

Full Length Research Paper

Selective anti-candidal action of crude aqueous pod extract of *Lecaniodiscus cupanioides*: a preliminary study on *Candida albicans* obtained from an AIDS patient

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Aqueous pod extract of *Lecaniodiscus cupanioides* was found to exhibit a substantial inhibitory action on both the laboratory strain and a clinical isolate of *Candida albicans* obtained from an AIDS patient. This action was selective as no other fungal or bacterial species tested was similarly inhibited. It is expected that this extract would have potentials as an herbal treatment of oral thrush manifested by immunosuppressed individuals.

Key words: *Lecaniodiscus cupanioides*, anti-candidal specificity, *Candida albicans*, oral thrush, AIDS.

INTRODUCTION

The advent of the AIDS pandemic in Africa has brought in its wake a dramatic increase in the impact of life-threatening fungal infections in the immuno-compromised patients. The prolonged use of azole derivatives and amphotericin B has culminated in an emerging prevalence of drug resistant strains of *Candida albicans* (Law et al., 1994; Kelly et al., 1997). Thus there is an exigent need for novel antifungal remedies, and higher plants remain a vital source of these new substances, especially in low resource countries (Iwu and Wootton, 2002).

Lecaniodiscus cupanioides, Planch ex Benth, Fam. Sapindaceae, is a tropical plant widely distributed in Africa and Asia (Evans, 1996). It is identified by various names in Nigeria, such as Ukpo (in Igbo), Utantan (in Edo), Kafi-nama-zaki (in Hausa) and Akika (in Yoruba). The plant is ethno-medically reputed to be useful in the treatment of wounds and sores, abdominal swelling caused by liver abscess, fevers, measles, and hepatomegaly as well as burns (Yemitan and Adeyemi, 2005). In their recent study, Yemitan and Adeyemi, (2005) have revealed the CNS depressant action of the plant. Other reports

by Adeyemi et al. (2005) have shown that while exhibiting analgesic properties, the aqueous root extract of the plant may also be used as a galactogen and a laxative.

Preliminary studies to establish the nature of the phytochemical composition of the aqueous pod extract have been conducted in the present investigation. The principal objective of this study, however, was to examine the antifungal action of the aqueous extract of the pod of this plant. It was found out that the antifungal activity was selective against *C. albicans*. It had no activity against other fungi and bacteria tested. Due to its marked anti-candidal activity, it was suggested that the extract could equally be active against *C. albicans* associated with oral thrush manifestations in AIDS patients. The clinical isolates intended to be used in testing the plant extract were, therefore, narrowed down to those obtained from AIDS patients manifesting oral thrush.

MATERIALS AND METHODS

Antimicrobial materials

Dried pods of *L. cupanioides* were collected in August 2005 from the environs of Nsukka in Enugu State of Nigeria. They were authenticated by Mr. A. Ozioko, the plant taxonomist of the Bioresources Development and Conservation Programme (BDPC), Nsukka. A voucher specimen of the pods has been deposited in the

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Table 1. Phytochemical analysis of aqueous pod extract of *L. cupanioides*.

Plant constituent	Relative abundance
Saponins	++++
Steroids	-
Resins	-
Fats and oils	-
Alkaloids	-
Proteins	++++
Glycosides	+++
Flavonoids	+++
Reducing sugars	++
Terpenoids	+
Tannins	++++
Carbohydrates	++++
Acidity	++

- = Absent
 + = Present in small concentrations
 ++ = Present in moderately high concentrations
 +++ = Present in high concentrations
 ++++ = Present in very high concentrations

Table 2. Inhibition zone diameters of varying concentrations of the plant extract against the test strains of *C. albicans*.

Concentration (mg/ml)	Mean IZD (mm)	
	Clinical isolate	Laboratory strain
100.0	12.0	
50.0	11.0	
25.0	9.5	
12.5	8.5	
6.25	7.5	
3.12	6.0	6.0

A concentration of 3000 IU/ml of nystatin produced a mean IZD of 23.1 mm and 22.7 mm against the clinical isolate and laboratory strain respectively; whereas a concentration of 10 µg/ml of clotrimazole produced a mean IZD of 36.5 mm and 25.5 mm respectively.

herbarium of the Department of Pharmacognosy of the University of Nigeria, Nsukka. Nystatin (Bulbei, India) and clotrimazole (Fari-dabad, India) were used as standard anticandidal drugs in comparison with the plant extract.

