Gram-positive and Gram-negative bacteria. The chloroform extract was found to be more active than the other extracts against all the test bacteria. MIC values were 2.0 and 3.0 mg/ml, respectively, for *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their respective drug-resistant strains. The sensitivity of the test bacteria varied with the species and strains. The study provides basis for the isolation and purification of antibacterial compound(s) from the seeds of *A. mexicana* L.

Key words: *Argemone mexicana*, antibacterial activity, disk diffusion method, drug resistant.

INTRODUCTION

The genetic ability of pathogenic bacteria to develop resistance against commonly used antibiotics is a major medical problem and challenge worldwide, posing a big threat to human society (Cohen, 1992; Neu, 1992; Yurdakok et al., 1997). This has necessitated a search for novel antibacterial substances from various natural sources, including flowering plants. A wide variety of plant secondary metabolites have been identified as active principles for the treatment of various ailments (Taylor et al., 2001; Ncube et al., 2008). Some plants have shown the ability to overcome resistance in such organisms which led the researchers' to isolate active principles and investigate mechanisms. A number of studies have been conducted for the selection of the crude plant extracts in a therapeutic treatment of bacterial infections (Ikram and Inamul, 1984; Izzo et al., 1995; Bhattacharjee et al., 2006).

The discovery and development of structurally novel chemical entities to control the multi-drug resistant pathogenic bacteria is desperately desired by the pharmaceutical industries and drug developers, which are looking towards the underexplored natural sources for developing the front line drugs. The use of the plant extracts and the phytochemicals can be of great significance in therapeutic treatments and could be helpful to curb the problem of these multi-drug resistant microorganisms. The growth of the multi-drug resistant *Pseudomonas aeruginosa* was inhibited by the extracts from *Caryophyllus aromaticus* (clove), *Syzygyum joabolanum* (jambolan), *Punica granatum* (pomegranate) and *Thymus vulgaris* (thyme) (Nascimento et al., 2000). World Health Organization (WHO) has also advocated that the medicinal plants would be the best source for obtaining a variety of drugs (Basso et al., 2005).

A number of plant secondary metabolites like alkaloids and flavonoids have been used as antiviral, antibacterial, antiamoebal and anticancer agents (Ahn, 1994; Silva et al., 1996; Iwu, 1999; Iwu et al., 1999; Scheck et al., 2006). The glycoside and saponins from *Quillaja saponaria* and *Acacia auriculoformis* were found to be antiprotozoal *in vitro* (Wallace, 2004). Phenolics and polyphenols are the other group of plant secondary metabolites that exhibit antimicrobial activity. Flavones, flavonoids and flavonols, synthesized by the plants in response to the microbial infection, have antimicrobial activities against a wide array of the microorganisms (Bennett and Wallsgrove, 1994).

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A. mexicana L. (Papaveraceae), possess the analgesic, narcotic, antispasmodic and sedative properties. The fresh yellow, milky, seed extract contains protein-dissolving substances which are effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches and also in dropsy and jaundice. In Mexico, the seeds have been used as an antidote to snake poisoning (Chopra et al., 1986; Bhattacharjee et al., 2006).

In view of the above, an attempt was made to evaluate the potential of *A. mexicana* L. seed extracts against standard bacterial strains as well as multi-drug resistant bacteria, isolated from hospital. Three Gram-negative (*P. aeruginosa*, *Escherichia coli* and *Salmonella typhi*) and three Gram positive (*S. aureus, Enterococcus* sp. and *Staphylococcus* sp.) bacteria were screened for therapeutic potential of extracts.

MATERIALS AND METHODS

Plant material

Seed-bearing healthy plants of *A. mexicana* L. were bought from a local herbal market, and the plant was authenticated at the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University (B.H.U.), Varanasi. The fresh seeds were collected from plants and washed thrice with distilled water and dried on blotting paper in laboratory at 37 ± 1°C for 24 h. After drying, seeds were powdered in a grinding machine.

Preparation of extracts

The powdered seed material was filled in a soxhlet apparatus and extracted with methanol for 3 - 4 days. The extract was taken out from the soxhlet and filtered using sterile filter paper (Whatmann No.1) in a conical flask and subjected to water bath evaporation.

The methanolic extract, a semi-solid gummy brown mass, was passed through silica gel column chromatograph eluting with different solvents of increasing polarity such as hexane, chloroform, methanol and water. The individual eluate was monitored by thin layer chromatographic technique and tested for the chemical nature of compounds such as alkaloid, steroid, tannin, flavonoid, saponin, glycoside etc. These eluates (extracts) obtained were stored in a refrigerator at 4°C for further experimentation.

