Evaluation of Antimicrobial Activity of *Argemone mexicana*

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Abstract:

The pathogenic multi drug resistant bacterial strains were tested against the extracts (aqueous, ethanol & methanol) of leaves and stem of *Argemone mexicana L* employing Agar well diffusion assay and turbidometric assay. It was found S.typhi exhibited least sensitivity towards the all extracts while P.aeuroginosa and E.coli were found to be most sensitive. The Agar well diffusion assay, Disc diffusion assay and Turbidometric assay were employed for the present study. The comparative analysis with standard antibiotics reveled that extract of leaves and stem of Argemone mexicana demonstrated effect against MDR bacterial strains.

Introduction: The present investigation carried out to assess the antimicrobial potential of *Argemone mexicana*. It is generally known as Pivala Dhotra, Yellow Poppy because of its yellow flower. The seeds of the *A. mexicana* are used as adulterant for oil. Traditional healers use *A. mexicana* to treat Malaria [10] Ayurveda reported that the plant is purgative, diuretic and destroys worms. It cures skin-diseases, leprosy and inflammation bilious fevers. Roots are equally used as antihelmenthic. Juice is used to cure opacity of cornea and ophthalmia. Seeds are purgative and sedative. In Mexico the seed is used as an antidote to snake poisoning and the fresh yellow milky seed extract contains protein-dissolving substances, effective in the treatment of warts, coldsores, cutaneous infections, skin diseases, itches and also dropsy and jaundice [12]. Our earlier investigation revealed the antimicrobial potential crude extract of *A. mexicana* [21].

Materials and Methods:

Preparation of the Disc: The discs of Whatman filter paper No.1 (5 mm) were taken and sterilized in autoclave at 15 lbs for 20 minutes. On these sterile discs the solvent extracts aliquots ranging 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl with respective concentrations of solvent extracts of *A. mexicana* plant material with micropipette and were allowed to soak. The discs were further used for experimental purpose.

Agar Well Diffusion Assay: Agar well diffusion assay was employed to check the susceptibility of the test microorganisms against solvent extracts of *A. mexicana*. Assay was conducted by using antibiotic sensitivity medium. The pH of the medium for bacterial cultures was maintained 7.1 and for fungi it was 6.8 respectively. The selected pathogens were seeded and plates were prepared by using standard spread plate technique. Five mm well was bored on the agar base with the help of cork borer. For determination of MIC of *A. mexicana* extract was taken by using micropipettes in five different aliquots i.e. 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl respectively. Then the plates were kept at 4^0 C for 5 minutes for proper diffusion. Afterwards the plates were incubated at 37^0 C for 24 hrs and the antimicrobial activity of various solvent extracts was recorded as inhibitory zone [2].

Turbidometric Assay: The turbidometric method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform distribution of an antimicrobial compounds. The turbidometric assay was employed to evaluate the sensitivity of the test pathogen in liquid culture. In this assay of the 24 hrs old culture (1ml) was inoculated in sterile nutrient broth (10 ml) and to this the 10^{-1}, 20^{-2}, 30^{-3}, 40^{-4}, and 10^{-5} dilutions of

solvent extract (1ml) were added and allowed to incubate for 24 hrs at 37^0 C. After incubation the growth in terms of turbidity of the bacterial cultures was measured spectrophotometrically at 600 nm [18]. The readings were compared with that of the controls.

Determination of Minimal Inhibitory Concentration (MIC): MIC was determined by both agar and broth dilution methods. For broth dilution tests, 0.1ml of standardized suspension of bacteria (106 CFU/ml) was added to each tube containing different concentrations of the aqueous extracts (02-50 µl/ml) and incubated for 24h at 37°C. In agar plating method dilutions having 02-50 µl of aqueous extracts was placed in the cups on the inoculated plate and incubated as mentioned above [9].

Comparative analysis of antimicrobial potential of various extracts of Argemone mexicana with Standard Antibiotics: The standard antibiotics used in the experimental design were procured from Himedia Laboratories, Pune. The standard antibiotics are pure drug utilized as active pharmaceutical ingredients. All the antibiotics were ultrapure grade. The multidisc of the differential Standard Antibiotics for Gram Positive and Gram Negative test pathogen were placed on the microbial lawn with help of the forceps. The discs were gently impregnated with forceps for proper diffusion. Plates were incubated at 37^0 C for 24 hrs. The results were recorded by measuring the inhibitory zone of respective standard antibiotics in mm. The recorded data was used for performing the comparative analysis with solvent extracts of *A. mexicana* [3].

