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ANTIFUNGAL ACTIVITY OF LEAF EXTRACT OF *CRASSOCEPHALUM CREPIDIODES* ON SELECTED DERMATOPHYTES AND *CANDIDA ALBICANS.*

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ABSTRACT

Effect of ethanolic and aqueous extracts of the leaf of *Crassocephalum crepidiodes* on some dermatophytes and *Candida albicans* was investigated using disc diffusion agar technique. The two extracts exhibited antifungal activity at 10mg/ml concentration against *Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporium audouinii,* but not against *Candida albicans.* There was significant difference ($p<0.05$) in fungicidal activity between the ethanolic and aqueous extracts, with ethanolic extract showing higher activity. The susceptibility profile of the dermatophytes tested was *T. mentagrophytes.* > *T. rubrum* > *M. audouinii.* The phytochemical studies of the extracts revealed that the aqueous extract lacked terpenes and anthraquinone while terpenes were absent in ethanolic extract.

KEY WORDS: Antifungal, Dermatophytes, Extract, Susceptibility.

INTRODUCTION

Seminal to the advancement in orthodox medicine, man had used local herbs to treat various diseases with great success. The upsurge of subcutanous mycoses in Nigeria and the emergence of fungal resistance to known antifungal drugs have necessitated the use of antimycotic drugs with elegance and complex formulations to treat these infections. However, such drugs are not cost effective and often requires high foreign exchange to import them into the country, hence the need to explore alternative and cost effective means of treating dermatophytoses. Furthermore, although fungal related infections may not be as common as bacterial infections, they are more difficult to treat especially in patients whose immunity has been compromised. These are some of the reasons the exploration and development of natural products with potent antifungal activity is important. For example, Kosuge *et al.* (1968) reported that 'rice bran ter' was highly effective on the treatment of eczema, while Ohigashi *et al.* (1972) reported on the antifungal properties of *Sapium japonicum.* There are also reports on the antifugal properties of some spices such as Clover and star anise seeds (Hitokoto *et al.,* 1980), *Allium sativum* (Prasad and Sharma, 1981) and *Allium cepa* (Sharma *et al.,* 1981).

In Nigeria, studies on antifungal property of medicinal plants have been reported by few Scientists. Ikenebomeh and Metitiri (1988) reported the antifungal property of *Casia alata.* Adekunle (2000) reported that crude extracts of *Brachystegia eurycoma* and *Richardia brasiliensis* exhibited antifungal activity on different species of *Aspergillus* and *Candida albicans;* while Ormenka and Osuoha (2000) reported antifungal property of grape fruit extract on *Aspergillus niger* and *Candida albicans.*

The phytochemical screening of some of the medicinal plants so far studied reveals that the extracts contain bioactive compounds such as 2-methoxy-1-4 naphthoquinone (Little, 1948), alkaloids terpenes, saponins, flavenoids (Adekunle, 2000) and limonene, linalol and pinene (Omenka and Osuoha, 2000). These bioactive substances are of much chemotherapeutic value and offer considerable information the world over in the establishment of natural plant provision.

Crassocephalum crepidiodes is an herbaceous plant with soft greenish stem, pinnate leaves with indented margins and small bell-shaped yellow to white flowers in whorled in florescence. The plant has no well developed tap root system, but grows well during rainy season, or under shade during dry season in any fertile soil. The plant is known as 'mkpefit' in Ibibio. Traditionally, the warm extract from leaves is used as concoction for the treatment of athlete's foot and other skin diseases. The warm extract is usually obtained by passing the leaves gently over flame to soften the tissue and then squeezing out the extract with hand.

Although there have been reports on antifungal properties of some important medicinal plants in Nigeria, the antimycotic acitivity of *C. crepidiodes,* another important medicinal plant in the country, has not been studied and reported in literature.

The aim of this study therefore is to investigate the antifungal activity of crude extract of the leaf of *C. crepidiodes* on selected dermatophytes and *Candida albicans.* The bioactive components of this plant part are also reported.

MATERIALS AND METHODS

Plant Materials

Leaves of *C. crepidiodes* were obtained from vegetable gardens around residential houses in Itam, Akwa Ibom State, Nigeria. The plant was identified using text, 'Forest-Our Divine Treasure' by Etukudo (2000). The identity of the plant was further authenticated by the Department of Botany, University of Uyo.