Qualitative assay

Sensitivity of bacterial strains to the commonly used antibiotics and to aqueous and organic extracts of *A. mexicana* seeds was assayed by the modified Kirby Bauer Disk Diffusion susceptibility method (Bauer et al., 1966). The bacterial strains (4 - 5 colonies) to be tested was suspended in 4 ml of normal saline (0.85%) and the density of suspension was adjusted to approximately 10^8 CFU ml^{-1} using a 0.5 M barium sulphate suspension as the turbidity standard. The surface of the sterile 3.8% MH (Mueller Hinton) agar in the petri dishes was dried and the test bacteria were inoculated separately with a sterile swab to obtain a bacterial lawn. High potency antibiotic disks (Hi-media) were placed on the MH agar to determine the inhibition zones produced by different antibiotics.

From each extract of seeds, 10 µl aliquots was aseptically transferred to sterile paper disks (6 mm diameter) prepared from Whatmann No.1 filter paper. The extract impregnated disks were placed directly on the bacterial lawn. After incubation of plates for 18 h at 37^0C the diameter of the inhibition zones was measured. Sterile distilled water and DMSO were taken as a control for aqueous and organic extracts, respectively. The dissolution of organic extracts was aided by 1% (v/v) DMSO and that of aqueous extracts with water which did not affect the growth of test bacteria.

Quantitative assay

Agar dilution method was used to determine the Minimum Inhibitory Concentrations (MICs) of extracts against test bacteria (National Committee for Clinical Laboratory Standards, 1997). The lowest concentration of an extract at which a test bacterium did not show any visible growth was taken as its MIC. Chloroform extract (1 ml) of different concentration was added to 19 ml of MH agar in order to achieve the concentration range of 1.0 mg to 10.0 mg/ml. Plates were dried and divided into sectors based on the number of organisms. Bacterial cultures grown overnight to population density of 10^8 $CFUmL^{-1}$ were applied to sectors, each marked for the inoculation of single test bacterium. Plates were observed following incubation at 37°C for 18 h.

Test organisms

The bacterial strains used for the study were obtained from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The strains, *E. coli*, *P. aeruginosa*, *S. typhi, S. aureus* and *Enterococcus* sp. were sensitive to antibiotics. The 2 strains *S. aureus* and *P. aeruginosa,* resistant to Gentamycin and Oflaxacin antibiotics, were isolated from hospital.

RESULTS AND DISCUSSION

The extraction of biologically active compounds from the plant material depends on the type of solvent used in the extraction procedure. The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol and water (Bisignino et al., 1999; Lourens et al., 2004; Parekh et al., 2005 Rojas et al., 2006). Most of the antimicrobial active compounds that have been identified were soluble in polar solvents such as methanol instead of water (Cowan, 1999; Parekh et al., 2006). The methanol extract of *A. mexicana* seeds was chromatographed over silica gel column eluting with different organic solvents with increasing polarity to separate the components in each solvent for their antibacterial property and chemical analysis.

The results for metabolites screening (Table 1) of seeds of *A. mexicana* extracted in different solvents revealed the presence of various metabolites in different solvents. Alkaloids and flavonoids were found in chloroform and methanol extracts, whereas, glycosides and saponins were present in methanol and water extracts. Steroids and tannins were detected in hexane and water extracts, respectively. A number of plant secondary metabolites like alkaloids and flavonoids have been reported as anticancer, antiviral, antibacterial and antiamoebal agent (Silva et al., 1996; Iwu, 1999; Iwu et

Table 1. Preliminary phytochemical screening of the aqueous and organic solvent extracts of the seeds of *A. mexicana.*

Tested group	Hexane extract	Chloroform extract	Methanol extract	Water extract
Tannins	-	-	-	+
Alkaloids	-	+	+	-
Glycosides	-	-	+	+
Saponins	-	-	+	+
Flavonoids	-	+	+	-
Steroids	+	-	-	-

+ = Present, - = absent.

Table 2. Sensitivity of the different bacterial strains to the various extracts (500 mg/ml) of the seeds of *A. mexicana*.

Bacterial sp.	Hexane extract	Chloroform extract	Methanol extract	Water extracts
E. coli	-	+ + +	-	-
P. aeruginosa	-	+ + +	+ +	-
Enterococcus sp.	-	+ + +	-	-
S. typhi	-	+ + +	+ +	-
S. aureus	-	+ + +	+ +	-
P. aeruginosa^{R}	-	+ + +	-	-
S. aureus^{R}	-	+ + +	-	-

- = No inhibition; ++ = inhibition zone ≤10 mm; +++ = inhibition zone > 10 mm; ^{R} = resistant strain.

al., 1999; Scheck et al., 2006) while glycosides and saponins have antiprotozoal activity (Wallace, 2004).

