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In vitro activity of selected medicinal plants against pathogenic fungi

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Abstract
The efficacy of eighteen crude plant extracts to control the growth of four phytopathogens *viz. Sclerotium rolfsii*, *Fusarium oxysporum*, *Alteraria solani* and *Phytophthora capsici* was evaluated *in vitro*. Assays proved that two plant extracts *Sida rhombifolia* and *Boerhavia diffusa* provided significant inhibition of mycelial growth of the fungi under investigation. While *S. rhombifolia* leaf extract inhibited the colony growth of *S. rolfsii*, *A. solani* and *P. capsici* upto 94.10%, 75.14% and 55.15% respectively; *B. diffusa* leaf extract was effective against *S. rolfsii* and *A. solani*, inhibiting the colony growth upto 86.82% and 79.13% respectively. Thus, the study demonstrated that both plants have the potential to be used as biocontrol agents to suppress the growth of some phytopathogens.

Keywords: botanical, phytopathogen, plant extract, antifungal, natural fungicides

Introduction
Over the centuries, man has struggled to protect the economically important crops against the invasion of insects, microbial pathogens, and other pests. Crop losses are visible in tropical or subtropical regions where the temperature is moderately higher than the other parts of the world due to fungal species. Pathogenic fungi are the leading infectious agents which causes detrimental changes during the developmental stages in plants. Fungal pathogens not only devastate crop growth and physiology but also, affect further transportation, post-harvest loss. Fungi can produce several types of mycotoxins (aflatoxins, ochratoxins, patulins, fumonisin, zearalenone, deoxynivalenol, etc.) in the stored food products which lands in economic loss in crop management and postharvest storage. Mycotoxins are mostly produced by ubiquitous, saprophytic fungal strains which exhibits a serious worldwide food safety problem with an estimate of about twenty-five percent of world's crop annually affected [1] To combat the fungal pathogenesis, several chemical and synthetic inputs have been showered to the crop plants which landed to environmental pollution accompanying with deterious side effects to human health. In course of time fungal species develops resistance to many synthetic fungicide's products also. There is a need for a search of an alternative less expensive and eco-friendly method to control fungal contamination both pre- and post-harvest [2]. Now, natural products are the best weapon for the survival of any type of problems regarding infection, pathogenesis, or protection from diseases. Due to their wide range of active secondary metabolites, easily biodegradable, less toxicity etc., medicinal plants are the possible substitutes to synthetic ones. Botanical fungicides are known to possess biocompatibility and rapid biodegradation [3] and structural diversity of natural products provides unlimited opportunities for novel biological antifungal agents. Many plant species have been reported to be effective in countering phytopathogenic fungi, such as *Thymus vulgaris* [4], *Cnidium monnieri* [5], *Syzygium aromaticum*, [6] *Allium sativum* [7], *Polygonum cuspidatum* [8], *Waltheria indica* [9]. The objective of this study is, therefore, to investigate the *in vitro* effect of aqueous extracts of certain selected medicinal plants on selected species of phytopathogenic fungi.

Materials and Methods
Plant materials
Plants with medicinal properties belonging to different families were sampled from sites around Hesaraghatta Lake and Gene Field Bank, IIHR-ICAR, Hesaraghatta, Bangalore. Aerial parts of selected medicinal plant species were collected. The collected plants were carried in polythene bags to the laboratory and fresh leaves from healthy plants were amassed. The fresh leaves were surface sterilized with 0.01% mercuric chloride, rinsed with double distilled water, cleaned, and dried in the oven at 50˚C for 7 days. The dried material of each plant species was ground into a powdered material using a blender and the mince was sealed in polyethylene bags until extraction.

Preparation of plant extracts
For investigations, crude plant extracts were prepared by soaking ten grams of the ground material with 100mL of sterile distilled water with stirring for 72 hours. Then the

mixture was filtered through double layers of muslin cloth and finally filtered through Whatman filter paper No. 41 to remove leaf debris and obtain a clear filtrate. The filtrate was stored at 4˚C until further use.

Fungal cultures

Phytopathogenic fungal cultures of *Sclerotium rolfsii*, *Fusarium oxysporum*, *Alteraria solani* and *Phytophthora capsici* were provided from the culture collection of Division of Plant Pathology, IIHR, Bangalore. The cultures were preserved in slants containing potato dextrose agar (PDA) except *P. capsici* which was preserved using carrot agar (CA) till used.

