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Appraisal of antifungal activity of *Aloe vera*

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Abstract

In vitro studies were carried out to evaluate the antifungal activity of *Aloe vera* shoot extract in aqueous (polar) and organic (non-polar) solvents against few pathogenic species of genus *Alternaria* viz., *A. alternata*, *A. citri* and *A. tenuissima.* The assessments revealed that *Aloe vera* contained substantial antimicrobial efficacy. The shoot aqueous extracts caused significant inhibition in growth and biomass production of the three tested fungi. In case of n-hexane extraction the inhibitory effect was found to be variable with the applied concentration.

Keywords: *Aloe vera, Alternaria alternata, A. citri* and *A. tenuissima*.

Introduction

Natural products or plants derived compounds contribute to a great extent in fight against pathogenic microorganisms (Vyvyan, 2002). The biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against fungal activities. Manohar *et al*. (2001) analyzed *Lippia junelliana* (Mold.) Tronc. against *Alternaria solani*, *Sclerotium cepivorum*, and *Colletotrichum coccodes*. Dubey *et al*. (2000) tested *Ocimum gratissimum*, *Zingiber cassumunar*, *Cymbopogon citratus*, and *Caesulia axilliaris* against *Aspergillus flavus*. Similarly Singh *et al*. (1998) determined fungitoxicity of extracts from 11 higher plants against a range of fungi based on sugarcane pathogens. There are reports that residues of *Melia azedarach*, *Eucalyptus citriodora* and *Alstonia scholaris* contain antifungal acids, tannins and flavonoids (Falahati, 2005). Lettuce (*Lactuca sativa*) incorporation into soil reduced the disease severity against root and stem rot disease of cucumber (*Cucumis sativus*) caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Pavlou and Vakalounakis, 2004).

Aloe vera L. is a member of liliaceae family. It is the most widely accepted and used for various medical, cosmetic and nutraceutical purposes (Klein and Penneys, 1988; Haller, 1990). The fresh leaves of *A. vera* are used to obtain two components: a bitter, yellow liquid fraction (exudate) and a mucilaginous pulp from the parenchymatous tissue. The liquid fraction constituents are largely phenolic in nature (Reynolds, 1985). It also has a high content of 1, 8-dihydroxianthraquinone derivatives (*Aloe* emodin) and their glycosides (aloins), which are used as cathartics (Morton, 1977). The pulp contains carbohydrate polymers (glucomannans or pectic acid) and other organic and inorganic components (Grindlay and Reynolds, 1986). *A. vera* is reported to contain mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals (Newall *et al*., 1996). The main active constituent of *A. vera* plant extract is aloine, an anthraquinone heteroside (Bruneton, 1993).

The goal of present investigation was to evaluate the antifungal potential of aqueous and organic solvent extracts of *Aloe vera* against three plant pathogenic fungi viz., *Alternaria alternata*, *A. citri* and *A. tenuissima* and to determine the extract concentrations that can inhibit mycelial growth and development of these species.

Materials and Methods

Collection of Plant material

Fresh shoots of *Aloe vera* were collected from Experimental Station, Mycology & Plant Pathology Department, University of the Punjab, Lahore, Pakistan and washed thoroughly under running tap water, dried with blotting paper and cut into small pieces. The soluble ingredients of the plant material were then extracted by solubilization in water and n-hexane as different solvents.

Aqueous extraction

Aqueous extraction of water soluble ingredients of plant material was carried out

according to Bajwa *et al.* (2004). A 50% w/v stock solution of plant extract was obtained by soaking the crushed plant materials in sterilized distilled water for 48 h at 30±2 °C. It was then passed through muslin cloth and finally through Whatman Filter Paper No.1 under aseptic conditions.

n-hexane extraction

The n-hexane extraction of the active ingredients was carried out according to Alkhail (2005). The test plant was crushed and extracted by macerating 50 g of plant material in 100 mL of n-hexane for 24 h. The extract was filtered by passing through Whatman filter paper No.1. n-hexane extract was evaporated to 2 mL and the volume was made up to 100 mL with sterilized distilled water.

The lower concentrations of 2, 4, 6 and 8% of both aqueous and n-hexane extracts were prepared by adding appropriate quantity of sterilized distilled water. The extracts were stored at 4 °C in pre-sterilized flasks. To avoid contamination and prospective chemical alterations, the extracts were used within 3-4 days.

Antifungal bioassays

Aqueous and organic solvent extract bioassays were carried out in liquid medium. The basal medium employed to grow fungi was 2% malt extract (ME) medium in 250 mL conical flasks. To avoid bacterial contamination, antibacterial Chloromycetin capsules @ 1 capsule 100 mL^{-1} of medium were used. To 80 mL of ME, 20 mL of each of 2–8% extract of *A. vera* was added. Control received the same quantity of distilled water. Inoculum discs of 5 mm diameter, obtained from 7-day old actively growing fungal cultures of *A. alternata*, *A.citri* and *A. tenuissima* were transferred to flasks aseptically and incubated at 25±2°C. The mycelial biomass from triplicate samples for each treatment was collected on pre-weighed filter papers after 10 days. Their dry weight yield was determined after 24 h oven drying at 60 °C (Bajwa *et al.*, 2006).