The sensitivity of bacterial strains to various extracts (Table 2) revealed that the seeds' extract in the chloroform was inhibitory to the test organisms *E. coli*, *P. aeruginosa*, *Enterococcus* sp., *S. typhi*, *S. aureus* and also for the resistant strains *P. aeruginosa*^{R} and *S. aureus*^{R}. Methanol seed extracts were observed inhibitory to *P. aeruginosa*, *S. typhi*, and *S. aureus*. The chloroform extract of seeds was found more (> 10.0 mm) inhibitory in comparison to methanol extracts. However, no inhibitory activity was observed in the water and hexane extracts of seed.

A. mexicana seed extract in water had no antibacterial property. The water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics are only important as antioxidant compounds (Yamaji et al., 2005; Nang et al., 2007). Different concentrations of the seed extracts in the chloroform were tested to find out the inhibition zone for the bacterial strains. The lowest concentration (1.0 mg/ml) was not effective against any of the test organisms. However, inhibition zones were observed at the increasing concentrations (10.0 mg/ml - 50.0 mg/ml). Results revealed that the concentrations dependent inhibition zone in the test organisms produced by chloroform extracts with minimum inhibition at lowest concentration (10.0 mg/ml) and maximum inhibition at highest concentration (50.0 mg/ml) taken in the study (Table 3). The sensitivity of the test bacteria to a particular concentration of chloroform extract varied with the species/strains, which can be attributed to difference in the permeability of bacterial cell wall or membrane and/or other species or strain specific characteristics of bacteria.

On the basis of above results, it was desired to find out the inhibition potential of the seed extract (1.0 - 10.0 mg/ml) to establish the minimum inhibitory concentrations (MIC) for the test organisms. The minimum inhibitory concentrations of the chloroform extracts of seeds against test bacteria varied from 2.0 to 5.0 mg/ml (Table 4). The lowest MIC (2.0 mg/ml) with zone of inhibitions (7.0, 9.0 and 9.0 mm) were observed in *E. coli*, *S. aureus* and *S. aureus*^{R} respectively. The minimum inhibitory concentration (3.0 mg/ml) with zone of inhibitions (9.0, 10.0 and 8.0 mm) was observed against *P. aeruginosa*, *S. typhi* and *P. aeruginosa*^{R}, respectively. The highest MIC (5.0 mg/ml) with zone of inhibition (8.0 mm) was recorded in *Enterococcus* sp. It was interesting to observe that extract was effective against strains which were sensitive or insensitive to antibiotics to Gentamycin and Oflaxacin and also MIC values were same for sensitive strain and their respective strain which were insensitive to antibiotics. MIC provides an idea of the effectiveness of an active extract or a compound against a microorganism. There is inverse relationship between MIC of an extract or a compound and the sensitivity of a microorganism to it.

The results of the study provides basis for subsequent bioactivity- guided fractionation of the extracts of the

Table 3. Evaluation of the various concentration of the chloroform extracts for inhibition zone against the different bacterial strains.

Organism	Chloroform extracts (mg/ml)					
	1.0	**10.0**	**20.0**	**30.0**	**40.0**	**50.0**
E. coli	0.0*	13.0	19.0	22.0	23.0	25.0
P. aeruginosa	0.0	13.0	16.0	17.0	19.0	20.0
Enterococcus sp.	0.0	12.0	14.0	15.0	16.0	19.0
S. typhi	0.0	14.0	16.0	17.0	19.0	21.0
S. aureus	0.0	16.0	21.0	22.0	26.0	27.0
P. aeruginosa^R	0.0	12.0	14.0	16.0	18.0	19.0
S. aureus^R	0.0	14.0	18.0	20.0	24.0	26.0

*Values are Inhibition zone (mm).

Table 4. The zone of the inhibition and MICs of chloroform extracts against the different bacterial strains.

Organism	Inhibition zone (mm)	MIC (mg/ml)
E. coli	7.0	2.0
P. aeruginosa	9.0	3.0
E. Sp.	8.0	5.0
S. typhi	10.0	3.0
S. aureus	9.0	2.0
P. aeruginosa^R	8.0	3.0
S. aureus^R	9.0	2.0

seeds of *A. mexicana* and isolation of pure antibacterial compound(s).

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