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In-vitro Assessment of Antifungal Activity of *Aloe vera* Leaf Powder Extracts Against Banana Pseudostem Rot Fungi, *Marasmiellus* spp.

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Abstract: Being an important medicinal plant, *Aloe vera* is important in industrial perspective as well as traditional usage. The antifungal activity of A. *vera* leaf powder extracts were assessed against banana pseudostem rot fungi, *Marasmiellus* spp. Leaf powder with acetone and ethanol extracts of 20, 200, 400, 1000 and 2000 µL were administered to assess the inhibition of colony growth of *Marasmiellus* spp. The experiment was conducted in a CSompletely Randomized Design. In *A. vera* acetone extract, first day after inoculation, inhibition percentage of the *Marasmiellus* spp was higher (74.53 %) in 2000 µL and the lowest percentage (23.53 %) of inhibition was obtained in 20 µL of *A. vera* acetone extract. The similar highest and lowest percentage of inhibition were observed in second, third and fourth day after inoculation. In *A. vera* ethanol extract, complete (100 %) inhibition was observed in 1000 µL and 2000 µL extracts. The lowest inhibition (2.26 %) was recorded in 20 µL of *A. vera* ethanol extract. Among the 1000 µl and 2000 µL leaf extracts, ethanol extract had the highest inhibition percentage compared to *A. vera* acetone extract. These findings are useful to prepare the extracts of *A. vera* leaf powder for the management of *Marasmiellus* spp.

Keywords: : *Aloe vera*, Antifungal activity, Banana pseudostem rot, Colony inhibition, *Marasmiellus* spp

1. Introduction

Aloe vera has the inherent ability to induce toxic effects on mycelial growth and proliferation of selected fungi by producing aromatic substances, most of which are phenols or their oxygen substituted derivatives. These substances serve as plant's natural defense mechanisms against predation by microorganisms (Prashar *et al.*, 2011). The antimicrobial effects of *A. vera* have been attributed to the plant's natural anthraquinones such as aloe emodin, aloetic acid, aloin,

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anthracine, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbaloin, and resistannol. In relatively small concentrations together with the gel fraction, these anthraquinones provide analgesic, antibacterial, antifungal, and antiviral activity, in high concentrations they can be toxic (George *et al.*, 2009). Antifungal activity of *A. vera* was reported against *Aspergillus flavus* and *Aspergillus niger*. Arunkumar and Muthuselvam (2009) reported that maximum antifungal activity is observed in acetone extracts when compared to aqueous and ethanolic extracts. Among the two fungal organisms maximum growth suppression was observed in *A.flavus* than *A.niger*.

Bajwa and Shafique (2007) investigated that, the highest inhibitory effect of fungal (Genus *Alternaria*) biomass is achieved with aqueous extract of the tested plant species than n-hexane extracts that may be attributed to the presence of main active constituent of *A. vera* plant extract, the aloine, an anthraquinoneheteroside. The n-hexane extract exhibited least inhibitory activity. This was associated with the presence of nutritional compounds present in the n-hexane extract that stimulated fungal growth and masked the inhibitory effect. Antifungal activity of *A. vera* against *A. flavus* was investigated with six different solvents such as acetone, ethanol, water, methanol, chloroform and ethyl ether have been used for the extraction from *A. vera* fresh leaves. Acetone extract of *A. vera* is used as an effective antifungal agent to inhibit the growth of *A. flavus* compared to other solvents (Babaei *et al.*, 2013). In agriculture, the banana crop loss due to *Marasmiellus* spp. has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. To rectify this detrimental effect *Aloe vera* leaf powder extracts are used to control some of the plant pathogens as an eco-friendly manner.

2. Materials and Methods

2.1 Initial Preparation of Plant

Freshly collected *A. vera* leaves were surface sterilized with 70 % ethanol. Later, they were chopped into small pieces without removing gel and were allowed to sun drying for 3 days. After drying, leaf parts were powdered using an electric grinder.

2.2 Separation of Extracts

A. vera leaf powder of 30 g was mixed with 100 mL of ethanol and acetone separately and kept in room temperature for 72 h. Each mixture was filtered through Whatman No .1 filter paper.

2.3 Fungus Species

Marasmiellus spp. fungus was isolated from diseased banana pseudostem in Thirunelvely. Fungus was cultured in vitro on Potato Dextrose Agar (PDA).