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Source of Microorganisms

The dermatophytes and *Candida albicans* used in this study were clinical isolates obtained from the Microbiology and Parasitology Laboratories, University of Calabar Teaching Hospital, Calabar, Nigeria. The organisms were propagated on Biotech brand of Sabouraud dextrose agar (SDA) and thereafter stored on slants of the same medium and preserved in the refrigerator at 4°C until the commencement of the test. The SDA medium was prepared according to manufacturer's description.

Preparation of Leaf Extract

About 600g of fresh leaves of *C. crepidiodes* were obtained from the plant between the hours of 6.00 and 7.30 a.m in the month of June. The leaves were rinsed in clean water to remove some extraneous materials from the leaf surfaces and drained. Thereafter they were chopped with clean kitchen knife and homogenized with Commercial waring blender, model MS 223.

The homogenate was divided into two portions, each weighing 150g. The first part was suspended in 350ml of 70% aqueous ethanol solution and allowed to soak for 48 hours, while the second part was soaked in 350ml of distilled water for 48 hours. The resulting solutions were filtered through a funnel lined with Whatman No. 1 filter paper, with the aid of vacuum pump. The residues were resuspended two times in 50ml of 70% aqueous ethanol or distilled water for 2 hours each. The final extracts were sterilized by membrane filtration using seitz membrane filter with 0.45µ pore diameter. The sterilized extracts were evaporated under pressure using a Bibby rotary evaporator RE 100 to produce powder. A 10.0mg/ml concentration of the crude ethanolic and aqueous extracts were made and assayed for antifungal activity.

The pH of the extract was measured with a portable pH meter (Griffin England).

Assay of Antifungal Activity

The antifungal activity of both aqueous and ethanolic extracts was assayed using disc diffusion agar technique as described by Madigan *et al.*(1997). Spores or conidia of the test organisms were transferred with sterile moistened wire loop from SDA slant into sabouraud dextrose broth, mixed well and incubated 25°C for 72 hours. Cell counts were determined after every 6 hours with clinical haemocytometer. When cell population of 10^6 cells/ml was obtained, the incubation was stopped.

With the aid of sterile micropipette, 100µl of each fungal suspension under test was seeded on six different SDA plates and spread with sterile glass spreading rod. Sterilized Whatman No. 1 filter paper discs, measuring 5mm in diameter were soaked in each of the crude extracts being assayed for 3 hours. Four of the soaked discs were placed at 5mm-spaced points on each plate containing the test organisms using sterile forceps. Four controls were set up as follows: The first contained the fungal inoculum with discs soaked in known standard antimycotic drugs, canesten, griseofulvin and nystatin at 100mg/ml respectively. The second and third controls comprised paper discs soaked in distilled water and 70% ethanol respectively, while the fourth control had only sterilized discs which were not soaked in water, ethanol or extracts.

All the tests were carried out in triplicates and their mean measurements calculated. Both the experimental and control plates were incubated at 25°C for 48 hours and zones of inhibition round the paper discs quantified by direct measurements of their diameters from the edge of the paper discs.

Phytochemical Screening

The phytochemical studies were carried out on the extract using the methods described by Culier (1982), Sofowora (1984) and Gundiaza (1985). The plant was tested for the presence of alkaloids, tannins, saponins, flavenoid, anthraquinone and terpenes.

Statistical Analysis

Student t-test statistic as described by Phillips (1973) was employed to test for significance in fungicidal activity of ethanolic and aqueous extracts.

