Phytochemical screening and in vitro anticandidal activity of extracts and essential oil of *Curculigo pilosa* (Schum and Thonn) Engl. Hypoxidaceae

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*Curculigo pilosa* is commonly used for herbal preparations as a purgative and also in the management and treatment of hernia, infertility and gonorrhea in Southwestern Nigeria. Owing to reported resistance of *Candida albicans* to toxic expensive anticandidal agents such as azoles and its implication for promoting opportunistic fungal infections of immunosuppressed patients, the anticandidal activity of *C. pilosa* was studied. The phytochemical screening of its powdered rhizomes was done using standard procedure. The extracts and essential oil were prepared using Soxhlet and Clavenger-type apparatus respectively. Ten *C. albicans* isolates from vagina cotton swabs were obtained from three hospitals in Ibadan, Nigeria. The isolates were tested against extracts and essential oil for any anticandidal activity using agar-well diffusion method. The minimum inhibitory concentration (MIC) was determined using broth dilution method. The phytochemicals found in *C. pilosa* were alkaloids, saponins, tannins, cardenolides and traces of anthraquinones. The ethanol extracts (500 mg/ml) and undiluted essential oil exhibited anticandidal activity while the water extract (1000 mg/ml) was inactive against isolates. The MIC exhibited by the ethanol extract against the tested isolates range between 0.020 and 1.500 mg/ml. The isolation and identification of the active compounds of *C. pilosa* could lead to the discovery of anticandidal phytomedicine.

**Key words:** *Curculigo pilosa, Candida albicans, phytochemical screening, extracts, essential oil, anticandidal activity.*

INTRODUCTION

The prevalence of *Candida albicans* in candidiasis has been reported by many authors. Osho (2000) studied the antimicrobial effects of some medicinal plants on *Candida* species isolated from human oral mucosa and reported that *C. albicans* constituted 64.8% of the 128 isolates in the six species of *Candida* obtained by him. Other species encountered in the study were *C. tropicalis, C. glabrata, C. krusei, C. stellatoidea* and *C. parapsilosis*. The species most frequently causing human candidiasis are *C. albicans, C. tropicalis* and *C. glabrata* whilst the others may also be of medical importance (Jones, 1985). The global human immunodeficiency virus (HIV) epidemic has resulted in an increase in severely ill immunocompromised hospitalized patients, accompanied by more reports of fungal infections. The most common fungal pathogens associated with invasive disease in humans are opportunistic yeasts (e.g. *Candida albicans*) (Toscano and William, 1999). Unfortunately the limited number of antifungal agents available in the market is toxic, expensive and *C. albicans* has developed resistance to commonly used antifungals (Perea et al., 2001). Due to this reason, there has been a search for newer generation of drugs to combat such complex mycotic pathogens. This has attracted the researchers to search for new antifungal agents of herbal origin which are relatively economically affordable, safer and easily available to common men (Rai et al., 2003).

*Curculigo pilosa* belongs to Hypoxidaceae and is an herbaceous plant with stout, erect rhizomes bearing a cluster of grass-like leaves to 60 cm long and flower shoots to 20 cm at the end of the dry season. It is found...
in seasonally marshy savanna. It is widely dispersed from Senegal to West Cameroun and over much of tropical Africa and Madagascar (Burkill, 1985). In Nigeria, it is found in Mubi, Abuja, Igboho and Erin-odo (UIH). In the Yoruba traditional medicine of Southwestern Nigeria C. pilosa is used as a purgative as well as for the management and treatment of hernia, infertility, genital infections and sexually transmitted infections especially gonorrhoea.

A survey of literature indicates that many investigators have studied herbal antifungal agents in recent past. Giordani et al. (2001) reported the in vitro susceptibility of C. albicans to Euphorbia characias latex using the macrobroth dilution method. Runyoro et al. (2006) reported that twenty-eight (28) out of the sixty-three (63) aqueous methanolic extract, belonging to 27 plant species and constituting 48% of the Tanzanian medicinal plants collected exhibited activity against C. albicans. Ajaiyeoba and Sama (2006) reported that the leaf and stem redistilled hexane and ethanol extract of Capparis thonningii showed inhibitory activity against C. albicans and Aspergillus flavus. The concentrations of extract used were 250, 500 and 1000 mg/ml.

This work examined the antifungal activity of C. pilosa against 10 clinical isolates of C. albicans, to produce scientific insight for the use of the plant in ethnobotany and widen the spectrum of activity against Candida.

MATERIALS AND METHODS

Plant material
Fresh rhizomes of C. pilosa were purchased from a local market in Ibadan, Nigeria in the month of July and were identified in the University of Ibadan Herbarium (UIH). The rhizomes were thoroughly washed with tap water, air-dried, ground into powder, weighed and stored in an air-tight glass container for further use.