2.4 Evaluation of Antifungal Activity of A. vera Extracts by Diffusion Plate Method

$$C.G.I. = \frac{Colony\ diameter\ in\ control\ group - Colony\ diameter\ in\ the\ treatment}{Colony\ diameter\ in\ control\ group} \times 100$$

Where, C.G.I (%)= Colony Growth Inhibition Percentage

To evaluate the antifungal activity of ethanol and acetone extracts of *A. vera*, diffusion plate method (Babaei *et al*., 2013) was used. Different quantities (0, 20,100, 200,1000 and 2000 µL) of each extract (ethanol and acetone) were added separately into 20 mL PDA culture plates before solidifying of the medium and mixed well. After solidification of plates, by using cork borer of (0.5 mm diameter), *Marasmiellus* spp. was transferred to the center of the plate of each concentration and incubated at 28 ^{o}C. Three replicates were maintained. The growth of fungi (diameter in cm) was measured from one day after inoculation up to four days (Thiruchchelvan *et al.*, 2012). The fungal (colony) growth inhibition (CGI) was measured following Kawai *et al.*, (1998) formula as indicated below and the data were statistically analyzed using SAS 9.0 statistical software package following LSD mean separation procedure at 95% probability level.

3. Results and Discussions

Significant reduction of *Marasmiellus* sp. fungal growth was obtained in *A. vera*-acetone extract in comparison to control. In the experiment, 2000 µL of *A. vera* – acetone extract had the most positive impact on inhibition of *Marasmiellus* sp. among the treatments (Table 1; Plate 1).

Table 1. Colony growth inhibition percentage with different levels of *A. vera*- acetone extract up to 4 days after inoculation.

A.vera-acetone extract	Days After Inoculation			
	1	2	3	4
20µL	23.53^{e}	26.61^{e}	23.01^{e}	7.43^{e}
100µL	27.47^{d}	37.41^{d}	34.09^{d}	11.88^{d}
200µL	37.25^{c}	48.92^{c}	42.05^{c}	18.13^{c}
1000µL	55^{b}	65.46^{b}	51.42^{b}	49.58^{b}
2000µL	74.53^{a}	74.81^{a}	71.59^{a}	59.79^{a}

All the values are means of three replicates; Mean values having same alphabet in a column indicate the values are not significantly different according to the LSD at 95 % confidence interval.

For all concentrations of *A. vera* acetone extract, the highest colony growth inhibition was obtained one day after inoculation. Inhibition of colony growth was gradually increased while increasing the concentration of extract. All treatments were significantly different among each other.

One day after inoculation, the highest colony growth inhibition (74.53 %) was obtained in 2000 µL of *A. vera* – acetone extract whereas the, lowest colony growth inhibition (23.53 %) was recorded in 20 µL *A. vera* – acetone extract. Similar pattern of change was observed two, three and four days after the inoculation. Considering the ability of rapid growth rate of the fungus *Marasmiellus* spp. in banana plants grown in Jaffna peninsula, inhibition of fungal growth can greatly help to reduce the disease incidence of banana. Findings of this study would help to provide the usefulness and effectiveness of extract of

A.vera to reduce the fungal infections. Some specific compounds are isolated from the *A. vera* using particular solvents, due to their different solubility of various compounds. Different solvents have the specified fungal activity (Babaei *et al*., 2013) thus, another experiment was set with *A. vera* ethanol extract.

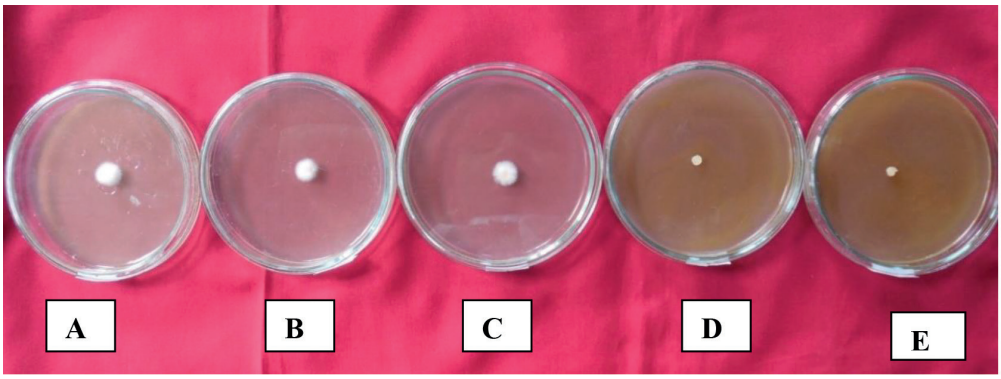

Plate 1 : Colony growth of *Marasmiellus* sp. in different quantities of *A. vera*- acetone extract; A− *A.vera* acetone leaf powder extract 20 µL; B −*A.vera* acetone leaf powder extract 100 µL; C−*A.vera* acetone leaf powder extract 200 µL; D − *A.vera* acetone leaf powder extract 1000 µL; E− *A.vera* acetone leaf powder extract 2000 µL.

