Article 124

Antimycotic Activity of *Commelina cyanea*

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In an attempt to establish the antifungal properties of Commelina cyanea against Epidermophyton floccosum, fresh leaves and stem of Commelina cyanea were harvested, air-dried and ground to powdered form. Crude extracts were obtained from the plants using cold distilled water and methanol as solvents. Phytochemical screening was done on both the extracts and powder using conventional methods. Sensitivity test was done using the agar dilution method against Epidermophyton fluccosum as test isolate. The MIC of susceptible extract was established using the multiple agar dilution tube technique. The methanolic extract was found to be sensitive with an MIC of 6.25mg/ml while complete resistance was registered with the aqueous extract. Various phytochemicals identified in the test extracts were reported simultaneously.

KEYWORDS
Commelina cyanea, antimycotic activity, *Epidermophyton floccosum*.

INTRODUCTION

The indiscriminate use of antifungal agents has resulted in the development of microbial resistance to these agents. In addition, the toxicity and residual effect of these agents remains a great limiting factor that has led to the emerging of natural plant extracts for treatment of various fungal infections [1,2,3,4,5].

Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other compounds, reported to have in vitro antifungal properties. A series of molecules with antifungal activity against different strains of fungus have been found in plants, which are of great importance to man [4,5,6,7].

Commelina cyanea has been used in North West Cameroon as indigenous medicine for treatment of dermatomycosis. Evaluation of antifungal activities of *Commelina cyanea* against dermatophytes may be useful in determining it as an alternative source of an antifungal agent in future.

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MATERIALS AND METHODS

Fresh leaves and stems of *Commelina cyanea* were harvested from within Bamenda II Sub-Division in the month of June 2012. The harvested plant were rinsed properly in sterile distilled water, dried in shadow and then ground into powdered form.

Aqueous Extraction

100g of the powdered plant was put in clean and sterile 1000ml conical flasks containing 600ml of cold sterile distilled water. The flasks were shaken intermittently for 24 hours. The material was then filtered and the filtrate was distributed into three clean, sterile evaporating dishes and was evaporated to dryness in an oven at 60°C [8].

Methanolic Extraction

50g of the powdered plant was wrapped in Whatman no.1 filter paper and was placed in soxhlet extractors. 250ml of methanol was used for the extraction. The extract so obtained was finally dried in the hot air oven to remove all the solvent. The crude extract was weighed and stored in sterile universal bottles at 4°C in a refrigerator [8].

Phytochemical Screening

Phytochemical screening for resins, alkaloids, saponins, tannins, glycosides, and flavonoids was done on both the extracts and powder using conventional methods [8,9].

Test for Resins

To 0.5g of each sample (Aqueous extracts, menthol extract and powdered plant) was added 5ml of boiling ethanol. The solutions were filtered through Whatman no.1 filter paper and the filtrates were diluted with 4ml of 1% aqueous HCl. The formation of a heavy resinous precipitate indicated the presence of resins [8].

Test for Alkaloids

0.5g of each sample was stirred with 5ml of 2N HCL in a steam bath. The solutions were filtered and 1ml of each filtrate was tested with a few drops of Dragendorff``s reagent and another 1ml was treated with Wagners reagent. Formation of a precipitate was an indication of the presence of alkaloids [8].

Test for Saponins

0.5g of each sample was stirred with water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins [8].

Test for Tannins

0.5g of each sample was stirred with 10ml of boiling distilled water and filtered. 0.5ml of 6% ferric chloride added to the filtrate. A deep green coloration indicated the presence of tannins. The second portion of the filtrate was treated with a few ml of iodine solution. A faint bluish coloration confirmed the presence of tannins [8].

Test for Glycosides

0.5g of each sample was stirred with 10ml of boiling distilled water. This was filtered and 2ml of the filtrate was hydrolised with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution were added to 2ml of Benedict`s qualitative reagent and boiled. A reddish brown precipitate showed the presence of glycosides [9]

Test for Flavonoids

0.5g of each sample was dissolved in 2ml dilute NaOH solution. A few drops of concentrated H2SO4 were added. The presence of flavonoids was indicated when the solution became colorless [9].

Preparation of Fungi Suspension

Pure culture of *Epidermophyton floccosum* was obtained from Science for Life Foundation laboratory. The isolate was inoculated into brain heart infusion broth and incubated at room temperature for 72 hours after which it was sub cultured onto SDA slants to test for purity. The purified isolate was Sub-cultured onto Potato Dextrose Agar slopes and incubated for 5 days at 25oC to ensure maximum sporulation. With the aid of a sterile Pasture pipette the colonies on the surface of the culture were gently washed with sterile distilled water and the culture tube was vortexed with sterile glass beads to break up the moulds. This gave a uniform suspension that was allowed to stand for a few minutes so that larger hyphal segments had time to settle. The top homogenous layer was diluted to obtain the desired inoculum density [10].

Susceptibility Testing (Agar Gel Dilusion Method).

The dried plant extracts were dissolved in sterile distilled water to obtain a 400mg/ml solution and sterilized by filtration through a 0.45um membrane filter. Various dilutions were made in sterile molten SDA in test tubes to obtain 200mg, 100mg, 75mg, 50mg, 25mg, 12.5mg and 6.25mg concentrations which were kept in a slanting position at room temperature for solidification. The slopes so obtained were inoculated with 50ul of a uniform suspension of the broth culture of *Epidermophyton floccosum* and all slopes were incubated at 25°C and were checked on daily bases for growth. Controls were set along the side. For positive control grisofulvin incorporated into SDA slants at concentrations of 500ug/ml was used, while for negative control plain SDA slopes were used. As such absence of growth was considered positive, while growth was considered negative. The MIC was considered to be the lowest concentration that gave no growth.

RESULTS

The phytochemical analysis of both aqueous and methanolic extract of the plant *Commelina cyanea* showed the presence of the various phytochemicals (Table1).

The test isolate was completely resistant to aqueous extract at all concentrations applied in the present studies. The lowest concentration of the methanolic extract that inhibited the test isolate was 6.25mg/ml. (Table 2).

DISCUSSIONS

The methanolic plant extracts of the plant *Commelina cyanea* have proven to contain considerable useful properties as well as bioactive phytocompounds which were resins, alkaloids, saponins and glycosides. The antimicrobial activity of alkaloids and saponins can be explained by the fact that they are not only capable of complexing extracellular proteins but also break the microbial membranes causing cell death [7]. This explains the inhibitory activity against the test organism *E. floccosum.*

Based on the above results, this plant may provide a potential alternative source of antimycotics. However, further work needs to be done on the in-vivo susceptibility and toxicity.

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TABLES

Table 1: Phytochemicals analysis of *Commelina cyanea* plant Extract

Test for	**Results**		
	Aqueous Extract	**Methanolic Extract**	**Powder**
Resins	-	++	+
Alkanoloids	+	+	+
Saponins	+	+++	++
Glycosides	-	++	-
Flavonoids	-	-	-
Tanins	-	-	-

Key: + = Present
++ = Highly present
- = Absent

Table 2. The MIC value in mg/dl of *Commelina cyanea* on *E. floccosum*.

Organism	Aqueous extract(mg/ml) MIC							Methanolic extract(mg/ml) MIC							Griseofulvin (ug)
	200	100	75	50	25	12.5	6.25	200	100	75	50	25	12.5	6.25	500
E. flocossum	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

FIGURES

Fig.1 *Commelina diffusa*