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ANTIMICROBIAL ACTIVITY OF ARGEMONE *MEXICANA* LINN (FLOWERS)

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Abstract:
The aim of the present study was to examine the antimicrobial effect of the sample from the ethylacetate fraction of flowers of Argemone Mexicana Linn. This sample was shown to possess antimicrobial activity against bacteria and fungi, viz. Six bacterial strains were Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus substilis, Lacto bacillus and two fungal strains Curvularia lunata and Candida albicans by using disc diffusion method. The anti bacterial activity of the sample from ethyl acetate fraction is almost comparable with standard solvent control Chloramphenicol. The anti fungal activity is almost comparable with standard solvent control Fluconazole. The results obtained from this study indicate that A. mexicana is a potential source of antimicrobial and thus could prevent many diseases.

Keywords: *Argemone Mexicana Linn; antibacterial activity; antifungal activity; diffusion method; Chloramphenicol;*

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INTRODUCTION:

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [1]. The uses of herbal treatment are one of the possible way to treat diseases caused by multi drug resistant bacteria. Though Many Pharmaceuticals industries have produced a number of antibiotics from several years but in many cases it was observed that the cultures were showing resistance against the medicines [2]. To prove efficiency the plant extract used as a drugs against different types of pathogens [3-8]. 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 [9,10].

Argemone mexicana L. (Papaveraceae), commonly known as Prickly Poppy in English and Premathandu in Tamil found in Mexico and now has widely naturalised in the United States, India, Bangladesh and Ethiopia. It occurs as wasteland weed in almost every part of India [11, 12]. In Mexico, the seeds have been used as an antidote to snake poisoning [13] *Argemone mexicana Linn*. is also widely well known around the world for its medicinal property to treat several diseases: in India, decoction of the leaves are indicated for the treatment of bacterial infections in the Ayurvedic medicine [14]. In West Africa, A. mexicana is used as uncomplicated malaria remedy [15]. The studies indicated that the traditional medical practitioners recommend utilization of the leave plant mainly for the treatment of malaria and the liver disorders [16].

The present study was undertaken to evaluate the antibacterial potentials and phytochemical analysis of *Argemone mexicana* flowers extract against some selected bacterial species with the possible use as a genuine antimicrobial agent in pharmacological industries.

MATERIALS AND METHODS:

Collection of Flowers

Fresh flowers of *Argemone mexicana* Linn were collected from Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India, during the month of January and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. DP004 dated: 22/01/2016). St.Joseph's College (Campus),Trichy, Tamil Nadu, India.

Extraction and Fractionation

Fresh flower (1kg) of *Argemone mexicana* Linn collected at Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

Antimicrobial Procedure

Screening of Antibacterial Activity

Bacteria Tested:

Four bacterial strains were used throughout investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of Inoculums:

Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that were incubated without agitation for 24 hrs at 37^{o}C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0x10^6 colony forming units (CFU/ml).

Antibacterial Susceptibility Test:

The disc diffusion method (Bauer et al., 1966) was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The compound of concentration 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml were loaded on 6 mm sterile disc. The loaded disc were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic *Chloramphenicol* of concentration 1mg/ml was used as positive control [17].

***Table No. I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Argemone mexicana* Linn**

S.No	Organisms	Zone of inhibition(mm)				
		Standard (*Chloramphenicol*)	Sample Concentration (mg/ml)			
			20	**30**	**40**	**50**
1	*Salmonella typhi*	20	7	10	12	18
2	*Escherichia coli*	24	8	10	15	17
3	*Enterococcus faecalis*	27	8	9	12	14
4	*Bacillus cereus*	26	6	8	12	14
5	*Bacillus substilis*	17	0	9	10	12
6	*Lacto bacillus*	23	6	9	12	14

