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Preliminary Report on Management of HIV/AIDS-Associated Opportunistic Skin Infections with Phytoderma™, a Natural Myco-Based Cream

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Abstract

Despite the introduction of Highly Active Antiretroviral Therapy (HAART), opportunistic skin diseases continue to be prevalent and persistent in human immunodeficiency virus-infected individuals. Patients in this study were already receiving antiretroviral therapy for more than 2 years presented clinically with skin malaise previously treated unsuccessfully with conventional antifungal and chemotherapeutic agents. An exit pool system where patients consented to be involved in the study was applied. One hundred (100) out of five hundred and thirty (530) HIV/AIDS patients screened, representing 18.7%, were found with cutaneous infectious fungal infections. Of the 100 HIV/AIDS infected patients with cutaneous infectious fungal infections; *Tinea pedis* and *Tinea unguium* had prevalence rate of 60%, *Tinea capitis* 25%, *Tinea corporis* 15% respectively. Twenty (20) of the patients in the study had dark/ash color skin depigmentations with swollen hard knobs identified as Kaposi sarcoma Continuous culture of scrapings from the skin, head scalp, finger and toe nails from HIV/AIDS infected patients on malt extract, sabaraud dextrose agars in triplicates as well as KOH microscopy revealed the presence of *Trichophyton rubrum*, *Trichophyton Mentagraphyte*, *Candida albicans* and *Microsporium spp*. Bulk Methanol and water extracts of Ganoderma lucidum sporophore and whole mushroom fruit of *Termitomyces titanicus* in a 100% concentration inhibited mycelia growth of all the tests fungi *in vitro* as compared to growth of mycelium on methanol and water control plates with ketoconazole 200 mg/ml (undiluted). Crude methanol and water extracts of *Ganoderma lucidum* and *Termitomyces titanicus* significantly, *invitro*, exhibited inhibition against mycelia spread of *Trichophyton rubrum*, *Trichophton mentagrophytes*, *Microsporium spp* in 4 weeks. The methanol and water crude extracts were formulated into petroleum base cream named Phytoderm™. Phytochemical analyses of extracts from *Ganoderma* and *Termitomyces* indicated flavonnoids, saponins, carbohydrates and cardiac Glycosides. The cream administered to 100 patients with 95% efficacy for all skin infections including herpes and Kaposi sarcoma.

Keywords: Herpes; Retroviral; Antifungal; Immunology; *Ganoderma lucidum*; HIV/AIDS

Introduction

Infectious fungal skin diseases are increasing globally in patients with human immunodeficiency virus (HIV/AIDS) and even in those receiving antiretroviral therapy [1,2]. Although the disease burdens due skin infections are potentially high, they are hardly considered as major public health problems in literature and at the global health stage compared to malaria, tuberculosis and some neglected tropical diseases [1,3-5] in Cameroon and most Sub Saharan Africa countries, skin infections due to fungi are increasingly competing on the scale with opportunistic infections associated with HIV/AIDS [1,6-7] long term use of imidazole antifungal derivatives such as ketoconazole, fluconazole and econazole are becoming ineffective to treat many of the skin infections seen in these countries and these have been blamed largely on the unavailability of established antifungal gram guided prescription in health centers in these countries [8]. Similarly, other antifungal drugs such as griseofulvin, nystatin, diflucan, benzoic acids, and salicylic acid have also been over prescribed in sub Saharan Africa and over the years; leading to the development of resistant strains of many fungal species [9-10]. These treatment challenges couple with the unpleasant side effects that the patients receiving this treatment have to go through calls for the need to continuously search for new antifungal leads and alternative or complementary measures. Interestingly, [5] and other researchers reported that there are a number of indigenous mushrooms that could play a role in the treatment of skin infections. A number of studies have also shown that many medicinal mushrooms such as *Ganoderma lucidum* have a wide range of medicinal applications [10,11]. A number of medicinal plants in Africa have potent antifungal activities [4,8,9]. No studies have been reported on the antifungal activity of *Ganoderma lucidum* especially on strains from HIV/AIDS patients. Little effort has been made to actually translate this indigenous knowledge in Africa for clinical and other biotechnological applications [2]. The main of objectives of this study were to 1) report the prevalent skin diseases amongst HIV/AIDS patients in North west Region of Cameroon receiving antiretroviral drugs but with a clinical presentation of a skin disease; 2) carry out in vitro antifungal screening of some plants and local mushrooms commonly used by regional traditional doctors in Cameroon 3) attend to skin diseases; and to test the performance of Phytoderm™ creams on HIV/AIDS patients with a range of skin problems.

