Studies on the effects *Cymbopogon citratus, Ceiba pentandra* and *Loranthus bengwelensis* extracts on *species of* dermatophytes

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ABSTRACT: Alcohol and water extracts of *Cymbopogon citratus, Ceiba* _pentandra and *Loranthus bengwelensis* were investigated for anti-fungal properties and phytochemical constituents. The anti-fungal examination was by disk diffusion and agar dilution techniques, while the phytochemical constituents were investigated using standard chemical methods. Results showed that all the extracts inhibited the growth of standard and local strains of the organisms used, namely *Epidermophyton flocosum, Microsporum canis, Trichopyton rubrum* and *Candida albicans.* Some of the extracts had fungicidal effects while others had fungistatic effect on the organisms. The treatments were significantly different (P = 0.05). The minimum inhibitory concentration of the extracts against the tested microorganisms ranged between 150mg/ml and 50mg/ml. Comparisons were made with ketoconazole at 1mg/ml. The alcohol extracts were found to be generally more effective than the water extract for *C. pentandra* while the reverse was the case for the *C. citratus* and *L. bengwelensis* extracts. (P = 0.05). The phytochemical analysis revealed the presence of saponins, tannins, fats and oils, alkaloids and phenol but absence of cardiac and cyanogenic glycosides. The presence of saponins and phenols were inferred as being responsible for the anti-fungal properties of the extracts. [The Journal of American Science. 2008;4(4):58-67]. (ISSN: 1545-1003).

Keywords: *Cymbopogon citratu; Ceiba pentandra; Loranthus bengwelensis;* dermatophytes.

1. INTRODUCTION

Plants have been know to synthesize a variety of chemical substances, such as phenolic compounds, terpenes, steroids, alkaloids, glycosides, fats and others. The also synthesize secondary metabolites, which are of no apparent importance to the plants life. However, these have been found to have profound effects on animal systems with therapeutic properties (Abdul 1986). With current advanced technology, plants are being analyzed and their therapeutic abilities investigated more intensely. Today these methods have been used to isolate an ever increasing number of medicinal substances from plant sources. These medicinal plants are the sources of important drugs of the modern world. Some of these include guanine from *Cinchonabark*, reserpine from *Rauwoiffia* root, digitoxin from *Digitalis* leaf, atropine from *Belladonna* root and leaf, hyoscyamine from *Hyoscyanamus* and Datura leaves and roots, conine from *Conium* leaf, morphine from Opium capsule, sennosides from *Cassia* leaves, colchicina from *Colchicum* corn and vineristine and vinblastine from *Catharanthus* root are but a few (Abdul 1986). UNCTAD/GATT (1974) showed that about 33% of drugs produced in developed countries are derived from plants. According to some sources, 80% of present day medicines are directly or indirectly derived from plants (Myers, 1982). Astonishingly, these large quantities of modern drugs come from less than 15% of the plants which are known to have been investigated pharmacologically.

Cymbopogon citratus commonly called lemon grass is an aromatic, perennial grass belonging to the family grimneae. It is a tropical plant, grown as an ornamental in many temperate areas with maximum a height of about 1.8m and its leaves 1.9cm wide covered with a whitish bloom. Like other members of the genus, *citratus*, yields citral, a volatile oil with strong lemon fragrance. It is used in manufacture of perfumes, coloured soaps and synthesis of vitamin A. Folk medicine in certain parts of Nigeria use the essential oil as an insect repellant (Soforowa 1970). In certain medications, it is used for mental illness (Ebomoy, 1986). It is an antifungal, antitoxicant and deodorizing agents (Obiora 1986). In combination with other herbs, it has large use as cure for Malaria (Gbile 1986).

