



# AMERICAN JOURNAL OF PHARMTECH RESEARCH

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## A Comparative study on Antimicrobial Activity of *Argemone mexicana* Linn. and *Aerva javanica* (Burm.F.) Juss. Ex. Schult.,

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### ABSTRACT

The bacterial and fungal inhibitory efficiency of the *Argemone mexicana* Linn. (Papaveraceae) and *Aerva javanica* (Burm.f.) Juss. Ex. Schult. (Amaranthaceae) were evaluated against bacterial species like *Staphylococcus aureus*, *Streptococcus pyrogens*, *Klebsiella pneumonia* and *Escherichia coli* and two fungal species such as *Aspergillus niger* and *Penicillium notatum*. The susceptibility of the microorganism to the extracts of these plants was compared with each other and with selected antibiotics. All these plants were effective against three of the pathogenic microorganisms. This *in vitro* study corroborated the antimicrobial activity of the selected plants used in folklore medicine.

**Key words:** Antibacterial activity, Antifungal activity, Disc diffusion method, Well diffusion method.

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Received 30 November 2013, Accepted 20 December 2013

Please cite this article in press as: Anand SP. *et al.*, A Comparative study on Antimicrobial Activity of *Argemone mexicana* Linn. and *Aerva javanica* (Burm.F.) Juss. Ex. Schult. American Journal of PharmTech Research 2014.

## INTRODUCTION

A multitude of herb species and other plant materials have been described for the treatment of various diseases throughout the world <sup>1</sup>. Some of the plants which are being used for the treatment of ailments have received scientific or medicinal scrutiny and even the WHO expert committee recommends that this area warrant further attention <sup>2</sup>. Approximately 20% of the plants are found in the world have been submitted to pharmacological and other related biological test. A substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources<sup>3</sup>. These are the natural and safer source of phytochemicals to fight against new strains of micro organisms. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus it anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections <sup>4</sup>.

The use of plant extracts which include the important phytochemicals, that shows antibacterial properties, may be importance in therapeutic treatments. In the past few years, a number of studies have been done in different countries to prove such efficiency <sup>5-8</sup>. Plant derived phytoalexin <sup>9</sup>, isothiocyanates <sup>10</sup>, allicins, anthocyanins <sup>11</sup> and essential oils <sup>12</sup> tannins and polyphenols and terpenoids <sup>13-14</sup> have demonstrated antibacterial and antifungal activities. These compounds are bacteriostatic influencing on lag phase, growth rate and maximum growth of microorganisms.

In view of this fact, current study has been carried out on *Argemone mexicana* Linn. and *Aerva javanica* (Burm.f.) Juss. Ex. Schult., the medicinally valuable plants. *Argemone mexicana* belongs to the family Papaveraceae, is commonly known as Bramathandu in tamil. Ayurveda reported that the plant is purgative, diuretic and destroy worms. It cures skin – diseases, leprosy, inflammation bilious fever, anthelmintic, opacity of cornea, ophthalmic, antidote to snake poisoning and also dropsy and jaundice <sup>15</sup>. *Aerva javanica* belongs to the family Amaranthaceae, is commonly known as Perumpolia in tamil. In traditional medicine the parts of plants are used for treatment of swelling, headache, rheumatism and kidney problems. The plant was reported as anthelmintic, diuretic, demulcent and have antimicrobial and hepatoprotective activities also been demonstrated.

Previous studies also found and proved the antimicrobial activity of *Argemone mexicana* and *Aerva javanica* but limited to seeds and leaves of these plants. However, an attempt has been made in the present study to compare the antimicrobial activity of the whole plant extract of both

medicinally valuable plants.

## MATERIALS AND METHODS

### Microbial Strains

Bacterial strains *Escherichia coli* MTCC-443, *Pseudomonas aeruginosa* MTCC-7441, *Staphylococcus aureus* MTCC-766 and *Klebsiella pneumonia* MTCC-109 and fungal strains *Aspergillus niger* (MTCC-322) and *Penicillium notatum* (MTCC-5476) were obtained from the microbial type culture collection (MTCC), Chandigarh, India. The cells from lyophilized vials were transferred into nutrient agar slants and potato dextrose agar slants respectively and preserved at 4°C in refrigerator.