Statistical analysis

Standard errors of means of three replicates of each treatment were computed using computer software Microsoft Excel. All the data were analyzed by analysis of variance (ANOVA) using computer software SPSS. Following the ANOVA, Duncan's Multiple Range (DMR) Test was applied to separate the treatment means using Computer software COSTAT.

Results and Discussion

The data tested through ANOVA revealed (Table 1) that the effect of test fungi (F), extract type (E) and extract concentration (C) were highly significant ($P \leq 0.001$) for fungal biomass production. The interactive effect of E × C was also highly significant for the studied parameter. However, the interactive effects of F × E, F × C and F × E × C were insignificant.

Effect of aqueous extract of *Aloe vera* on fungal biomass production

The results obtained from biomass assays of all the test species in different concentrations of aqueous extract of *A. vera* are presented in Fig. 1. In general aqueous extract were found more inhibitory to test fungal growth than n-hexane extracts. The antifungal effect of all the concentrations of aqueous extracts was significant against all the three test fungal species except for 2% extract against *A. tenuissima*. The relative intensity of this effect, however, was found to vary with the species involved, as well as the concentration of the extract employed. There was a gradual decrease in biomass of all the three test fungal species as the concentration of extract was increased from 2–6% and an increase thereafter. Relatively more toxicity of aqueous extracts was recorded against *A. tenuissima*. There was 33 - 93%, 25 – 82% and 43 - 82% reduction in biomass of *A. tenuissima*, *A. alternata* and *A. citri*, respectively due to various concentrations (2–6%) of aqueous extracts of *A. vera*. The 8% aqueous extract concentration revealed a contrasting effect as it caused a significant increase in fungal dry biomass production in case of *A. alternata* and *A. citri* while the increase in growth rate was insignificant for *A. tenuissima*.

Effect of n-hexane extract of *Aloe vera* on fungal biomass production

The *in vitro* antifungal potential of *Aloe vera* plant extracted by n-hexane solvent is presented in Fig. 2. A variable effect of various concentrations was recorded for all the test species. In case of *A. alternata*, all the concentrations significantly reduced the fungal biomass production. Amongst these 4% concentration was the most effective in suppressing the biomass production up to 63%. Though n-hexane extract caused a significant reduction in biomass production of *A. citri* as compared to control but it was proved to be

comparatively less toxic than other test species examined. The highest antifungal activity of n-hexane extract was recorded against *A. tenuissima* where maximum suppression of fungal growth was recorded in 8% (82% reduction) followed by 2 and 4% (63 and 56% reduction) concentrations, 6% concentration caused negligible reduction in fungal biomass production.

In the present study, two types of extracts of *Aloe vera* were used against pathogenic species of genus *Alternaria*. The results of this conceptual study clearly reflect that *Aloe* has inherent ability to induce toxic effects on mycelial growth and proliferation of these fungi. The relative intensity of this effect, however, varies with the species involved, as well as the concentration of the extract employed. These findings are inline with the work conducted by Casian *et al.* (2007) where hydroalcoholic extracts of fresh leaves of *Aloe vera* were tested against the mycelial growth of *Botrytis gladiolorum*, *Fusarium oxysporum* f.sp. *gladioli*, *Heterosporium pruneti* and *Penicillium gladioli* on Czapek-agar medium. The minimum fungicidal concentration (MFC) of plant extract was 80 µL mL^{-1} in case of *B. gladiolorum* and 100 µL mL^{-1} in case of *F. oxysporum* f.sp. *gladioli*, *H. pruneti* and *P. gladioli*. Various plant extracts e.g., *Cicer arietinum* (Bajwa *et al.*, 2006), *Parthenium hysterophorus* (Bajwa *et al.*, 2004) and *Magnolia grandiflora* (Ahmed and Abdelgaleil, 2005) etc. have also been examined for their antifungal activity with the objective of exploring environmentally safe alternatives of plant disease control.

In the present study the highest inhibitory effect of fungal biomass was achieved with aqueous extract of the tested plant species that may be attributed to the presence of main active constituent of *A. vera* plant extract, the aloine, an anthraquinone heteroside (Bruneton, 1993). The n-hexane extract exhibited least inhibitory activity. This might be associated with the presence of nutritional compounds present in this extract that stimulated fungal growth and masked the inhibitory effect (Levin *et al*., 1988).

Response of fungal biomass to the extracts was found to be species specific. The species specificity of phytotoxins has also been demonstrated for other plant species (Ahmad and Abdelgaliel, 2005; Braga *et al.*, 2007). Toxicity is assumed to be associated with the presence of strong electrophilic or nucleophilic systems. Action by such systems on specific positions of proteins or enzymes would alter their configuration and affect their activity (Macias *et al*., 1992).

The general trend illustrated by the test species was almost similar. Likewise, Bajwa *et al.* (2001) found inhibitory potential in aqueous extracts of three asteraceous allelopathic species against growth of *Aspergillus niger*.

