Research Article

Antifungal Activity of Phytochemicals of *Sida acuta* (Burm F.) on Mycoflora of Sorghum: *Sida acuta* to Control Sorghum Mycoflora

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ABSTRACT

Aim: To investigate the antifungal activity of phytochemicals from *Sida acuta* on sorghum mycoflora. The current study aimed to determine that *Sida acuta* root, stem and leaf extracts have antifungal properties. **Materials and Methods:** The stems, leaves and roots of *Sida acuta* were dried and powdered and the phytochemicals using the Soxhlet technique with 50% ethanol. Among the fungi expressed on sorghum seeds, the test organisms were screened and isolated as pure cultures and subjected to the antimicrobial assay using the well diffusion method. **Results:** The control of the pathogenic test fungus is interesting to notice. Leaf and stem extracts have shown a good zone of inhibition. Leaf extract inhibited *Claviceps* species and *Macrophomina* species, and leaf and stem extract inhibited *Fusarium thapsinum* and *Colletotrichum* species the most. **Conclusion:** *Sida acuta* (Burm f.), one of the weed plants in the Family Malvaceae, is known for its medicinal and antimicrobial properties. The ability of phytochemicals to reduce the incidence of the seed mycoflora of sorghum, a fibre-rich staple food grain, was investigated. Sorghum crops, in particular, struggle to battle fungal infestations in North Karnataka. As a result, *Sida acuta*'s antimicrobial properties help suppress many fungi, including *Macrophomina* species. It causes diseases such as charcoal rot and seedling blight of sorghum. Under high temperatures and low soil moisture, this fungus can cause notable yield losses in crops such as soybean and sorghum; *Fusarium thapsinum*, the causal agent for grain mould; *Colletotrichum* species, which causes seedling blight and seed rot; and *Claviceps* species, which causes sorghum ergot disease. The two pathogens that cause sorghum plant disease, *Macrophomina* species and *Claviceps* species, were tested for antifungal activity. This study's endeavours might yield a useful alternative to chemical fungicides if the dosage of phytochemicals is standardized and applied both as a therapy and a preventive strategy to monitor the seed mycoflora.

Keywords: *Sida acuta* (Burm f.), Phytochemicals, Extracts, Seed mycoflora, Antimicrobial, Zone of inhibition.

INTRODUCTION

Macrophomina species cause root rot and seedling blight disease in sorghum and soybeans, commercially significant horticultural crops. This pathogen causes seedling blight and rot in jowar and other diseases in crops, including stem blight, damping off and wilt. *Claviceps* species affect several types of grass, especially cereal crops. The fungal mycelium interferes. With the fruit or seed setting. Hence, nutrients were lost due to the formation of spores in place of fruit.[2] The toxic compounds produced by the genus *Macrophomina* and *Claviceps* result in the maximum loss of agricultural produce. Phytotoxins can cause various diseases in

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mammals when they eat contaminated food grains.[21] Mycotoxin, fumonisin produced by *Fusarium moniliforme* is cancer-promoting.[1] Chemical fungicides were employed in horticulture and agriculture to manage fungal diseases. Since they remain in the soil for a long duration, even if they are used to suppress pathogens, they can often cause adverse effects on the health of plants, animals and the environment. Hence, a need for bio-safe control of phytopathogens. Recent studies have shown the importance of bioactive compounds from plant extract and endophytic fungi in controlling plant pathogens.[38] Studies have shown that, based on morphology and phylogeny, genes were isolated and studied as *Colletotrichum fruticola* cause sorghum leaf diseases.[3,37] The idea of promoting the fungi toxic phytochemicals from vastly growing weed plants in many developing countries is still in laboratory studies and utilized by only a few local farming communities. Still, standardization of the phytochemical extracts as fungitoxic drugs to control fungus on sorghum seeds is needed. Production of phytochemical botanical fungicides needs expertise in dosage, phyto-toxin residue analysis and estimations. Flavonoids, epicatechin and taxifolin were isolated and found effective against *Macrophomina* species.[7,23,39] Present study with commonly available weed plant *Sida acuta* on open bare fields and in abandoned wastelands.[37] Many useful properties of *Sida acuta* have been investigated by many research teams regarding the anthelmintic, wound healing and demulcent with all such backgrounds about the plant; the present study is regarding the utilization of antifungal activity of *Sida acuta* in making an effective phytotoxin against the fungal disease-causing pathogens of sorghum.[28]