Ceiba pentandra is a tree and belongs to the family *Bombaceae*. It is commonly called silk cotton tree or kapok tree. In Nigeria, it is known in Hausa as *rimi* in Igbo as *akpu* and in Yourba as *araba*. It is reputed to be the largest tree of the West African region and originated from tropical America. The light seed was purported to have been blown across the Atlantic. This plant has been used in traditional medicine for several ailments. The bark contains a blackish mucilaginous gum, that swells in water and resembles

tragacanth. Folk medicines in Nigeria use the bark for the treatment of infections. It is astringent and is used in India and Malaya for bowel complaints. The bark is believed to also contain tannin. In West Africa, it is generally used in the treatment of diarrhoea.

Loranthus bengwelensis commonly referred to as Mistletoe belongs to the family *loranthaceae*. It is a semi parasitic shrub (Dutta, 1979) growing on citrus trees and developing sucking roots or haustorias. It is used traditionally for the capture of birds by the very sticky gums produced by the seed. In certain parts of Northern Nigeria, it is used for the treatment of various skin diseases and for the extraction of certain oils used in soaps and creams. Several works have highlighted the enormous potentials available in the use of plants and plant products for therapeutic purposes. Unfortunately however many of the reported claims on the efficacy of these plants and plant products are based on the untested word of traditional medicine healers. While efforts have been made to collate such information from these healers and from other sources, available documented evidence to support these claims are scarce and inadequate (Soforowa, 1982). This paucity of scientific evidence has informed the development and execution of this study to analyse *C. Citratus, C. Pentendra* and *L. bengwelensis* phytochemicaly and to determine if the plant extracts possess any inhibitory, action against the selected species of dermotophytes. The dermatophytes are a group of taxonomically related fungi which have affinity for cornified epidermis, hair, horn, nails and feathers where they produce dermatophilic infections.

Much is known about the sources of infection but very little is known of the factors that determine susceptibility or resistance of host, or prevalence. However, Kam *et al* (1997) stated that changes in climatic conditions affect prevalence of Tinea capitis, an infection of the scalp and hair with any of the dermatophytes. It is probably the most common fungal infection occurring before puberty. Tinea cruris a dermatophyte infection of the groin and upper thigh, is commonly seen after puberty especially in men. This may be caused by several species including *Epidermoplyton floccosum, Trichopyton mentagrophytes* and *T.rubrum.* Itching is a very common feature and change secondarily to persistent scratching and rubbing as in lichenification is commonly observed (David, 1983). Tinea pedis and manum, dermatoplyte infections of the hand and feet are rare before puberty; they are caused by T. *rubrum* and *T.mentagrophytes* with occasional involvement of other dermatophytes like microsporon canis etc.

All these infections are treated with antibiotics, mainly broad-spectrum antimycotics, usually by topical application with various degree of success. Today so many antimycotics have been brought into use, and some abused leading to development of resistance. This is a problem which only development of new drugs can check (Broston, 1988). The skin assumes enormous importance in a person's self image and its importance, as an organ of sexual attraction is well known. May people believe moreover, with some reason that the skin reflects the underling state of health and often seek reassurance on this point. There is the need to therefore give greater attention to developing more anti fungal (anti-dermatophilic) drugs so as to effectively check the increasing prevalence of these infections. Especially in the tropics and subtropics, where the climate make people more susceptible to the infections.

2. MATERIALS AND METHODS

a. Collection of Samples

The plant samples were obtained from Rasau village behind the students village hostel university of Jos, Nigeria, during the mid rainy season. They were kindly identified by professor Akueshi of Botany Department, university of Jos Nigeria and confirmed by Taxonomists at the Federal College of Forestry Jos, Nigeria.

The collected samples which included 400g of *Cymbopogon citratus* excluding roots, 400g of *Loranthus bengwelensis* made up of 200g each of stem and leaves, and 400g of *Ceiba pentandra* made up of 200g each of roots and bark.

b. Preparation of Extracts

The plant samples were oven dried at 50^0C for 48hours. On cooling they were subsequently ground separately by means of a clean pestle and mortar into a very fine powder. These were stored in airtight containers separately and refrigerated until required for use.

