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Yeast strains isolated from HIV-seropositive patients in Cameroon and their sensitivity to extracts of eight medicinal plants

K. A. Yongabi[1]*, W. F. Mbacham [2], K. K. Nubia[3] and R. M. Singh[4]

[1]Phytobiotechnology Research Foundation, P.O. Box 921, Bamenda, Cameroon.
[2]Biotechnology Research Centre, University of Yaounde 1, Cameroon.
[3]2723S Norfolk St San Mateo, CA94403.
[4]College of Pharmacy, Illm Academy of Higher Learning, 17-18 Knowledge Park, Greater Noida, India.

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A total of 530 HIV-seropositive patients, undergoing treatment at the Phytobiotechnology Research Foundation (PRF) Clinic from May 2007 to May 2008, were screened for yeast infection using various specimens. Of the total number of patients, 318 were females and 212 were males. The specimens comprised 550 stools specimens, 422 oral swabs, 98 sputum specimens, 60 vaginal swabs and 25 urine specimens. These were aerobically cultured on Sabouraud dextrose and potato dextrose agar media. A total of 79.6% of the patients indicated presence of yeast in oral specimens, while 28.3% indicated the presence of yeasts in stool specimens. *Candida albicans* was the sole isolate from urine and vaginal swabs. *Geotrichium candidum* was solely isolated from stool specimens (18.75%), while *Cryptococcus neoformans* (5%) was also isolated from sputum specimens. Bulk methanol extracts of *Magnifera indica* (mango) seeds, *Aspilia africana* (African iodine) leaves, *Ageratum* conyzoides (goat weed/king grass) leaves, *Allium sativum* (garlic) bulb, *Vernonia amygdalina* (bitter leaves), *Khaya senegalensis* (drywood mahogany) seeds, *Moringa oleifera* (drum stick/horseradish) and *Persea americana* (avocado) seeds exhibited appreciable growth inhibition of *Candida* spp. and *Geotrichium* spp. The results indicated that yeast infections are prevalent in HIV/AIDS patients and can be controlled with natural products.

Key words: Yeast, AIDS, seropositive, methanol extracts, medicinal plants.

INTRODUCTION

Mycotic infections constitute a public health problem in Cameroon, and its role in public health is not well researched. Similarly, this phenomenon has also been noted in Nigeria by Gugnani (1982) who noted that mycoses are not usually classified among notifiable diseases. Thus, there is a dearth of data on the incidence, morbidity and mortality due to mycotic infections. In this respect, Wozuzu et al. (1982) reported that mycoses by their very nature are not dramatic, traumatic nor fatal like some bacterial infections and, as such, are mostly ignored or taken lightly by patients and public health authorities. Yet, fungal infections, especially yeast infection, are playing an increasingly important role in the morbidity and mortality of patients with HIV/AIDS (Yongabi et al., 2000a).

*Corresponding author. E-mail: yongabika@yahoo.com.

Candida spp. causes widespread infections such as oral thrush, oesophagitis, enterocolitis, hepatitis, pneumonia, meningitis, encephalitis, septicaemia, arthritis, endocarditis, urinary tract infections and vaginitis. Most studies have been directed at cutaneous mycosis rather than systemic mycosis, especially in Africa (Gugnani, 1982; Wozuzu, 1982; Yongabi et al., 2000a, 2000b). However, systemic yeast infections are implicated in the morbidity of AIDS-defining infection, even when the patients are on antiretroviral interventions.

Unfortunately, there is increasing resistance of fungi to imidazole derivatives (personal communication with health authorities in Cameroon), while prescription of antifungal drugs is hardly based on proper diagnosis and antifungal sensitivity tests. This, coupled with long-term usage of these drugs, has resulted in resistant fungal strains amongst the population. The imperative to develop or

identify new antifungal drugs is unequivocal, especially from plants sources. Many studies have reported on medicinal plants with promising anti-yeast activity (Borone and Tansey, 1977; Moore and Atkin, 1977; Yongabi et al., 2000a, 2000b).

These studies need to be intensified both locally and internationally. Many traditional practitioners in Cameroon have used decoctions from *Persea americana* (avocado), *Agerantum conyroides* (goat weed), *Moringa oleifera* (horseradish) and *Vernonia amydalina* (bitter leaves), amongst other, to treat and manage fungal infections. In this paper, we report on the prevalent yeast isolates from HIV/AIDS patients in Cameroon and also report on their sensitivity to methanol extracts of eight medicinal plants used by traditional practitioners in managing fungal infections.

MATERIALS AND METHODS

Criteria for specimen collection

The patients opted to be treated with a naturopathic therapy that was developed at the Phytobiotechnology Research Foundation (PRF) research station. Specimen collection and tests were selected based on the complaints given by the patients, as well as paraclinical examinations. Patients without symptoms and signs of fungal infections were excluded from this study.

