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ISSN 0189-6016©2009ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *GLADIOLUS DALENII* VAN GEEL (IRIDACEAE)

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Abstract

Bulb extracts of *Gladiolus dalenii* reportedly used in the treatment of fungal infections in HIV/AIDS patients in the Lake Victoria region were tested for antifungal activity using the disc diffusion assay technique. Commercially used antifungal drugs, Ketoconazole and Griseofulvin (Cosmos Pharmaceuticals) were used as standards. Dichloromethane (CH₂CL₂)/Methanol (MeOH) in the ratio 1:1. Soluble extracts showed antifungal activity against *Aspergillus niger*. Direct bioautography on silica gel Thin Layer Chromatography (TLC) and appropriate spraying agents were used to identify the active component in the extract. The activities of both the extracts were higher than that of Griseofulvin. CH₂CL₂ soluble extract in addition showed ability to delay sporulation in *A.niger*. The active group of compounds in the extracts was identified as alkaloids, which offer immense potential for development of new and valuable pharmaceutical products.

Key words: *G. dalenii*, *Aspergillus niger*, Antifungal activity

Introduction

Traditional medicine has been practiced since time immemorial by almost all cultures for treatment of numerous human diseases in many parts of the world (Palombo 2006; Dasilva and Hoareau, 1999). About 80% of rural populations in Africa use traditional medicine, mostly plant preparations, for their primary healthcare (WHO 2002-2005; Moshi 2005). About 20% of patients, who seek conventional medical care, first consult traditional healers (Dejong, 1991). In Kenya, like other African countries, traditional medicine is practiced by nearly all ethnic groups in the treatment of skin related problems, gastrointestinal diseases, sexually transmitted diseases, malaria, wounds, eye infections, measles and snake bites among others (Otieno *et al.*, 2007; Lukhoba *et al.*, 2006 ; Owuor and Kisangau, 2006 ; Mukiyama, 2005 ; Kariba *et al.*, 2001).

Gladiolus dalenii is one of the plants used by local communities in the Kenyan Lake Victoria Basin to treat various infections such as meningitis, malaria, diarrhoea, ulcers and HIV related fungal infections. Hot water extracts of freshly chopped or dried bulb is drunk to treat ulcers. Dried powdered bulb is sniffed to treat meningitis. A pinch of the same in water is drunk for the treatment of malaria and diarrhoea.

This study was carried out to establish the claimed therapeutic efficacy of *G. dalenii* against opportunistic fungal infections in HIV/AIDS patients with respect to *A.niger*.

Materials and methods

Plant material

The leaves and powdered bulb were collected from Chesikaki village in Bungoma, Kenya in September 2007. The specimens were authenticated by a qualified plant taxonomist in the University of Nairobi, and a voucher specimen (SLO 308) deposited at Nairobi University Herbarium (NAI).

Microorganism

Aspergillus niger, a human fungal pathogen, was used in the susceptibility assays. Stocks were maintained on Sabourad's Dextrose Agar (SDA) slants at 4°C prior to use for antifungal tests.

Preparation of crude extracts

20g of air dried powdered bulb was extracted by cold solvent percolation with dichloromethane/methanol (1:1) according to standard extraction methods (Harborne, 1998). The powdered plant material was mixed thoroughly with the solvent, left to stand for 24 hrs, and decanted (this was repeated twice). The filtrates were pooled and filtered using a Buchner funnel. After evaporation of the solvents, dichloromethane and methanol extracts were obtained. These were made up to desired weight/volume for bioassay analyses.

Preliminary screening for antifungal activity

Antifungal bioassay was carried out using disc diffusion method (Belboukhari and Cheriti, 2006). Discs cut from Whatman filter paper No.1 using a cork borer of diameter 1.2 cm were soaked for two hrs in each extract reconstituted with the extracting solvents serially diluted at concentration 1mg/100µl. The impregnated discs were then aseptically transferred into Sabourad's Dextrose Agar (SDA) plates freshly inoculated with 0.5ml spore suspension of the test fungus. Griseofulvin and Ketoconazole antifungal drugs served as standards at similar concentrations, while discs impregnated with the extracting solvent served as controls. The plates were prepared in duplicates and then sealed with parafilm to avoid contamination and any possible drying up, and incubated in humid conditions at 25°C. The plates were observed for presence of zones of inhibition around the discs from day 3 to 5.

Direct Bioautography

In order to identify the active compound/s in each extract, commercially prepared Thin Layer Chromatography (TLC) plates made from silica gel were developed and the separated spots were seen visible under the UV lamp. Freshly developed TLC plates were sprayed with previously prepared suspension of *A. niger* in peptone media and incubated under humid conditions at 25°C (Hostettmann and Marston 1994; Horvath Gy et al., 2002) and zones of inhibition noted.

Preliminary characterization of active compounds

Active CH₂CL₂ and MeOH extracts were tested for the presence or absence of five classes of compounds (Table 1) using appropriate spraying agents and UV detection according to Chowdhury et al., (2008) and Harborne, (1998).

