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Antifungal activity of methanolic and ethanolic leaf extracts of medicinal plants

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ABSTRACT

The following plants were screened for thew study *viz.*, *Ocimum sanctum* (*Tulsi*), *Withania somnifera* (Ashwagandha) and *Asparagus racemosus* (Shatavari) which were traditionally used in India to treat various diseases. In evaluating antioxidant property and phytochemical analysis, all three plants were screened for antifungal activity. It was evaluated using Well diffusion method. The extracts were tested against fungus *Aspergillus niger*. Inhibition of fungal growth was investigated using PDA well diffusion method. The contents of total flavonoid compounds in crude methanolic and ethanolic extracts obtained from *Ocimum sanctum*, *Withania somnifera* and *Asparagus racemosus* leaves.

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INTRODUCTION

Since the ancient times, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Rios and Recio, 2005). These infections may be locally within the dermis and some can subsequently become generalized as a blood infection. Because of the side effect and the resistance that pathogenic micro-organisms build against the antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Sour, 2000). According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants have been investigated for better understanding their medicinal properties. Medical uses of plants range from the administration of roots, barks, stems, leaves and seed to the use of extracts and decotion from the plants (Ogbulie *et al.*, 2007).The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries (Daniel and Hopf, 2000).Generally the domestic

medicinal plants like *Tulsi* (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), *Shatavari* (*Asparagus racemosus*), are highly used in domestic purpose. These plant extracts have secondary metabolites of phytochemicals that have too many pharmacologically active substances which are useful to make drug to treat against various infectious diseases caused by fungi. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. These are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties (Miliauskas *et al.*, 2004). In this study, the antifungal activities of selected plants, *Tulsi* (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Shatavari (*Asparagus racemosus*), were tested against selected fungal (*AspegilIus niger*) culture, respectively.

MATERIAL AND METHODS

The fresh leaves and petals were collected from the Nursery of the School of Forestry and Environment, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad which were surface sterilized simply by washing under tap water and Distilled water and dried in shed for 20 days. After drying, leaves and petals were grounded in a grinder mixer to powdered form and stored for further use. The antifungal activity of plant leaves was tested against the following fungus *viz.*, *Aspergillus niger* which was collected from Microbial Culture Collection Bank, Sam Higginbottom Institute of Agriculture, Technology and Sciences. The culture was sub cultured on nutrient agar slants and stored at 4^0C till use. Plant extracts were prepared using two organic solvents, *viz.*, Ethanol and Methanol. The antifungal activity of *Ocimum sanctum* (*Tulsi*), *Withania somnifera* (Ashwagandha) and *Asparagus racemosus* (Shatavari) leaves was evaluated against *Aspergillus niger* in methanolic and ethanolic extract by using Well diffusion method. Total flavonoid content (Morena *et al.*, 2000) was also determined for *Ocimum sanctum* (*Tulsi*), *Withania somnifera* (Ashwagandha) and *Asparagus racemosus* (Shatavari).

RESULTS AND DISCUSSION

Plant extracts were prepared from dried powdered samples. Both the methanolic and ethanolic extracts were taken to study the antifungal activity of leaves of *Ocimum sanctum* (*Tulsi*), *Withania somnifera* (Ashwagandha) and *Asparagus racemosus* (Shatavari), against one test organism *viz.*, *Aspergillus niger* which is pathogenic for human being and cause severe diseases. Distilled water was taken as control. Well diffusion method was used in this present study in order to get the antifungal properties of the different plant extracts against the test organism.

Result for antifungal activity of *Ocimum sanctum* in methanolic and ethanolic extracts :

The following figures and tables clearly indicated that the zone of inhibition for methanolic extract of

Table 1 : Antifungal activity of *Ocimum sanctum* against *A. niger*

Solvent / Fungal species	Methanolic extract (Zone of inhibition in mm)	Ethanolic extract (Zone of inhibition in mm)	Distilled water (control) (Zone of inhibition in mm)
A. niger	20 ± 1.0	18 ± 1.0	00