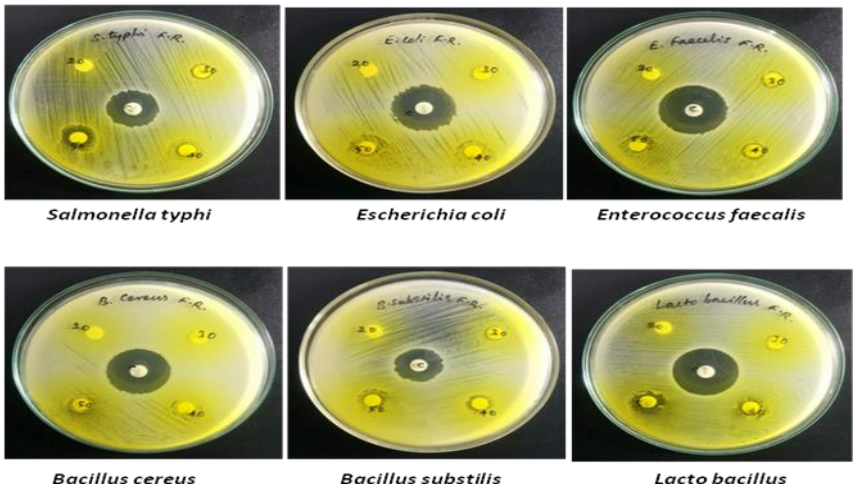
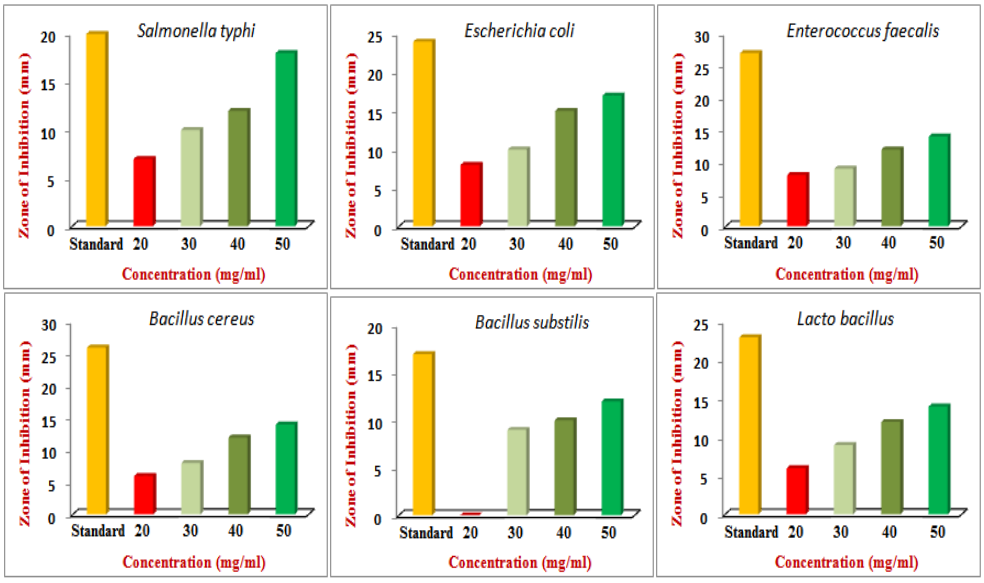


***Fig. I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Argemone mexicana* Linn**



***Graph No.1: Graphical representation of anti bacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Argemone mexicana* Linn. *(Standard: Chloramphenicol, concentration 1 mg/ml*)**

Screening of Antifungal Activity

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 105 CFU/ml.

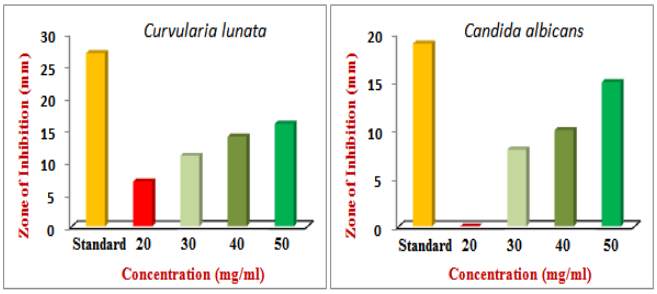
Determination of Antifungal Activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with sample solution and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hrs. The diameters of zone of inhibition observed were measured.

Table No. II: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of Argemone mexicana Linn

S.No	Organisms	Zone of inhibition(mm)				
		Standard *(Fluconazole)*	Sample Concentration (mg/ml)			
			20	30	40	50
1	*Curvularia lunata*	27	7	11	14	16
2	*Candida albicans*	19	0	8	10	15

Fig. II: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of Argemone mexicana Linn



Graph No.2: Graphical representation of antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of Argemone mexicana Linn (Standard: Fluconazole, concentration 1 mg/ml)

RESULTS AND DISCUSSION:

In the present study, *Argemone mexicana* Linn flowers were screened for antimicrobial activity and compared with standard drug. It is evident from the data presented in Table I that the compound isolated from the ethyl acetate fraction of *Argemone mexicana* Linn flowers possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 7 mm, 8 mm, 8 mm, 6 mm, 0 mm and 6 mm for 30 mg/ml as 10 mm, 10 mm, 9 mm, 8 mm, 9 mm and 9 mm for 40 mg/ml showing 12 mm, 15 mm , 12 mm, 12 mm, 10 mm and 12 mm for 50 mg/ml as 18 mm, 17 mm, 14 mm,14 mm, 12 mm and 14 mm for the test sample against *Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus substilis* and *Lacto bacillus* respectively when compared with standard drug *Chloramphenicol* showing 20 mm, 24 mm , 27 mm, 26 mm, 17 mm and 23 mm zone of inhibition respectively.

Then it is evident from the data presented in Table II that the test sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 7 mm and 0 mm, for 30 mg/ml as 11 mm and 8 mm, for 40 mg/ml as 14 mm and 10 mm, for 50 mg/ml as 16 mm and 15 mm for the test solution against *Curvularia lunata and Candida albicans* respectively when compared with standard drug Fluconazole showing 27 mm and 19 mm of inhibition respectively.

CONCLUSION:

This present study suggests that the sample isolated from the ethylacetate fraction of *Argemone Mexicana* Linn flowers possesses bioactive compounds with antibacterial activity against the bacterial strains. It is also suggest that *A.mexicana* Linn used for the treatment of disease caused by some bacteria tested in this study. These *A.mexicana* plant extract can be used to formulate the new antibacterial drugs against the diseases.

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