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Determination of antifungal potential of *Sida acuta*

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Abstract

Sida acuta has been widely used for its various pharmacological properties. Hence, in the current study we aimed to determine the antifungal activity for leaf, stem and root extracts of *Sida acuta*. Ethanol (50%) was used successively for extraction of active principles from the dried powdered leaves, stem and root of *Sida acuta*. The antifungal screening was done with two plant pathogens viz. *Fusarium oxysporum* ATCC 26225 and *Colletotrichum gloeosporioides* ATCC 20358 as test microorganisms. In the agar-well diffusion assay highest zone of inhibition in diameters were recorded with leaf and stem ethanol extracts of *S. acuta*. Both microorganisms were markedly affected by all the three extracts under study. In conclusion, findings of this study demonstrated that all parts of *S. acuta*, particularly the leaf and stem possessed antifungal property, and which ethanolic extract of leaf and stem parts of *S. acuta* could be used to control plant diseases as a safe alternative option to chemical fungicides.

Keywords: *Sida acuta*, antifungal, *F. oxysporum*, *C. gloeosporioides*

Introduction

Various factors are responsible for changes in which food becomes less palatable or even toxic to consumers these changes may be accompanied by alterations in taste, smell, appearance or texture. Numerous microbial defects of crops are characterized by the types of microorganisms responsible for their deterioration [1]. The *Fusarium oxysporum* species complex embraces a variety of strains ubiquitously present in soils. Interactions between plants and the root-colonizing fungus *Fusarium oxysporum* can be neutral, beneficial, or detrimental for the host. *F. oxysporum* is infamous for its ability to cause wilt, root-, and foot-rot in many plant species, including many agronomically important crops. Fusarium wilt is one of the major diseases caused by pathogenic *F. oxysporum* strains. Wilts are a major threat for agriculture [2], and *F. oxysporum* ranks among the 10 most devastating fungal plant pathogens worldwide [3]. Besides wilt disease some strains can also cause foot- or root-rot resulting in serious yield losses in affected crops [4]. *F. oxysporum* produces micro- and macroconidia and chlamydospores that can remain viable in infected soils for decades, thereby frustrating crop rotation schemes [5]. Pathogenicity of *F. oxysporum* is host-specific, as typically strains infecting one plant species do not cause disease in others. Based on this host-specificity, pathogenic strains have been classified into so-called *formae speciales* (f. spp.), of which over 100 have currently been describe [6]. The evolved *F. oxysporum* pathogens can give rise to devastating crop losses, *Fusarium* wilt disease of banana, caused by *F. oxysporum* f. sp. *cubense*, being a prime example [7, 8].

Colletotrichum gloeosporioides (Anthracnose) is one of the most common *Colletotrichum* fungal plant pathogens. It causes bitter rot in variety of crops worldwide, particularly perennials in the tropical regions [9]. Some of the important host plants include Sorghum, Wheat and Maize. It produces substantial amount of pre- and post-harvest loss in these crops worldwide. It acts as a secondary invader of injured tissue, but can also survive as a saprophyte. Recently, the genus *Colletotrichum* was nominated as the eighth most important group of phytopathogenic fungi in the world, based on perceived scientific and economic significance [3]. The prominence of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc and the anthracnose disease it causes to horticultural produce present a mounting threat to global agriculture. More than 50% of losses of fresh fruits and vegetables are caused by *Colletotrichum* species [10, 11].

Efforts have been made to control plant diseases using plant extracts [10-16]. They gave evidences that the plant extracts are effective bioagents against a wide range of plant pathogens viz., fungal, bacterial and viral pathogens. Plant seed oils had been also used to control plant pathogens [17-21]. Plant extracts of many higher plants like neem have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials [21]. Some plants contain components that are toxic to pathogens when extracted from plant and applied on infected crops. These components are called botanical pesticides or botanicals. Commonly used botanicals include plant extracts such as neem (*Azadirachta indica*, A. juss) and garlic (*Allium sativum*); and essential oils such as nettle (*Urtica* spp.), rue (*Ruta graveolens*, Linn), thyme (*Thymus vulgaris*, Linn), and tea tree (*Melaleuca alternifolia*) [22]. Plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [23]. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the plant pathogens. These groups of compounds show antimicrobial effect and serve as plant defence mechanisms against pathogenic microorganisms [24]. The underlying mechanisms are not clearly understood, but involvement of induced resistance is considered [25]. These bioagents are nonpolluting, cost effective, non-hazardous and can be prepared with available materials in the field. *Sida acuta*, the common wireweed, is a species of flowering plant in the mallow family, Malvaceae. It is believed to have originated in Central America, but today has a pantropical distribution and is considered a weed in some area [26]. With this background present study was carried out with the main purpose to determine the antifungal activity of *S. acuta* for leaf, stem and root extracts against fungal pathogens of Sorghum.