MATERIALS AND METHODS

Sida acuta (Burm F.) plant was collected from different areas of Bengaluru, Karnataka, India. The plant sample was made using fresh plant parts rinsed with clean water. Leaves, stems and roots were cut into pieces with a knife before being oven-dried at 75ºC for 12 hr to remove all moisture. The dried root, stem and leaf materials were made to a fine powder using mortar and pestle and later in an Omega blender (USA). The ethyl alcohol extract of the plant was made by powdering a sample of the leaf, stem and root in 10 mL of ethanol. Then it was filtered with Whatman filter paper. The extract was concentrated with a rotary evaporator and the solvent was allowed to evaporate. The concentrated extract was kept in an 2 mg airtight container in the refrigerator at 20ºC until needed for analysis.[4]

A diluted sample was prepared for the antifungal activity assay: The sample was prepared by dissolving 100 mg/mL of powdered leaf, stem and root samples in 50% ethyl alcohol each.[14] For the preparation of the standard, itraconazole 1 mg/mL was made using sterilized water. 50% ethyl alcohol was used as a control. The pure cultures of microorganisms were validated by the Microbial Type Culture Collection Centre. The fungal isolates include *Claviceps* species and *Macrophomina* species, *Fusarium thapsinum* and *Colletotrichum* species. Preparation of media for the culturing or isolating the fungi is done using potato dextrose agar and potato dextrose broth. The media was prepared using the measuring balance and measured chemicals were dissolved in distilled water and later sterilized in an autoclave at 121ºC under 760 mmHg. The antifungal activity of the plant extracts in different concentrations was investigated using the agar well diffusion method.[13] Refined and standardized by much research. The suspension of the inoculum was prepared using 0.85% NaCl or the saline solution in which the fungi were grown in potato dextrose broth for 47-49 hr at 28ºC and the dilution of the standard inoculum size was adjusted to 1-2 x 106 CFU/mL. The fungal suspension (0.1 mL) was inoculated into PDA Petri plates using a sterile, non-toxic cotton swab with a wooden applicator. 5 mL diameter wells were punched in the agar and filled with 20 µL (2 mg) and 10 µL (1 mg) of *S. acuta* extracts, respectively. 20 µL (20 µg) of Itraconazole, a commercial antifungal drug, was employed as a reference standard, while 20 µL of 50% ethanol served as a control. Plates containing *S. acuta*, reference standard and control were incubated at 27±2ºC for 48-72 hr. After incubation, the treated plates were examined for a zone of inhibition surrounding the wells. The zone of inhibition was measured in millimetres and documented.

RESULTS

The antifungal assay using a well diffusion method, this method validates and quantify the zone of inhibition in the assay performed. The inhibition zone for the fungal isolates differed for different parts of extracts like root; stem and leaves are summarized in Table 1 and shown in Figures 1 to 4. At a concentration of 2 mg, the leaf extract inhibited *Claviceps* species and *Macrophomina* species, the inhibition zone was 13±0.2 mm and 12±0.3 mm, respectively. At 1 mg concentration, the leaf inhibited *Claviceps* species and *Macrophomina* species by 11±0.3 mm for both species. At 2 mg, the stem exhibited the maximum inhibition against C. species and *Macrophomina limbalis*, measuring 11±0.2 mm and 12±0.3 mm. At 1 mg

