

## STUDY ON ANTIFUNGAL ACTIVITY OF AERIAL PART OF *ARGEMONE MEXICANA* LINN

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Article Received on  
31 March 2017,

Revised on 21 April 2017,  
Accepted on 11 May 2017

DOI:10.20959/wjpr20176-8558

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### ABSTRACT

Our culture is rich in herbal drugs. These herbal plants have the tendency to produce many kinds of secondary metabolites in severe conditions. These metabolites are polyphenols, flavonoids, terpenoids, steroids, quinones, alkaloids, polysaccharides and so on. Antifungal activity of aerial part of *Argemone mexicana* was also performed on different extracts of *Argemone mexicana* against *Fusarium moniliforme* and *Aspergillus niger*. Ethyl acetate extract of *Argemone mexicana* showed significant results against *Fusarium moniliforme* which was further followed by ethanolic extract of *Argemone mexicana*. Antifungal activity of EAEAM may be due to the

flavonoids, aromatic acids, and its esters. The strong antifungal activity of *Argemone mexicana* may be due to high total phenolic and flavonoid contents.

**KEYWORDS:** Antifungal activity, *Argemone mexicana*, Aerial part, Ethanolic extract.

### INTRODUCTION

Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins. More than 300 fungal metabolites are reported to be toxic to human and animals. The main toxic effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppressant. Plant metabolites appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides.<sup>[1]</sup>

Plants normally produce various secondary metabolites not only to adapt to their environment but also to defend themselves against biotic or abiotic stress, such as high light intensity, extremely high or low temperature, high salinity, drought and natural enemies. These metabolites are polyphenols, flavonoids, terpenoids, steroids, quinones, alkaloids, polysaccharides and so on. Studies have shown that from 9% to 16% of patients with gastrointestinal disorders use alternative remedies, with the highest rates in patients with irritable bowel syndrome, often considered to have a component of functional etiology.<sup>[2]</sup>

The *Argemone mexicana* plant is commonly grown plant and used as medicinal preparation. The herb native of west Asia, grows in Uttar Pradesh, Hoshiarpur and Jullundur in Punjab, Rajasthan and Madhya Pradesh. *Argemone mexicana* belongs to Kingdom- Plantae, Division- Magnoliophyta, Order- Ranunculales, Family- Papaveraceae, Genus- *Argemone*, Species- *Argemone mexicana*. It is erect prickly herb abounding throughout, in areas up to 1,500m elevation on road side and waste places.<sup>[3]</sup>

*Argemone mexicana* has number of health benefits like hepatoprotective activity<sup>[4]</sup>, antioxidant activity<sup>[5]</sup>, *in vitro* antitrichomonal activity<sup>[6]</sup>, peripheral analgesic activity<sup>[7]</sup>, antibacterial and antifungal activity<sup>[8]</sup>, anthelmintic activity<sup>[9]</sup>, *in vitro* anti-cancer activity<sup>[10]</sup>, antidiabetic activity<sup>[11]</sup>, and wound healing activity.<sup>[12]</sup> The whole plant is good tonic, depurative. The flowers are bitter, digestive, astringent, and stomachic. The root are useful in guinea-worm infestation, skin diseases, leprosy, pruritus.<sup>[13]</sup> It possess antifungal activity and used in the treatment of fungal infection. Hence the present study was undertaken with the aim of exploring the antifungal activity of *Argemone mexicana* linn.

## MATERIAL AND METHODS

### Plant material

The plant was identified on the basis of its vernacular name Mexican poppy and its morphological characteristics. The aerial part of identified *Argemone mexicana* linn was collected from near OPJS University, Churu, Rajasthan.

The plant was identified and authenticated by **Dr. Zia ul Hasan**, Prof. Botany Saifia Science College, (Barkatulla University) Bhopal (M.P.). Voucher specimen number is **317/Bot/Saifia/2012**.

The method was carried out according to the NCCLS guidelines.

### Screening by cup plate method

This method is based on diffusion of antifungal component from reservoir hole to the surrounding inoculated Sabouraud dextrose Agar medium, so that the growth of fungus is inhibited as zone around the hole. Two fungi were selected viz. *Fusarium moniliforme* and *Aspergillus niger*.

#### A. Preparation of inoculum

The suspension of fungus was prepared as per Mac-Farland nephelometer standard.<sup>[14]</sup> A 24hr. old culture was used for the preparation of fungus suspension.