Materials and Methods

Collection of sample

Matured *S. acuta* was collected from an abandoned farm land in and around Shivamogga district, Karnataka India.

Preparation of sample

The fresh plant parts were washed with clean water and oven dried at a temperature 65 °C for 12 hours. The leaves, stems and roots of *S. acuta* were later cut into bits with knife and then oven-dried at a temperature of 70 °C for 12 hours to remove all moisture. The samples were grounded in a mortar with a pestle, and then in a blender (Omega, USA) into powdered form.

Extraction of plant materials

Ethanol extraction

The ethanol extract of the plant was prepared using the powdered sample of the leaf, stem and root in 100mL of ethanol individually by soxhlet extraction. Thereafter filtered using Whatman filter paper. The extract was then concentrated using rotary evaporator and allowed the solvent to evaporate. The concentrated extract was stored in an air tight container in a refrigerator at 20 °C until it is required for analysis [27].

Sample preparation

The sample was prepared by dissolving 100 mg/mL powdered sample of the leaf, stem and root in 50% of ethanol individually.

Standard antifungal preparation

Itraconazole (1 mg/mL) was prepared in sterile water. 50% ethanol was used as control.

Organisms used

The pure cultures of the microorganisms were obtained by standard blotter method to screen pathogens expressed in all the collected sorghum samples (Rabi FSH₃, Rabi SPB86, Kharif CSH varieties) followed by isolation of *Fusarium oxysporum* and *Colletotrichum* sp. The fungal isolates include *Fusarium oxysporum* ATCC 26225 and *Colletotrichum gloeosporioides* ATCC 20358.

Preparation of media

Potato dextrose broth (PDB) and potato dextrose agar (PDA) used were prepared according to manufacturers' instructions as indicated on the product label. The quantities required were measured using a weighing balance (in grams) into a conical flask and dissolved in the appropriate volume of water using a measuring cylinder. The media were properly mixed and sterilized by autoclaving at 121 °C for 15 minutes at 760 mmHg.

Antifungal activity

Antifungal activity of the extract of *S. acuta* was studied using the agar well diffusion method as described by Perez [28]. Fungal organism from growth on potato dextrose broth incubated at 27 ± 2 °C for 48 h were suspended in saline solution (0.85% NaCl) and adjusted to a standard inoculum size to 1-2 x 10⁶ CFU/mL. Fungal suspension (0.1 mL) was used to inoculate PDA petriplates with a sterile non-toxic cotton swab on a wooden applicator. Five millimeters diameter wells were punched in the agar and filled with 20 µL (2 mg) and 10 µL (1mg) of *S. acuta* extracts individually. 20 µL (20µg) of Itraconazole a commercial antifungal compound was used as reference standard and 20 µL of 50% ethanol was added as control. The treated plates with *S. acuta*, reference standard and control were incubated at 27 ± 2 °C for 48-72 hrs. After incubation the treated plates were observed for zone of inhibition around the wells. Zone of inhibition was measured in millimetre (mm) and recorded.

Results

The antifungal assay in this study was performed by agar well diffusion method so that it could be qualified and quantified by zone of inhibition in diameters. The zone of inhibition observed for different extracts against the test organisms are summarized in Table 1 and Figure 1 & 2. The zone of inhibition revealed that the leaf and stem extract had the highest inhibition against *F. oxysporum* and *C. gloeosporioides* being 12±0.2 mm at 2 mg concentration for leaf. In stem zone of inhibition showed 12.5±0.3 and 12 ±0.3 against *F. oxysporum* and *C. gloeosporioides* respectively. The root extract showed highest inhibition at 2mg against *F. oxysporum* and *C. gloeosporioides* being 11 ± 0.1 mm and 12±0.3 mm respectively.