Results and Discussion

Evaporation of the organic solvent yielded two extracts: Dichloromethane and Methanol soluble extracts. These were labelled as Ac1 and Ac2 respectively and antifungal activity tests carried out.

Table 2 showed that both the extracts and Ketoconazole (K), a commercial antifungal drug exhibited antifungal activity against the test fungus while Griseofulvin (G) drug showed no activity. Ac2 showed higher activity than Ac1, whereas Ketoconazole showed the highest activity.

Based on preliminary TLC screening by direct Bioautography and appropriate reagents, the active antifungal compounds in both the test extracts were identified as alkaloids.

The petridishes with the extract Ac1 (Dichloromethane soluble extract), recorded delayed sporulation of the filamentous fungus up to day eight (8) whereas the plate containing methanol soluble extract (Ac2) had a dense sporulation of the fungus from day 5.

Table 1: Thin Layer Chromatography analyses of the antifungal compounds

Phytochemicals	Spraying reagent	Appearance of the positive spot.
Flavonoids	25% aqueous solution Basic lead acetate.	Spots fluoresce in long-wave UV light.
Alkaloids	Dragendorff	Orange
Sapogenins	Antimony chloride in concentrated hydrochloric acid.	Violet.
Terpenoids	Antimony chloride in chloroform.	Green
Quinones	Exposure to ammonia fumes.	Red, orange, yellow, brown.

(Adapted from Chowdhury et al., 2008.)

Table 2: % mean Inhibition zones of the extracts/drugs

Extract/ drug	% mean inhibition
CH ₂ CL ₂ soluble extract (labeled Ac1)	69.44
MeOH soluble extract (Ac2)	75.00
Griseofulvin drug (G)	0.00???
Ketoconazole (K)	90.28
Negative Control (C)	12.50

Re-culturing and incubation of spores and mycelia from both the plates with the different extracts Ac1 and Ac2 resulted into normal growth and sporulation. Regarding the incubation period and activity, there was no significant difference (p value = 0.393) recorded in activities of the extracts as far as the incubation period was concerned, however in both the extracts, the mean percentage activity was highest on the 3rd day.

This study shows that CH₂CL₂ and MeOH extracts of *G. dalenii* possess antifungal activity against *A. niger*. The opportunistic fungus appeared susceptible to activities of the extracts as shown by the large zones of inhibition (Table 2.) at a concentration of 1mg/100µl. The delayed sporulation induced by dichloromethane soluble extract was an interesting observation which has not been previously reported. Steenkamp et al., (2007) reported no activity of water and methanol bulb extracts of this plant against *Staphylococcus aureus*, and *S. epidermidis* bacteria; this was in contrast to reports by Fawole et al., (2008) who found out that dichloromethane extracts of this plant were active against *Bacillus subtilis* and *S. aureus*, while ethanol extracts were active against *Candida albicans* but inactive against *Escherichia coli*. Antiamoebic activity of bulb extracts of this plant has also been reported by Moundipa et al., (2005). The antimicrobial activities reported confirm why extracts of this plant is used in traditional medicine to treat wounds, eye infections, ear infections, headache, dysentery, diarrhoea, stomach upset and gonorrhoea (Fawole et al., 2008 ; Yiniger et al., 2008 ; Hutchings and Staden, 1994 ; Arnold and Gulumian, 1984). Burkill, (1985) reported its usage in veterinary medicine. Other ethnomedicinal usage include treatment of arthritis, rheumatism, laxative, pains associated with menstruation, paralysis, convulsions, spasms, vermifuges, wound dressing, antidote

