IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 3, December 2022
Impact Factor: 6.252

Study of Invitro Anti-Fungal Activity of *Argemone mexicana* against Fungi like *Aspergillus niger* and *Candida albicans*

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Abstract: *Plants measure wealthy supply of medication for antifungal and antiviral properties. These properties are employed by plants to safeguard themselves from foreign particles or pathogens. With the advancement of techniques currently we tend to carry out extracting the chemicals and victimisation to cure plant and animal infection. The in vitro antifungal activity of binary compound extract from Argemone mexicana plants utilized in ancient drugs for the treatment of varied diseases. Extract effectivity was evaluated victimisation the agar well diffusion assay against two fungi i.e. Aspergillus niger and Candida albicans. Zone of inhibition against fungi was studied. The importance of those ends up in reference to ethnobotanical information which is mentioned.*

Keywords: Antifungal, *Argemone Mexicana, Candida albicans,* Zone of inhibition

I. INTRODUCTION

A variety of microorganisms as well as fungi, bacteria, viruses, harbour the soil. Out of those microorganisms, few area units useful for the soil, some don't damage the soil in any respect neither have the benefit of it, however a number of them have the potential to cause major injury to plant growth moreover because the quality of soil. Soil borne illness will be a serious limitation for plants. The infective agent might stay dormant within the soil and may become morbific once more as shortly because the host is out there. Preventive measures taken for the soil to avoid contamination and healthy environmental conditions will be effective for healthy plant growth. Fungi are indirectly to be blamed for allergic or cytotoxic disorders due to the assembly of mycotoxins or allergens. Generally, for the management of phytopathogenic fungi use of artificial fungicides is finished. These chemical fungicides have tremendous effects on the plant as well as on the animals feeding on them Hence, there has been an increasing demand to create use of natural merchandise which will act as antifungal agents resulting in less injury to the surroundings and living organisms. Biologically active compounds found in plants are a lot safer than the artificial fungicides. Hence, extracts and oils of medicative plants has been used since it contains a great deal of secondary metabolites as compared to the other plants.

II. MATERIALS AND METHOD

2.1 Materials

A. Fungi Used

1. Aspergillus niger

Isolation: *Aspergillus* culture was obtained by doing agar plate method and the culture was observed under microscope and sub-culturing was done to obtain pure cultures of *Aspergillus niger*.Seven days old culture of fungiwas used.

Macroscopic Characters: The colony is black in colour and the reverse surface is white to light yellow in colour.

Microscopic Characters: Hypha is septate and hyaline. Conidiophore is present and arises from the basal foot. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia 3.5-5.0 µm in diameter, dark brown to black and rough-walled.

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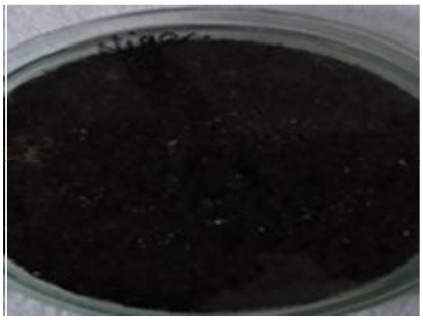
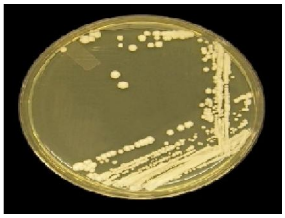


2. *Candida albicans*

Isolation: Candida culture was obtained by doing agar plate method which gave white colonies. The colony was then sub-cultured and candida albicans pure cultures were obtained. Seven days old culture of fungiwas used.

Macroscopic Characters: Colonies are white to cream in colour.

Microscopic Characters: Abundant branched pseudohyphae and true hyphae with blastoconidia are present. The blastoconidia are formed ingrape-like clusters along the length of the hyphae. Terminal chlamydoconidia may be formed with extended incubation



B. Plants Used

Argemone mexicana:

Species of poppy found in Mexico and now widely naturalized in many parts of the world It has bright yellow latex. It is poisonous to grazing animals, and it is rarely eaten, but it has been used medicinally by many peoples. *Argemone mexicana* seeds contain 22–36% of a pale yellow non-edible oil, called *argemone oil* or *katkar oil*, which contains the toxic alkaloids sanguinarine and dihydrosanguinarine. Four quaternary isoquinoline alkaloids, dehydrocorydalmine, jatrorrhizine, columbamine, and oxyberberine, have been isolated from the whole plant of *Argemone mexicana*. Many other alkaloids such as argemexicaines A and B, coptisine, cryptopine, allocryptopine and chelerythrine have also been found in this plant. The seed pods secrete a pale yellow latex when cut open. This argemone resin contains berberine and protopine.

