

## Original Article

# Studies on Antifungal Activity and Brine Shrimp Toxicity *Argemone mexicana*

Nahar A<sup>1</sup>, Habib MA<sup>2</sup>, Awal MA<sup>3</sup>, Rahman AKMSM<sup>4</sup>, Mondol MAG<sup>5</sup>, Alam MS<sup>6</sup>

### Abstract

The present study determined the antifungal activity of the root of extracts *Argemone mexicana* against 7 fungi using dichloromethane and methanol as solvent. Among these pathogens, 3 were dermatophytes and the rest 4 were nondermatophytes. The dichloromethane extract of *Argemone mexicana* exhibited moderate to good activity at concentration of 200 µg/disc but at concentration of 50 µg/disc, the same extract showed no activity against the tested fungi. The methanol extract were also inactive against the tested pathogens. In brine shrimp toxicity test, it was observed that LC50 value of the dichloromethane extract of *Argemone mexicana* was 22.35 µg/ml. From these findings it is indicative that dichloromethane extract of *Argemone mexicana* is biologically active and may have antifungal principles that could be useful in fungal diseases.

**Key words:** *Argemone mexicana*, Antifungal activity, Brine shrimp toxicity.

### Introduction

Fungal infections are common health problem throughout the world. The diseases are precipitated by various factors such as illiteracy, unawareness, over crowding, ignorance, warmer climate, low socio-economic status and poor hygienic conditions. Treatment of fungal infections needs long time to cure. So the poor people of rural areas cannot continue the treatment and are more sufferers. Bangladesh has a good source of medicinal plants due to our favorable climate. Most of these grow wild in the wastelands, jungles, roadsides and forests without any cultivation. The traditional practitioners claimed the effectiveness of various plant parts for the ailment of different fungal infections. Literature survey revealed that different plant extracts and their isolated compounds have been found

to be effective against various species of fungus that are responsible for fungal diseases<sup>1,2</sup>. *Argemone mexicana* Linn. locally named as Shialkanta is a spiny herbaceous plant grows annually in waste lands in all parts of the country. The stems of the plants are 0.3-1.2 meter in height. Leaves are thistle-like, green, spiky and oblong. Flowers are yellow, Sepals-3, Petals- 6. Ovary is prickly, one celled and ovules numerous. Fruits are prickly, oblong, opening by 4-6 valves. Seeds are numerous. Different parts of the plant are used for antifungal, anthelmintic, diuretic and purgative properties. It is also useful in jaundice, whooping cough, warts, tumors, cancers and eye diseases. The plant contains chemical constituents such as alkaloids, protopine and barberine, resine, allocryptonine, tannin, benzophenanthridine, sanguinerine and glucosides<sup>3,4,5</sup>.

1. Dr. Ainun Nahar, Lecturer, Department of Pharmacology, Rajshahi Medical College, Rajshahi.
2. Professor (Dr.) Anwar Habib, Professor & Head, Department of Pharmacology, Rajshahi Medical College, Rajshahi.
3. Professor (Dr.) M. A. Awal, Professor & Head, Department of Pharmacology, North Bengal Medical College, Sirajgonj.
4. Dr. A.K.M. Shahidur Rahman, Assistant Professor, Department of Pharmacology, KYAMC, Enayetpur, Sirajgonj.
5. Professor (Dr.) M. A. Gafur Mondal, Professor & Head, Department of Blood Transfusion, KYAMC, Enayetpur, Sirajgonj.
6. Professor M. Shah Alam, Department of Botany, Rajshahi University, Rajshahi, Bangladesh.

**Address of correspondence:** Professor (Dr.) M. A. Awal, Department of Pharmacology, NBMC, Sirajgonj.  
Mobile: +88-01712896846; E-mail:drawal\_ru@yahoo.com

Brine shrimp toxicity (lethality bioassay) test utilized by pharmacologists and natural product chemists in the detection and isolation of plant constituents with a variety of biological activities. The brine shrimp lethality assay was proposed by Michael<sup>6</sup> and later developed by Vanhaecke<sup>7</sup>. It is based on the ability to kill laboratory-cultured *Artemia nauplii* brine shrimp. It has the advantages of being rapid, inexpensive and simple method<sup>8</sup>. The conventional brine shrimp lethality bioassay was used for the assessment of toxicity of DCM extract of root of *Argemone mexicana*. Scientists are now engaged to explore bioactive constituents of medicinal plants with the hope of adding new compounds for the treatment of various diseases. These findings used as a guide in our continuing search for new natural antifungal agents from folk medicinal plants. The present study was to evaluate the antifungal activities along with the toxicity study of root extracts of *Argemone mexicana*.