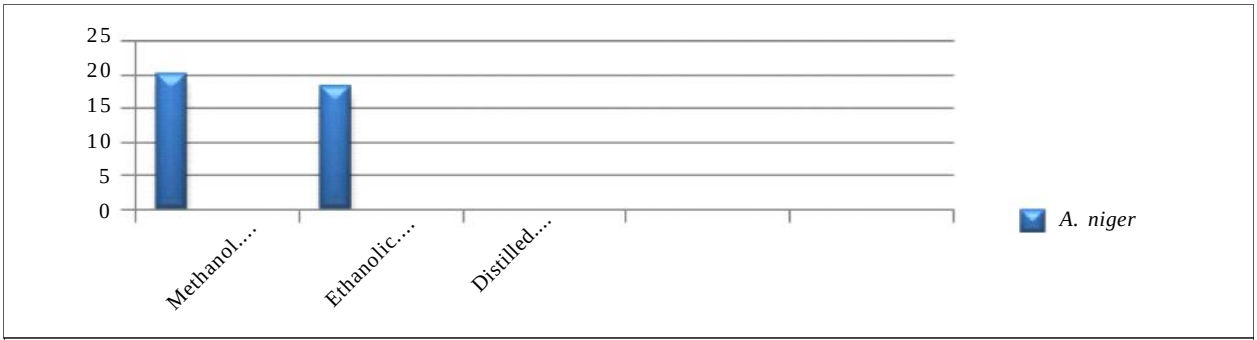

Fig. 1 : Antifungal activity of *Ocimum sanctum* against *A. niger*

In methanolic extract **In ethanolic extract**

Fig. 2 : *Ocimum sanctum* against *A. niger*

Ocimum sanctum was maximum against *A. niger* whereas ethanolic extract showed minimum zone of inhibition against *A. niger*. Distilled water (control) showed no zone of inhibition (Table 1; Fig. 1 and 2).

The zone of inhibition for methanolic and ethanolic extract gave maximum 20 ± 1.0 mm and the minimum zone of inhibition 18 ± 1.0 mm against *A niger* was observed in extract of *Ocimum sanctum* (Fig. 2).

Result for antifungal activity of *Withania somnifera* in methanolic and ethanolic extracts :

The following figures and tables clearly indicated that the zone of inhibition for methanolic extract of *Withania somnifera* was maximum against *A. niger* whereas ethanolic extract showed minimum zone of inhibition against *A. niger*. Distilled water (control) showed no zone of inhibition (Table 2; Fig. 3 and 4).

Table 2 : Antifungal activity of *Withania somnifera* against *A. niger*

Solvent / Fungal species	Methanolic extract (Zone of inhibition in mm)	Ethanolic extract (Zone of inhibition in mm)	Distilled water (control) (Zone of inhibition in mm)
W. somnifera	12 ± 1.0	11 ± 1.0	00

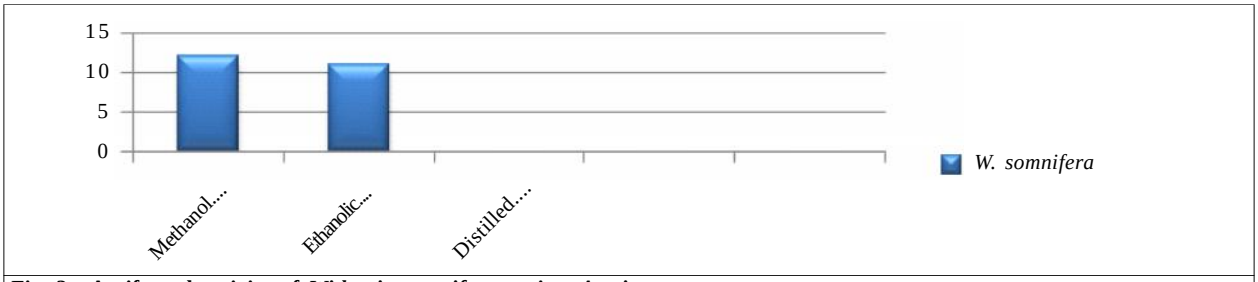

Fig. 3 : Antifungal activity of *Withania somnifera* against *A. niger*

In methanolic extract **In ethanolic extract**

Fig. 4 : *Withania somnifera* against *A. niger*

The zone of inhibition for methanolic and ethanolic extract gave maximum 20 ± 1.0 mm and the minimum zone of inhibition 18 ± 1.0 mm against *A niger* was observed in extract of *Withania somnifera* (Fig. 4).