RESULTS

The fungicidal activity of the plant extracts on the test organisms is shown in Table I. The extracts inhibited the growth of all the dermatophytes tested, but not *Candida albicans*. The control plates without the incorporation of the extracts gave good growth of the organisms. Generally, *Trichophyton mentagrophytes* was most susceptible followed by T. *rubrum*, while *M. audouinii* was least sensitive. Comparatively, the *Trichophyton* species were more markedly affected by the extracts than the *Microsporum* species. This tables also shows the zones of inhibition (in millimeters) of the aqueous and ethanolic extracts of *C. crepidiodes*

Table 1: Fungicidal activity of ethanolic and aqueous extracts of the leaf of *Crassocephalum crepidiodes*

Organism	Zone of inhibition (mm)					
	Controls	Aq. Extract (10mg/ml)	Ethanol Extract (1umg/ml)	GRF (100mg/ml)	CAN 100mg/ml	NST (100mg/ml)
T. mentagrophyte	0.00 ± 0.00	16.14 ± 0.24[a]	21.50 ± 0.31	13.75 ± 0.18	12.00 ± 0.10	10.54 ± 0.88
T. rubrum	0.00 ± 0.00	14.75 ± 0.13	20.63 ± 0.45	10.50 ± 0.03	0.00 ± 0.00	9.24 ± 0.01
M. audeunii	0.00 ± 0.00	10.40 ± 0.17	11.34 ± 0.64	0.00 ± 0.00	7.52 ± 0.23	8.73 ± 0.47
C. albicans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	14.20 ± 0.14	16.75 ± 0.14	20.17 ± 0.05

Controls consist of discs soaked in 70% alcohol, water and unsoaked discs.

GRF: Griseofulvin; [a] values are mean ± standard deviation from three replications.
CAN: Canesten;
NST: Nystatin.

Table 2: Bioactive compounds of the extracts of the leaf of *C. crepidiodes*

Bioactive Compounds	Aqueous Extract	Ethanolic Extract
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Flavenoids	+	+
Anthraquione	-	-
Terpenes	-	-

+: Present;
Absent.

compared to those of griseofulvin, nystatin and canesten. The ethanolic extract exhibited higher antifungal activity than the aqueous extract at the concentration used. Moreso, both extracts inhibited all the dermatophytes originally resistant to the commercially available antimycotic drugs (griseofulvin, nystatin and canesten) used in this study, but failed to inhibit *C. albicans.*

The results of the phytochemical screening of the plant are presented in Table 2. As indicated, the aqueous extract of the leaf of *C. crepidiodes* had all the bioactive compounds tested for except anthraquinone and terpenes, whereas the ethanolic extract showed the presence of tannins, flavenoid anthraquinone, saponin and alkaloids.

The statistical anlysis using T-test to compare the fungicidal activities of ethanolic and aqueous extracts gave significant difference ($P<0.05$) in all treatments between the aqueous and ethanolic extracts with ethanolic extract showing higher activity.

DISCUSSION

The results obtained from this study indicated that all the dermatophytes except *Candida albicans* were inhibited by the aqueous and ethanolic extracts of the leaf of *C. crepidiodes.* There were no morphological malformations in both the experimental and control cultures of *C. albicans.* A similar observation was made by Aboul-Enein (1963) in *Candida albicans* treated with a number of substituted phenyl hydrazone derivatives.

Ikenebomeh and Metitiri (1988) also reported that extract of *Casia alata* at various dilutions did not inhibit the growth of *C. albicans.* Therefore, *C. albicans* might be resistant to some antimycotic agents as also observed in this study. It is possible that higher concentrations of *C. crepidiodes* extract than that employed in this test may be inhibitory to *C. albicans*

The higher antimycotic activity of the crude extracts when compared with commercially available standard antifungal drugs showed that the extracts might contain more potent active principles than their synthetic counterparts. Furthermore, the plant material was not subjected to the same rigorous treatment usually encountered in pharmaceutical companies during extraction, hence the active ingredients might not have been distorted.

The phytochemical screening of the leaf extract showed the presence of bioactive compounds. These compounds might have exerted inhibitory effect on the dermatophytes tested, as medicinal plants have been confirmed to contain pharmacologically active principles such as alkaloids flavenoids, terpenes, anthraquinone, tannins and saponins (Ebana *et al.* 1993; Itah, 1997 and Adekunle, 2000). It is clear from the foregoing that some medicinal plants, including the ones in this study, have good medicinal properties hence they can be exploited by pharmaceutical industries.

The crude extracts of *Crassocephalum crepidiodes* inhibited some medically important dermatophytes and may therefore be used as alternative topical application for the treatment of some subcutaneous mycoses such as *Tinea pedis, Tinea coporis* and *Tinea capitis,* which are caused by these dermatophytes.

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