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C. Culture Used

1. Liquid Culture Medium: Potato Dextrose Broth

Potato: 200g, Dextrose: 20g, Distilled water: 1000ml

Peeled and chopped potatoes were boiled in distilled water, till the water became starchy. Solution was filtered through muslin cloth and the volume was raised to 1000 ml by adding distilled water. Filtrate was then transferred to a conical flask and dextrose was added. The flask was plugged and autoclaved at the pressure of 15 lbs./sq. inch at 121°C for 20 minutes. The pH of the media was checked (5.6 ±0.2). Streptomycin was added before pouring the plates.

2. Solid Culture Medium: Potato Dextrose Agar

Potato: 200g, Dextrose: 20g, Distilled water: 1000ml, Agar: 14gm

Peeled and chopped potatoes were boiled in distilled water, till the water became starchy. Solution was filtered through muslin cloth and the volume was raised to 1000 ml by adding distilled water. Filtrate was then transferred to a conical flask and dextrose and agar were added. The flask was plugged and autoclaved at the pressure of 15 lbs./sq. inch at 121°C for 20 minutes. The pH of the media was checked (5.6 ±0.2). Streptomycin was added before pouring the plates.

D. Other Requirements

Petri plates, Conical flasks, Micropipettes, cork borer, forceps, nichrome loop, Laminar Air Flow, etc. The entire experiment must be carried out in Aseptic conditions with sterilized glasswares.

2.2 Method

A. Aqueous extract preparation from *Argemone mexicana*:

Leaves were crushed and soaked in Distilled water and kept on shaker overnight (150 rpm for 24 hours). Next day the mixture was filtered using muslin cloth. The extract was stored at 4°C.

Extract of Different Concentration:

Concentration (%)	Dried powder (gm)	Distilled water(ml)
10%	10	100
20%	20	100
30%	30	100
40%	40	100
50%	50	100

B. Testing of Plant aqueous Extract of *Argemone mexicana*:

1. In Liquid Culture Medium:49 ml of Potato dextrose Broth was added to each conical flask and 1 ml of plant extract was added to it. The total volume should be 50 ml in each conical flask. A control flask was maintained to compare the growth. The flasks were plugged and autoclaved for sterilization of Potato dextrose broth and extract.

Preparation of the concentrations of aqueous extract of *Argemone mexicana*:

Concentration g/ml (wt/vol)	Volume of extract(ml)	Volume of broth (ml)
Control	0	50
10	1	49
20	1	49
30	1	49
40	1	49
50	1	49

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After 7 days of incubation, the fungal growth was observed

Biomass Estimation: The flasks filtrate was separated by filtration with Whatmann filter paper. After filtration, the mat left on the filter paper was dried in oven (180°C for 2 hours). The dry weight was taken and biomass was calculated.

2. On Solid Culture Medium: The antifungal activity on Solid medium was studied using Agar well diffusion method. In this method, sterilized PDA is poured into the petri-plates aseptically then allowed to cool. About 5 mm diameter well (reservoir) was made in the centre of the petri-plate using a sterilized cork borer. The plates were then inoculated with fungal discs at equidistant radii. About 100 µl of the plant extract of respective concentration was filled in the well by using sterilized micropipettes.A control petri-plate was maintained without extract for comparison.Petri-plates were incubated for 7 days to observe the zone of inhibition.

III. OBSERVATIONS AND RESULTS

1. In liquid culture medium:

Control	Excellent	++++
10	Very Good	+++
20	Good	++
30	Fair	+
40	Poor Growth	+
50	No Growth	-

***Argemone mexicana* extract on *Aspergillus niger*:**

Concentration of Extract	Weight of Whatmann filter paper (g)	Weight of paper + Biomass (After drying)	Weight of Biomass (g)	Visual Fungal growth in PD Broth
Control	0.830	1.612	0.782	++++
10%	0.820	1.517	0.697	++++
20%	0.822	1.484	0.662	+++
30%	0.831	1.478	0.647	++
40%	0.821	1.440	0.619	+
50%	0.823	0.874	0.051	-

