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Antifungal Activity of the Plant Extracts of *Alstonia scholaris*, *Argemone maxicana* and *Datura alba* to Control Growth of Opportunistic Fungus (*Aspergillus niger*)

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ABSTRACT

The present investigation was carried out to observe the antifungal activity of *Alstonia scholaris*, *Argemone maxicana* and *Datura alba*. For this purpose effect of different alcoholic extract concentration was observed on growth performances of *Aspergillus niger* on 5th and 7th day. Our result shows that alcoholic extract concentrations inhibit radial growth of this fungus. Results also indicate that inhibition of fungal growth increase with the increase in the concentration of alcoholic extracts.

Keywords: Medicinal plants extracts, Antifungal activity, Alcoholic extract, *Aspergillus niger*.

INTRODUCTION

Herbal medicines used against various fungal diseases. Antifungal activity of natural plant extract and pure compound can be detected by inhibition of various microflora like yeast, fungi by samples that are placed with them. About 100,000 species of fungus are present in the environment and more than 100 of them are pathogenic in human⁶. Many of the Pharmaceuticals like opium, aspirin, digitalis, quinine etc have a long history of usage as herbal remedies. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants^{4,8}. Plants and their extracts have been used all over the world in folk medicines and the use of extracts has been supported by the isolation of antifungals from the plants. Many plants produce secondary metabolites. These metabolites may serve as potent antimicrobial agents and thus may be useful for human beings. It has been estimated by the World Health Organization (WHO) that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care¹. Lupeol and Epicatechin have been identified in the methanol extract of *Alstonia scholaris*. This extract has shown antioxidant and anticancer effect. It also showed significant antimicrobial effect against *Staphylococcus aureus* and gram negative organisms like *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas auriginosa* and *Candida albicans*⁹. Traditionally herbal medicines provide an interesting, largely unexplored source of potential new drugs¹⁰. Antifungal activity of eight medicinal plants extract (*Aloe vera*, *Ocimum sanctum*, *Cenetella asiatica*, *Piper betle*, *Calotropis gigantea*, *Vitex negundo*, *Ocimum basilicum* and *Azadirachta indica*) was assayed by agar well diffusion method on plant pathogenic fungus (red rot disease causing agent) *Colletotrichum falcatum*. The result revealed that the extract of eight medicinal plants showed significant reduction in growth of *C. falcatum*⁷. Antony et al., 2012 have reported that butanolic extract of bark of the *Alstonia scholaris* have potent anti-tubercle effect and anti-*Mycobacterium tuberculosis* potential and it was concluded that it is a promise for future therapeutic interventions. Therefore present investigation has

been aimed to evaluate the antifungal activity of alcoholic extracts of three medicinal plants against the pathogenic fungus viz. *Aspergillus niger*.

MATERIAL AND METHODS

Sample Collection

Samples for the following medicinal plants were collected from district Saharanpur & Shivalik belt of Uttar Pradesh as well as from Garhwal hills of Uttarakhand, India.

1. *Alstonia scholaris*
2. *Argemone maxicana*
3. *Datura alba*

The freeze-dried pathogenic fungi *Aspergillus niger* was obtained from Forest Pathology Division, Forest Research Institute, Dehradun. The cultures were maintained on Sabouraud Dextrose Agar (SDA) slants and kept refrigerated until used. The SDA plate cultures were inoculated from the slants and incubated at $25 \pm 1^\circ\text{C}$ for 7 days.

Plant Extract Preparation

For the preparation of various plant extracts 5 gm of fresh plant part was washed 2-3 times with distilled water and then treated with 0.1 % HgCl_2 solution for sterilization. After surface sterilization plant samples were ground in mortar and pestle with 50 % methanol. The homogenized liquid was filtered and centrifuged at 5000 rpm. The supernatants were used as test extract & make up into 20 ml using 50 % methanol. Further, the extract was diluted into different concentrations, i.e. 10 %, 25 % and 50 %. 20 ml of SDA (Sabouraud Dextroes Agar) culture medium with 5 ml of the above concentration of the extracts were poured in sterile petriplates and allowed to solidify. In the control same volume of distilled water (in place of experimental material) was mixed in appropriate amounts.

Fungal Inoculation

For antifungal activity mycelia discs of 5 mm diameter were cut from the periphery of 7 day old culture of the test organisms and were aseptically inoculated upside down on the surface of the SDA medium in plates. Inoculated petriplates were incubated at $25^\circ\text{C} \pm 10\text{C}$ and observation were recorded at 5th and 7th day.

After 5th and 7th day of incubation, observations were recorded on the basis of colony diameter (cm) on medium and percent inhibition of radial growth was calculated using following formula:

$$\% \text{ Growth Inhibition} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treated sets} \times 100}{\text{Colony diameter in control}}$$

OBSERVATIONS AND RESULT

The present investigation was carried out to observe the antifungal activity of *Alstonia scholaris*, *Argemone maxicana* and *Datura alba*. For this purpose effect of different alcoholic extracts concentrations with (10%, 25% and 50%) were observed on the growth performances of *Aspergillus niger* causing human skin diseases are given in Table 1.

