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### ANTIFUNGAL EFFECT OF SELECTED MEDICINAL PLANTS EXTRACT AGAINST GROWTH OF PATHOGENIC FUNGUS *ASPERGILLUS FLAVUS*

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**Abstract:** The aim of this study is to evaluate the fungicidal property of *Alstonia scholaris*, *Argemone maxicana*, *Datura alba*, *Solanum nigrum* and *Solanum xanthocarpum*. For this purpose effect of different alcoholic extract concentrations were observed on growth performances of *Aspergillus flavus* on 5<sup>th</sup> and 7<sup>th</sup> 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.

**Keywords:** Medicinal plants, Antifungal activity, Alcoholic extract, pathogenic Fungus, *Aspergillus flavus*.



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## INTRODUCTION

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 causes superficial diseases of the skin or appendages.<sup>1</sup> More than 300 fungal metabolites are reported to be toxic to man and animals. The main toxic effects are carcinogenicity, genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression.<sup>2</sup> Various medicinal plants have been used for years in daily life to treat diseases all over the world. According to World Health Organization (WHO) more than 80% of the world population relies on traditional medicines for their primary healthcare needs.<sup>3</sup> Plants based natural products traditionally known to combat microbial infections are expected to play a big role in this regard.<sup>4</sup> India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases. Crude extracts of some well known medicinal plants are used to control some of the plants pathogens.<sup>5</sup> 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. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine.<sup>6-7</sup> 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.<sup>8</sup> *Argemone mexicana* seed extract are effective against cutaneous infection, skin diseases, warts, sores, dropsy, jaundice and antibacterial.<sup>9</sup> All *Datura* plants contain tropane alkaloids such as scopolamine, hyoscyamine, and atropine, primarily in their seeds and flowers. *Datura* has been used for centuries in some cultures as a poison and as a hallucinogen.<sup>10</sup> *Datura metel* is known for its use in fever with catarrh, cerebral complications, diarrhea, skin diseases, antiseptic, animal bites, antihelminthic and in herpetic diseases, and also has healing potential on burn wounds. *Datura metel* extracts were reported to have hypoglycemic and antimutagenic properties. It is also known for its larvicidal activity against the third instars larvae of *Culex quinquefasciatus* antibacterial activity against burn pathogens and antifungal activity against various plant pathogens.<sup>11</sup> *Solanum nigrum* is herb is antiseptic, antidysentric and diuretic in nature and used in the treatment of cardiac, skin disease, psoriasis, herpi virus and inflammation of kidney and have antimicrobial agent kills or inhibit the growth of microorganisms such as *Echerichia coli*, *Bacillus subtilus*, *K. pneumonia*, *V. cholera*, *Micrococcus luteus*, *Salmonella typhiurium*, *Aspergillus niger*, *A. flevus* *A. fumigatus*,

*Candida albicans*, to cure liver disorders, chronic skin ailments (Psoriasis and ringworm) fevers, diarrhoea, eye diseases, hydrophobia. They are also useful in otopathy, ophthalmopathy, rhinopathy and hepatitis.<sup>12</sup> *Solanum nigrum* has been used as the important ingredient for herbal formulations in India, namely Liv.-52, which is mainly used for treating liver diseases.<sup>13</sup> Fruits of *Solanum xanthocarpum* were investigated for hypoglycemic activity in rats and mice. Its fatty oil also possesses significant antifungal activity. Major chemical constituents present in plant Solasodine, Steroidal alkaloids,  $\beta$ -Solamargine, Cycloartenol, Norcarpesterol, Cholesterol, Solanocarpine, Carpesterol, Atropine, Diosgenin and  $\beta$ -Sitosterol, 3-Hydroxy-3 Methyl Glutaryl COA Reductase, Solanine, Glycosides, Phenolics, Flavonoids, Steroid and Saponins.<sup>14</sup> 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.<sup>15</sup> 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.<sup>16-17</sup> Traditionally herbal medicines provide an interesting, largely unexplored source of potential new drugs.<sup>18</sup> Therefore the present work is an extension to the antimicrobial work and aimed at investigating the antifungal activity of the alcoholic extracts of five medicinal plants.