Evaluation of antifungal activity of plant extracts

The fungicidal efficacy of the plant extracts against the fungal pathogens was assessed by employing Poisoned Food Technique. A requisite amount of the extract was dissolved into sterile Potato Dextrose Agar (PDA) medium and Carrot Agar (CA) medium to obtain the desired concentrations. The media was then poured into sterile Petri dishes and allowed to solidify. For the positive control, suitable chemical fungicides at the recommended dose (Table 1.) were included in the media. Also, a set of negative controls were prepared. Discs of 4 mm diameter of the actively growing fungal culture were transferred aseptically to the centre of the poured Petri dishes of the treatment and control sets. The treatment and control sets were incubated at 25±2˚C for seven days. The fungal colony diameter of the treatments was measured and compared with the control sets and the percentage of mycelia inhibition was calculated using the following formula [10, 11, 12].

$$I = \frac{[C - T]}{C} \times 100$$

Where, I = Percent Inhibition
C = Colony Diameter in Control
T = Colony Diameter in Treatment

Table 1: Fungicides used for fungicidal analysis

Fungi	Fungicide	Recommended Dose (%)	Active Ingredient (%)
S. rolfsii	Blightox	0.2	50
F. oxysporum	Carbendazim	0.1	50
A. Solani	Carbendazim	0.1	50
P. capsici	Aliette	0.2	80

Results

The data pertaining to the antimicrobial potential of the botanicals on the mycelial growth has been illustrated in Tables 2, 3, 4 and 5 and Fig. 1. Table 2 shows that of the eighteen plant extracts screened, eight plant extracts were effective in inhibiting the growth of the four phytopathogens. Among these eight, four crude extracts were found to be more effective in inhibiting the growth. The study shows that all the four crude plant extracts inhibited the growth of the four different fungal phytopathogens (Table 3-6) of the four plant extracts, *S. rhombifolia* was found to be most effective in inhibiting the growth of all the four phytopathogens. Maximum inhibition was observed in *S. rolfsii* (94%) followed by *A. solani* (75%) and *P. capsici* (55%) in *S. rhombifolia* plant extracts. The second most effective extract was found to be *B. diffusa*, inhibiting *S. rolfsii* (87%) and *A. solani* (79%). Although the leaf extract of *S. acuta* was found to be moderately effective against *P. capsici* suppressing its growth to 36%, it was strongly effective against *S. rolfsii* and *A. solani* inhibiting their mycelial growth with 74% and 68%, respectively. In contrast, *V. negundo* extract was not as effective against the phytopathogenic fungi except growth of *S. rolfsii*, suppressing the growth to 70% (Fig. 1 a & b) (Fig 2 a & b). Also, another crucial observation made was regarding the pattern of growth of the fungi. As they were unable to grow radially due to the presence of the extract, the mycelia began to grow vertically. This pattern of growth was very prominent in *A. solani* and *F. oxysporum*.

Table 2: Antimicrobial activity caused by plant extracts

Plant extract	Plant extract	*S. rolfsii*	*F. oxysporum*	*A. solani*	*P. capsici*
1	*Terminalia bellarica*	-	-	-	-
2	*Sapindus laurifolius*	-	-	-	-
3	*Tribulus terrestis*	-	-	-	-
4	*Sida acuta*	+	+	+	+
5	*Trichodesma indicum*	-	-	-	-
6	*Madhuca longifolia*	-	+	-	-
7	*Solanum indicum*	-	-	-	-
8	*Plumbago zeylanica*	-	-	-	+
9	*Caesalpinia crista*	-	-	-	-
10	*Gmelina arboria*	-	+	-	+
11	*Saraca asoca*	-	-	-	-
12	*Sida rhombifolia*	+	+	+	+
13	*Gymnema sylvestre*	-	-	-	-
14	*Boerhavia diffusa*	+	+	+	+
15	*Clerodendron sp.*	-	-	-	-
16	*Salacia chinensis*	-	-	-	-
17	*Vitex negundo*	+	+	+	+
18	*Embelia ribes*	-	-	-	+

(+) susceptible (-) not susceptible

Table 3: Effect of plant extracts on growth of *S. rolfsii*

Plant Species	Mean Diameter of Mycelial Growth of (cm)		
	DAY 3	DAY 5	DAY 7
S. acuta	0.83	1.45	2.23
S. rhombifolia	0.50	0.50	0.50
B. diffusa	0.58	0.70	1.12
V. negundo	1.75	2.18	2.58