Phytochemical screening
The powdered plant material was screened for the presence of natural products using standard procedures in the laboratory of the Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria.

Preparation of extracts and essential oil
Water extract: 200.0 g of the dried powdered rhizome was soaked in 1000 ml of sterile distilled water for 48 h. The mixture was filtered and the filtrate was freeze dried. 5 g of the extract was reconstituted in 5 ml sterile distilled water to obtain a solution of 1000 mg/ml, which was used for the antifungal screening.

Ethanol extract
500 g of powdered sample was extracted in 1.5 litre of ethanol (95 % w/v) for 24 h using Soxhlet apparatus. The extract was transferred into sample holder of the rotary vacuum evaporator, where the extract was concentrated to dryness at 50°C and then air-dried to constant weight. The extract was refrigerated at 4°C prior to use. 5 g of the extract was reconstituted in 10 ml sterile distilled water to obtain a solution of 500 mg/ml, which was used for the antifungal screening.

Essential oil
Essential oil was extracted from 300 g of the plant sample (4 h) by hydrodistillation using a Clavenger - type apparatus designed to the British pharmacopoeia specification (1980). The essential oil was stored in the refrigerator at 4°C prior to use. The undiluted oil was used for the antifungal screening.

Identification of C. albicans isolates
The C. albicans isolates were identified according to the methods used by Gbadamosi and Egungomi (2008).

Screening of plant extract for antifungal activity
The extracts were tested for their antifungal activity using agar well diffusion method. Each was suspended in sterile malt extract broth (Difco Laboratories, USA), incubated at 35 ± 2°C for 18 h.

Different concentrations of each isolate were prepared from the broth in sterile distilled water to give a range of concentrations at 10⁻¹ to 10⁻⁶ colony forming unit (cfu) per ml. One millilitre of each concentration was added and thoroughly mixed with 19 ml of sterile liquid Mueller Hilton agar (LAB M, UK.) and poured into sterilized Petri dishes (100 mm in diameter). The agar was left to solidify, from each of these plates 9 mm diameter wells were cut out from the agar using sterile cork-borer. Each of these wells was filled with 50 μl of plant extract using a micro pipette. The plates were left at room temperature, long enough for diffusion of the extract into agar. Subsequently, the plates were incubated at 35 ± 2°C for 18 - 36 h. Zones of inhibition were measured in millimetres. A control plate containing the test organism without any plant extract was also incubated. Each examination was carried out in triplicates for all isolates.

Minimum inhibitory concentration (MIC) of ethanol extract
The MIC was also determined using broth dilution method. The dilutions of the ethanol extract to be tested were prepared in 5.0 ml volumes of sterile nutrient broth to give a range of concentration from 5,000 to 0.020 mg/ml. After preparation of suspensions of test organisms Ca. 10⁻⁶ organisms per ml, 0.1 ml was added to the extract/broth dilutions (Atalay et al., 1998). For control experiment, 200 mg tablet of metronidazole (May and Baker, Nigeria) was dissolved in 200 ml of sterile distilled water to give a concentration of 1 mg/ml. The dilutions of metronidazole to be tested were prepared in 5.0 ml volumes of sterile nutrient broth to give a range of concentration from 1 to 0.020 mg/ml, that was used for the MIC test. After 18 h incubation at 35 ± 2°C, the tubes were then examined for growth.

Assay of essential oil by agar-well diffusion method
All overnight cultures of isolates were grown in malt extract broth at 35 ± 2°C for 18 h. The inoculum load was adjusted to 1 x 10⁶ organisms per ml using serial dilution method prior to use. 1 ml of this concentration of inoculum was added and thoroughly mixed with 19 ml of sterile liquid Mueller Hilton agar and poured (aseptically) into sterilized Petri-dishes. The agar was allowed to solidify. From each plate 9 mm diameter wells (two wells per Petri
RESULTS AND DISCUSSION

The percentage yields of the extracts were 17.83% (ethanol), 22.76% (aqueous) and 0.17% (essential oils). The phytochemicals in C. pilosa extracts are shown in Table 1. All isolates were identified as C. albicans. The aqueous extract of C. pilosa showed no antifungal activity. Table 2 shows the inhibitory activity of the ethanol extract on C. albicans isolates. The extract was active on 9 out of 10 tested isolates. The highest activity was on isolate C10 with an inhibition zone of 52.00 mm at $10^{-6}$ cfu/ml inoculum load, the least activity was on isolate C6 with a diameter of inhibition of 12.50 mm at an inoculum concentration of $10^{-2}$ cfu/ml. Thus the ethanol extract of C. pilosa was most active on isolate C10 and least active on isolate C6, while it was inactive on isolate C4 at all inoculum concentrations used. The result of the MIC tests is presented in Table 3. The essential oil of C. pilosa exhibited inhibitory activity against all screened isolates of C. albicans with inhibition zones of 31.00 - 59.00 mm. The oil was most active on isolate C6 and least active on isolates C3 and C8 (Table 4).