### Preparation of Inoculums

The young actively growing cells were generated by growing cells in nutrient broth for 24 hours at 37°C. The cell suspensions were diluted with nutrient broth to provide initial cell counts of about  $3 \times 10^8$  CFU/ml. While an aliquot of 1ml was used for antimicrobial test. An overnight grown culture of fungus was used for antifungal activity tests.

### Plant Collection

Plant specimens of *Argemone mexicana* Linn. and *Aerva javanica* (Burm.f.) Juss. Ex. Schult., were collected from, Boda Hill, Namakkal, District, Tamil Nadu, India. After collection, plant parts were rinsed with running tap water, cut into small pieces and kept under shade until drying. After proper drying plant material was pulverized into powdered form by a grinding machine.

### Preparation of Extracts

For cold plant extracts, 3 solvents namely, methanol, ethanol and distilled water were used individually for 3 lots of powder-mass of whole plants. 50 gm of plant powder were extracted with 300 ml of (methanol, alcohol and dis. Water) solvents for 72hrs by Soxhlet extractor. Then the extracts with different solvents were evaporated using rotary evaporator. Extracts were transferred into pre-weighed sample containers and were stored. Later it was used for antibacterial activity and antifungal activity.

### Screening for Antibacterial Activity

#### Disc Diffusion Method

Disc diffusion method was used to test the antibacterial activity of the extracts against several bacteria. The essential leaf extracts were used for studying their antibacterial activity. A loopful of bacterial strains were inoculated into 5ml of nutrient broth and incubated for 24hrs at 37°C to get active strain. Nutrient agar plates were prepared by pouring 20ml of molten media into sterile

petriplates. After solidification of media, inoculums of MTCC strains were swabbed uniformly and the inoculums was allowed to dry for 5 minutes.

The different concentrations of extracts loaded disc was 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° C for 24 hrs. At the end of incubation, zones formed around the disc were measured with transparent ruler in millimetre. Based on the diameter of the zone of inhibition, antibacterial susceptibility was ranked <sup>16</sup>.

### **Agar Well Diffusion**

The bacterial suspensions were prepared equal to the turbulence of Mac far land 0.5 standard and were cultivated (100 µl) on nutrient broth. After that 6mm diameter were punched in agar plate . The standard antibacterial agent streptomycin (10mg/ml) was tested in the same manner as control. All the plates were incubated at 37° C, for 18 -24 hours. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed <sup>17-18</sup>. Each test was preformed twice and the average of the results was taken. The solvent (ethanol 50%) was not affected the growth of any of the bacteria.

### **Screening for Antifungal Activity**

The plant extracts were assayed for antifungal activity against the fungal strain obtained from microbial type culture collection . This fungus was grown on PDA plate at 28° C and maintained with periodic sub-culturing at 4° C.

Every 15ml of sterile potato dextrose agar medium was poured into sterile petridishes after flaming the top of the conical flask. 5 ml of either crude or aqueous extract of each plant was added. The solution inhibition each petridish was gently swirled and allowed to solidify. The extract amended medium in the petridishes were inoculated separately at the centre with each test fungus and incubated at room temperature for 14 days (a sterile needle was used to inoculate fungal strains to these petridishes). The medium without any extract served as control. Incubation period of 24 – 48 hrs at 28 °C was maintained for observation of antifungal activity of plant extracts. The complete antifungal analysis was carried out under aseptic condition.

## **RESULTS AND DISCUSSION**

### **Antibacterial Activity**

The antibacterial activity of the plant extracts from *Argemone mexicana* and *Aerva javanica* was studied against bacterial strains using agar disc diffusion method (Plate – 1 & Table - 1). The methanol extracts of *Argemone mexicana* plant showed maximum activity against

*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Streptococcus pyrogens*. The alcoholic extracts of this plant showed maximum activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and *Streptococcus pyrogens*. The antimicrobial activity of methanol extracts showed more effective followed by alcoholic extract and aqueous extracts against all the bacterial strains. A similar study was obtained by Indranil Bhattacharjee reported that methanolic extract of *A. mexicana* leaves and seeds showed greater antibacterial activity against some species of gram positive and gram negative pathogenic bacteria than aqueous extracts<sup>19</sup>.