A clear zone shown by the activity of Ac1

A clear zone shown by the activity of Ac2

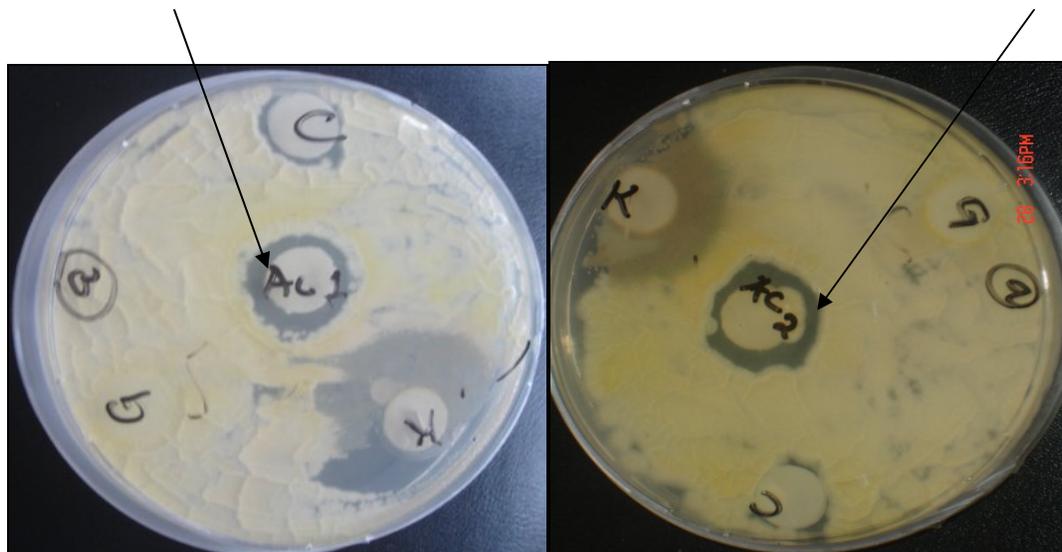


Figure 1: Antifungal activity of the two crude extracts from *Gladiolus dalenii*.
 Ac1- CH₂CL₂ soluble extract; Ac2- MeOH soluble extract; G- Griseofulvin;
 K- Ketoconazole; C- Negative Control,

Lack of sporulation by *A.niger* in
 Plate with Ac1 extract

Dense sporulation by *A.niger* in
 plate with Ac2 extract

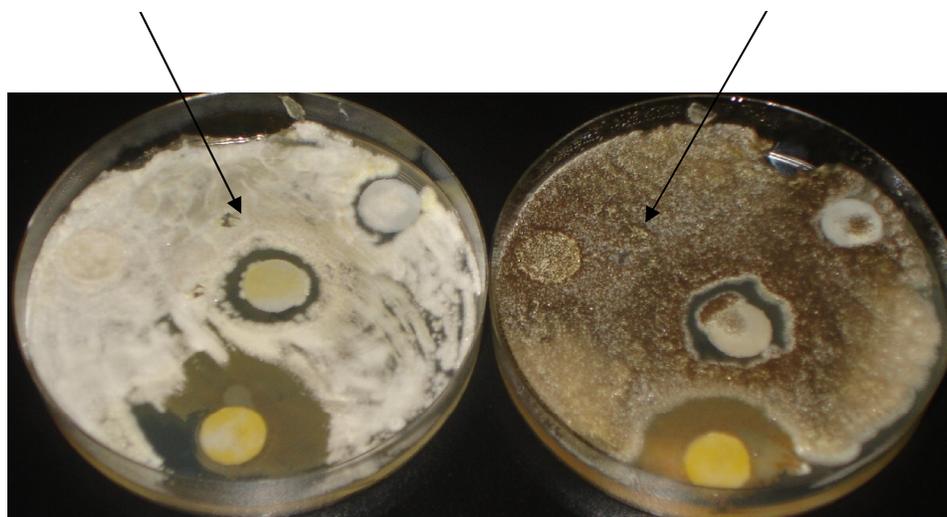


Figure 2 : Spore inhibitory properties of Ac1

Ac1- CH₂CL₂ soluble extract,
 Ac2- MeOH soluble extract

against venomous snake and spider bites or stings and naso-pharyngeal infections. Burkill, (1985) also reported its usage to make a drink, and as general food. In this regard, the activity of this plant and its ability to inhibit sporulation in *A.niger* may have been reported in this study for the first time.

Significance of the results of this study is in the control of opportunistic fungus such as *Aspergillus niger* in immuno-compromised individuals. The fungal growth may be terminated completely at higher concentration of the active ingredient of this plant. Ability to delay sporulation as exhibited in these results is an indication that this plant has the potential to reduce allergies in susceptible individuals by ensuring no spores are produced. This finding suggests that extracts from *G. dalenii* could be used successfully for the management of Aspergillosis.

Preliminary screening results show that alkaloids are responsible for the antifungal activity in this plant. This finding is partly in agreement with that of Burkill (1985) who reported that alkaloids are the main phytochemical component in this plant, but did not mention anything about the antifungal activity of this plant.

Conclusions and Recommendations

Results of this study indicate that it is important to carry out taxonomic verification and biochemical analysis studies on medicinal plants known to have ethnomedicinal uses. Further analysis of *G. dalenii* offers possibilities of developing plant-based compounds to help combat the lung problems caused by *A. niger*, one of the frequently encountered fungal infections in immunocompromised patients. Only *in vitro* methods were used in assessing the antifungal activity of the crude extracts of *G. dalenii*. Further investigation using bioassay guided fractionations to isolate and identify the pure compounds responsible for the antifungal activity and spore inhibitory ability are recommended. *G. dalenii* is a herb in nature and is able to grow fast, with the advantage of being an ornamental plant, possibilities of cultivation in large scale for commercial exploitation are recommended.

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Dedication

Within the month that the final draft of this paper was completed and ready for submission, one of the authors, Prof. G.M. Siboe succumbed to cancer he had struggled with for years. This paper is only part of how his profound knowledge in research goes on; because it is in the hearts and minds of those who knew him. This paper is therefore dedicated to him, and the other authors have chosen to include his name in the authors' list due to his invaluable contribution.

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