concentration, the stem extracts have shown less inhibition viz., *Claviceps* species and *Macrophomina* species were inhibited by 9±0.1 mm and 6±0.1 mm respectively. At 2 mg, the root extract inhibited *Claviceps* species and *Macrophomina limbalis* at a rate of 11±0.3 mm and 7±0.1 mm respectively. The root extract at 1 mg dosage did not inhibit *Claviceps* species or *Macrophomina* species. Table 1 also shows that the 2mg of leaf, stem and root extracts inhibited *Fusarium thapsinum* and *Colletotrichum* species the most, with a zone of inhibition of 12±0.3 mm for the leaf at a dose of 2 mg for both fungi. At 1 mg leaf extract concentration, less inhibition of 9±0.1 mm was found for both fungi. The zone of inhibition in the stem revealed 12±0.3 and 11.5±0.2 against *Fusarium thapsicum* and *Colletotrichum* species, respectively. The 1 mg concentration of stem extracts is 10±0.1 mm and 8±0.1 mm. At 2 mg concentration, the root extract inhibited *Fusarium thapsicum* and *Colletotrichum* species by 12±0.1 mm and 9±0.3 mm. The 1 mg concentration of root extracts is 6±0.1 mm and 7±0.1 mm.

The inhibitory action of the phytochemical constituents determines the pharmaceutical and antimicrobial nature of the experimental plant.[26] The inhibition zone resulted from sorghum phytopathogenic fungal pure cultures tested by extracts of the *Sida acuta* plant are summarized in Table 1, Figures 1 to 4.

Antifungal activity against *Claviceps* species and *Macrophomina* species in all the extracts of *S. acuta* concentrations with MIC of 1mg and maximum concentration of 2 mg. Only in the "root extract" sample "minimum inhibitory concentration" was not 1 mg, which did not show any inhibition. The leaf extracts phytochemical treatment results showed a satisfactory inhibition of the phytopathogens, *Claviceps species* and *Macrophomina* species. This shows the leaf extracts have the highest alkaloids.[11] Thus, ethanol extracts of *Sida acuta* have many antifungal properties and can be exploited for the treatment of plant fungal diseases. It would be an eco-friendly, cost-effective, organic management of plant pathogens.

DISCUSSION

The present study investigated the antifungal activity of plant parts of *Sida acuta* using well diffusion method. Here, inhibition zones controlling the growth of fungal strains were obtained at two different concentrations (1 mg and 2 mg) of plant extracts derived from leaves, stems and roots of *Sida acuta*. Varied degrees of inhibitory effect were seen against the disease-causing fungal pathogens of sorghum like *Claviceps* species, *Macrophomina* species, *Fusarium thapsinum and Colletotrichum* species. The findings suggest that these plant extracts have potential antifungal properties that could be further explored for their use in controlling other fungal infections.[26] Here the minimum inhibitory concentration was 1mg. According to the current study, *Sida acuta* is an excellent substitute for chemical fungicides. The goal of this effort is to standardize the preparation of extracts obtained from *Sida acuta* leaves, stems and roots. The work promotes the well diffusion inhibitory assay for all other sorghum infecting fungi. Previously, *Aspergillus niger, Sporothrix schenckii and Candida albicans* were used in the *in vitro* well diffusion method to assess the antifungal impact of a poly-herbal formulation.[27] Numerous studies have demonstrated that plant extracts may replace chemical fungicides and bactericides.[15,19]

According to another study, the ethanolic extracts of *Moringa oleifera* Lam. against isolates of *Candida albicans, Escherichia coli* and *Staphylococcus aureus*

Table 1: Phytochemical effect on the phytopathogenic organisms.

