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Full Length Research Paper

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

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in seasonally marshy savanna. It is widely dispersed from Senegal to West Camerouns 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 gonorrhea.

A survey of literature indicates that many investigators have studied herbal anticandidal 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 anticandidal 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 anticandidal 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 anticandidal 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 anticandidal screening.

Identification of *C. albicans* isolates

The *C. albicans* isolates were identified according to the methods used by Gbadamosi and Egunyomi (2008).

Screening of plant extract for anticandidal activity

The extracts were tested for their anticandidal 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 10^{-6} 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

Table 1. Phytochemical screening of rhizome of *C. pilosa.*

Phytochemical constituents	Powdered rhizomes
Alkaloids	+
Anthraquinones	±
Cardenolides	+
Saponins	+
Tannins	+

+ = Present; ± = trace amount present

Table 2. Inhibitory behaviour of ethanol extract of rhizome of *C. pilosa* against *C. albicans* isolates at different concentrations of inoculum.

C. albicans isolate code	Inoculum load (cfu/ml) / zone of inhibition (mm)					
	1.0×10^{-1}	1.0×10^{-2}	1.0×10^{-3}	1.0×10^{-4}	1.0×10^{-5}	1.0×10^{-6}
C1	*20.00 ± 0.00^{a}	17.50 ± 3.53^{a}	19.00 ± 1.41^{a}	16.50 ± 2.12^{a}	17.00 ± 2.82^{a}	19.00 ± 1.41^{a}
C2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	25.00 ± 0.00^{b}	17.50 ± 2.12^{b}	25.00 ± 0.07^{b}	25.00 ± 0.00^{b}
C3	19.00 ± 1.41^{a}	21.50 ± 4.95^{a}	22.50 ± 2.12^{ab}	27.00 ± 1.41^{abc}	32.50 ± 3.53^{bc}	35.00 ± 0.07^{c}
C4	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
C5	21.00 ± 1.41^{a}	21.00 ± 1.41^{a}	20.00 ± 0.00^{a}	23.50 ± 0.70^{a}	25.00 ± 0.07^{a}	23.50 ± 2.12^{a}
C6	15.00 ± 0.00^{a}	12.50 ± 0.70^{a}	19.00 ± 1.41^{a}	18.50 ± 4.95^{a}	27.50 ± 3.53^{b}	36.00 ± 0.00^{c}
C7	21.00 ± 1.41^{a}	22.00 ± 0.00^{a}	25.50 ± 0.70^{b}	25.00 ± 0.00^{b}	35.50 ± 0.00^{c}	50.00 ± 0.00^{d}
C8	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	20.00 ± 0.00^{a}	22.50 ± 3.53^{ab}	27.50 ± 3.53^{b}	27.50 ± 3.53^{b}
C9	19.50 ± 0.70^{a}	19.50 ± 0.70^{a}	24.50 ± 0.70^{b}	24.50 ± 0.70^{b}	29.00 ± 1.41^{c}	27.00 ± 1.41^{bc}
C10	19.50 ± 0.70^{a}	19.50 ± 0.70^{a}	22.50 ± 0.70^{a}	20.50 ± 0.70^{a}	35.50 ± 0.70^{b}	52.00 ± 2.82^{c}

Diameter of the cork borer = 9.00 mm.
Values represent Mean ± SD. (n = 3).
Values in the same column followed by the same letter are not significantly different ($p > 0.05$) from each other. They differ significantly ($p \leq 0.05$) with values that do not share a similar letter.
0.00 = Resistant.

dish) were cut from the agar using sterile cork-borer, each of these wells was aseptically filled with 50 µl of undiluted essential oil of plant samples or sterile nutrient broth (control). The plates were incubated at 35 ± 2°C for 18 - 38 h and zones of inhibition were recorded in millimetres (mm) (Jennie et al., 2003).

Statistical analysis

Analysis of variance and comparison of means were carried out on all data using Statistical Analysis System (SAS). Differences between means were assessed for significance at $P \leq 0.05$ by Duncan's multiple range test (DMRT).

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 anticandidal 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 puragative 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.*

Test drug	***C. albicans* isolates / minimum inhibition concentration (mg/ml)**									
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
C. pilosa	0.020 ± 0.00	0.100 ± 0.00	0.100± 0.00	0.100± 0.00	0.020 ± 0.00	0.100± 0.00	0.020 ± 0.00	0.020 ± 0.00	0.020 ± 0.00	1.500 ± 0.00
Metroni-dazole	0.040 ± 0.00	0.040 ± 0.00	0.040 ± 0.00	0.020 ± 0.00	0.040 ± 0.00	0.040 ± 0.00	0.040 ± 0.00	0.020 ± 0.00	0.020 ± 0.00	0.020 ± 0.00

Values represent Mean ± SD. (n = 3).