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Methodology

Criteria for specimen collection

The patients opted to be treated using naturopathic therapy that is developed at PRF research station. PRF is a registered research Non-Governmental Organization with the Cameroon Government and registered with the European Union. Specimen collection and tests were selected based on the complains given by the patients as well as Para- clinical examinations. Patients' without symptoms and signs of fungal infections were excluded from this study. The map the study area is shown below.

Map of bamenda: Bamenda is the third main city in Cameroon with an estimated surface area of 30 km^2 and with a estimated population of two million people with a growth rate of 4.9% growth rate per annum. For the past three years, the HIV/AIDs prevalence in Cameroon has been estimated as greater than 4% with the North West region where Bamenda (Figure 1) is the capital city ranked highest prevalence in the country

Specimen collection

The patients were all HIV/AIDS patients whose status was confirmed at the Bamenda regional hospital and were receiving HAART, and re-confirmed at the PRF Clinics, using Enzyme linked immunosorbent assays (ELISA) using a commercial Bioline cassette (Tigsun Diagnostics Co. Ltd). A total of 530 HIV sero-positive patients were screened for opportunistic skin infections. Scrapings were collected from the affected part of the skin using sterile scalpel and Blade and specimens properly labeled (Figure 2) using a bold marker and serial number assigned to represent each patient's name.

Microscopic examination and culture of skin scrapings from patients

The specimens were appropriately processed using the KOH method [2,12] and portions of each specimen were cultured aseptically by the placing methods onto Potato and Sabouraud dextrose agars and incubated at room temperature at 27°c for 72 hours according to methods described by [2,3]. Plates were examined for morphological and micro morphology.

Sources, identification and processing of mushroom and plant materials

The *Ganoderma sporophore* was harvested from decaying trees in Boyo Division of North West Cameroon and the plants and parts were selected based on their ethno folkloric history. These plants have been used by the rural people in Northern Nigeria and many parts of rural Cameroon to manage and treat skin diseases. The plants were all collected in villages around Bamenda, North West Cameroon and Voucher samples taken and identified by botanists at the University of Dschang, Cameroon. The plant materials were sun-dried under light filtered under shade for three weeks (Figures 5 and 6). This was to ensure that potential active ingredients should not undergo denaturation due to sun rays. This was done in the dry season as well as in the wet season they were then pulverized in a mortar using a pestle, sieved using sieves of 3 mm mesh and stored in brown khaki envelopes for further studies.

Extraction procedures

The sun-dried fifty grams (50 gm) plant material was added to 250 mL each of methanol and water (1:5 w/v) in 250 beakers (Pyrex) for each plant powder and allowed to extract for 72 hours [7]. The extracts were filtered using Whatman filter paper no 1 (Whatman, UK) and the filtrate solvent was evaporated under vacuum using rotary evaporator at 55°C and the resulting dried extracts were stored in sterile screw capped bottles and kept at room temperature.

Determination of antifungal activity of the extracts.

Agar diffusion method according to [8] was employed. Zero point (0.2) g of the plant extracts was reconstituted in 5 ml of distilled water and methanol. Each of the extracts was incorporated in 15 ml of each of the agars in molten state and allowed to solidify. A 6 mm of steel borer was used to bore 2 week culture of each of the fungal isolates and carefully placed into each of the bored well at the center of the plate and placed at room temperature for the fungi to grow and spread out. A control set up by introducing the extracting solvent (methanol and water) into the different wells as well as 0.2 ml of ketoconazole 200 mg was also used.

The plates were incubated at room temperature of 27°C for 72 hours. The development of inhibition of mycelia spread containing the extract indicated the anti-fungal activity of the plant extracts against the test organism. The differences between the inhibition rates of the mycelia spread observed for the test (Figures 3 and 4) and that of the control was recorded as actual diameter of zones of inhibition caused by the plant extract.