Preparation of extracts was done by modifying methods used by Akinyaju *et al* (1986).

Hot Water Extract (HWE) of the respective plant samples was made by dissolving 150g, of the powdered plant sample in 200mls of distilled water for 4 hours. It was then further extracted using the soxhlet apparatus for a further 2hrs. The resulting infusion was filtered using Watman # 1 filter paper. The filtrate was then subjected to gentle evaporation using a hot plate. The resulting paste was then scrapped

onto a watch glass where it was allowed to evaporate to dryness in an oven at 60^0C. The HWE was then ground, weighted and stored in the powdered form in an (AE) airtight container in a refrigerator until required.

The Alcohol Extract was made by soaking 100g of each powdered plant material in a solution of 200ml and 100ml of 95% ethanol and methanol respectively. The mixture was allowed to stay for 48hrs. It was stirred at 12hr. intervals by means of sterile glass rod. The resulting liquid was filtered using Watman # 1 Filter paper. The filterate was evaporated gently to dryness and weighed. It was stored in the same condition as HWE. The pH was determined using the pH meter (Phep 3 microprocessor pH meter by Hanna inst. Co). This indicated the level of acidity or alkalinity of the aqueous and ethanol extracts.

c. Phytochemical Screening of Extracts: The standard methods of analysis employed were adapted from those used by Iwu (1982), Emeruwa (1982) and Onwuliri (1996).

d. Isolation and Identification of test Organisms: The test organianisms *microsporum carnis, Trichophyton rubrum and Epidermophyton, floccosum* were isolated from children with dermatophyte infections in Gingiri primary school Jos Nigeria. These were then compared with stock cultures obtained from to dermato philosis research centre, National Institute for Veterinary research Vom Nigeria. The criteria used for identification and isolation were as those described by Rebel and Taplin (1970) and Campbell (1980).

e. Preliminary Screening for Anti fungal properties: Each of the extracts was individually reconstituted using minimal amounts of extracting solvent and further diluted with buffered glycerol. They were tested neat (without dilution), 200mg/ml, 100mg/ml and 50mglml as in Abide and Irobi (1993). These concentrations of the extract were impregnated into 7mm diameter sterile disks punched out of Wattman filter paper No 1. These sterile disks are replaced on sabouraud dextrose Agar plates amended with chloramphenicol at 0.05mg/l. These plates had previously been inoculated with 1ml of standard solution of fungal spore containing approximately 103 spores/1ml of sample organism and incubated at 25^0C for 10 days. The presence of zones of inhibition around each of the discs after the period of incubation was regarded as the presence of antimicrobial action while the absence of any measurable zones of inhibition was interpreted as absence of antimicrobial action.

f. Minimum Inhibitory Concentration (MIC): The MIC of the extracts was determined by incorporating various concentrations of the extracts into the culture media (chloramphenicol amended Sabouraud Dextrose Agar). Final extract concentrations of 150 to 30mg/ml of media, was made and poured. A 1ml Standard solution of fungal spores (103 spores/ml) was added into each of the tubes and incubated for 10days at 25^oC. Positive control tubes containing only the growth medium and a test organism per tube were also set up. The minimum inhibition concentration was regarded as the lowest concentration of extracts that did not permit any visible growth when compared with that of the control tubes.

g. Minimum Cidal Concentration

Samples from tubes used for the MIC assays which did not show growth after the period of incubation was diluted 1:4 with fresh media and 50ml amounts sub-cultured on fresh medium as in Rotimi *et al* (1988). The Minimum Fungicidal Concentrations (MFC) was regarded as the lowest concentration of the extracts that did not permit any fungal colony growths after seven day of incubation.