**Table 1: Antibacterial activities of *Argemone mexicana* and *Aerva javanica* extract using different solvents by diffusion methods**

S. No	Tested Organisms	Extracts	<i>Argemone mexicana</i>			<i>Aerva javanica</i>		
			Disc diffusion method Inhibition Zones (cm)	Agar diffusion method Inhibition Zones (cm)	well	Disc diffusion method Inhibition Zones (cm)	Agar diffusion method Inhibition Zones (cm)	well
1.	<i>Staphylococcus aureus</i>	Streptomycin	3.0 ± 0.5	3.1 ± 0.6		3.0 ± 0.5	3.1 ± 0.6	
		Alcohol	1.3 ± 0.6	2.0 ± 0.6		<b>1.8 ± 0.7</b>	<b>1.7 ± 0.4</b>	
		Methanol	<b>1.7 ± 0.9</b>	<b>2.0 ± 0.4</b>		0.8 ± 0.1	1.0 ± 0.2	
		Aqueous	0.3 ± 0.1	0.3 ± 0.1		0.2 ± 0.0	0.4 ± 0.2	
2.	<i>Streptococcus pyrogens</i>	Streptomycin	2.6 ± 0.3	3.5 ± 0.2		2.6 ± 0.3	3.5 ± 0.2	
		Alcohol	0.4 ± 0.2	1.7 ± 0.0		<b>0.8 ± 0.1</b>	<b>2.3 ± 0.7</b>	
		Methanol	<b>0.5 ± 0.3</b>	<b>2.0 ± 0.9</b>		0.7 ± 0.3	1.5 ± 0.5	
		Aqueous	-	0.5 ± 0.4		-	0.6 ± 0.4	
3.	<i>Escherichia coli</i>	Streptomycin	2.0 ± 0.2	3.0 ± 0.1		2.0 ± 0.2	3.0 ± 0.1	
		Alcohol	1.0 ± 0.6	1.9 ± 0.9		<b>1.5 ± 0.8</b>	<b>2.0 ± 0.7</b>	
		Methanol	<b>1.3 ± 0.8</b>	<b>2.0 ± 0.3</b>		1.1 ± 0.7	2.6 ± 0.9	
		Aqueous	-	0.3 ± 0.0		0.3 ± 0.0	0.5 ± 0.3	
4.	<i>Klebsiella pneumonia</i>	Streptomycin	2.0 ± 0.3	3.0 ± 0.1		2.0 ± 0.3	2.4 ± 0.7	
		Alcohol	<b>1.3 ± 0.5</b>	<b>1.5 ± 0.1</b>		<b>1.5 ± 0.7</b>	<b>3.0 ± 0.9</b>	
		Methanol	0.7 ± 0.4	1.7 ± 0.5		0.9 ± 0.3	1.0 ± 0.4	
		Aqueous	-	0.1 ± 0.2		-	0.4 ± 0.3	

The alcoholic extracts of *Aerva javanica* plant showed maximum activity against *staphylococcus aureus* followed by *Klebsiella pneumonia*, *Escherichia coli* and *Streptococcus pyrogens*. The methanol extracts showed maximum activity against *Escherichia coli* followed by *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus pyrogens*. The antibacterial activity of alcohol extracts showed more effective followed by methanol extracts against all the bacterial strains. A similar result was obtained by Ashish Seti that alcoholic extract of *Aerva tomentosa* whole plant showed broad-spectrum of activity against both gram positive and gram negative

bacteria<sup>20</sup>. The aqueous extract of whole plant of *Aerva javanica* and *Argemone mexicana* showed low range of activity against all tested organisms when compared to other extracts.