The phytochemical analysis of the plant material revealed the presence of alkaloids, traces of anthraquinones, cardenolides, saponins and tannins (Table 1). Many vegetable drugs owe their therapeutic action to phytochemical constituents (Oliver-Bever, 1986). Many well known purgative drugs such as aloes, senna and others contain di-tri or tetra-hydroxymethyl anthraquinones which occur in the plants either free or in the form of glycosides (Oliver, 1960). This finding justifies the use of C. pilosa as a purgative.

The extraction of the plant sample with water and
Table 3. Minimum inhibitory concentration (MIC) of ethanol extract of rhizomes of *C. pilosa*.

<table>
<thead>
<tr>
<th>Test drug</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pilosa</em></td>
<td>0.020 ± 0.00</td>
<td>0.100 ± 0.00</td>
<td>0.100 ± 0.00</td>
<td>0.100 ± 0.00</td>
<td>0.020 ± 0.00</td>
<td>0.100 ± 0.00</td>
<td>0.020 ± 0.00</td>
<td>0.020 ± 0.00</td>
<td>1.500 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.040 ± 0.00</td>
<td>0.040 ± 0.00</td>
<td>0.040 ± 0.00</td>
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</table>

Values represent Mean ± SD. (n = 3).

Table 4. Inhibitory behaviour of essential oil of rhizome *C. pilosa* on *C. albicans* isolates.

<table>
<thead>
<tr>
<th>Test oil</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pilosa</em></td>
<td>37.00 ± 1.80</td>
<td>42.50 ± 1.80</td>
<td>31.00 ±1.80</td>
<td>43.50 ± 1.80</td>
<td>43.50 ± 1.80</td>
<td>59.00 ± 1.80</td>
<td>37.50 ± 1.80</td>
<td>31.00 ± 1.80</td>
<td>52.50 ± 1.80</td>
<td>32.50 ± 1.80</td>
</tr>
</tbody>
</table>

Diameter of the cork borer = 9.00 mm.
Values represent Mean ± SD. (n = 3).

Ethanol gave different percentage yields of extracts, which did not have any relationship with the anticanicidal activity of the plant. Although the yield of aqueous extract was higher, the extract was inactive on *C. albicans*. That the ethanol extract exhibited a relatively high degree of anticandidal activity while no activity was shown by the aqueous extract is significant. This finding can be correlated with the traditional preparation of herbs in which alcoholic drinks are used to extract the active plant components.

Based on the results of antimicrobial screening, it is evident that the ethanol extract of *C. pilosa* was very active (90%) on *C. albicans*. That the ethanol extract exhibited a relatively high degree of anticanicidal activity while no activity was shown by the aqueous extract is significant. This finding can be correlated with the traditional preparation of herbs in which alcoholic drinks are used to extract the active plant components.

Based on the results of antimicrobial screening, it is evident that the ethanol extract of *C. pilosa* was very active (90%) on *C. albicans* isolates (Table 2). As shown in Table 3 the ethanol extract of *C. pilosa* gave the MIC values (0.02 – 1.5 mg/ml) and metronidazole inhibited all the tested isolates with varied MIC values (0.02 - 0.04 mg/ml). The MIC of ethanol extract of *C. pilosa* on *C. albicans* isolate C1 and C7 was 0.02 mg/ml, a value which was lower than the MIC of metronidazole (0.04 mg/ml). Also the essential oil showed varied degree of anticanicidal activity. The oil of *C. pilosa* exhibited 100% anticanicidal activity against all isolates (Table 4). A great many of essential oil have a slight antibiotic action and are used in the treatment of infections (Oliver, 1960).

Other pharmacological activities of *C. pilosa* have been reported. Palazzino et al. (2000) isolated two benzyl benzoate diglucosides, piloside A and piloside B and a glucosyl-fused norlignan, pilosidine, previously obtained as tetra-o-methyl derivative from the rhizome of *C. pilosa*. Pilosidine showed facilitating effect on adrenaline evoked contraction. Also Cometa et al. (2001) reported the reversible hypertensive effect of total extract of *C. pilosa*, its butanolic fraction (0.5 - 100 microg) and the most active compounds structurally similar to adrenaline, pilosidine (10 µg – 1 mg/kg) in anaesthetized rat.

**Conclusion**

The significant anticanicidal activity exhibited by the ethanol extract and essential oil of *C. pilosa* is an indication that active compounds from this plant could be a source of anticanicidal agent. Also tincture, ointment, cream and soap could be prepared from the plant for treatment of candidiasis and fungal infections of the skin. The results from this work form a basis for isolation and identification of phytochemical compounds responsible for the observed anticanicidal activity.

**REFERENCES**