Label on plate	Test Compounds	Concentration per well	Zone of Inhibition (mm)			
			Claviceps species	Macrophomina limbalis	Fusarium thapsinum	Colletotrichum. species
Standard	Itraconazole	20 µg	15±0.3	13±0.3	14±0.3	15±0.3
C2	Control 50%	50%	-	-	-	-
C1		-	-	-	-	
L2	Leaf extract 1 mg	2 mg	13±0.2	12±0.3	12±0.3	12±0.3
L1		11±0.3	11±0.3	9±0.1	9±0.1	
R2		2 mg	11±0.3	7±0.1	12±0.1	9±0.3
R1	Root extract	1 mg	-	-	6±0.1	7±0.1
S2	Stem Extract	2 mg	11±0.2	12±0.3	12±0.3	11.5±0.2
S1		1 mg	9±0.1	6±0.1	10±0.1	8±0.1

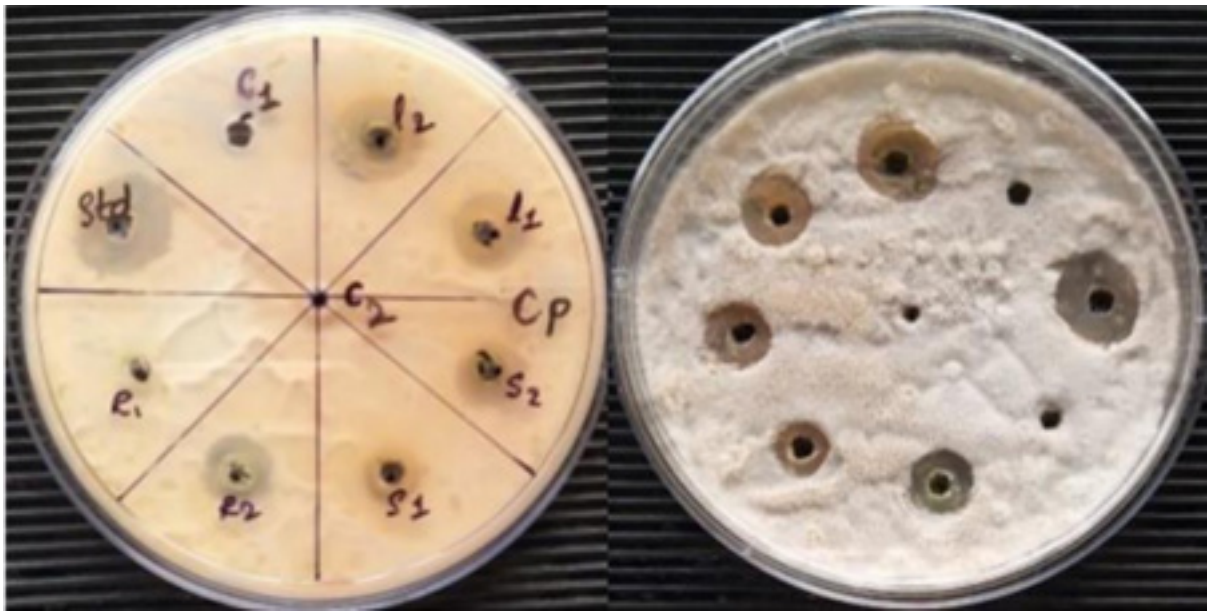

Figure 1: Inhibitory activity of phytochemicals of *Sida acuta* against *Claviceps* species.