Specimen collection

The patients were all HIV/AIDS-positive and their status was confirmed at the PRF Clinics. A total of 530 HIV-seropositive patients was screened for opportunistic yeast infections (318 were females and 212 were males). Stool specimens (550) were collected using clean plastic containers provided by the patients. Oral swabs (422) were taken using sterile swab sticks. Vaginal swabs (75) were collected at the clinic using appropriate methods (Cheesbrough, 1984). Sputum specimens (98) were collected using sterile McCartney bottles given to each patient presenting with a productive cough. Urine specimens (50) were collected aseptically by giving to male patient's sterile Bijou bottles and sterile McCartney bottles to the female patients. Mid- stream urine (MSU) was collected in each case and patients were adequately advised on collection procedures.

Microscopic analysis and stool culture

Stool specimens were examined macroscopically for their form and consistency. A fecal smear was made in 0.9% saline and examined for yeast cells on grease-free microscope slides under the x10 and x40 objectives. Portions of the stool specimens were then aseptically streaked onto Potato dextrose and Sabouraud dextrose agar media, and the agar plates were incubated at 27^{o}C for 72 h.

Microscopic examination and culture of oral, urine, sputum and vaginal swabs

The urine specimens were centrifuged at 3000 rpm and examined microscopically using standard laboratory techniques (Cheesbrough, 1984). The oral and vaginal swabs were rinsed directly with 0.9 ml of saline, after which smears were made and examined directly under the x10 and x40 objectives. The sputum specimens were processed using the KOH method (Cheesbrough, 1984) and portions of each specimen were cultured aseptically by streaking onto Potato dextrose and Sabouraud dextrose agar media, followed by incubation of the agar plates at 27^{o}C for 72 h. Isolates on agar plates were examined for morphological and micromorphology, and their biochemical properties was also determined (selective ability to ferment different types of sugar and produce gas, as well as germ tube formation) according to the methods of Rhode and Hartman (1980).

Sources, identification and processing of plant materials

The plants and parts thereof were selected based on their ethnofolkloric history. These plants have been used by rural people in northern Nigeria and many parts of rural Cameroon to manage and treat skin diseases, as well as gastrointestinal disorders and opportunistic diseases, especially in HIV/AIDS patients. The plants were collected in villages around Bamenda, north-west Cameroon. Voucher specimens were taken and identified by botanists at the Abubakar Tafawa Balewa University, Bauchi, Nigeria. The plant material was sun-dried for a week, except for bulbs of *Allium sativum*, which were later dried in the oven at 25^{o}C for an additional week. The plant material was then pulverized in a mortar using a pestle, sieved through 3-mm mesh and then stored in brown khaki envelopes for further studies.

Extraction procedures

Fifty grams (50 g) of the dried plant material was added to 250 ml of methanol (1:5 [w/v]) in 250-ml beakers (Pyrex) and allowed to extract for 72 h (Irobi and Daramola, 1993). The extracts were filtered using Whatman filter paper No 1 (Whatman, UK) and the filtrate solvent was evaporated under vacuum at 55^{o} C using a rotary evaporator. The resulting extracts were stored in sterile screw-capped bottles and kept at room temperature.

Determination of antifungal activity of the extracts

The agar diffusion method, according to Collins et al. (1995) was used. Briefly, 0.2 g of the plant extracts was reconstituted in 5 ml of distilled water and methanol. Wells, 6 mm diameter, were made in Potato dextrose agar with a stainless steel cork borer. Cultures of the test yeasts, (*Candida albicans* and *Geotricum candida*), grown for 18 h in mycological peptone water, were inoculated onto the agar and 0.2 ml of the extracts was added to each well. Controls comprised extraction solvent (methanol) and 0.2 ml of ketoconazole (200 mg). The agar plates were incubated at 27^{0}C for 72 h. The development of zones of inhibition around the wells containing the extract was taken to indicate the antifungal activity of the plant extract against the test organism. The differences between the zones of inhibition observed for the test and that of the control was recorded as actual diameter of zones of inhibition caused by the plant extract.

RESULTS AND DISCUSSION

Direct microscopy analysis of stool specimens from HIV-seropositive patients showed that 150 of the 530 specimens (28.30%) had yeast cells, and 80% of these were positive on yeast culture (Table 1). *C. albicans* had the highest frequency of occurrence (62.5%), followed by *G. candida* (18.75%) and *Saccharomyces cerevisiae*

Table 1. Prevalence of yeast isolated from stool specimens of HIV-seropositive patients at the PRF Research Clinic.

Total number of specimens of HIV-seropositive patients analyzed	Number of specimens with yeast cells (direct microscopy)	Positive culture cases
530	150 (28.30%)	80 (15.09%)

Table 2. Prevalence of yeast isolated from oval swabs from HIV-seropositive patients at the PRF Clinic.