3. RESULTS AND CONCLUSION

The percentage extractions lied between 11% and 3% for the alcohol extracts and between 6.96% and 5.10 for the hot water extracts. The lowest was 3% for HWE of *C. citratus* (table 1). The result also showed that alcohol extracts of *C. pentandra* and *L. bengwelensis gave* higher yields than their respective water extracts, while for *C. citratus* the reverse was the case. This justifies the use of water as solvent in folk medicine for preparing some decoctions for cure of skin diseases. That *C. bengewlensis* and *C. pentandra* showed more alcohol soluble constituents or secondary metabolites than water soluble ones also supports the use of alcohols in the preparation of certain herb medicaments in folk medicine. The pH values for the water extracts are within the range of 7.4 and 3.7; this variation is large and might be responsible differences in activity observed amongst of the various aqueous extracts on the test organisms. The values

of the alcohol extracts are within the range of 6.4 and 4.9; the variation amongst the alcohol extracts is small which limited might account for the limited variation of the effects of the extracts on the test organisms. The pH values of the water extracts show that pH of all the HWE extracts are in the acidic range (Table 1) and it is likely that the antimicrobial activity of the active principle in the plant HWE extracts are effective at an acidic pH.

Phytochemical studies of the plants extracts showed the presence of tannins, saponins, alkaloids, fats an oils, phenol, steroids and carbohydrates in both the water and alcohol extracts. All extracts showed the presence of glycosides, but none showed the presence 0 and C glycosides. (Table 2). Hence Ebana, *et al.* (1991) suggested that antimicrobial activity of plants may be due in part to the presence of phytoalexins.

The results of the antimicrobial sensitivity showed that inhibition of microbial growth was greater at high concentrations of the plant extracts and, less inhibition was observed as the concentration was lowered. This shows that the effectiveness of the extracts is directly related to the concentration of the extracts. The crude extracts of *L. bengwelesis* showed the highest inhibition against the test microorganisms (tables 3-5). This implies that the inhibitory compound in the *L. bengwelensis* extracts are either more efficacious, or exist in higher concentrations. The lowest inhibition was observed for *C. citratus,* although the neat alcoholic extract performed quite impressively. This might be because the inhibitory compound is more soluble in alcohol than water. In all the preliminary screening, the least inhibitory effect was observed on *C. albicans.* The *E. floccosum* showed the highest inhibition for the water extracts of *L. bengwelensis.* In the comparative antifungal susceptibility assay, the extracts were used at concentrations of 150mg/ml for both the alcoholic and water extracts. ketoconazole was used for comparison at 1mg/ml concentration. Ketoconazole produced the highest inhibitory effect on all the test organisms followed by the water extract of *L. bengwelensis* and then the alcoholic extract of *C. pentandra* respectively (table 6). The higher values observed for the ketoconazole could be explained by the pure nature of the chemical compound, as against that of the plant extracts which are still crude and impure. The minimum inhibition concentration levels at which the crude extracts inhibited the test organisms are very important. They are normally used to evaluate the efficacy of chemotherapeutic agents under standard conditions. Inhibitory concentrations of 100mg/ml and below are considered promising in the antimicrobial screening of crude plant extracts. The reasons for the high MIC values of these extracts examined may either be due to the fact that the extracts used in the experiment were in the crude form, or that the active compound(s) are present in very low concentrations. It could also be as a result of antagonistic action of the component compounds in the extracts. Thus retarding their activity in the crude extracts. Purification of the extracts therefore may drastically reduce the MIC values. This therefore gives support to a certain degree, the traditional medical use of the plants evaluated for treating superficial fungal infections. This also reinforces the concept of ethno-botanical approach to the screening of plants as potential sources of bioactive substances against disease causing pathogenic fungi as very promising.