Table 2. Colony growth inhibition percentage with different levels of *A. vera* –ethanol extract up to 3 days after inoculation.

A. vera ethanol extract	Days After Inoculation		
	1	2	3
20 µL	2.26^{d}	8.42^{d}	7.48^{d}
100 µL	17.29^{c}	20.15^{c}	13.67^{c}
200 µL	30.075^{b}	31.5^{b}	20.28^{b}
1000 µL	100.00^{a}	100.00^{a}	100.00^{a}
2000 µL	100.00^{a}	100.00^{a}	100.00^{a}

All the values are the means of three replicates; Figures having same alphabet in a column indicate the values are not significantly different according to the LSD at 95 % confidence interval.

Marasmiellus sp. growth was suppressed in ethanol extract than control. Ethanol extracts of *A. vera* at 1000 µL and 2000 µL showed complete inhibition of *Marasmiellus* spp. among the treatments. For all concentrations of *A. vera* ethanol extract, the highest colony growth inhibition was obtained one day after inoculation. Inhibition of colony growth was gradually increased while increasing the concentration of leaf powder extracts. One day after inoculation, the highest colony growth inhibition (100 %) was obtained in 1000 µL and 2000 µL of *A. vera* - ethanol extracts and the lowest colony growth inhibition (2.26 %) was obtained in 20 µL *A. vera*-ethanol extract. Similar pattern of change was observed two, three and four days after inoculation (Table 2).

While comparing the results of the two experiments, *A. vera* ethanol extract had the highest colony inhibition than acetone extract. In Jaffna peninsula, banana is the one of main orchard crop grown by the farmers and fungal diseases are severe and affect the yield. Therefore, management of *Marasmiellus* sp. is essential to obtain high yield of banana. Results obtained from this study will help to formulate better management strategy to inhibit the fungal growth and finally to reduce the disease incidence of banana aiming at improving the economic status of the people in Jaffna peninsula.

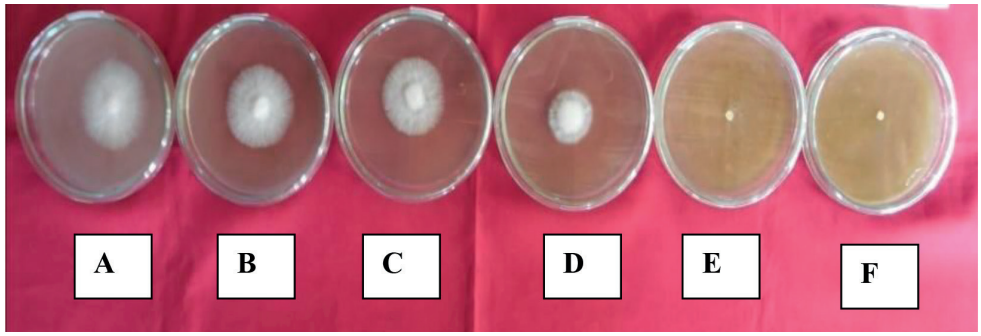

Plate 2: Colony of *Marasmiellus* sp. in *A.vera*- ethanol extract; A−Control; B−*A. vera* ethanol leaf powder extract 20 µL; C−*A. vera* ethanol leaf powder extract 100 µL; D−*A. vera* ethanol leaf powder extract 200 µL; E−*A. vera* ethanol leaf powder extract 1000 µL; F−*A. vera* ethanol leaf powder extract 2000 µL

4. Conclusions

The results obtained from in vitro assessment of antifungal activity of *A. vera* extracts showed varying levels of antifungal properties against banana pseudostem rot fungus, *Marasmiellus* spp. The complete colony growth inhibition was achieved in 1000 µL of ethanol- *A. vera* leaf powder extract. These antifungal properties of *A. vera* extracts indicate that *A. vera* is a valuable natural resource of antifungal agent in addition to other properties. Further studies should be carried out to analyze phytochemical compounds present in various *A. vera* leaf

powder extracts and to detect minimum inhibitory concentration of extracts against major pathogenic fungi.

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