**Antibacterial Activity of *Argemone mexicana* Methanolic Extract  
by Disc Diffusion Methods**



*Staphylococcus aureus*



*Escherichia coli*



*Klebsiella pneumonia*

**Antibacterial Activity of *Aerva javanica* Alcoholic Extract  
by Disc Diffusion Methods**



*Staphylococcus aureus*



*Klebsiella pneumonia*



*Escherichia coli*

**Figure 1: Antibacterial activity of *Argemone mexicana* and *Aerva javanica* methanolic extract by disc diffusion method**

**Antifungal activity**

The antibacterial activity of the plant extracts from *Argemone mexicana* Linn and *Aerva javanica* was studied against fungal strains using streak plate method. The maximum antifungal activity against *Penicillium notatum* was demonstrated by methanol extract and followed by *Aspergillus niger* by *Argemone mexicana* plant. The *Aerva javanica* plant showed maximum antifungal activity against *Penicillium notatum* was demonstrated by alcoholic extract and followed by *Aspergillus niger*. The aqueous extract of whole plant of *Aerva javanica* and *Argemone mexicana* showed low range of activity against all tested organisms when compared to other extracts. (Table – 2).

**Table 2: Effects of *Argemone mexicana* and *Aerva javanica* extracts using different solvents concentration on various fungi by streak plate method**

S. No.	Microorganisms	Extracts	Growth rate of organisms on different concentration of extracts					
			<i>Argemone mexicana</i>			<i>Aerva javanica</i>		
			Control	50%	100%	Control	50%	100%
1.	<i>Penicillium notatum</i>	Alcohol	+++	+	-	+++	++	+
		Methanol	+++	++	+	+++	+	-
2.	<i>Aspergillus niger</i>	Alcohol	+++	-	-	+++	+	-
		Methanol	+++	+	+	+++	-	-

+++ : High range; ++ : Moderate range; + : Low range; - : Nil, Control : Without plant extract

The results showed that the antimicrobial activity of both plants were comparable with the results of previous researches using extracts of other plant species of the genus *Aerva*, *Plumbago zeylanica*, *Acne vulgaris*, *Boerhavia diffusa*, *Tinospora cordifolia*, *Clitoria ternate*, *Hygrophila auriculata*, *Pergularia daemia* and *Zehneria scabra*<sup>21-25</sup>. These observations showed that the organic solvents extraction method is better for isolation of antibacterial compounds belonging to these secondary metabolite groups such as alkaloids, flavanoids, tannins, phenolic compounds, phytosterols, saponins, glycosides, etc., have been reported for their antimicrobial activity<sup>26-27</sup>. Fungi cause multiples infections examples, infection of blood, liver, lungs and mouth, etc.,<sup>28-29</sup>. *Aspergillus niger* typically cause infectivity in lungs and has also been detected on skin of burnt injuries<sup>30</sup>. Our results showed that both plants are inactive against fungal strains and thus exhibit very poor activity. The results revealed that inhibitory effects of test samples was dose dependent as the concentration increased the zone of inhibition was also increased which was evidenced by Uma Reddy<sup>31</sup>. These observations may be attributed to two reasons they are the nature of biological active components whose activity can be enhanced in the presence of solvent and the stronger extraction capacity of solvents could have produced greater number of active constituents responsible for antibacterial activity.

These results expose that the plants has quite a number of chemical constituents, which are responsible for the many pharmacological actions. The plant extract were more effective but further *in vivo* study on purified active compounds have to do to determine toxicity and adequate dosage for proper administration.

## CONCLUSION:

The present study suggested that, extracts prepared from *Argemone mexicana* and *Aerva javanica* have great potential as antimicrobial agent against bacterial pathogens and they can be used as alternative medicine in the treatment of bacterial infections. This study supports the use

of these medicinal plants preparations can be used as antimicrobial agent to prevent or control the bacterial infections.

#### ACKNOWLEDGMENTS:

The authors are thankful to the University Grant Commission (UGC), New Delhi for the financial assistance under the Major Research Project Scheme [UGC F.NO.41-440/2012 (SR)]

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