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

Test oil	***C. albicans* isolates (10^{-6} cfu/ml) / zone of inhibition (mm)**									
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
C. pilosa	37.00 ± 1.80	42.50 ± 1.80	31.00 ±1.80	43.50 ± 1.80	43.50 ± 1.80	59.00 ± 1.80	37.50 ± 1.80	31.00 ± 1.80	52.50 ± 1.80	32.50 ± 1.80

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 anticandidal 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 anti-candidal 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 metroni-dazole (0.04 mg/ml). Also the essential oil showed varied degree of anticandidal activity. The oil of *C. pilosa* exhibited 100% anticandidal 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 being reported. Palazzino et al. (2000) iso-lated 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 anticandidal 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 anticandidal 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 anticandidal activity.

REFERENCES

Ajaiyeoba EO, Sama W (2006). PHCOG MAG: Research Article Phytochemical and Antimicrobial studies of *Capparis thonningii* and *Capparis tomemtosa*. Pharmacognosy Magazine. ISSN: 09731296, 2(6).

Atalay S, Brian MJ, Murat E (1998).The *in-vitro* antibacterial activity of Turkish medicinal plants. J. Ethnopharmacol. 67:79-86.

British Pharmacopoeia (1980). Her Majesty´s Stationary Office. 2:109.

Burkill HM (1985). The useful plants of West Tropical Africa. 2nd edition, vol. 2. Royal Botanic Gardens Kew.

Cometa MF, Palazzino G, Galef C, Palmery M (2001). Studies on vasoconstrictor activity of *Curculigo pilosa* extract and of its isolated compounds. Farmaco, 56(5-7): 353-356.

Gbadamosi IT Egunyomi A (2008). *In-vitro* anticandidal activity of extracts and essential oil of roots of *Plumbago zeylanica* Linn. (Plumbaginaceae). Nig. J. Bot. 21(2): 286-297.

Giordani R, Julien T, Muriel M, Patrick R (2001). Enhanced antifungal activity of ketoconazole by *Euphorbia characisa* latex against *Candida albicans.* J. Ethnopharmacol. 78:1-5.

Jennie RH, Jenny MW, Heather MA (2003). Evaluation of Common Antibacterial screening methods utilized in Essential oil research. J. Essential Oil Res.15: 428-433.

Jones JM (1985). *Candidiasis.* In: Warren K.S. and Mohamound A.A.F., (eds.), Tropical Geographical Medicine. McGraw Hill Book. Co. NY. p. 903.

Oliver-Bever B (1986). Medicinal plants in Tropical West Africa. Cambridge University Press (1st edition). Library of Congress catalogue card numbers 85-5952. p. 149.

Oliver B (1960). Medicinal plants in Nigeria. The Nigeria College of Arts, Science and Technology. pp. 93-124.

Osho A (2000). Antimicrobial effects of some medicinal plants on Candida species isolated from human oral mucosa. Ph.D thesis, University of Ibadan, Ibadan, Nigeria.

Palazzino G, Galeffi C, Federici E, Delle Monache F, Cometa, MF, Palmery M (2000). Benzylbenzoate and norlignan from *Curculigo pilosa:* structural analysis and *in vitro* vascular activity. Phytochemistry. 55(5):411-417.

Perea S, Lopes-Ribat JL, Kirkpatrick WR, MCAtee RK, Santillan RA, Martinez M, Calabarese D, Sanglard D, Partterson TF (2001). Prevalence of molecular mechanisms of resistance to azole antifungal agents in *Candida albicans* strains, displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. Antimicrobial Agents Chemotherapy. 45:2676-2684.

Rai MK, Deepak A, Wadegaonkar P (2003). Plant derived-antimycotics; potential of *Asteraceous* plants, In: Plant-derived antmycotics; Current Trends and Future Prospects, Haworth Press, New York, London, Oxford, pp. 165-185.

Runyoro KB, Deborah M, Olipa D, Cosam CJ, Zalcaria HM (2006). Screening of Tanzanian medicinal plants for anticandidal activity. BMC Complementary and Alternative Medicine 6:11.

Toscano CM, William RJ (1999). Edipemiology and Clinical Aspects of Unusual Fungal Nosocomial Infections. Clinical updates in fungal infections. Volume II, Issue 1.Retrieved 22 January, 2007 from www.adelaide.edu.au/candidiasis.htm. *Candidiasis* School of molecular and Biomedical Science. The University of Adelaide, Australia 5005. UIH. University of Ibadan Herbarium.