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RESEARCH ARTICLE

SCREENING OF MEDICINAL PLANTS EXTRACTS TO CONTROL GROWTH OF OPPORTUNISTIC FUNGUS (*ASPERGILLUS FLAVUS*)

***Vijai Malik**

Department of Botany M.S. College Saharanpur (U.P.) India

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ABSTRACT

The aim of this study is to evaluate the fungicidal property of *Alstonia scholaris, Argemone maxicana* and *Datura alba*. For this purpose effect of different alcoholic extract concentrations were observed on growth performances of *Aspergillus flavus* on 5th and 7th day. Result shows that alcoholic extract concentrations inhibit radial growth of this fungus. Result also indicates that inhibition of fungal growth increase with the increase in the concentration of alcoholic extracts.

INTRODUCTION

India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases. Various medicinal plants have been used for years in daily life to treat diseases all over the world. Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. Medicinal uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts from the plants. These plant extracts are source of many potent and powerful drugs. 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* (Prince and Prabakaran, 2011). 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 (Antony et al., 2012). 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 care (Akerele, 1993). More than 100,000 species of fungi are known to cause diseases in plants about 50 species causes diseases in humans and about as many cause in the animals most of them superficial diseases of the skin or appendages (George, 1997). 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 (Cragg *et al.*, 1997 and Shu 1998). Traditionally herbal medicines provide an interesting, largely unexplored source of potential new drugs (Udgirkar *et al.,* 2012). Therefore the present work is an extension to the anti-microbial work and aimed at investigating the antifungal activity of the alcoholic extracts of three medicinal plants.

**Corresponding author: Vijai Malik*
Department of Botany M.S. College Saharanpur (U.P.) India

MATERIALS AND METHODS

Sample Collection

Samples for the following medicinal plants were collected from district Saharanpur & Shiwalik 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 flavus* 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 ± 1°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 25^0 C ± 1^0C and observations 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:

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

RESULTS AND DISCUSSION

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 flavus* causing human skin diseases are given in Table 1.

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

Result in table 1 shows that the various alcoholic extract concentrations of root, shoot and seed causes inhibition of the radial growth of this opportunistic fungi. Thus in 10%, 25% and 50% alcoholic root extract the radial growth values are 86.9%, 78.2% and 69.5% of control respectively at 7th day of growth. Table 1 further shows that like root extracts the growth is further inhibited in various concentrations of shoot extract and seed extract. Thus, at 7th day in 50% seed extract the growth is 50.0% as compared to control.

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

Table 1 shows that alcoholic extract from *Argemone maxicana* of different concentrations inhibit the growth of *Aspergillus flavus.* 10%, 25% and 50% root extract of this plant retards radial growth of this fungi in culture medium by 18%, 43% and 47% of the control respectively at 7th day of growth. Observation further shows that like root extract growth is also inhibited in the presence of shoot and seed extract under culture medium. Result further shows that the growth of this fungus inhibited more in presence of higher alcoholic concentrations as compared to lower aqueous concentrations of extract of various plant parts.

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

Results of effects of various concentrations of alcoholic extracts of *Datura alba* plant parts on the radial growth of *Aspergillus flavus* given in Table 1. Growth studies done at 5th and 7th day, reveals that growth is more inhibited at 7th day in higher alcoholic extract concentration. Thus, at 7th day root, shoot and seed alcoholic extract inhibits growth of *Aspergillus flavus* by 50.0%, 56.0% and 42.8% of control respectively in 50% alcoholic extract concentration. Likewise, growth of this fungus at 5th day in seed extract is 77.7%, 66.6% and 55.5% in 10%, 25% and 50% alcoholic concentration of seed respectively. Table 1 also shows that like seed extract, root and shoot extract also shows inhibitory effect on growth of this fungus, however, inhibition increases with the increase in concentrations.

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

Days	*Alstonia scholaris*			*Argemone maxicana*			*Datura alba*		
	Root	Shoot	Seed	Root	Shoot	Seed	Root	Shoot	Seed
Growth in Control 0% extract									
5th	1.8	1.6	1.5	1.6	1.8	1.6	1.9	2.0	1.8
7th	2.3	2.5	2.0	2.8	2.8	2.9	2.6	2.5	2.8
Growth in 10% alcoholic extract									
5th	1.4	1.3	1.0	1.3	1.2	1.2	1.6	1.7	1.4
7th	2.0	2.0	1.7	2.0	2.2	1.9	2.2	2.2	2.0
Growth in 25% alcoholic extract									
5th	1.2	1.0	0.8	1.0	1.1	1.0	1.4	1.4	1.2
7th	1.8	1.7	1.6	1.6	2.0	1.6	1.6	1.8	1.7
Growth in 50% alcoholic extract									
5th	1.1	0.9	0.7	0.8	0.8	0.9	1.0	1.2	1.0
7th	1.6	1.6	1.0	1.2	1.3	1.1	1.3	1.4	1.2

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 flavus*. So this antifungal property provides a scientific basis for the use of these plants as suitable antifungal agent. Extracts of these plants can be used against infection caused by *Aspergillus flavus.* This study also

encourages that these plant should be cultivated in large scale to increase the use of these plant in traditional medicine. Plants based natural products traditionally known to combat microbial infections are expected to play a big role in this regard (Cowan, 1999). Results with different alcoholic extract concentrations of *Alstonia scholaris, Argemone maxicana* and *Datura alba* on the radial growth of pathogenic fungi like *Aspergillus flavus,* clearly shows that alcoholic extract concentration inhibits radial growth of this opportunistic fungus. Result also indicates that inhibition of fungal growth increases with the increase in the concentration of alcoholic extracts.

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