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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF *ARGEMONE MEXICANA* LEAVES

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Abstract

A total of 6 bacteria, two Gram positive (*Bacillus subtilis* MTCC 619 and *Staphylococcus aureus* MTCC 2940*),* four Gram negative bacteria (*Escherichia coli* MTCC 118*, Pseudomonas aeruginosa* NCIM 2074*, Proteus vulgaris* NCIM 2817*, Salmonella typhi* NCIM 2501) and 4 fungi (*Aspergillus niger* NCIM 589*, Fusarium moniliforme* NCIM 1099*, Candida albicans* NCIM 3100 and *Mucor plumbeus* NCIM 984) were tested for antibacterial and antifungal activity against methanolic extracts of leaves of *Argemone mexicana* by agar well diffusion method. Among different concentrations (40µl, 70µl, 100µl) of leaf extracts studied 40µl showed no activity against bacteria and fungi. Whereas, 100µl showed maximum inhibition against all the test organisms under investigation. While, 70µl showed activity against *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* and all the fungi under study.

Key words: *Argemone mexicana,* antibacterial, antifungal activity.

Introduction

Plants have always been a rich source of lead compounds (e.g. morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine). Many of these lead compounds are useful drugs in themselves (e.g. morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anaesthetics developed from cocaine). Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree, and the antimalarial agent artemisinin from *Artemisia annua*. Plants provide a large bank of rich, complex and highly varied structures which are unlikely to be synthesized in laboratories. Furthermore, evolution has already carried out a screening process itself whereby plants are more likely to survive if they contain potent compounds which deter animals or insects from eating them. The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments. According to the WHO (Santos *et al.*1995), medicinal plants would be the best source for obtaining a variety of drugs. About 80% populations of the developed countries use traditional medicines, derived from medicinal plants. Therefore, such plants should be investigated thoroughly to determine their structural and functional properties, as well as the efficiency of various parts (Ellof, 1998). Even today, the number of plants that have been extensively studied is relatively very few and the vast majority has not been studied at all.

Argemone mexicana (Mexican Poppy, Mexican Prickly Poppy or Cardosanto), an annual herb with bright yellow sap is used as a medicinal plant in several countries. In Mexico, the seeds are considered as an antidote to snake venom. In India, the smokes of the seeds

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are used to relieve toothache. The fresh yellow, milky seed extract contains protein-dissolving substances, effective in the treatment of warts, cold sores, cutaneous infections, skin diseases, itches, and also dropsy and jaundice (Chopra *et al*. 1986).

The plant contains alkaloids as beriberin, protopine, sanguinarine, optidine, chelerythevine, out of which berberine plays a vital role in treatment of trachoma and leishmaniasis (Watt and Breyer Brandwijlei, 1962). Cheng *et al* (2003) reported two new protopines agemexicainies A and B and the anti HIV alkaloids 6-acetonyldihydrochelerythevine from *Argemone mexicana* which contains various fatty acids. *Argemone mexicana* is used by traditional healers in Mali to treat malaria (Willcox *et al*., 2007)

The plant is diuretic, purgative and destroys worms and it is also effective in wound healing. The whole plant is analgesic, antispasmodic, possibly hallucinogenic and sedative (Emboden, 1979; Chevallier, 1996). It contains alkaloids similar to those in the opium poppy (*Papaver somniferum*) and so can be used as a mild pain-killer (Chevallier, 1996).

The present study was aimed to determine the role of methanolic extract of leaves of *A. mexicana* for potential antibacterial and antifungal activity, against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), four Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Salmonella typhi)* and four different fungi (*Aspergillus niger, Candida albicans Fusarium moniliforme, and Mucor plumbeus).*

Materials and methods

Collection of plant material - The leaves of *A. mexicana* were collected in fresh polythine bags from tribal area of papikondalu in Khammam district, Andhra Pradesh, India and brought to laboratory. The leaves were initially rinsed with distilled water to remove soil and other contaminants and dried on paper towel in laboratory at 37 ± 1^{o} C for 24 h.

Methanol extract – The leaf material after drying, was ground in a grinding machine in the laboratory then 50g was weighed and mixed with 200 ml of a methanol in separating funnel and mixed several times by shaking, the mixture was kept for 24 h at room temperature. After 24h the mixture was filtered through Whattman no. 1 filter paper and the filtrate was adjusted to the required concentration (50 ml of methanol for the residue of 50 g of powdered leaf material). The extracted liquid was subjected to rotary evaporation in order to remove the methanol and stored in refrigerator for further study.