Preparation of myco-phyto based cream

The organic extracts (200 mg each) of *Ganoderma lucidum*, *Urtica*

Figure 1: Map of bamenda

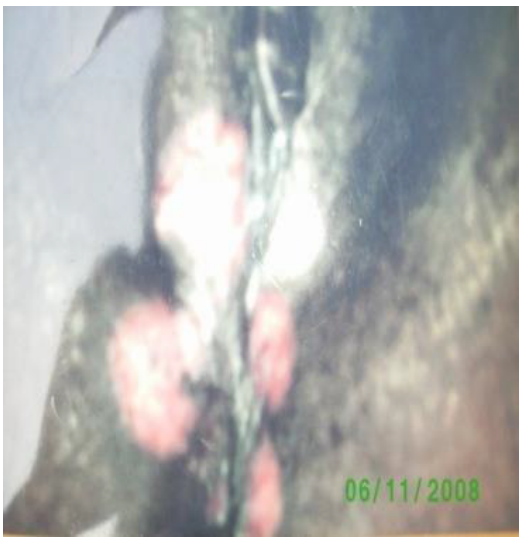

Figure 2: Genital herpes on the vaginal of an HIV Patient before treatment with plant and mushroom extract based cosmetics

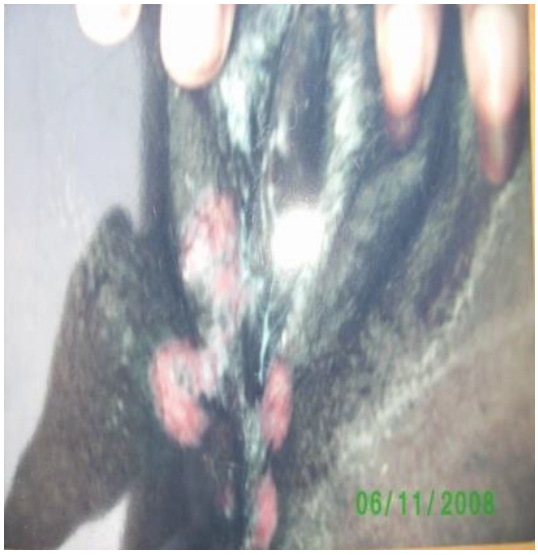

Figure 3: Genital herpes one week after treatment with plant and mushroom extract based cosmetics

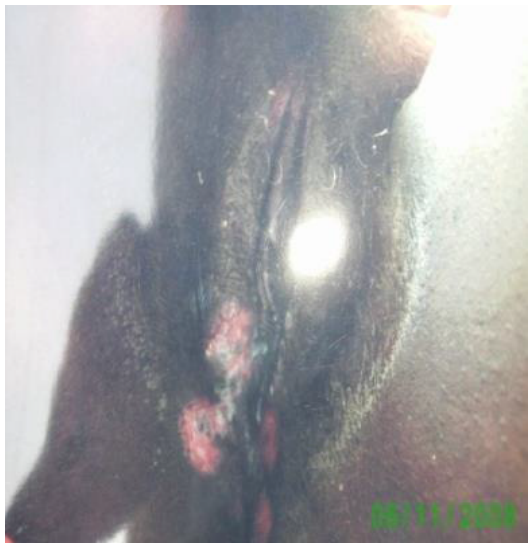

Figure 4: Genital herpes two weeks after treatment with plant and mushroom extract based cosmetics

Figure 5: Genital herpes three weeks after treatment with plant and mushroom extract based cosmetics

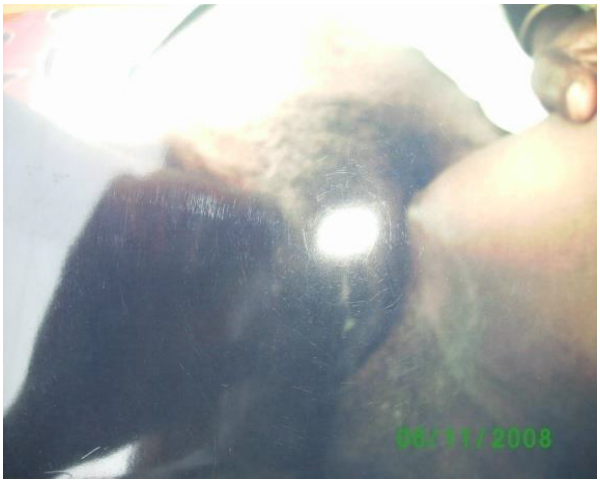

Figure 6: Genital herpes three weeks and half after treatment with plant and mushroom extract based cosmetics

Figure 7: *Ganoderma lucidum* growing wild in situ, methanol extracts of it used in the cream

dioica and persea Americana were blended into 200 grams of Petroleum jelly following standard chemical techniques reported previously by [8]

Preparation of medicated soap: The organic extracts (200 mg each) of *Ganoderma lucidum*, *Urtica dioica* and persea Americana were blended into 5 liters of caustic solution at the point saponification of caustic soda and bleached palm oil. Standard organic chemistry protocols as described by [8] were applied.