Table 4: Effect of plant extracts on growth of *F. oxysporum*

Plant Species	Mean Diameter of Mycelial Growth of (cm)		
	DAY 3	DAY 5	DAY 7
S. acuta	2.15	2.63	3.68
S. rhombifolia	1.25	1.72	3.25
B. diffusa	1.90	2.40	3.82
V. negundo	2.23	2.57	3.82

Table 5: Effect of plant extracts on growth of *P. capsici*

Plant Species	Mean Diameter of Mycelial Growth of (cm)		
	DAY 3	DAY 5	DAY 7
S. acuta	0.62	0.73	0.88
S. rhombifolia	0.00	0.45	0.62
B. diffusa	0.60	0.63	0.67
V. negundo	0.67	0.71	0.77

Table 6: Effect of plant extracts on growth of *A. solani*

Plant Species	Mean Diameter of Mycelial Growth of (cm)		
	DAY 3	DAY 5	DAY 7
S. acuta	1.42	1.80	1.73
S. rhombifolia	0.65	0.92	0.33
B. diffusa	0.54	0.83	1.12
V. negundo	1.78	2.07	2.70

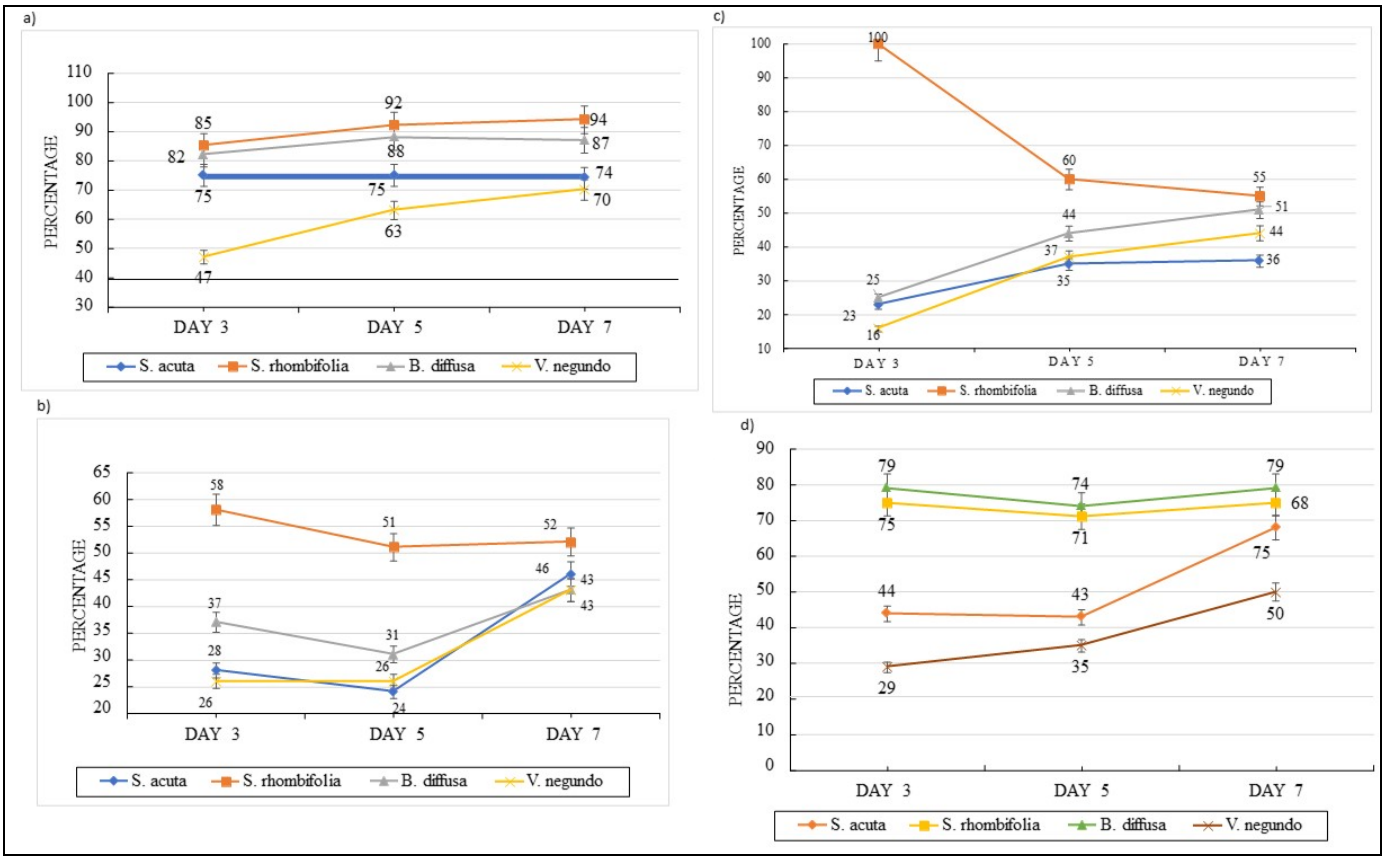

Fig 1: *Effect of plant extracts on the growth of S. rolfsii, F. oxysporum, P. capsica, A. solani. Numbers above each line represents the percentage of inhibition of mycelial growth.*

