In-vitro Anti-microbial assay of seed of Argemone mexicana L. An anti-infective agent

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ABSTRACT

The present study was designed to evaluate the antibacterial activity of the aqueous, extracts of the seed of Argemone mexicana L. (Papaveraceae) using agar well diffusion method against common strains of bacterial species, namely, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus and Staphylococcus aureus. 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. The highest inhibition zone observed for A. mexicana seed against Bacillus cereus was 20.05 mm. Antifungal Activity in *Candida albicans* and *Aspergillus niger* were done and shows maximum inhibition. This research suggests that natural produces obtained from A. mexicana L. may provide to the evolution of novel antimicrobial agents.

Key words: *Argemone mexicana, antimicrobial activity, plant extracts, agar well diffusion method, medicinal plant, bacteria.*

INTRODUCTION

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases[1]. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. The worldwide emergence of *Escherichia coli, Klebsiella pneumoniae, Haemophilus* and many other ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections[2].

Candida albicans, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis. Alarmingly, the incidence of nosocomial candidemia has risen sharply in the last decade. All this has resulted in severe consequences including increased cost of medicines and mortality of patients. Thus, in light

of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance[3]. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains. For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief[4]. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent.

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity. There are several reports on the antimicrobial activity of different herbal extracts.

MATERIALS & METHODS

Collection and identification of plant materials

The fresh roots, stem and leaf were collected in January 2018 from Nagarcoil ghats Ghats of Tamil Nadu, India and dried at 36°C for 10 days. The plant specimens were identified and confirmed by botanical department of WCC Nagarcoil.

Test microorganisms

In the research, we have used bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* were used for bioassay. The pure strains were procured from Biomedical Engineering Research Foundation, Salem, Tamilnadu, India. The organisms were maintained on nutrient agar media at 4oC and sub cultured for 24 h before use[1,5].

Preparation of plant extracts

Fresh plant were collected and cleaned. The whole plants were air dried in the laboratory at room temperature 36°C for 10 days. Through this process, the seeds were peeled out from the flower and the seeds were collected and ground into powder form. The powder was stored in airtight bottles at room temperature before extraction[6].

Anti microbial assay

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in centimetres[1,6].

REAGENTS

1**. Muller Hinton Agar Medium** (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2**. Nutrient broth** (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient

medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

3. **Gentamycin** (standard antibacterial agent, concentration: 20mg / ml)

PROCEDURE

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains such as *Pseudomonas aeroginosa, Bacillus subtilis , Staphylococcus aureus and Klebsiella pneumoniae*. Wells of approximately 10mm was bored using a well cutter and 25 µl , 50 µl and 100 µl of sample was added to the well.The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).Gentamycin was used as a positive control.

ANTIFUNGAL ACTIVITY

In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown species of fungus such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of 50 µl and 100 µl concentration were added, the zone of inhibition was measured after overnight incubation and compared with that of standard antibiotics.

RESULTS & DISCUSSION

The results of antimicrobial activity of aqueous extract of Argemone mexicana are given in Table 1 and showed wide spectrum of screening. When the four extracts were compared with other and with that of standard antibiotic gentamycin.

The extract obtained using aquous extract shows 9mm of inhibitory zone against gentamycin in *Pseudomonas aeroginosa, 12mm in bacillus subtilis table 2 ,14mm in Staphylococcus aureus table 3 and 15mm in Klebsiella pneumoniae table 4 in* 100 µl volume of samples.

Table 1. Inhibitory action against Pseudomonas aeroginosa

Sample	Volume of Sample (µl)	Zone of inhibition (cm)
Gentamycin		**2.7**
	25µl	**NIL**
	50 µl	**NIL**
	100 µl	**0.9**

Table 2. Inhibitory action against Organism Bacillus subtilis

Sample	Volume of Sample (µl)	Zone of inhibition (cm)
Gentamycin		**2.5**
	25µl	**NIL**
	50 µl	**0.8**
	100 µl	**1.2**