Result for antifungal activity of *Asparagus racemosus* in methanolic and ethanolic extracts :

The following figures and tables clearly indicated that the zone of inhibition for methanolic extract of *Asparagus racemosus* was maximum against *A. niger* whereas ethanolic extract showed minimum zone of inhibition against *A. niger*. Distilled water (control) showed no zone of inhibition (Table 3; Fig. 5 and 6)

The zone of inhibition for methanolic and ethanolic extract gave maximum 11 ± 1.0 mm and the minimum zone of inhibition 10 ± 1.0 mm against *A. niger* was observed in extract of *Asparagus racemosus* (Fig. 6).

Results for total flavonoid content (TFC) :

The contents of total flavonoid compounds in crude methanolic and ethanolic extracts obtained from *Ocimum sanctum*, *Withania somnifera* and *Asparagus racemosus* leaves are presented in Table 4 and Fig. 7. The results were reported as Quercetine Equivalents (QE) mg/g extract.

Table 5 showed that the highest concentration of

Table 3 : Antifungal activity of *Asparagus racemosus* against *A. niger*

Solvent / Fungal species	Methanolic extract (Zone of inhibition in mm)	Ethanolic extract (Zone of inhibition in mm)	Distilled water (control) (Zone of inhibition in mm)
A. racemosus	11 ± 1.0	10 ± 1.0	00

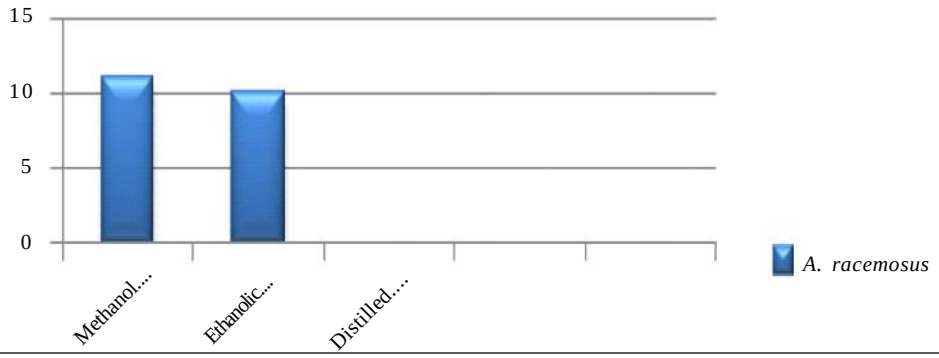

Fig. 5 : Antifungal activity of *Asparagus racemosus* against *A. niger*

In methanolic extract **In ethanolic extract**

Fig. 6 : *Asparagus racemosus* against *A. niger*

Table 4: Standard values of quercetine at different concentrations

Standard	Concentration(mg/ml)	O.D. at 415 nm
Quercetine	0.02	0.224
	0.04	0.450
	0.06	0.666
	0.08	0.854
	0.10	1.054

Table 5 : Total flavonoids content of methanolic and ethanolic extracts of various plant samples under study

Plant material	Plant part used	Concentration of plant extract (mg/ml)	O.D. at 415 nm (Methanolic extract)	O.D. at 415 nm (Ethanolic extract)	Total flavonoid (mg QE/g extract)
O. sanctum	Leaves	1.0	1.241	1.506	0.29
W. somnifera	Leaves	1.0	0.992	1.306	0.30
A. racemosus	Leaves	1.0	1.201	1.238	0.31

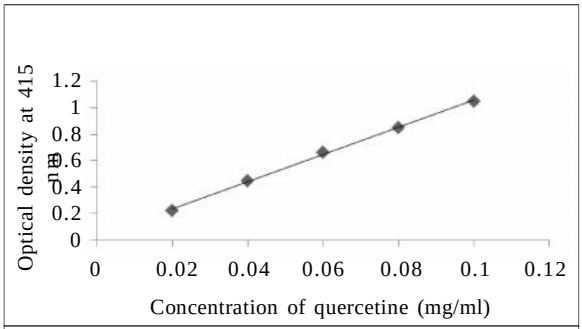

Fig. 7 : Standard graph of quercetine

total flavonoids was 0.31 mg QE/g present in the methanolic and ethanolic extract of *A. racemosus* at 1.0 mg/ml concentration, whereas the lowest concentration of total flavonoids was 0.29 mg QE/g in methanolic and ethanolic extract of *O. sanctum* at 1.0 mg/ml concentration. While in case of methanolic and ethanolic extract of *W. somnifera*, the total flavonoids content was found to be 0.30 mg QE/g at 1.0 mg/ml concentration.

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