A suspension of fungus was made in a sterile isotonic solution of sodium chloride and the turbidity was adjusted such that it contained approximately  $1.5 \times 10^6$  cells/ml. It was obtained by adjusting the optical density (650 nm) equal to 1.175% barium chloride in 100ml of 1% sulphuric acid.

#### B. Sample preparation

The twenty extracts of *Argemone mexicana linn* were dissolved in DMSO to get a concentration of 200mg/ml.

#### C. Culture medium

Sabouraud dextrose Agar medium (Hi Media) was used for preliminary antifungal activity. The medium was prepared by dissolving in water and autoclaving at  $121^{\circ}$  C for 15 minutes.

#### D. Standard Preparation

Fluconazole standard was prepared at a concentration of 10  $\mu$ g / ml in sterile distilled water.

#### Nutrient Medium

**Table: 1 Sabouraud's Agar Medium \***

Sl.No	Ingredients	Weight in g
1	Dextrose	50
2	Peptone	20
3	Agar	30
4	Distilled water	q. s. 1000 mL

\*pH 5.6 was maintained for nutrient media.

This medium was used for both sub culturing and also for estimating the antifungal activity. The pH of the medium plays an important role for the growth of fungi. Acidic medium

favours the growth but excess of acid will not allow agar to solidify. Hence, the pH of medium was adjusted using 0.1% lactic acid.

### E. Antifungal screening of extracts

The extracts (Ethyl acetate, Ethanol and Aqueous) of *Argemone mexicana* were subjected to standardized antifungal screening procedure by agar gradient method.

### F. Preparation of assay medium

The above mentioned quantities of different ingredients were accurately weighed and dissolved water. The medium so prepared was sterilized by autoclaving at 121<sup>0</sup>C for 15 minutes.

### Working procedure

An inoculum was prepared by suspending a single isolated colony in about 5 ml of normal saline. This is mixed slowly to achieve a smooth suspension. Later, one drop of tween 20 was added for filamentous fungi and the mould was broken by shaking. A sterile cotton swab was moistened in the inoculum suspension and excess of moisture was removed by rolling the cotton swab on the inside of the tube, above fluid level 30 ml of sterile hot Sabouraud's agar medium was poured in each plate and allowed to harden on a level surface. The surface of Sabouraud's agar medium plate was streaked with the help of moistened cotton swab in all the direction ions.

The surface of Sabouraud's agar plate was dried out 28<sup>0</sup>C. Later, 4 bores per plate were made using sterile cork borer. The above procedure was carried out in aseptic condition and 0.1 ml test solution was added to the respective bore and 0.1 ml fluconazole was taken as standard reference. A control using DMSO was maintained in each plate. The plates were incubated at 28<sup>0</sup>C for 48 hr. Later the values of zones of inhibition were recorded in triplicate and reported in Standard Error Mean ( $\pm$  SEM).