## MATERIALS AND MEDTODS

### *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*
4. *Solanum nigrum*
5. *Solanum xanthocarpum*

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  $25 \pm 1^\circ\text{C}$  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%  $\text{HgCl}_2$  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 Sabouraud Dextrose Agar (SDA) 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  $25^\circ\text{C} \pm 1^\circ\text{C}$  and observations were recorded at 5<sup>th</sup> and 7<sup>th</sup> day. After 5<sup>th</sup> and 7<sup>th</sup> 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*, *Datura alba*, *Solanum nigrum* and *Solanum xanthocarpum*. 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 7<sup>th</sup> 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 7<sup>th</sup> 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 7<sup>th</sup> 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 5<sup>th</sup> and 7<sup>th</sup> day, reveals that growth is more inhibited at 7<sup>th</sup> day in higher alcoholic extract concentration. Thus, at 7<sup>th</sup> 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 5<sup>th</sup> 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.

#### ***Antifungal activity of Solanum nigrum on Aspergillus flavus***

Table 1 shows that the alcoholic extract from *Solanum nigrum* of root, shoot and seed is inhibitory to the growth of this fungus. Further shows that with the increase in concentrations the rate of growth inhibition also increases. Thus, 10%, 25% and 50% alcoholic root extract of *Solanum nigrum* the radial growth of this fungus in culture medium 17%, 34% and 50% of the control respectively at 7<sup>th</sup> day of growth. Observation further shows that like root extract growth is also inhibited in the presence of shoot and seed alcoholic extract under culture medium. Result further shows that the growth

of this fungus inhibits more in presence of higher concentrations as compared to lower concentrations of extract.

### ***Antifungal activity of Solanum xanthocarpum on Aspergillus flavus***

Table 1 shows that alcoholic extract from *Solanum xanthocarpum* of different concentrations inhibit the growth of *Aspergillus flavus*. 10%, 25% and 50% seed extract of this plant retards radial growth of this fungi in culture medium by 76.66%, 60.00% and 46.66% of the control respectively at 7<sup>th</sup> day of growth. Observation further shows that like seed extract growth is also inhibited in the presence of root and shoot 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.

**Table 1 : Antifungal activity of *Alstonia scholaris*, *Argemone maxicana*, *Datura alba*, *Solanum nigrum* and *Solanum xanthocarpum* on growth performance of *Aspergillus flavus*.**

Days	<i>Alstonia scholaris</i>			<i>Argemone maxicana</i>			<i>Datura alba</i>			<i>Solanum nigrum</i>			<i>Solanum xanthocarpum</i>		
	Root	Shoot	Seed	Root	Shoot	Seed	Root	Shoot	Seed	Root	Shoot	Seed	Root	Shoot	Seed
<b>Growth in Control 0% extract</b>															
5 <sup>th</sup>	1.8	1.6	1.5	1.6	1.8	1.6	1.9	2.0	1.8	1.9	1.8	1.8	1.8	1.9	2.0
7 <sup>th</sup>	2.3	2.5	2.0	2.8	2.8	2.9	2.6	2.5	2.8	2.4	2.6	2.3	2.6	2.7	3.0
<b>Growth in 10% alcoholic extract</b>															
5 <sup>th</sup>	1.4	1.3	1.0	1.3	1.2	1.2	1.6	1.7	1.4	1.5	1.4	1.3	1.0	1.3	1.5
7 <sup>th</sup>	2.0	2.0	1.7	2.0	2.2	1.9	2.2	2.2	2.0	2.0	2.1	2.0	1.7	1.9	2.3
<b>Growth in 25% alcoholic extract</b>															
5 <sup>th</sup>	1.2	1.0	0.8	1.0	1.1	1.0	1.4	1.4	1.2	1.3	1.1	1.0	0.8	1.0	1.2
7 <sup>th</sup>	1.8	1.7	1.6	1.6	2.0	1.6	1.6	1.8	1.7	1.6	1.5	1.6	1.2	1.5	1.8
<b>Growth in 50% alcoholic extract</b>															
5 <sup>th</sup>	1.1	0.9	0.7	0.8	0.8	0.9	1.0	1.2	1.0	1.0	1.0	0.9	0.7	0.9	1.0
7 <sup>th</sup>	1.6	1.6	1.0	1.2	1.3	1.1	1.3	1.4	1.2	1.2	1.3	1.2	1.1	1.2	1.4

## DISCUSSION AND CONCLUSION

Studies on herbal plant extracts showed that the various solvent extracts 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.<sup>4</sup> Results with different alcoholic extract concentrations of *Alstonia scholaris*, *Argemone maxicana*, *Datura alba*, *Solanum nigrum* and *Solanum xanthocarpum* on the radial growth of pathogenic fungi like *Aspergillus flavus*, clearly shows that alcoholic extract concentration inhibits radial growth of this opportunistic fungus. Result indicates that inhibition of fungal growth increases with the increase in the concentration of alcoholic extracts.

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