Table 1: ANOVA for effect of different concentrations of aqueous and n-hexane extracts of *Aloe vera* on biomass of three test fungal species.

Sources of variation	df	SS	MS	F values
Treatments	29	1.82	0.063	5.08^{*}
Test fungi (F)	2	0.387	0.194	15.61^{*}
Extract type (E)	1	0.163	0.163	13.16^{*}
Concentration (C)	4	0.739	0.185	14.88^{*}
F × E	2	0.010	0.005	0.410^{ns}
F × C	8	0.184	0.023	1.854^{ns}
E × C	4	0.318	0.079	6.416^{*}
F × E × C	8	0.028	0.003	0.279^{ns}
Error	60	0.744	0.0124	
Total	90			

*, significant at P≤ 0.001.
ns: non-significant

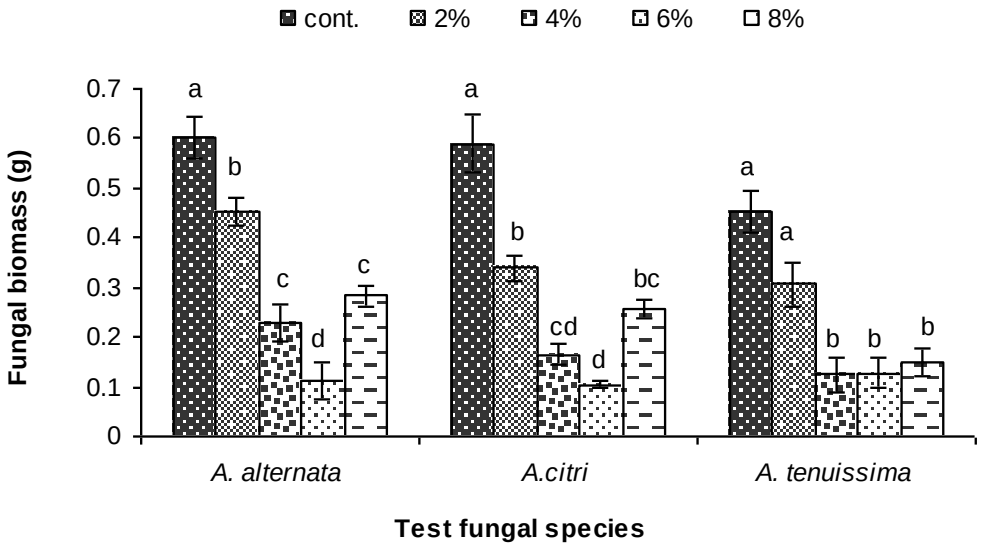

Fig. 1: Effect of different concentrations of aqueous extract of *Aloe vera* on dry biomass production of *Alternaria alternata*, *A. citri* and *A. tenuissima* after 10 days of incubation.
Vertical bars show standard errors of means of three replicates.
Values with different letters show significant difference (P≤0.05) as determined by DMR test.

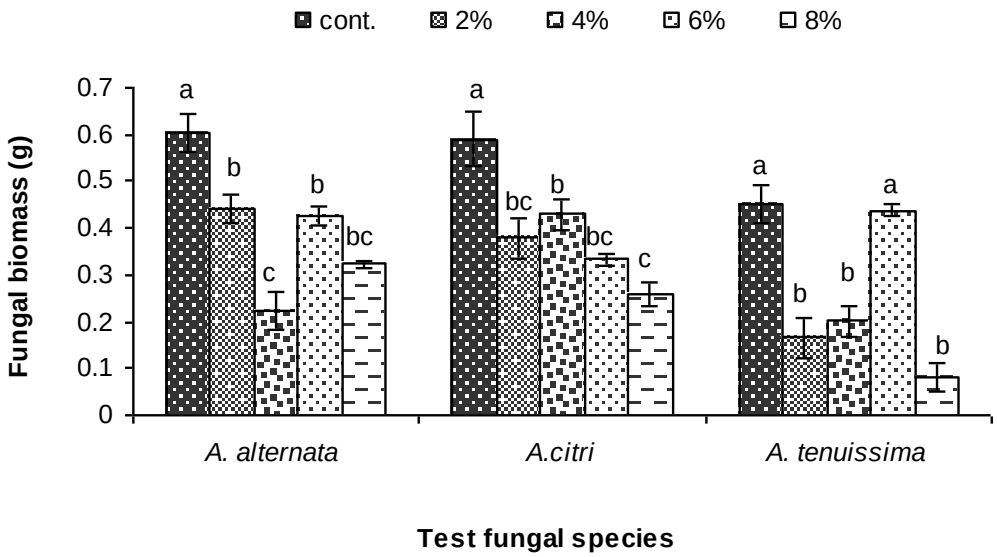

Fig. 2: Effect of different concentrations of n-hexane extract of *Aloe vera* on dry biomass production of *Alternaria alternata*, *A. citri* and *A. tenuissima* after 10 days of incubation.
Vertical bars show standard errors of means of three replicates.
Values with different letters show significant difference (P≤0.05) as determined by DMR test.

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