## Materials & Methods

### Preparation of the extracts

The root part of *Argemone mexicana* was collected from Rajshahi locality and taxonomically identified by Professor ATM Naderuzzaman (now retired), Department of Botany, Rajshahi University. Adhering dirt's and soils from the roots were removed without washing and cut into small pieces. The plant parts were dried in a well ventilated room by spreading on a table without heaping the materials in one place. Dried parts then grinded into powder by grinder machine, preserved in a paper bag and labeled properly. The dry powder was extracted in two conical flask using dichloromethane and methanol as solvent, shaken repeatedly for 24 hours. Extracts then filtered and the process repeated twice more. The extracts were concentrated to dryness (semisolid masses) by rotary evaporator at 30°C under reduced pressure and stored in suitable sized glass bottles. Antifungal screening was carried out taking these extracts of *Argemone mexicana* (Table-1).

Name of plant	Parts of plant used	Solvent used	Amount of powder used	Yielded extracts
<i>Argemone mexicana</i>	Root	DCM	250 grams	25.14 mg
		Methanol	250 grams	15.32 mg

**Table-1:** Weight of extracts from powdered plant materials of *Argemone mexicana* by cold extraction

process using dichloromethane (DCM) and methanol as solvent.

## Antifungal screening

Antifungal potency of the extracts was tested by disc diffusion method<sup>9</sup>. Seven fungi namely *Trichophyton rubrum*, *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium* species, *Fusarium* species and *Microsporum gypseum* were selected for this investigation. The medium (Potato dextrose agar) was poured into sterile petridishes and the inoculum was adjusted to contain 105 to 107 fungi per ml. The extracts were dissolved in solvents (dichloromethane and methanol) to obtain a concentration of 50 µg/µl. The discs (6 mm in diameter) were prepared by sterile filter paper and dried in an oven to remove moisture. The solutions were applied on the dried filter paper discs by micropipette to obtain 50 and 200 µg of extracts in each disc. Fluconazole was kindly donated by Square Pharmaceuticals Limited, dissolved in water and were used as standard (50µg/disc). The discs were then placed on the petridishes seeded with the medium containing inoculum and allowed to diffusion in a refrigerator at 4°C for 24 hours. The petridishes were then incubated at 30°C for 48-72 hours and the zones of inhibitions observed were measured (Table-2).

Tested fungi	Diameter of zone of inhibition (in mm.)		
	DCM extract (200 µg/disc)	DCM extract (50 µg/disc)	Standard Fluconazole (50 µg/disc)
<i>T. rubrum</i>	15	-	14
<i>C. albicans</i>	12	-	17
<i>A. flavus</i>	08	-	16
<i>A. fumigatus</i>	13	-	-
<i>Penicillium</i> spp.	11	-	-
<i>Fusarium</i> spp.	13	-	6
<i>M. gypseum</i>	11	-	10

**Table-2:** In-vitro antifungal activity of crude DCM and methanol extracts of root part of *Argemone mexicana* against the tested pathogens.

*T. rubrum* = *Trichophyton rubrum*, *C. albicans* = *Candida albicans*, *A. flavus* = *Aspergillus flavus*, *A. fumigatus* = *Aspergillus fumigatus*, *Penicillium* spp. = *Penicillium* species, *Fusarium* spp. = *Fusarium* species, *M. gypseum* = *Microsporum gypseum*, - = No inhibition, mm = millimeter.