Table 1: Inhibitory activity of *S. acuta* against test organisms

Label on plate	Test compounds	Conc. Per well	Zone of inhibition (mm)	
			<i>F. oxysporum</i>	<i>C. gloeosporioides</i>
Std	Itraconazole	20 µg	15 ± 0.5	14 ± 0.5
C2	Control	50%	-	-
C1		50%	-	-
L2	Leaf extract	2 mg	12 ± 0.2	12 ± 0.2
L1		1 mg	9 ± 0.1	8 ± 0.2
S2	Stem extract	2 mg	12.5 ± 0.3	12 ± 0.3
S1		1 mg	9 ± 0.2	8 ± 0.2
R2	Root extract	2 mg	11 ± 0.1	12 ± 0.3
R1		1 mg	8 ± 0.1	8 ± 0.2
Figure Reference	Fig 1	Fig 2		

Std-Standard, C-Control, L-Leaf, S-Stem, R-Root

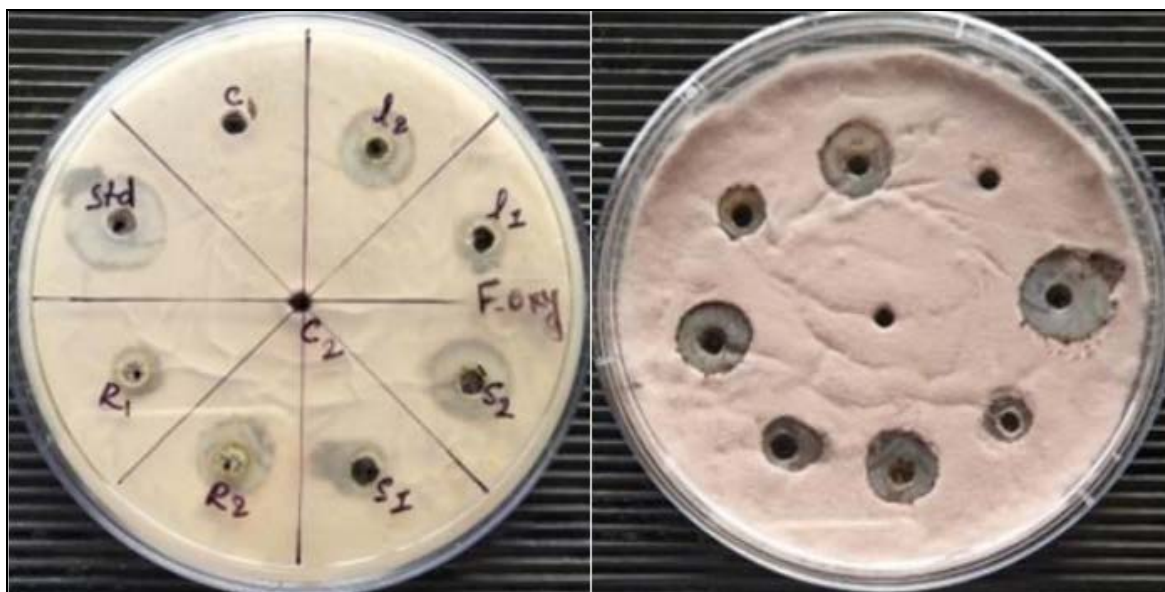


Fig 1 (a & b): Inhibitory activity of test compounds against *F. oxysporum*

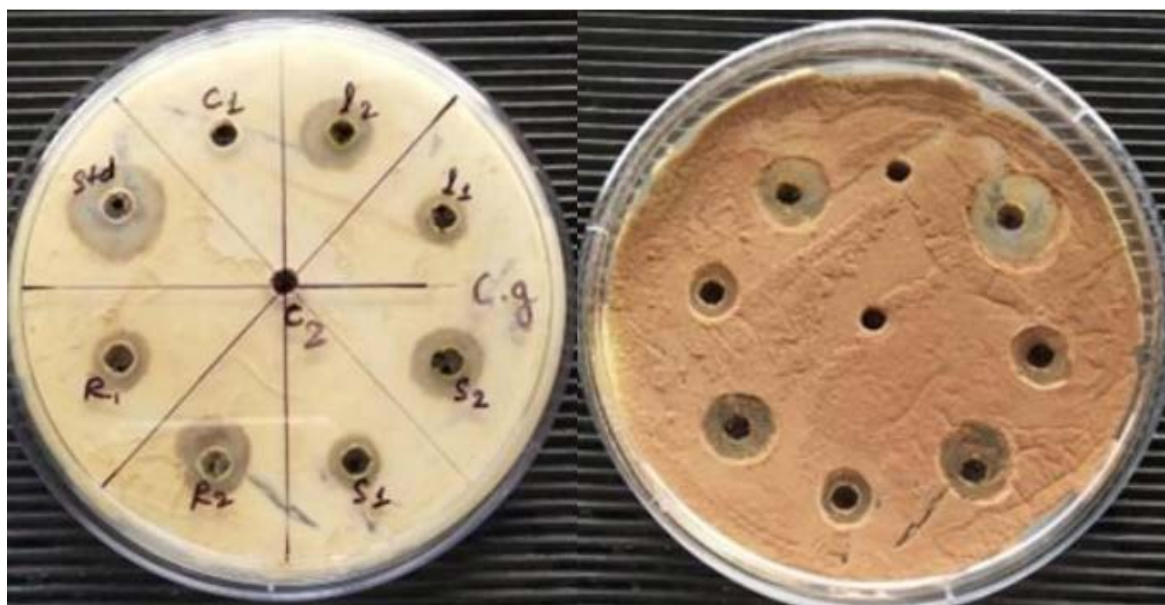


Fig 2 (a & b): Inhibitory activity of test compounds against *C. gloeosporioides*

Discussion

The ultimate aim of this research is to develop safe alternative control strategies to reduce dependency on synthetic fungicides. Several plant extracts are known to play an important role in the management of plant diseases [16, 29, 30]. They act directly or indirectly against plant pathogens, either to inhibit fungal growth and multiplication or by inducing

resistance in crop plants. In the present study there was considerable antifungal activity against *F. oxysporum* and *C. gloeosporioides* in all the extracts of *S. acuta* concentrations with maximum activity at 2 mg. It was evident from several reports that plant extracts are effective biocontrol agents against a wide range of plant pathogens [16, 29, 30]. Plants have the ability to synthesize

aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [23]. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against pathogens. These groups of compounds show antimicrobial effect and serve as plant defence mechanisms against pathogenic microorganisms [24]. Plants of Meliaceae family, especially neem, contain at least 35 biologically active principles of which nimbin and azadirachtin are the most active insecticidal ingredients and are present predominantly in the seeds, leaves and other parts of the neem tree [31]. The active ingredients of neem constitute mostly of triterpenoides, e.g., nimbin, nimbicidine, azadirachtin etc [32]. The inhibitory effect of the plant extracts might be attributed to the presence of antifungal components, i.e., Azadirachtin in *Azadirachta indica*, Artemesin in *Artemisia annua*, Caratenes in *Ocimum sanctum*, Emodin in *Rheum emodi* and Eucalyptol in *Eucalyptus globulus* [33].