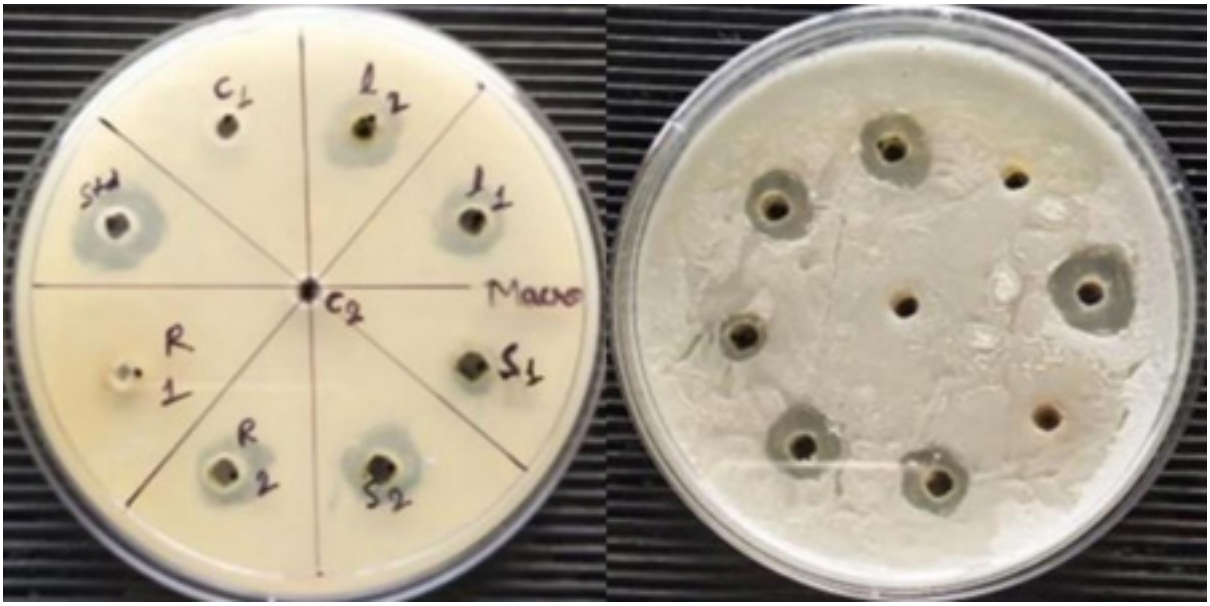

Figure 2: Inhibitory activity of plant extracts against the *Macrophomina* species.

demonstrated notable antibiotic activity against test pathogens, indicating the need for these extracts to be improved and standardized to serve as alternate sources of antimicrobial medications.[10] In addition to the plant's vegetative components, fruit extracts and the reproductive branch or inflorescence have also demonstrated fungicidal qualities. According to a study, the fruits and leaves of *Cerbera odollam* contain antifungal properties that make them a viable substitute for synthetic fungicides.[11,31,36] Using agar-well diffusion methods, the antifungal properties of *Chromolaena odorata* against *Phytophthora megakarya* were assessed in one of the studies, along with the determination of the lowest inhibitory doses of the extracts.[12] These extracts' exceptional fungicidal properties support the notion that plant extracts can be used as herbal fungicides and act as a basis for the development of novel, all-purpose herbal fungicidal formulations.[8,24] The following examples show similar evidence for the antifungal action of *Sida* and other plant extracts. *Sida cordifolia* leaf extract inhibited *Cryptococcus neoformans* at a dosage of 2 mg, as well as *Candida albicans*.[5,25] Similar to the current experiment results, leaf extracts from *Sida acuta* have been demonstrated to inhibit *Claviceps* at a

