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Assay of Antimicrobial Activity of *Sida acuta* (Burm. F.) by Well Diffusion Method

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A B S T R A C T

Sida acuta is a shrub indigenous to pantropical regions. *Sida acuta* belongs to the mallow family, Malvaceae. The plant is widely used for its various pharmacological properties. Therefore, in the current study antifungal activity for leaf, stem and root extracts of *Sida acuta* were carried out. Ethanol (50%) was used successively for extraction of active principles from the dried powdered leaves, stem and root. The antifungal screening was done with two plant pathogens *viz. Claviceps purpurea* MTCC 2334 and *Macrophemina phaseolina* MTCC 10576 as test microorganisms. In the agar-well diffusion assay, highest zone of inhibition in diameters were recorded with leaf ethanol extracts of *S. acuta*. Among the plant extracts, leaf has shown better inhibitory activity against the test organisms followed by stem and root. Both microorganisms were markedly affected by all the three extracts under study. Findings of this study, therefore, showed that all parts of *S. acuta*, particularly the leaf, possessed antifungal property, and hence which can be used in development of fungicide.

Introduction

Macrophomina phaseolina is an important soil-borne plant pathogen that causes diseases over 500 plant species including economically important crops such as legumes, sunflower, cotton, sorghum and vegetables. Generally, it causes charcoal rot disease in various crops; it also causes other diseases such as seedling and stem blight, damping off and wilt. It has vast distribution in tropical and subtropical countries (Shifa *et al.*, 2018) *Claviceps purpurea* is a phytopathogenic fungus infecting a broad range of grasses including economically important cereal crop plants. The fungus infects exclusively the young ovaries of the host plants. After successful colonization the ovary is replaced by fungal mycelium and production of conidia begins. The infection cycle ends with the formation

of a sclerotium, the resting structure of the fungus (Shaw, 1980 and Mantle and Luttrel, 1980). Ergot alkaloids, the best characterized secondary metabolites of *C. purpurea* are produced exclusively in the sclerotia tissue. These toxins are historically important as they affect the central nervous system of mammalians and were the reason for severe intoxications in the past, caused by consumption of contaminated bread (Tudzynski *et al.,* 2014).

Several strategies are being adopted to control fungal plant pathogens by synthetic fungicides. No doubt, these fungicides are effective in controlling plant diseases but they also pose severe hazards to human health and cause environmental pollution by accumulation in soil and water (Awla *et al.,* 2017). It necessitates alternative environmentally friendly strategies for management of phytopathogens. Many recent studies have shown that crude plant extracts as well as purified compounds isolated from various plant species are very effective in the control of fungal plant pathogens (Javaid *et al.,* 2018; Javaid *et al.,* 2018; Khurshid *et al.,* 2018).

Studies have shown that plant extracts of *Chenopodium* spp., *Sennaoccidentalis* and *Cirsiumarvense* can control growth of *M.phaseolina* and charcoal rot of mungbean (Javaid and Amin, 2009; Banaras *et al.,* 2017; Javaid *et al.,* 2017; Javaid *et al.,* 2017; Javaid *et al.,* 2018). From Azadirachta and Mango leaves, three flavonoids (–)-epi-catechin, (−)-epicatechin-3-*O*-β-glucopyranoside and 6-(phydroxybenzyl)taxifolin-7-*O*-β-d-glucoside were isolated and found effective against *M.phaseolina* (Kanwal *et al.,* 2011, 2010).

Sida acuta belongs to the genus Sida, of the mallow family, Malvaceae. It is commonly known as stubborn weed. It is an erect, branched, small perennial shrub with a woody tap root, and hairy branches up to 1 m high (Akodundu and Agyakwa, 1998). It is predominant in roadsides, waste areas, grazing land, disturbed land and abandoned farmlands. Traditional medicine has started gaining credence over the last decade. Different parts as well as whole plants are used in folk medicine for treatment of different afflictions. A wide range of medicinal uses of *S. acuta* have been extensively documented. The use of *S. acuta* in treatment of asthma, renal inflammation, colds, fever, headache, ulcer and worm infections in regions around Central America has been reported (Caceres *et al.,* 1987). The leaves are used for their diuretic, demulcent, anthelmintic and wound healing properties (Mohideen *et al.,* 2002). Moreover, it is used as a medicine in treatment of liver disorders, urinary disease, nervous disorder, blood disorder, biliary disease (Sreedevi *et al.,* 2009). Any substance that kills or inhibits the growth of microorganisms with negligible side effects on the host is considered as an antimicrobial (Ekpo and Etim, 2009). In addition, it could be natural or man-made. With these viewpoints present study was designed to conduct with the main objective to determine the antifungal property of *S. acuta* for leaf, stem and root extracts against the selected 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 (Jose and Radhamany, 2013).