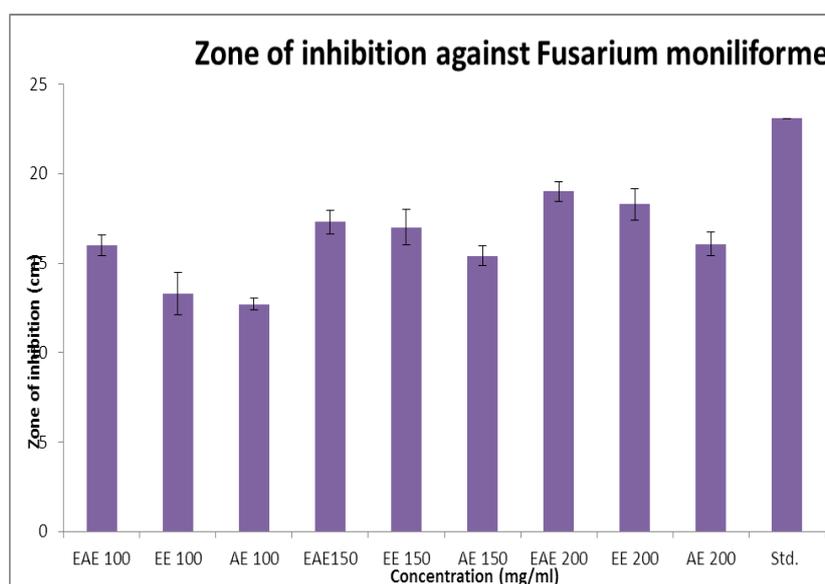
## RESULTS

**Table: 2 Evaluation of antifungal activity**

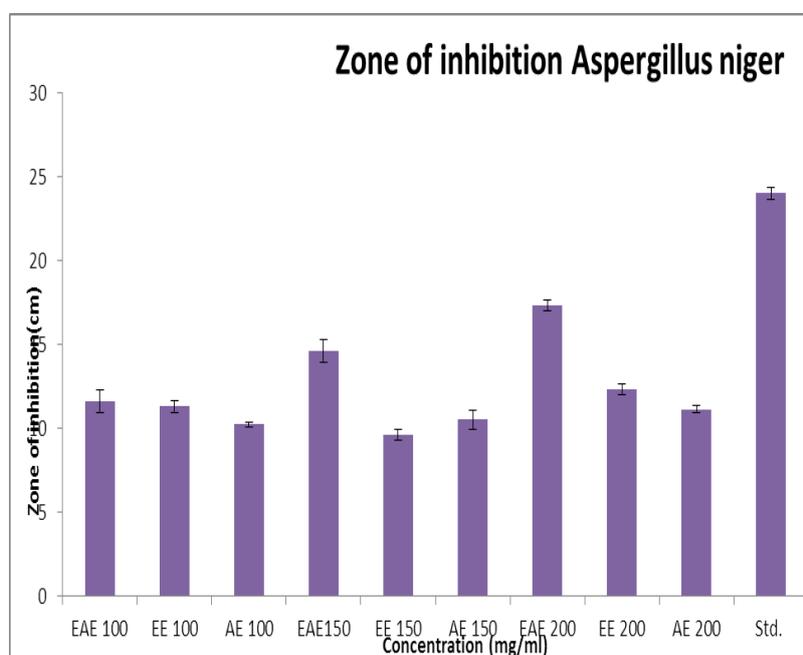
Plant	Treatment	Dose (mg/ml)	Zone of inhibition (mm) (Mean $\pm$ SEM)	
			<i>Fusarium moniliforme</i>	<i>Aspergillus niger</i>
<i>Argemone mexicana</i>	Ethyl acetate extract	100	16.1 $\pm$ 0.56**	11.7 $\pm$ 0.67*
		150	17.5 $\pm$ 0.67*	14.9 $\pm$ 0.66*
		200	19.1 $\pm$ 0.58**	17.5 $\pm$ 0.35**
	Ethanol extract	100	13.6 $\pm$ 1.21**	11.2 $\pm$ 0.33*

		150	17.2±1.11**	9.5±0.32**
		200	18.6±0.89**	12.1±0.32*
	Aqueous extract	100	12.9±0.36*	10.21±0.11**
		150	15.45±0.55**	10.54±0.56*
		200	16.10±0.65**	11.17±0.22**
	Fluconazole	2mg/10ml	23.3± 0.01**	23.9±0.34*

Each value represent Mean±SEM, n=5. One-way ANOVA followed by Dunnet test through Instat software, compare all vs. standard applied. Statistically significant at \*\*P<0.01, \*P<0.05.



Graph: 1 Graphical representation of Antifungal activity against *Fusarium moniliforme*



Graph: 2 Graphical representation of Antifungal activity against *A. niger*

## DISCUSSION

Antifungal activity was also performed on different extracts of *Argemone mexicana* against *Fusarium moniliforme* and *Aspergillus niger*. Ethyl acetate extract of *Argemone mexicana* showed significant results against *Fusarium moniliforme* which was further followed by ethanolic extract of *Argemone mexicana*. In case of antifungal activity of different extracts against *Aspergillus fumigatus*, ethyl acetate extract of *Argemone mexicana* was more effective followed by ethanolic extract of *Argemone mexicana*.

## CONCLUSION

Two fungi were selected *Fusarium moniliforme* and *Aspergillus niger*. From the above, it was concluded that the ethyl acetate extract of *Argemone mexicana* has best antifungal activity against *Fusarium moniliforme* and *Aspergillus fumigatus*. Antifungal activity of EAEAM may be due to the flavonoids, aromatic acids, and its esters. The strong antifungal activity of *Argemone mexicana* may be due to high total phenolic and flavonoid contents. There are numerous questions yet to be answered concerning chemical compositions and antifungal properties of Indian *Argemone mexicana* and further research is required for clarification.

## ACKNOWLEDGEMENT

The author express gratitude to his guide Asst. prof. Dr Anil Middha, Faculty of Pharmacy, OPJS University, Churu, Rajasthan, for his kind support.

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