Administration of myco-phytocream to patients recruited in the study (placebo group)

HIV/AIDS patients from PRF clinics and other local clinics in the North West Region of Cameroon were enrolled for follow up and management of their condition with Phytoderm™, a myco-phyto-based therapy, developed at the Phytobiotechnology Research Clinic, Bamenda, Cameroon. Patients with skin diseases were screened using thorough appropriate culture tests for proper etiologic identification

Figure 8: Mushroom extract based cream

Micro morphology	Frequency of Occurrence
Spores	80%
Macro conidia	70%
Micro conidia	90%
Mycelia Strands	60%
Keratinized epithelial cells	100%

Frequency----------Average of three Microscopic fields

Table 1: Microscopy of Skin scraps from clinical specimens of HIV patients (KOH Method)

Crude Extract	Nature of Extract	Parts extracted and country mushroom was harvested
Methanol extracts(Ganoderma lucidum)	Brown sticky solid.	Stipe and sporophore (Cameroon)
Water extracts (Ganoderma lucidum)	Leather-like black solid	Stipe and Sporophore (Country)
Methanol extract (Termitomyces titanicus)	Brownish liquid	Whole crop (Cameroon)
Water extract (Termitomyces titanicus)	Brown liquid	Whole crop (Cameroon)

Table 2: The nature of extracts

Extract	TR	TM	MC	CA
Methanol	8 mm 9 mm	7 mm 8.8 mm	6.8 mm 8 mm	8.5 mm 9.2 mm
Water	14.5 mm	14.0 mm	13.7 mm	12.3 mm
Methanol Control				
Water Control	14.8 mm	13.9 mm	14.3 mm	12.4 mm
Ketoconazole 200mg control	8 mm	6.8 mm	8.5 mm	no growth
Pure culture of test fungi inoculated at same time and with no extract/drug	15.2 mm	16.2 mm	16.4 mm	16 mm

CA------- Candida albicans (does not grow with mycelium)

TR-------- Trichophyton rubrum

TM------- Trichophyton mentagrophyte

MC----- Microsporium canis

Table 3: Antifungal activity of Methanol and Water extracts (Mean Mycelia spread in 4 weeks) of *Ganoderma lucidum*

and then given the herbal medicated cream. They were properly educated on the mode of administration and asked to report on a weekly basis for observation and follow up. Each patient received a container of Phytoderm ™ which was 100 grams excluding the weight of the container, 5 grams was administered daily in the proportion of 2.5 grams twice daily (Figure 8) for three weeks maximum therapeutic period. Follow up were terminated when the infection on the skin was resolved but patients still report for follow up on HIV management.

None of the patients recruited in this study actually dropped out. As the cream was given at no cost, coupled with the fact that fungal infection persist, the patients all complied with the regiments.

As the diagnosis of the skin infection was based on qualitative observation, the likelihood of lapses in diagnosis cannot be ruled out.

Results and Discussion

The results indicated in (Table 1) show the various and prevalent micro morphological structures fungi display in its pathogenesis on the host. These Micro morphologies are similar to reports by [12].

The results show that Micro conidia were found in all the specimens and typical of all the fungal isolates (Figure 7). Both micro conidia and macro conidia demonstrated invasiveness as demonstrated by presence of keratinized epithelial cells and debris and is typical of fungal infections on the skin [13].

The infectiology and immunology of cutaneous infections amongst HIV patients in Cameroon has not been previously reported. In (Table 2), the nature of extracts from the stipe and sporophore of *Ganoderma lucidum* (Gl) and *Termitomyces titanicus* (Tt) indicated a dark brown sticky extract for G1 and brownish liquid for Tt. This has not been previously reported.