Test organisms

Strains of *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa* NCTC 6570 were used in the study. The untyped strains of *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella paratyphi*, *Aspergillus niger* and *Candida albicans* used were those identified and preserved in our laboratories. Efforts were made at obtaining clinical isolates of *C. albicans* from AIDS patients. Most of the patients met during the study were already receiving antiretroviral therapy in the Bishop Shanahan Hospital, a missionary hospital in Nsukka, and did not manifest obvious oral thrush. However, within the period available for the study, we obtained one clinical isolate of *C. albicans* from an AIDS patient. With detailed identification procedures (Douglas,

2003), the isolate was confirmed to be *C. albicans*. It was sustained in our laboratory by weekly subculturing into fresh Sabouraud's dextrose agar slants, and incubated at room temperature (ca. 30°C). Cultures of the bacteria, aged 18 – 24 h, were employed in the tests, while cultures of *C. albicans* or *A. niger*, aged 72 h, were used. Each culture was harvested with 10 ml of sterile normal saline and the turbidity of the microbial suspensions was adjusted to an optical density of 0.50 at 530 nm, and used as such in the subsequent tests.

Preparation of the extract

The pods were dried overnight in an oven (Gallenkamp, England) at 60°C to ensure total elimination of absorbed moisture. The dried pods were then milled and an 80 g quantity of the powdered material was macerated for 24 h with one litre of distilled water. The resulting aqueous extract was filtered and the filtrate stored in an amber coloured bottle. A volume of 50 ml of the fluid extract was allowed to evaporate to dryness at room temperature. It was thereby found out that 1 ml of the sample corresponded to 40 mg of the dried extract.

Phytochemical screening

The aqueous extract was evaluated for the presence of alkaloids, glycosides, carbohydrates, saponins, flavonoids, tannins, resins, proteins, oils, steroids, terpenoids, as well as acidic compounds. Standard phytochemical procedures and tests were used as reported in a handbook on medicinal plants (Iwu, 1993).

Antimicrobial activity

All the organisms used in the tests were exposed to pre-determined concentrations of the aqueous extract of *L. cupanioides* as shown in Table 2, and the inhibition zone diameters (IZDs) were determined. The agar-cup diffusion method of evaluation was employed for the preliminary screening. The minimal inhibitory concentrations (MICs) of the aqueous plant extract, as well as the reference drugs, against *C. albicans* were determined by the broth dilution method (NCCLS, 1997). Each activity test was replicated at least seven times.

Statistical analysis

The comparison of data obtained for the various drug substances was conducted using a 95% confidence interval in the Student's t-test.

RESULTS

The results of the preliminary phytochemical analysis of the aqueous plant extract are shown in Table 1. It can be deduced that high concentrations of saponin, proteins, tannins and carbohydrates occur in the extract. Glycosides, flavonoids and reducing sugars appear to be present in moderate quantities.

It is noteworthy that the aqueous pod extract of *L. cupanioides* produced no measurable activity against the various bacteria used in the tests. It also had no activity against *A. niger*. But its inhibitory action on both the lab-

Table 3. Minimum inhibitory concentration (MIC) of the pod extract of *L. cupanioides* and reference standards against the test strains of *C. albicans*.

Organism: <i>C. albicans</i>	Mean MIC (\pm SD)		
	Clotrimazole (μ g/ml)	Nystatin (IU/ml)	Plant extract (mg/ml)
Clinical isolate	1.66 (\pm 0.43)	44.04 (\pm 16.60)	2.88 (\pm 1.41)
Laboratory strain	1.05 (\pm 0.17)	63.31 (\pm 7.11)	44.67 (\pm 3.34)

oratory strain and clinical isolate of *C. albicans* was remarkable. It appears that its action is selectively anti-candidal. Table 2 illustrates that the susceptibility of *C. albicans* against the plant extract is not only appreciable, but also concentration dependent. There was no significant difference between the susceptibility of the clinical isolate and that of the laboratory strain. However, there is obviously a significant difference between the activity of the plant extract and those of the reference drugs.