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 authenticated from Microbial Type Culture Collection and Gene Bank (MTCC). The fungal isolates include *Claviceps purpurea* MTCC 2334 and *Macrophemina phaseolina* MTCC 10576

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 (1990). 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 10^6 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 (2mg) 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 and Discussion

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 was observed for different extracts against the test organisms are summarised in Table 1 and Figure 1 and 2. The results of zone of inhibition revealed that the leaf extract had the highest inhibition against *C. purpurea* and *M. phaseolina* being 12 ± 0.2 mm and 11 ± 0.3 mm respectively at 2 mg concentration.

At 1mg concentration the leaf showed inhibition against *C. purpurea* and *M. phaseolina* being 10 ± 0.2 mm for both organisms. The stem had highest inhibition at 2 mg being 10 ± 0.2 mm against *C. purpurea* and *M. phaseolina*. The root extract showed highest inhibition at 2mg against *C. purpurea* and *M. phaseolina* being 10 ± 0.2 mm 10 ± 0.3 mm. Root extract at 1 mg concentration did not show inhibition against *C. purpurea* and *M. phaseolina*.

The therapeutic values of plants depend on their chemical components which produce specific pharmacological activities on the human and animal body.

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

Label on plate	Test compounds	Conc. Per well	Zone of inhibition (mm)	
			C. purpurea	***M. phaseolina***
Std	Itraconazole	20 µg	14 ± 0.5	12 ± 0.3
C2	Control	50%	-	-
C1		50%	-	-
L2	Leaf extract	2mg	12 ± 0.2	11 ± 0.3
L1		1mg	10 ± 0.2	10 ± 0.2
S2	Stem extract	2mg	10 ± 0.2	10 ± 0.2
S1		1mg	8 ± 0.2	7 ± 0.2
R2	Root extract	2mg	10 ± 0.2	10 ± 0.3
R1		1mg	-	-
Figure Reference			Fig 1	Fig 2

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

Fig.1a & b Inhibitory activity of test compounds against *C. purpurea*

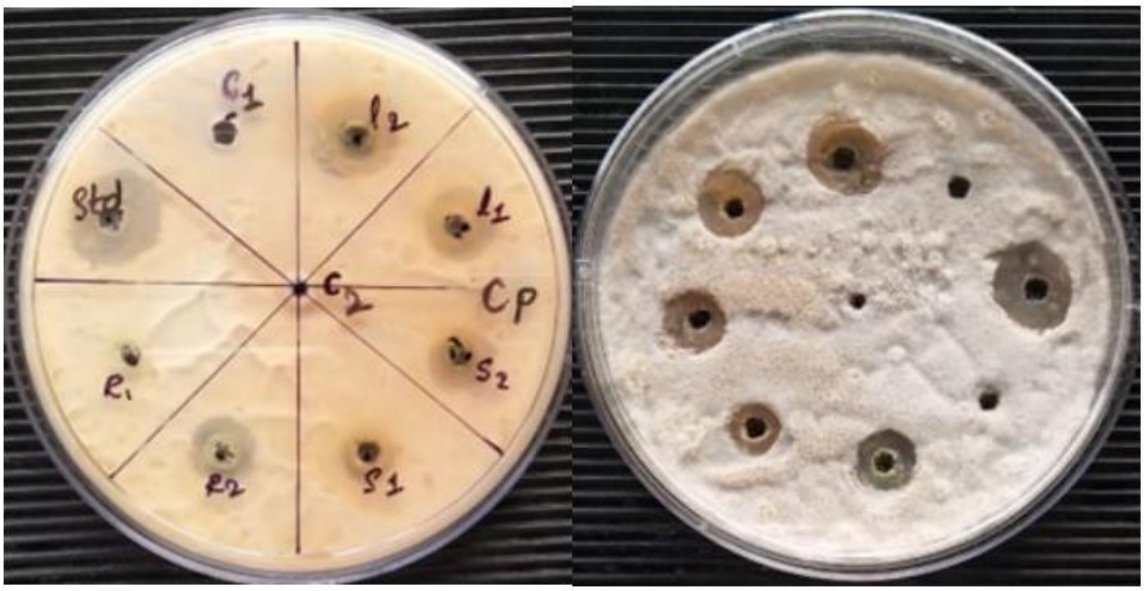
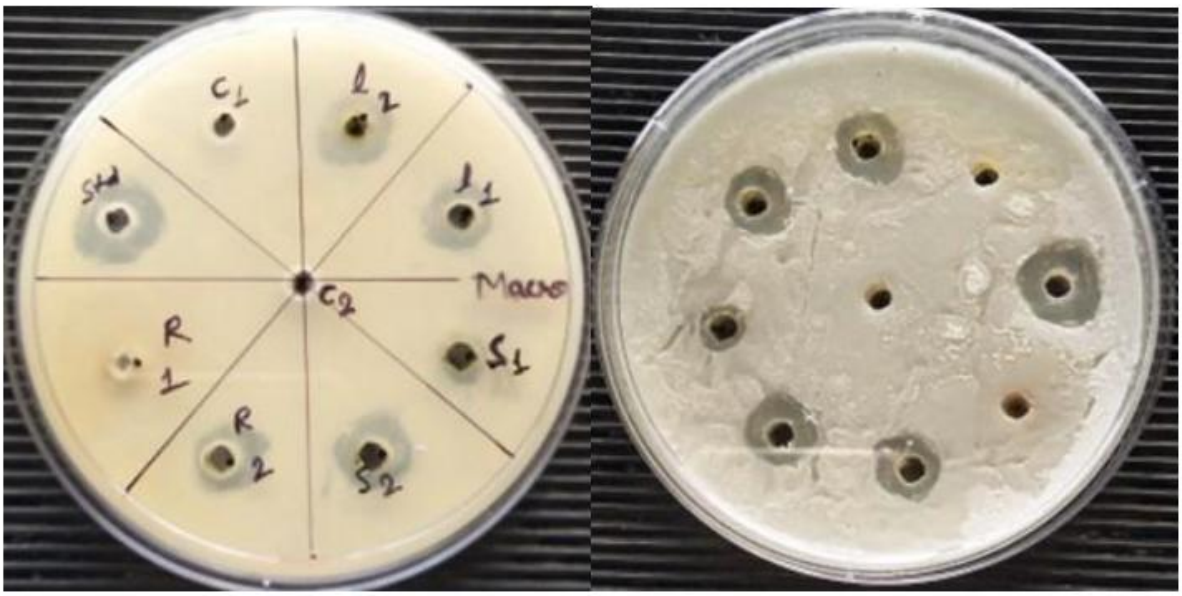