Figure 1. Antibacterial activity of Argemone mexicana

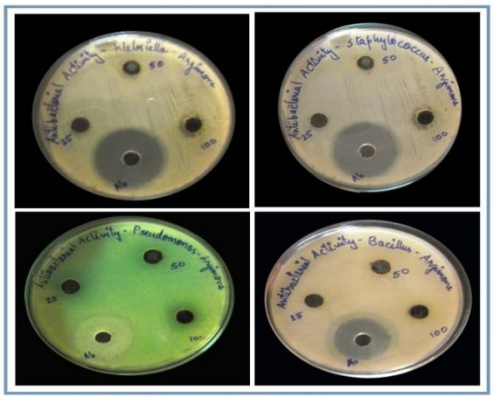

Table 3. Inhibitory action against Organism Staphylococcus aureus

Sample	Volume of Sample (µl)	Zone of inhibition (cm)
Gentamycin		3.0
	25µl	NIL
	50 µl	0.9
	100 µl	1.4

Table 4. Inhibitory action against Organism Klebsiella pneumoniae

Sample	Volume of Sample (µl)	Zone of inhibition (cm)
Gentamycin		2.7
	25µl	0.4
	50 µl	0.6
	100 µl	1.5

Inhibitory action against fungal Organism Candida albicans shows 6mm and Aspergillus niger shows 8mm against the fungal agent Clotrimazole are shown in table 5,6.

Table 5 . Inhibitory action against fungal Organism Candida albicans

Sample	Zone of inhibition (cm)
Clotrimazole	1.7
50	Nil
100	0.6

Figure 2. Antifungal activity of Argemone Mexicana

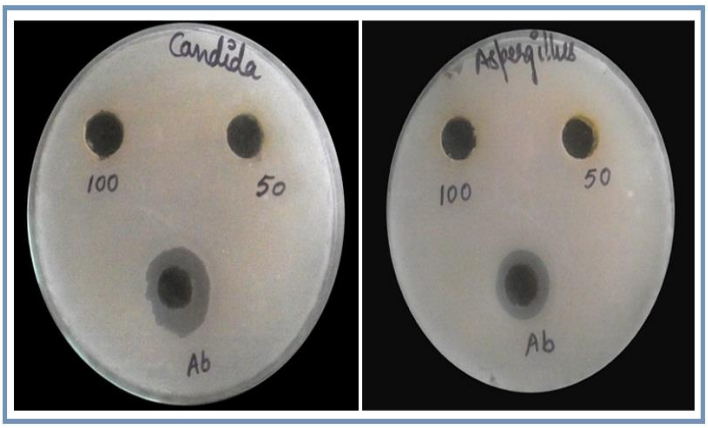

Table 5 . Inhibitory action against fungal Organism Aspergillus niger

Sample	Zone of inhibition (cm)
Clotrimazole	1.3
50	Nil
100	0.8
Note:	0.1 gm in 1ml DMSO

The result of the present work is found to be directly correlated with the observations of earlier researchers. Other details are needed to isolate and characterize the biotherapeutic potentials to evolve current antimicrobial medicines. A wide variety of antibiotics are commonly used for the Treatment of infections and wounds caused by bacteria. In recent years, multiple drug resistance, a threat to mankind has caused an urgent need for the search of innovative ways to control bacterial pathogens. Hence, natural antibiotics are in process of being discovered as alternative to synthetic products.

Though the study produced moderate zones of inhibition which was comparable to that of the stated references; it can further be performed with better methodologies to improve the results and to increase the effectiveness of argemone seed extract against virulent strains.

CONCLUSION

Increased frequency of administration and decreased effectiveness of antibiotics against common isolates has led to development of resistant strains *Pseudomonas aeroginosa, Bacillus*

subtilis , Staphylococcus aureus and Klebsiella pneumoniae. Hence, antimicrobial agents with minimal side effects preferably from natural products are necessary. Grape seed extract showed satisfactory antibacterial effect in this study, however the antibacterial activity could be enhanced by improving the method of extract preparation like incorporation of organic acids to grape seed extract or extraction of specific components like ethanolic and phenolic fraction using sophisticated equipments. Hence, this study may serve as a base to further researches in this field. Once the well sophisticated study can did, it can be used in clinical settings as an alternative or supplementary to antibiotics for external wound healing ointments.

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