Aspergillus niger grown in PD Broth + *Argemone mexicana* aqueous extract

***Argemone mexicana* extract on Candida:**

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Concentration of Extract	Weight of Whatmann filter paper (g)	Weight of paper + Biomass (After drying)	Weight of Biomass (g)	Visual Fungal growth in PD Broth
Control	0.831	1.809	0.978	+++++
10%	0.882	1.158	0.276	+++
20%	0.825	1.078	0.253	+++
30%	0.812	0.988	0.176	++
40%	0.822	0.895	0.073	++
50%	0.841	0.871	0.030	-

Candida albicans grown in PD Broth + Argemone mexicana aqueous extract

2. In Solid Culture Medium

Argemone mexicana extract on *Candida*

Concentration of Extract	Inhibition zone observed on the 8th day (cm)		
	I	II	Average
Control	0.2	0.6	0.4
10%	0.1	0.8	0.45
20%	0.1	0.2	0.15
30%	0.1	0.6	0.35
40%	0.1	0.2	0.15
50%	0.3	0.4	0.35

IV. RESULTS

Results showed that A*rgemone mexicana* was effective as an antifungal extract since it showed Zone of Inhibition. Although, *Aspergillus niger* was resistant, still it did show reduction in biomass at higher concentrations of plant extracts. *Candida* on the other hand was very sensitive to plant extracts and hence growed slowly.

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A. On Liquid Culture Medium:

For aqueous extract of A*rgemone mexicana:*

The growth of *Aspergillusniger* was completely inhibited at 50% concentration of aqueous extract. Other concentrations showed fair amount of growth.The growth was very slow for *Candida* on aqueous extract on 10%, 20% - 40% showed great inhibition whereas, 50% didn't show growth at all. Hence, MIC was found in 50%.

B. on solid culture medium:

*Aspergillus niger*is resistant to the plant extracts (Aqueous & Ethanolic). When inoculated on equidistant radii it tends to overgrow in the reservoir in between too. Hence, agar well diffusion method was not an effective method to test the inhibition for *Aspergillus. niger*. The agar well diffusion method showed a clear zone of inhibition on higher concentrations of both Aqueous and Ethanolic extracts for *Candida*.

For aqueous extracts of *Argemone mexicana*:

As compared to the control plate and other concentrations (10%, 20%, 40%), *Candida albicans* showed very clear inhibition in 30% and 50% concentrations.

V. DISCUSSION

Plants are susceptible to pathogenic fungal attacks which causes major loss of yield and damage to the quantity and quality of its product. To overcome this problem chemical fungicides were used. The usage of this chemical compounds results in accumulation of toxinogenic chemicals in the plant and has adverse effects. To avoid these problems and promote healthy growth of plants use of chemical fungicides are eradicated and natural fungicides which has minimal or no side effects are used.According to the research, *Argemone mexicana* consists of active constituents whichis known to show inhibition for fungal activity against murine pulmonary aspergillosis and candiadis.For testing the efficiency of aqueous extracts two types of methods were performed - 1. Liquid culture media method (Broth method) and 2. Solid culture media method (Agar well-diffusion method). On Liquid culture media for aqueous extract showed complete inhibitory effect on both fungus on 50% concentrations and reduction in mat at 40% concentration.On solid culture media inhibitory zones were observed at 50% concentration in *Candida* for aqueous plant extracts and *Aspergillus niger* did not show a zone of inhibition.

VI. CONCLUSION

The above experiment has concluded that that Aqueous plant extract of Argemone mexicana is effective against the both pathogenic fungi i.e., *Aspergillus niger* and *Candida albicans*. *Aspergillus niger* showed more resistance at most concentrations. *Candida* being sensitive to the antifungal compounds present in theextracts shows considerable decrease in biomass and showed complete inhibition at the highest concentrations used (50%). The variations in the effects of plant extracts on inhibiting the pathogenic fungi was due to the variable concentrations of plant extract we used. Also, the of solvent used for plant extract preparation has an effect on inhibition.Studies indicate that the higher the concentration of plant extract the higher is effect of inhibition. Higher concentrations of the plant extract show tremendous reduction in *Aspergillus niger* growth and complete inhibition for *Candida*. *Aspergillus niger* does not show zones of inhibition even on higher concentrations whereas, *Candida* does.

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