Fig.2a & b Inhibitory activity of test compounds against *M. phaseolina*



There was considerable antifungal activity against *C. purpurea* and *M. phaseolina* in all the extracts of *S. acuta* concentrations with maximum activity at 2 mg except for root extract at 1 mg did not show inhibition. This indicated that plant extract hinders the growth of microorganisms at a higher level.

The leaf extract gave the highest inhibition against *C. purpurea* and *M. phaseolina*. This might be due to the alkaloids occurred highest in the leaf which is thought to be the major active component, with others being detected in the lesser extent. This implied that the ethanolic leaf extract of *S. acuta* could be used for manufacture of drugs against *C. purpurea* and *M. phaseolina*. Besides, the negative environmental impacts of fungicides are intensively increasing every day. Thus, the alternative methods for reducing fungicides' use are being developed including plant extracts, as one of the effective methods that incorporate natural antifungal substances. Some plants contain certain components that are toxic to plant pathogens, namely, botanical pesticides or botanicals (Dubey *et al.*, 2008). In fact, natural products have proved to be potential sources of environmentally safe antimicrobial agents which could be useful in plant protection and plant disease control (Wang *et al.*, 2004).

Resistance can be systemically induced in some susceptible plants by the application of certain chemical substances as well as the pre-inoculation with pathogenic or non-pathogenic microorganisms (Kuc, 1982). In this subject, plant extracts have been found to effectively control a wide range of plant pathogens through inducing a defence response in the infected plants (Srivastava *et al.*, 2011). Studies on the antifungal activity of botanical extracts to protect plants from diseases have received much attention (Bhuvaneshwari *et al.*, 2015).

The mode of action of abiotic inducers against plant pathogens might occur as a secondary messenger enhancing the host defence mechanisms (Geetha and Shetty, 2002), either by increasing the activity of peroxidase, or by the synthesis of new peroxidase isozymes isoforms, by the accumulation of the phenolic compounds (Hassan *et al.*, 2007), or through inhibition of some antioxidant enzymes and catalases thereby leading to the production of elevated amounts of H_2O_2 (Radwan *et al.*, 2008) In addition, abiotic inducers also enhance resistance through direct effects on the development and survival of the pathogens or indirect effects on plant metabolism with subsequent effects on the pathogen-food supply (Khan *et al.*, 2003).

To the best of our literature knowledge this is the preliminary pilot study to determine the antifungal activity of *S. acuta* against plant pathogens *viz.C. purpurea* and *M. phaseolina*. 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. Hence, the bioactive agents could be isolated and incorporated in the synthesis of fungicides.

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