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Short Communication

Antifungal effect of *Calotropis procera* stem bark on *Epidermophyton flocosum* and *Trichophyton gypseum*

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The antifugal activities of aqueous extract of *Calotropics procera* was determined against *Epidermophyton flocosum* and *Tricophyton gypseum* using agar diffusion techniques. The crude extract of *C. procera* showed activity on *E. flocosum* and *T. gypseum* at 4.0 mg/ml. The result of minimum inhibitory concentration (MIC) was 0.5 and 0.9 mg/ml and that of minimum fungicidal concentration (MFC) was 2.0 and 4.0 mg/ml, respectively. The result of the Ames test indicated that the crude extract is not mutagenic. Phytochemical screening of the crude extract revealed the presence of saponin, tannins, sequiterpene and alkaloids. The results of the study suggest that *C. procera* stem could be a potential source of chemotherapeutic drugs for the treatment of tinea associated with *E. flocosum* and *T. gypseum*.

Key words: *Calotropis procera, Epidermophyton flocosum, Trichophyton gypseum,* mutagencity.

INTRODUCTION

Dermatophytes are fungi that colonize skin, hair and nails of the living host. These fungi posses greater invasive properties than those causing superficial infections but they are limited to the keratinized tissues (Baron, 1996). They cause a wide spectrum of disease that range from guild scaling disorder to one that is generalized and highly inflammatory (Baron, 1996).

Dermatophytic fungi exist throughout the world. Although some species are endemic, they tend to spread rapidly to non-endemic regions (Talaro, 2005). Dematophytoses endure today as a serious health concern not because they are life threatening but because of the extreme discomfort, stress, pain and the unsightliness they cause (Talaro, 2005). Antimicrobial agents (antibiotics) remain the alternative in the treatment of disease arising from microbial infections. A major problem encountered with antibiotics in clinical use is drug resistance, which mostly leads to treatment failure (Mosses et al., 2006). Other problems with antibiotics include toxicity high cost, low cost efficacy, etc. This necessitates a continuous search for new antimicrobial agents.

Medicinal plants have no doubt remained the major sources of both orthodox and traditional medicine worldwide. Accordingly, attention of scientists and researchers have been attracted towards developing new antibiotics that will curtail the increasing drug resistance among microorganisms (Edith et al., 2005). Schimmer et al. (1994) reported that plants used for traditional medicine generally contain a number of compounds which may be a potential natural antimicrobial combination and which may serve as an alternative, effective, cheap and safe antimicrobial agents for treatment of common microbial infections.

This study attempts to determine the antifugal effect of *Calotropis procera* stem bark extract on *Epidermophyton flocosum* and *Trichophyton gypseum*. The result would enable more rational exploitation of the plant in both traditional and orthodox medicine.

MATERIALS AND METHOD

Collection of plant material

The stem bark of *C. procera* was collected in Minna, Niger State of Nigeria. The plant was identified in collaboration with Botanists and Crop Production Department, Federal University of Technology, Minna.

Preparation of extract

The plant materials were dried in ambient temperature, ground and macerated in distilled water for 48 h, then filtered and the filterate concentrated by rotary evaporator.

Table 1. Susceptibility pattern of *Epidermophyton flocosum* to aqueous crude of *Calotropis procera.*

Aqueous extract concentration (mg/ml)	Zones of inhibition of (mm)	Control griseofluvim ointment concentration (mg/ml)	Zone of inhibition (mm)
5	13	5	20
4	10	4	13
3	05	3	04
2	0.75	2	05
1	0.12	1	02

Table 2. Susceptibility pattern of *Trchophyton gyseum* to aqueous crude extract of *Calotropis procera.*

Aqueous extract concentration (mg/ml)	Zones of inhibition (mm)	Control griseofluvim ointment concentration (mg/ml)	Zone of inhibition (mm)
5	12	5	16
4	8	4	9
3	4.5	3	5
2	1.8	2	4
1	0.11	1	1.9

Phytochemical screening

The phytochemical analysis of the aqueous extract of *C. procera* was determined for tannins, saponins, anthraquuinone and sequiterpene, as described by Sofowora (1993).

Test organisms

The isolates (i.e *E. flocosun* and *T. gyseum*) were obtained from the General Hospital, Minna. The organisms were grown on sabouraud dextrose agar 30^{o}C until they were jugged to have formed maximal numbers of conidia (Odds et al*.,* 1998). The conidia were suspended in sterile physiologic saline containing a drop of tween 80 in 100 ml, determination of colony forming unit (cfu) per milliliter was done by plating dilutions of the suspensions on sabouraud dextrose agar (oxoid) in replicates (Odds et al*.,* 1998).