Table 1: Percentage yield and pH of the Plant extracts

	AE		HWE	
	%	pH	%	pH
C. citratus	3.00	4.9	5.10	7.4
C. pentandra	7.98	6.4	5.74	3.7
L. bengwelensis	11.00	5.9	6.96	4.2

Key: AE = Alcohol Extract

HWE = Hot Water Extract

Table 2: Phytochemical Constituents of Water and Alcohol Extracts of *C. citratus*, *C. pentandra* and *L. bengwelensis*

Plant	*C. citratus*		*C. pentandra*		*L. bengwelensis*	
Extracts	AE	HWE	AE	HWE	AE	HWE
Plant constituents						
Fats/oils	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Starch	-	+	-	-	+	+
Reducing sugar	+	-	+	+	+	-
Glycosides	+	+	+	+	+	+
0 and C Glycosides	-	-	-	-	-	-
Flavenoids	+	+	+	+	+	+
Steroids/terpenoids (Libernlans test)	+	+	+	+	+	+
Alkaloids: Dragendoff	-	-	-	-	+	-
Mayers reagent	-	-	-	-	+	-
Wagners reagent	-	-	-	+	+	-
Hagers reagent	-	-	-	-	-	-
Phenol	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+

Key: + = Present
- = Absent
AE = Alcohol extract
HWE = Water extract

Table 3: MIC for *C. pentandra* Extracts

	Concentration of extracts in mg/ml								
	150	100	80	70	60	50	40	30	MIC
Water Extracts									
Microorganisms									
E.floccosum	-	-	+	+	+	+	+	+	80mglml
M.canis	-	-	-	-	+	+	+	+	70
T.rubrum	-	-	+	+	+	+	+	+	100
C. albicans	-	-	-	-	-	+	+	+	60
Alcohol Extracts									
E. floccosum	-	-	+	+	+	+	+	+	100
M. canis	-	-	+	+	+	+	+	+	80
T. rubrum	-	-	-	-	-	-	+	+	50
C. albicans	-	-	-	-	-	-	+	+	50

Key: + = tubes showed at least one colony of growth

\- = tubes that showed absence of growth

Table 4: MIC for *C. Citratus* Extracts

	Concentration of Extracts of mg/ml								
	150	100	80	70	60	50	40	30	MIC
Water Extracts									
Microorganism									
E. floccosum	-	-	-	-	+	+	+	+	70
M. canis	-	-	-	+	+	+	+	+	80
T. rubrum	-	+	+	+	+	+	+	+	150
C. albicans	-	+	+	+	+	+	+	+	150
Alcohol Extracts									
E. floccosum	-	-	-	+	+	+	+	+	80
M. canis	-	+	+	+	+	+	+	+	150
T. rubrum	-	+	+	+	+	+	+	+	150
C. albicans	-	+	+	+	+	+	+	+	150

Key: + = Tubes showed at least one colony of growth

\- = Tubes that showed absence of growth

Table 5: MIC for *L. bengwelensis* Extracts

	Concentration of extracts in Mg/ml								
	150	100	80	70	60	50	40	30	MIC
Water Extracts									
Microorganism									
E. floccosum	-	-	-	-	-	-	+	+	50mglml
M. canis	-	-	-	-	-	-	+	+	50
T. rubrum	-	-	-	-	-	+	+	+	60
C. albicans	-	-	+	+	+	+	+	+	100
Alcohol Extracts									
E. floccosum	-	-	-	-	-	+		+	60
M. canis	-	-	-	-	-	-		+	50
T. rubrum	-	-	-	-	-	+	+	+	6
C. albicans	-	-	-	-	-	+	+	+	60
							+		
							+		

Key: + = Tubes showed at least one colony of growth

\- = Tubes that showed absence of growth

Table 6: Comparative Antifungal susceptibility Assay

Extracts (150mg/ml)	*C. citratus*		*C. pentrandra*		*L. bengwelensis*		Ketotonazole 1mg/ml
Microorganisms	HWE	AE	HWE	AE	HWE	AE	
E. floccosum	9	10	10	8.5	11	6	16
M. canis	7	7	9	8	12	9	17
T. rubrum	6	6	8	12	10	7	19
C. albicans	6.5	5	9	9	6	6	15

Key AE: Alcohol Extract, HWE: Hot Water Extract.

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