Discussion

In the present investigation, eighteen crude plant extracts were screened *in vitro* to evaluate their efficacy in suppressing the growth of phytopathogenic fungi (*Sclerotium rolfsii*, *Fusarium oxysporum*, *Alternaria solani* and *Phytophthora capsici*). Assays showed that, four were effective in inhibiting the growth of which two plant extracts provided significant inhibition of mycelial growth of the fungi under study and their sensitivity to a given plant extract varied significantly. Differing sensitivity levels of the tested fungi can be attributed to the type of the conidia or cyst formed as a protective mechanism to evade the toxicity of the extracts. Hu *et al.* (2017) [13] stated that the antifungal mechanism of plant extracts can form of transmembrane pores or ion channels on the cellular membrane, leading to the leakage of essential metabolites, and the disruption of the cell wall structure, interfering with cell wall synthesis. Antifungal efficiency of these plant

extracts might be due to ability of bioactive compounds to disrupt the plasma membrane and mitochondrial dysfunction inducing metabolic stagnation. Chen *et al.* (2018) [14] concluded that the antifungal effects found in *Curcurma elonga* could have involved in disruption of fungal cell membrane systems, specifically the inhibition of ergosterol synthesis and the respiratory chain. It is important to note that the total activity is dependent on the solubility of the plant materials in a specific solvent and the activity of such extract on the selected microorganisms [15]. Several authors have confirmed that *Sida rhombifolia* [16], *Boerhavia diffusa* [17], *Sida acuta* [18], *Vitex negundo* [20] exhibited antifungal activity. *B. diffusa* extracts showed significant antifungal activity, which may be related with the presence of anthraquinones, anthraquinone and its derivatives were reported to have antifungal activities [21, 22] The manner of antifungal activity of leaf extract of *Thevetia peruviana* might be mainly due to cell wall attack and withdrawal of cytoplasm in the hyphae and eventually death of the mycelium [22]. Such modifications induced by the exposure to plant extract components may interfere with enzymatic reactions of wall synthesis and affect fungal morphogenesis and development. A variation in the fungitoxic effects of the concerned plant species under study against the phytopathogens may be due to considerable differences in the biochemical constituents of the plant and variation in the fungal species itself [24, 25, 26]. In a study, *Candida krusei* and *Aspergillus flavus* growth was found to be suppressed when methanolic seed extract of *S. rhombifolia* was administered [27]. Similarly, ethanolic and chloroform extracts of *Sida rhombifolia* (1, 10, 50, and 100µg/ml) inhibited the growth of pathogenic fungal strain (*Aspergillus niger*) [28]. Baskaran *et al* (2011) [29] reported that ethanolic and methanolic extracts of *Boerhavia diffusa* suppressed the growth of *Aspergillus flavus, Aspergillus niger* and *C. albicans* which confirms the antifungal property. Petroleum ether extracts of *Vitex negundo* exhibited maximum inhibition of *S. rolfsii* than other solvent extracts [30]. Sathiamoorthy *et al.* 2006 [20] reported that new flavone glycoside isolated from *Vitex negundo* was found to have significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans*. In another study, Free flavonoids extracts of different parts of *Sida acuta* was found to be effective against *C. albican*s than bound flavonoid extracts [31]. *Aspergillus niger, Aspergillus flavon, Candida albicans* growth was found to be suppressed in water: ethanolic extract of *Vitex negundo* [32].

Conclusion

It can be concluded that to reduce the dependence on synthetic fungicides as well as reduce the production costs, plant extracts especially *S. rhombifolia* and *B. diffusa* may be used as natural fungicides to control phytopathogenic fungi. Combinatory formulations with various botanical extracts may pave way in standardization of natural fungicides. Further, characterization of active compounds in the plant extracts must be charted out to find out the exact mechanism of action responsible for antifungal activity. The present study indicated that application of plant extracts as biocontrol agents was found to be effective in controlling the mycelia growth and these extracts may be a potential alternative for their application as natural fungicides.

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