Result:

Table 1: Antibiotic sensitivity of five selected bacterial strains in Nutrient Agar Medium

	Diameter of inhibitory zone in mm				
Antibiotics Ug/ml	**S.aureus**	**E. coli**	**B. Subtilis**	**S. typhi**	**P.aeruoginosa**
Amoxycillin (30)	0	9	2	14	12
Amikacin (30)	21	0	18	8	25
Ciprofloxacin (5)	22	25	28	21	26
Chloramphenicol (30)	24	15	20	25	28
Cefadroxil (30)	30	16	0	0	25
Erythromycin (15)	15	0	22	0	20
Gentamycin (10)	20	21	20	0	20
Kanamycin (30)	10	8	20	0	26
Sparfloxacin (10))	18	20	25	10	30
Norfloxacin (10)	18	22	5	25	20

The antibiogram of some standard antibiotics was analyzed against the wild bacterial strains selected for study using disc diffusion assay. The table no.1 depicts the antibiotic sensitivity of the bacterial strains. The study reveals S. aureus was resistant to Amoxycillin while showed high sensitivity to Cefadroxil. E.coli exhibited resistance towards Cefadroxil and Amikacin where as peaked sensitivity was observed against Ciprofloxscin. B. Subtilis was found resistant to Cefadroxil and its optimal sensitivity was recorded against Ciprofloxacin

followed by Saprfloxacin. The maximum sensitivity of S. typhi was recorded against Chloraphenicol and Norfloxacin. It showed resistance towards Cefadroxil, Erythromycin, Gentamycin and Kanamycin. P. aeruginosa was found sensitive to all standard antibiotics applied in the present study, Sparfloxacin showed lethal effect on its growth.

Table No:2 Antimicrobial sensitivity assay of various extracts of Argemone mexicana against selected bacterial strains

Extracts of Argemone mexicana	**S.aureus**	**E. coli**	**B. Subtilis**	**S. typhi**	**P.aeruoginosa**
Aqueous extract of Leaf	12	15	10	10	14
Aqueous extract of Stem	10	12	12	8	14
Ethanol Extract of Leaf	8	18	15	10	12
Ethanol Extract of Stem	10	10	12	12	20
Methanol Extract of leaf	20	18	21	16	22
Methanol Extract of Stem	22	20	18	14	20

In the present study aqueous, ethanol and methanol extracts of leaves and stem of *Argemone mexicana* were used for analyzing antibacterial activity. Table no 2 illustrates the effect of various extracts on selected bacterial strains. The aqueous extract of leaves showed maximum effect on *E.coli* succeeded by *P.aeruoginosa* where as aqueous extract of stem exhibited optimal activity against *P.aeuroginosa* followed by *E.coli*. Ethanol extract of leaves exhibited maximum activity against *E.coli* and *B.subtilis*. *P.aeuroginosa* was found to be most sensitive towards ethanol extract of stem followed by *S.typhi* and *B.subtilis*. Methanol extract exhibited most antibacterial activity as compared to aqueous extract and ethanol extract. *P.aeuroginosa* was found to be most sensitive towards methanol extract of leaves succeeded by *S.aureus*. All the bacterial strains were found sensitive to various extracts *Argemone mexicana*.

Discussion:

Earlier a number of secondary metabolites like alkaloids and flavonoids of various plants had been reported to have anticancer, antiviral, antibacterial and antiamoebal properties (Cowan MM (1999), Bhattacharjee I et.al (2006)) while antiprotozoal activity of glycosides and saponins had been reported (Wallace, 2004). Singh S.K et.al had reported the antibacterial activity of seeds of Argemone mexicana (2009). Antibacterial properties of plant extracts has been employed in traditional medicines (Shanab BA et.al (2004)). The MDR strains of bacteria are constantly posing challenges for finding out new antibacterial compounds.

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