The underlying mechanisms of disease suppression by plant extracts are not clearly understood, but involvement of induced resistance is considered [34]. The phenomenon of inducing resistance in plants by biotic and abiotic compounds, such as some microorganisms, natural active ingredients (allicin, fulvic acid and eugenol), salicylic acid, phosphates, and plant oils, potentially offers an alternate, more environmentally approach to crops protection against infection with many diseases [35, 36]. These bioagents are nonpolluting, cost effective, non-hazardous and can be prepared with available materials in the field. The mode of action of abiotic inducers for controlling plant diseases may include acting as second messengers in enhancing the host defense mechanism [37], activating resistance by increasing the activity of peroxidase, the synthesis of new POD isoforms, the accumulation of the phenolic compound [38], or through inhibition of some antioxidant enzymes and catalases, thereby leading to production of elevated amounts of H₂O₂ [39] and finally enhancing resistance by direct effects on multiplication development and survival of pathogens or indirect effects on plant metabolism with subsequent effects on the pathogen food supply [40].

Conclusion

This is the preliminary pilot study which determined the antifungal activity of *S. acuta* against plant pathogens *F. oxysporum* and *C. gloeosporioides*. The phytochemicals present in leaf, stem and root of *S. acuta* exhibited antifungal property presenting it as a potent plant in treatment of plant fungal diseases. *S. acuta* plant extracts having resistance mechanisms may be useful to control plant diseases. On the basis of the results obtained during the experiment and reports of success of plant extracts in controlling plant pathogenic fungi, the tested plant extracts hold promise for the organic and ecofriendly management of plant diseases. The findings of these studies may become the foundation for the use of biocontrol agents as a safe and cost-effective control method against plant pathogens.

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