Test microorganism – Six bacterial cultures were used in the present study. Two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*), four Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris*) and 4 fungal cultures (*Aspergillus niger, Fusarium moniliforme, Candida albicans, Mucor plumbeus*)**.**All the tested strains were obtained from culture bank of our laboratory. The bacteria were grown in nutrient broth (Himedia, M002) at 37^{o}C and maintained on nutrient agar slants at 4^{o}C and fungal cultures were grown and maintained on potato dextrose agar slants at 4^{o}C

Agar-well diffusion method - The assay was conducted by agar well diffusion method (Collins, 1976). The bacterial strains were grown on nutrient agar at 37^{o}C for 18 h and were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). The suspension was used to inoculate 90 mm diameter petri. Wells (6 mm diameter) were punched in the agar and filled with the methanol extract dissolved in DMSO to get different concentrations viz. 40µl, 70µl, 100µl of the extract. Controls were maintained by inoculating pure DMSO, which did not affect the growth of microorganisms. Plates were incubated at 37^{o}C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were conducted in triplicates. The same method was followed for testing antifungal activity using potato dextrose agar medium.

Disc diffusion method – Disc diffusion method was followed by taking Kanamycin as standard antibiotic for bacteria and Flucanazole as standard for fungi. The surfaces of the media were inoculated with bacteria and fungi separately. High potency bio-discs (Himedia) were placed on the agar. After 18 h of incubation at a specific temperature (30 ± 1) ^{oC} for *B. subtilis* and 37^{o}C for *S. aureus, E. coli* and *P. aeruginosa*, and after 7 days incubation at 22 ± ^{oC} for *Aspergillus niger, Fusarium moniliforme, Candida albicans* and *Mucor plumbeus,* the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter.

Results and Discussion

In the present investigation six bacterial species viz. *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris and* 4 fungal species viz. *Aspergillus niger, Fusarium moniliforme, Candida albicans* and *Mucor plumbeus* were tested to determine antibacterial and antifungal activity of methanolic extract of *Argemone mexicana leaves.* The values given in the tables are the mean of the three sets of observations.

Methanol extract of leaves of Argemone *mexicana* at concentrations of 40µg, 70µl and 100µl were used to study anti bacterial and antifungal activity. The concentration of 40 µl could show no activity against different bacteria under study. 70µl concentration of leaf extract of *Argemone mexicana* could inhibit the growth of *Escherichia coli* to a maximum extend followed by *Proteus vulgaris* and *Staphylococcus aureus* while,

rest of the bacterial cultures viz. *Bacillus cereus Pseudomonas aeruginosa, Salmonella typhi* were resistant (table1). At 100µl concentration growth of all the six bacterial species were found to be inhibited. *E.coli* was found to be inhibited to maximum extent while, *Pseudomonas aeruginosa* was found to be inhibited to least extent. Kanamycin was used as standard antibiotic against all the six bacterial cultures under study and was found to inhibit all the six bacterial cultures at concentration of 30µg/ml. Whereas, *Escherichia coli, Staphylococcus aureus* and *Proteus vulgaris* were found to be more inhibited by leave extract of *Argemone mexicana* when compared to standard antibiotic Kanamycin.

Antifungal activity of leaf extract of *Argemone mexicana* was performed against four fungi and the results are presented in the table 2. All the concentrations 40µl, 70µl and 100µl inhibited the growth of four fungi under study. *F.moniliforme* and *Mucor plumbeus* was inhibited to maximum extent followed by *A. niger* and *C.albicans.* Flucanazole was used as standard antibiotic against fungi under study and found to inhibit all fungi under study at concentration of 10µg/ml. Leaf extract of Argemone *mexicana* was found to show maximum inhibition of all the fungi under study when compared to standard antibiotic Flucanazole.

Based on previous reports and present investigation it can be said that the plant possess wide range of anti bacterial and antifungal constituents. Further investigations should be carried out in finding other activities of the extracts of leaf, root and stem.

Table.1 **Antibacterial activity of methanolic extract of *Argemone mexicana* leaves and standard antibiotic Kanamycin**

Bacteria	**Diameter of the inhibitory zones (mm) against different concentrations of leaf extracts**			**Kanamycin**
	40µl	70µl	100µl	30µg/ml
Bacillus cereus	--	--	14	26
Escherichia coli	--	19	24	10
Pseudomonas aeruginosa	--	--	12	33
Proteus vulgaris	--	17	20	09
Salmonella typhi	--	--	08	12
Staphylococcus aureus	--	13	15	11

Table.2 **Antifungal activity of methanolic extract of *Argemone mexicana* leaves and standard antibiotic Flucanazole**

Fungi	**Diameter of the inhibitory zones (mm) against different concentrations of leaf extracts**			**Flucanazole**
	40µl	70µl	100µl	10µg/ml
Aspergillus niger	11	13	19	15
Fusarium moniliforme	39	40	43	12
Mucor plumbeus.	19	22	26	14
Candida albicans	09	12	14	14

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