## Toxicity study (Brine shrimp lethality bioassay)<sup>8</sup>

Brine shrimp lethality bioassay test is a convenient bioassay for active plant constituents. Eggs of *Artemia salina* Lech. were placed in one side of a small tank divided by a net containing 3.8 % NaCl solution (brine solution) as artificial seawater for hatching. Brine solution was prepared by dissolving 38.00 gm sea salt (commercial NaCl salt) in one liter (1L) of distilled water. In other side of the tank, a light source was placed in order to attract the nauplii. After two days of hatching period the nauplii were ready for the experiment. Then 3 mg of the extract was accurately measured and dissolved in 0.6 ml (600  $\mu$ l) of dimethyl sulfoxide (DMSO) as solvent to get a concentration of 5 mg/ml to prepare stock solutions (mortality of brine shrimp nauplii in this DMSO solution was zero).

From the stock solution 5, 10, 20, 40 and 80  $\mu$ l were placed with the help of micropipette in 5 different vials making the volume up to 5 ml by NaCl solution. The final concentration of the samples, in the vials became 5, 10, 20, 40 and 40  $\mu$ g/ml respectively. 10 brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO (solvent) plus brine solution up to 5 ml was used. After 24 hours of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. The resulting data were evaluated using probit analysis<sup>10</sup> (Table-3).

Test Sample	LC50 ( $\mu$ g/ml)	95% Confidence Limit		Regression Equation	$\lambda^2$ (df)
		Lower	Upper		
A. mexicana (Root)	22.35	11.97	41.70	$Y = 3.16 + 1.36 X$	0.16 (3)
Ampicillin trihydrate	5.14	2.57	10.28	$Y = 4.12 + 1.23 X$	0.15 (3)

**Table-3:** LC50 value of the crude DCM extract of root of *Argemone mexicana* and compared with standard Ampicillin trihydrate on brine shrimp nauplii.

(A. = *Argemone*,  $\mu$ g = microgram, ml = milliliter, LC50 = median lethal concentration (concentration at which 50% of the shrimp nauplii died),  $\lambda^2$  = Chi-square, df = degree of freedom).

## Discussion

Mycotic infections are major public health problems caused by different fungal pathogens. Some investigators reported that about 15-20% of all skin diseases patients had fungal infections<sup>11,12</sup>. Scientists are now engaged to explore new and potent bioactive principles from the plant source useful in fungal infections. In the continuation of new antifungal drug discovery we investigated the dichloromethane and methanol extracts of *Argemone mexicana* against<sup>7</sup> pathogenic fungi, namely *Trichophyton rubrum*, *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium* species, *Fusarium* species and *Microsporium gypseum*. In the present investigation we found moderate to good antifungal activity of the DCM extract of *Argemone mexicana* at concentration of 200  $\mu$ g/disc against the tested pathogens. Our results correlate with the results of some workers<sup>13,14</sup>.

Ndounga and Oumba<sup>13</sup> reported that oil of *Ocimum basilicum* leaf showed moderate antifungal effects against *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* and these results supported our present findings. We found that methanol extract of the plant were not effective against the tested fungi. This may be due to the absence of antifungal principles in this extract. In the present study different toxicities were observed using brine shrimp lethality bioassay and the mortality rate of brine shrimp was found to be increased with increased in concentration of the samples. DCM extracts of *Argemone mexicana* showed moderate activity with LC50 value of 22.35  $\mu$ g/ml. Activities for extracts are considered significant when LC50 values are = 30  $\mu$ g/ml<sup>8</sup>. There are many reports on cytotoxic properties of the extracts of various plants growing in different parts of this region. Rahman<sup>15</sup> showed that crude ethanol extract of whole plant of *Commelina bengalensis* and its three organic solvent fractions exhibited significant activity in the brine shrimp lethality bioassay test. The LC50 values were 14.12, 10.00, 10.00 and 19.95 g/ml for the crude ethanol extract, n-hexane, carbon tetrachloride and chloroform soluble fractions respectively. Jum and co-workers<sup>16</sup> revealed that ethanolic extracts of *Catheranthus roseus* (Whole plant), *Albizia procera* (Bark), *Calotropis gigantea* (Latex), and *Daucus carota* (Tap root) were found to have moderate toxicity against the larvae of brine shrimp. The LC50 values were 8.00, 6.00, 5.00 and 10.00 g/ml respectively. These results correlate the findings of the present study.

## Conclusion

On the bases of above studies it can be concluded that *Argemone mexicana* may play a beneficial role in the management of fungal infections. However, detailed study may be needed for this purpose.

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