Susceptibility test against *E. flocosum* and *T. gyseum*

This was performed as described by Cheesbrough (2000) with some modification. The spores of the test organism (1 x 10^{8} CFU/ml) were inoculated onto sabourand dextrose agar. Sterile cork borer was used to bore 5 holes on the agar plates, after which various concentrations ranging from (1 - 5 mg/ml) were introduced aspetically into the walls of the agar plates seeded with the test organisms and incubated at 30^{o}C for 5 days. Griseofluvin ointment was prepared in various concentrations ranging from 1 - 5 mg/ml and introduced into wells of different agar plates seeded with test organisms as the control. The plates were incubated at 30^{o}C for 5 days (Gbodi and irobi, 1993).

Ames test

The required suspension of *Salmonella typhimurium* was prepared (0.1 ml) and was introduced onto the surface of the base plate in replicate, using sterile syringe and needle. After the solidification of the agar, a sterile cork borer (6 mm) was used to make 3 holes equidistance and crude extracts introduced into the holes and the plates were incubated at room temperature for 72 h.

Minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was determined using tube dilution technique. Varying amounts of aqueous crudes extract (0.5 – 5.0 mg/ml) were aspetically introduced into ten (10) test tubes containing sabouraud dextrose broth and 10^{8} CFU/ml of the test organisms. The test tubes were incubated at 30^{o}C for 5 days. Control test tubes containing the test organisms and the sabouraud dextrose broth were set up in replicate. Minimal inhibitory concentration (MIC) was considered as the lowest amount of extracts that inhibited visible growth of the test organisms in the broth culture (Gbodi and Irobi, 1993).

Minimum fungicidal concentration (MFC)

This was determined by culturing little portion of the concentrated of the tube culture that showed no visible growth in the MIC determination. (A loopful of the diluted prepared sabourand dextroses agar plates in two replicates and incubated at 30^{o}C for 5 days). A control consisting of the test organisms inoculated of fresh medium was set up. Minimal fungicidal concentration was regarded as lowest concentration of the extracts that inhibit growth on the medium after the period of inoculation (Gbodi and Irobi, 1993).

RESULTS AND DISCUSSION

The result of phytochemical screening of the aqueous extracts of *C. procera* revealed the presence of alkaloids, saponin, tannins, anthroaquinone and sequiterpene. The aqueous crude extracts produced zone of inhibition in disc diffusion technique ranging from 0.11 - 13 mm (Tables 1 and 2). Minimum inhibitory concentration of the extract was 0.5 and 0.9 mg/ml for *E. flocosum and T. gypeum,* respectively, including the segment in the ab-

stract for clearity. The minimum fungicidal concentration was 2.0 and 4.0 mg/ml, respectively.

Until the late eighties, it was widely believed that superficial infection was no longer a threat to public health. Fungal infection seems especially controllable due to good hygienic condition, but development of fungal resistance to antifungal drugs is almost an inevitable consequence of their application (Ekhaise and Okoruwa, 2001). Microorganism that acquired resistance to a particular antimicrobial agent becomes clinical important, particularly when the use of individual drug is wide spread. Mechanism of drug resistance by microorganisms to antimicrobial agents can be categorized into the enzymatic modification in activation of the antibodies or receptor modification as well as limiting access of the drug to its susceptibile host of pathogen (Ekhaise and Aliyu, 2005).

C. procera plant is used by traditional medicine practitioner in Gwari communities for the treatment of ring worms. The result in this study revealed that the crude extracts of the plant have antifungal activity against the test organisms. The inhibitory action of the crude extracts was recorded even at very low dose, which is a clear indication that the crude extract contains active commencements that have antifungal properties. Similarly, the methanol crude extract of *C. procera* have antifungal activity against *Microsporum canis* and *Trichophyton rubrum* at 5.0 mg/ml concentration (Kuta, 2006). The test organisms in response to the antifungal effect of the extracts as shown by the zones of inhibition (0.12 – 13 mm) and (0.11 – 12 mm) and the low values for minimal inhibition concentration (MIC) and minimal fungal concentration (MFC) range from (0.5 – 0.9 mg/ml) and (2.0 – 4.0 mg/ml) for *E. flocosum and T. gypseum*, respectively, is an indication that the organisms are sensitive to the crude extracts. Gbodi and Irobi (1993) had reported that patients of dematophytoses in Nigerian are faced with the challenges of re-occurrence of ringworm infections. The fact that the crude extract inhibited the growth of the test organisms, the use of the extract for therapeutic purpose should be encouraged, though the crude extracts had less inhibitory effect in some cases as revealed in the results (Tables 1 and 2). The result of this study suggests that *C. procera* can be potential source of chemotherapeutic agents that can be used for the treatment of tinea diseases.

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