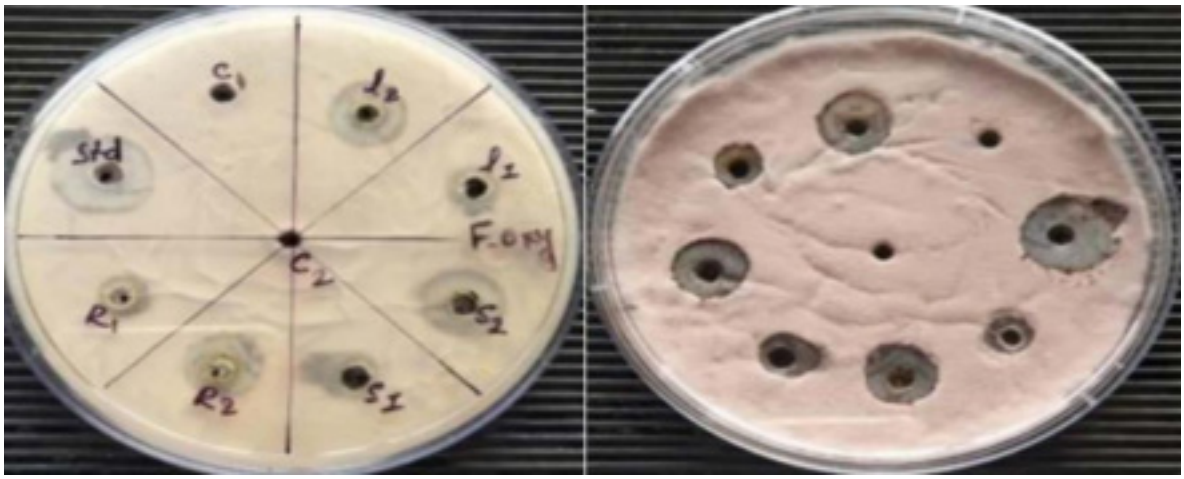

Figure 3: Inhibitory activity of phytochemical extracts against *Fusarium thapsinum.*

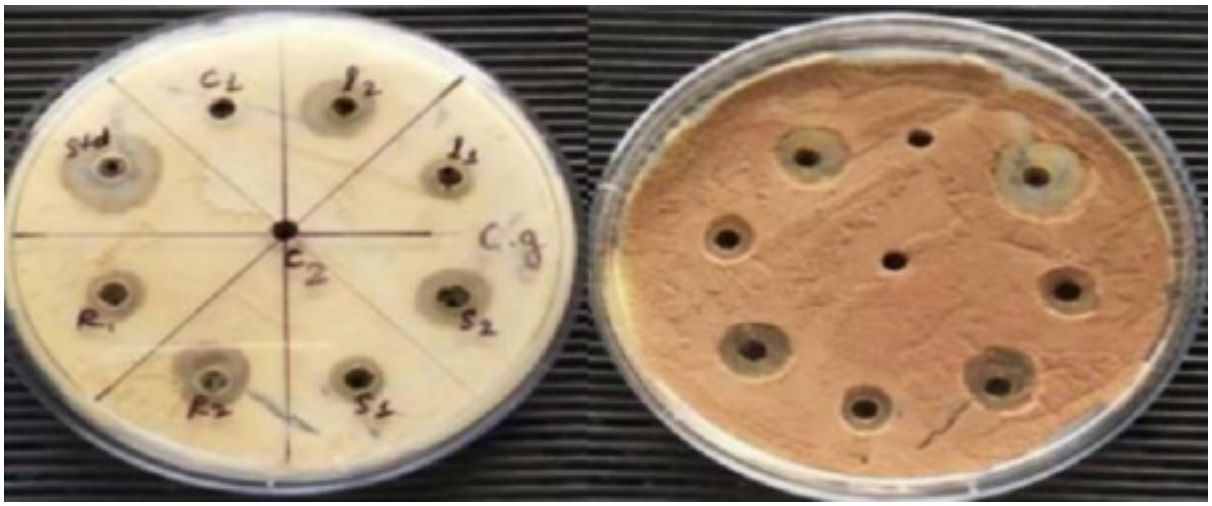

Figure 4: The inhibitory effect of phytochemical extracts on *Colletotrichum* species.