Antifungal activity of *Alstonia scholaris* on *Aspergillus niger*

Table 1 shows that in 10%, 25% and 50% alcoholic root extracts of *Alstonia scholaris* the growth of *Aspergillus niger* was 75.0%, 62.5%, 50% control respectively in 5th day old culture plate. Similarly, the growth is inhibited in presence of alcoholic shoot and seed extracts in culture medium. However, the inhibition of growth found more at higher concentrations. So in presence of 50% alcoholic shoot and seed extract the growth of *Aspergillus niger* is 43.3% and 48.3% of control respectively at 7th day. Nearly similar pattern of growth inhibition found in various other concentrations at both days of studies.

Antifungal activity of *Argemone maxicana* on *Aspergillus niger*

Observation shows that alcoholic extracts of *Argemone maxicana* plant part is inhibitory to the growth of *Aspergillus niger*. Results have shown that higher concentration of alcoholic extract is inhibitorier as compared to the lower concentration of alcoholic extracts.

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Thus, 50 % root extract causes 67 % growth inhibition at 7th day. Likewise, growth of *Aspergillus niger* on both day in various concentrations of seed extracts also inhibited. Thus, in 50 % alcoholic seed extract at 5th day and 7th day the growth is 50.0 % and 55.5 % of control respectively.

Table 1 also shows that shoot extract concentrations are also inhibitory to the growth of *Aspergillus niger*. Thus, in 10%, 25%, and 50% alcoholic root concentration the fungal growth at 7th day is 90.0%, 70.0% and 46.6% of control respectively.

Antifungal activity of *Datura alba* on *Aspergillus niger*

Result in table 1 shows that alcoholic plant part extracts of *Datura alba* are inhibitory to the growth of *Aspergillus niger*. Results have shown that inhibition of fungal growth increases with the increase in the concentrations of alcoholic extracts. Thus, 10% alcoholic seed extract causes 18.0% inhibition of *Aspergillus niger* growth at 7th day, however, this inhibition in 50% alcoholic seed extract at 7th day is ca. 52%. Root and shoot extracts of this plant also inhibits the growth of *Aspergillus niger* in culture medium. Thus, in 10%, 25%, and 50% alcoholic root extract the Fungal mycelial growth is 85.7%, 68.8% and 53.5% of control respectively at 7th day. Likewise, in 10%, 25% and 50% shoot extract concentration the mycelial growth is 83.3%, 66.6% and 46.6% of the control respectively at 7th day of growth.

Table 1: Antifungal activity of *Alstonia scholaris*, *Argemone maxicana*, *Datura alba* on growth performance of *Aspergillus niger*

Days	<i>Alstonia scholaris</i>			<i>Argemone maxicana</i>			<i>Datura alba</i>		
	Root	Shoot	Seed	Root	Shoot	Seed	Root	Shoot	Seed
Growth in Control 0% extract									
5 th	2.4	2.6	2.8	2.1	1.6	1.8	2.6	2.3	2.4
7 th	2.8	3.0	3.1	3.0	2.8	2.7	2.8	3.0	2.9
Growth in 10% alcoholic extract									
5 th	1.8	2.4	2.6	1.8	1.5	1.4	1.6	1.8	1.7
7 th	2.0	2.8	3.0	2.7	2.5	2.0	2.4	2.5	2.4
Growth in 25% alcoholic extract									
5 th	1.5	1.6	1.8	1.2	1.1	1.0	1.3	1.4	1.2
7 th	1.9	2.2	2.0	2.1	2.2	2.0	1.9	2.0	1.8
Growth in 50% alcoholic extract									
5 th	1.2	1.3	1.6	1.0	0.8	0.9	1.0	1.2	1.2
7 th	1.7	1.3	1.5	1.4	2.0	1.5	1.5	1.4	1.4

DISCUSSION AND CONCLUSION

Studies on herbal plant extracts showed that the various solvent extracts showed promising antimicrobial activity against fungal human pathogens. These extracts can be utilized for isolation and characterization of therapeutically active chemical constituents used in modern medicines. Alcoholic plant extract used here showed significant antifungal activity against *Aspergillus niger*. So this antifungal property provides a scientific basis for the use of these plants as suitable antifungal agent. This extract can be used against infection caused by *Aspergillus niger*. This study also encourages that these plant should be cultivated in large scale to increase the use of these plant in traditional medicine. Results with different alcoholic extract concentration of *Alstonia scholaris*, *Argemone maxicana* and *Datura alba* on the radial growth of pathogenic fungus like *Aspergillus niger*, clearly shows that alcoholic extract concentration inhibits radial growth of opportunistic fungi. Result indicates that inhibition of fungal growth increase with the increase in the concentration of alcoholic extracts.

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