The MICs of the plant extract as well as nystatin and clotrimazole are shown in Table 3. The effect of the unrefined nature of the plant extract as compared to the reference drugs is once more apparent. But there is obvious evidence that the plant extract has a substantial level of anticandidal activity. Comparatively, the clinical isolate seemed to be more sensitive to the plant extract than the laboratory strain. A similar trend was seen in the effect of nystatin, though in a different degree.

DISCUSSION

At the preliminary stage of the study, it is yet not clear which of the phytochemical constituents is dominantly responsible for the anti-candidal action recorded. In order to clarify this aspect, a more detailed investigation of the activity of the extract is currently on-going. This is also aimed at identifying specific active molecules in the extract. Following extensive chemical and spectral investigations, Adesegun et al. (2003) have reported that ethanol extracts of the plant contain the triterpenoids, 3-O-[α -L-arabinofuranosyl-(1 \rightarrow 3)- α -L-rhamnopyranosyl (1 \rightarrow 2)]-(α -L-arabinopyranosyl)-hederagenin and 3-O-[α -L-arabinopyranosyl-(1 \rightarrow 3)- α -L-rhamnopyranosyl (1 \rightarrow 2)]-(α -L-arabinopyranosyl) hederagenin]. But there is no evidence that the anti-candidal action resides in these compounds. However, a first approximation, based on other literature reports, may be to attribute this activity to the saponin or tannin component of the extract. It is probable that saponins exert anti-fungal activity via membranolytic actions, following the enzymatic (glycosidase) conversion of the saponin to their corresponding aglycones in the cell membrane vicinity (Vantquenne et al., 2002). On the other hand, tannins are known to inhibit extra cellular microbial enzymes, as well as cause deprivation of the substrate required for microbial growth (Bossche and Marichal, 1994). They are also known to exert direct action on microbial metabolism through inhibition of oxidative phosphorylation. A further mecha-

nism involving iron deprivation has also been suggested by Wheat et al. (1997).

The susceptibilities of the various strains of *C. albicans* to both the plant extract and standard anti-fungal agents were determined by the agar diffusion technique. While inter-strain differences in susceptibility were not statistically significant ($p < 0.05$), there were marked differences between the activities of the plant extract and those of the pure anti-fungal drugs. Such significant differences are normally present when crude (unpurified) plant extracts are compared with pure drugs that are already in clinical use (Yoder, 1982). Further purification of the plant extract would be expected to enhance the observed activity. Although the agar-cup diffusion method is commonly employed in preliminary susceptibility testing, it is not always dependable for accurate assessment and comparison. This is because of the high degree of interference inherent in this method, arising from drug diffusion problems (Dickert et al., 1981).

A more generally accurate method of assessment is the broth dilution technique. In this study, therefore, the broth dilution method was used in determining the activities measured as MIC. In using this method, higher degrees of inter-strain differences in susceptibility were observed. It would appear that the laboratory strain was more resistant to the plant extract and nystatin than the clinical isolate. These observations point to the fact that in order to make an affirmative conclusion on the activity of a drug (especially a new product) strains of the organisms from a wide variety of sources should be employed at the evaluation stage. These and other elucidation tests as well as detailed toxicological analysis and pilot formulations constitute some of the objectives of the on-going further studies.

This investigation has demonstrated that the aqueous pod extract of *L. cupanioides* exhibits remarkable inhibitory action on both the laboratory strain and clinical isolate of *C. albicans* obtained from an AIDS patient. These findings could provide a veritable basis for further evaluation of the extracts of *L. cupanioides* for possible use as herbal treatment of *C. albicans*-related oral thrush in AIDS patients. It should be borne in mind that several affordable, safe, effective and locally available botanical medicaments, based on ethnobotanical knowledge, are widely employed in many tropical communities for the treatment of ailments (Iwu and Wootton, 2002). They are obviously more accessible and affordable, to the econo-

mically challenged patients, than the refined allopathic medicines. From these preliminary studies, the potentials for probable therapeutic uses of *L. cupanioides* appear to be quite high.

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