dosage of 2 mg and to control wound bacterial isolates. When it comes to *Macrophomina*, the chloroform sub-fraction offers the strongest antifungal potential. Like this, the current study evaluates the phytochemicals in *Sida acuta* to suppress the microorganisms that cause sorghum illness. Tansy and sage exhibit the strongest antifungal effects against *Fusarium* species.[29] *Colletotrichum lindemuthianum* has been proven to grow less rapidly *in vitro* when plant extracts are used. There was no discernible difference in the sporulation and germination of conidia.[16] The extent of the sorghum pathogen's inhibited germination and sporulation drop can be monitored by repeating this experiment. If similar outcomes are observed, important fungal infections of sorghum can be controlled with herbal formulations based on *Sida acuta* phytochemicals. Significant antifungal activity against *Fusarium oxysporum* was demonstrated by methanolic extracts of *Chenopodium album* L., with the inflorescence extract displaying the highest activity.[6] The stem, root and leaf extracts of the readily available weed species *Sida acuta* have all demonstrated the *in vitro* suppression of fungal infections, which makes this study project still intriguing. The current research work uses simple well diffusion assay, which is the most common method for examining the antifungal activity of phytochemicals, as demonstrated in various studies.[25,26] The strengths of the well diffusion assay are simple in instrumentation. Thus, cost-effective and able to provide qualitative and quantitative data on the inhibitory effects of plant extracts against fungal pathogens.[5,18] However, limitations of the well diffusion assay lie in the variability in diffusion rates, which can affect the accuracy of results and the inability to precisely determine the Minimum Inhibitory Concentration (MIC) of the extracts, as highlighted in research on detection limits of antifungal compounds.[30,9] Another major drawback of the diffusion assay is that only *in vitro* results

may fail to give the same results *in vivo*.[18] Despite these limitations, the well diffusion assay remains a valuable tool in screening the antifungal potential of phytochemicals and validating traditional medicinal claims for combating fungal infections in plants and animals.[16,20,22]

CONCLUSION

The research on phytochemicals' antifungal potential provides a potentially novel line of defence against fungal infections, given the increasing resistance to conventional antifungal drugs. Significant antifungal and anticandidal qualities have been shown by phytochemicals and medicinal plants, offering stable alternatives to traditional drugs with less toxicity and adverse effects. Plant extracts have been shown in studies to be effective at inhibiting fungal growth, which implies that they may find application as natural, environmentally friendly treatments for fungal diseases in both medicine and agriculture. Furthermore, the identification of bioactive compounds from natural sources, such as polyphenols like rutin, kaempferol and quercetin, shows the range of ways phytochemicals combat fungus. This study highlights the necessity of looking at plant-derived goods as a practical way to address the global problem. This research is designed to develop safety management strategies to reduce reliance on chemical fungicides. Decreased pathogen pressure, ideal plant growth circumstances and the application of disease resistance (or tolerance) are highlighted as key components of integrated pest control systems. As this study has shown, *in vitro* antifungal activity was observed in *Fusarium thapsinum*, *Colletotrichum* species, *Macrophomina* species and *Claviceps* species. This must be tested in fields, with varied parameters like increase in germination, control of fungal diseases and infestation by insects. Many reports have shown that plant extracts are effective antibiotics against various plant diseases. In addition, the negative effects of fungicides on the environment are increasing. Therefore, another way to reduce chemical fungicide use has been created with botanical extracts. It is one of the best ways to incorporate natural antifungal medicine. Some plants contain toxic substances against plant diseases, such as phyto-pesticides or botanicals. In this context, plant extracts are effective in controlling various plant diseases by stimulating the immune response in plant pathogens. Thus, studies on the antimicrobial activity of plant extracts against plant pathogens have received widespread attention.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATION

Herewith we the authors of this original research paper declare that current research work has not been involved in harming anybody or any organism. Hence, any ethical approval is not applicable. As we have used weed plant material taken from the wastelands and not used any harmful chemicals that are hazardous to the environment. Hence, ethical approval of any kind is not obtained for the study.

We also declare that our present research work has no conflicting interests of any kind.

SUMMARY

Findings of this study demonstrated that all parts of *S. acuta*, particularly the leaf and stem possessed antifungal property, and with ethanolic extract of root, leaf, and stems of *Sida acuta* could be used to control plant diseases as a safe alternative option to chemical fungicides. In

this article, extraction of active principles from the dried powdered leaves, stem, and root using well diffusion assay has shown good inhibition of fungi like *Fusarium thapsinum, Colletotrichum, Claviceps* and *Macrophomina species*. So, bioprospecting the easily available weed plant *Sida acuta*, we can further do formulations out of this plant, to control the fungal infections in agricultural crops.

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