Extract	TR	TM	MC	CA
Methanol	10 mm 11 mm	8 mm 9.8 mm	7.4 mm 9 mm	9.5 mm 9.7 mm
Water	14.5 mm	14.0 mm	13.7 mm	12.3 mm
Methanol Control				
Water Control	14.8 mm	13.9 mm	14.3 mm	12.4 mm
Ketoconazole 200mg control	8 mm	6.8 mm	8.5 mm	no growth
Pure culture of test fungi inoculated at same time and with no extract/drug	15.2 mm	16.2 mm	16.4 mm	16 mm

CA------- Candida albicans (does not grow with mycelium)

TR-------- Trichophyton rubrum

TM------- Trichophyton mentagrophyte

MC----- Microsporium canis

Table 4: Antifungal activity of Methanol and Water extracts (Mean Mycelia spread in 4 weeks) of *Termitomyces titanicus*

Methanol Extracts	Water Extracts
Flavonoids detected	Flavonoids not detected
Alkaloids detected	Alkaloids not detected
Carbohydrates detected	Carbohydrates not detected
Tannins not detected	Tannins detected
Saponins not detected	Saponins detected
Glycosides not detected	Glycosides detected

Table 5: Preliminary phytochemical Screening of Ganoderma extracts

Methanol Extracts	Water Extracts
Flavonoids not detected	Flavonoids detected
Alkaloids not detected	Alkaloids not detected
Carbohydrates detected	Carbohydrates detected
Tannins not detected	Tannins not detected
Saponins detected	Saponins detected
Glycosides detected	Glycosides detected

Table 6: Preliminary phytochemical Screening of Termitomyces extracts

Kind of skin infection	No of patient	No cured	Duration of treatment	HIV/AIDs status	Percentage cured
Ringworm of scalp (Tinea capitis)	25	25	7-14 days	All HIV+	100%
Ringworm of skin/Tinea corporis	15	15	7-18 days	All HIV+	100%
Toe/Nail infection/. Tinea pedis and T unguium Tinea unguium	60	55	10-30 days	All HIV+	91.66%

Table 7: In vivo evaluation of plant and Mushroom based soaps and creams on Patients with a range of skin diseases at PRF Clinics

Type of skin problem	No of patients	No cured	Duration of treatment	HIV status	Percentage cured and Remarks
Kaposi Sarcoma	20	10, 7 still on the treatment	3 months to 6 months	All HIV+	50% 3 patients died all the plant extracts were taken orally as a Tincture and patients followed up for HIV/ treatment using immune therapy CD4 counts increased drastically
Herpes zoosters(shingles)	20	15	2 to 4	All HIV+	75%, CD4 counts increased significantly with concomitant immune therapy
Psoriasis	4	Not yet	-	None	Still on treatment but improving
Ectopic eczema skin depigmentation	4	Not yet	-	None	Still on treatment.

Table 8: Other types of skin problems managed with the natural product based cosmetics

The data in (Table 3) shows that the methanol extract of Gl demonstrated a greater mycelia inhibition on all the fungal isolate compared to a standard drug –ketoconale, both water and methanol controls. Termitomyces titanicus also demonstrated an inhibition of mycelia but lesser than with Gl (Table 4).

In a related study in [2] reported the activity of a number of medicinal plants extracts on yeast isolates from clinical specimens from HIV patients in Cameroon. The authors in that studied demonstrated that despite increasing fungal infections on HIV/AIDS patients, medicinal plants extracts could potentially provide useful leads toward development of effective antifungal chemotherapy.

In (Table 5), the results of the phytochemical screening revealed a rich mix of phytonutrients such as alkaloids, carbohydrates, glycosides and flavonoids. Both Gl and Tt had glycosides and carbohydrates detected in them (Table 6). *Ganoderma lucidum* has been described as one of the world's giant medicinal mushroom demonstrated a wide range of chemicals with wide range of medicinal [10,11,14] In all these reports, none has reported the potential antifungal application of *Ganoderma lucidum* in the treatment of skin diseases amongst HIV/AIDs patients [15-18]. In (Tables 7 and 8), [19-21] the mushroom based preparation Phytoderm™, was effective (95%) in the treatment of opportunistic cutaneous mycosis among HIV/AIDS patients in Cameroon, Central Africa [22-24]. The successful treatment of a number of Kaposi sarcoma cases [22-24], shingles is also promising, and this is the first report in the use of mushroom flora of Cameroon to attend to skin cancer [25-28]. The conclusion is drawn that these macro fungi [29,30] medicated cosmetics offer a comparatively cheaper safer and effective approach in managing skin disease in sub-Saharan Africa and more detail focus screening [31] for full exploitation is recommended.

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