Total number of specimens analyzed	Total number of specimens with yeast (direct microscopy)	Positive culture cases
530	122 (79.6%)	387 (73%)

Table 3. Prevalence of yeast isolated from sputum of HIV-seropositive patients at the PRF Clinic.

Total number of specimen analyzed	Total number of positive cases (KOH Microscopy)	Positive culture cases
98	39 (39.7%)	15(15.3%)

Table 4. Overall frequency of occurrence of yeast isolates by specimen.

Total number of HIV-seropositive patients	Isolate stool (80)	Sputum (98)	Oral (422)	Vagina (75)	Urine (50)
530	*Candida albicans* (62.5%) *Geotrichium candida* (18.75%) *Candida pseudotropicalis* (12.5%) *Saccharomyces cerevisiae* (16.25%)	*Candida albicans* (79.96%) *Cryptococcus neoformans* (2.04%)	*Candida albicans* (100%)	*Candida albicans* (100%)	*Candida albicans* (100%)

(16.25%). These results indicate that yeast infection is high in the gastrointestinal tract of HIV/AIDS patients. This finding is supported by earlier observations of Klein et al. (1984) and lake-Bakaar et al. (1988), both of whom noted not only the high prevalence of candidal infections amongst HIV/AIDS patients but also the role that it plays in the initial manifestation of acquired immunodeficiency syndrome.

C. albicans was the only yeast isolated in oral swabs (100%). This implies that oral thrush was very high amongst HIV/AIDS patients in Cameroon (73%), as indicated in Table 2. This finding correlates with the observations of Klein et al. (1984) who reported high frequencies of oral candidiasis in HIV/AIDS patients. *Cryptococcus neoformans* (2.04%) was also isolated from sputum specimens, together with *C. albicans* (79.96%) (Table 3). The results generally indicated that *C. albicans* strains are highly prevalent amongst HIV/AIDS patients in Cameroon (Table 4).

The eight medicinal plant extracts screened against *C. albicans* exhibited appreciable growth inhibition and was comparable to the use of ketoconazole (200 mg).

Amongst the extracts tested, *Allium sativum* (garlic) yielded the best results (Table 5). Previous reports generally shows that, *A. sativum* (garlic) is a broad spectrum antimicrobial agent. Deshpand et al. (1993) found inhibitory activity of *A. sativum* extracts on *Mycobacterium avium* complex isolates from HIV/AIDS patients. The results also showed that the widely used ketoconazole, which is the drug of choice for treating fungal infections, is less effective. This observation has also been reported by Smith et al. (1992) who observed fluconazole-resistant *Candida* spp. in HIV/AIDS patients. The extracts of *M. oleifera, P. americana* seeds *A. Sativum* (garlic) and *A. africana* inhibited *G. candida in vitro*. The methanol control did not exhibit any antifungal activity.

Conclusion

This study has shown that yeast infections are prevalent as opportunistic infections amongst HIV/AIDS patients in Cameroon, and, as such, should be considered amongst

Table 5. The antimicrobial activities of methanol extracts of eight medicinal plants against *Candida albicans* isolates.

Medicinal plants	Parts screened	Diameter zone of inhibition zone (mm)
Persea americana	Seeds	20.0
Aspilia africana	Leaves	11.0
Moringa oleifera	Seed	24.0
Allium sativum	Bulb	27.0
Agerantum conyzoides	Leaves	13.0
Vernonia amygdalina	Leaves	14.0
Magnifera indica	Seed	18.0
Khaya senegalensis	Seed	8.0
Ketoconazole (200 mg)	-	19
Methanol	-	0

Table 6. The antimicrobial activities of methanol extracts of eight medicinal plants against *Geotrichium candida* islolates.

Medicinal plants	Part screened	Diameter of zone (mm)
Persea americana	Seeds	27.0
Aspilia africana	Leaves	14.0
Moringa oleifera	Seeds	20.0
Allim sativum	Bulb	28.0
Agerantum conyzoides	Leaves	12.5
Vernonia amygdalina	Leaves	11.5
Magnifera indica	Seeds	17.0
Khoya sengalensis	Seeds	5
Ketocanazole (200 mg)	-	21
Methanol	-	0

the surrogate tests for monitoring the well-being of HIV / AIDS patients when following up on antiretroviral interventions. Moreover, the high prevalence of *Candida* isolates from sputum specimens suggests that physicians should be open-minded in the way that pulmonary infections are being treated since fungal infections are increasingly playing a key role in upper-respiratory tract infections in HIV/AIDS patients. More importantly, the *in vitro* antifungal activities of the plant extracts observed in this study has lend credence to the use of these plants in traditional medicine and some of these show promise for the possible future development of fungicides. This obviously requires in depth studies in the future.

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