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CONTENTS	i
EDITORIAL BOARD	ii
NOTICE TO AUTHORS AND CONTRIBUTORS	iii – iv
Information Needs and Sources for Resolution of Drug Problems by People Living with HIV-AIDS, Case Study of a Military Hospital Soremekun R. O. and Shonowo J. B.	213 – 217
Assessment, and Toxicological effect of Lead, Cadmium and Chromium in Blood Samples of some Female Cosmetic Users in a Tertiary Institution in Lagos Nigeria Samuel, T. A., Udomiaye A. E., Biobaku, A. and Samuel A. A.	218 – 223
Does Age of Traditional Medicine Practitioners (TMP) Influence Formulation Type in Herbal Management of Memory Loss? Oiseoghaede O. J., Ajayi A. Glory¹, Odukoya A. O. A. A. Sowemimo¹ and N. F. Mustapha	224 – 226
Antifungal Screening of <i>Ceiba petandra</i> and <i>Lannea kerstingii</i> Stem Bark Extract Cream Formulations Kola-Mustapha A.T., Aremu O. I.¹, Njiga N. S. and Ayotunde H.T.	227 – 232
Evaluation of the Disintegrant Efficiency of Bambara Groundnut (<i>Vigna Subterranea</i> L. Verdc) Eraga, S. O. and Iwuagwu, M. A.	233 – 239
The Development of Novel <i>Beilschmiedia manni</i> Seed Gum as Tablet Binder Aremu, O. I and Ogungbemi, A.E.	240 – 245
Effect of Efflux Pump Activity Inhibition on Antibiotic Resistance of Bacterial Isolates from Patients Attending Lagos University Teaching Hospital - A Pilot Study Adeluola A. O., Oyedeji K. S., Mendie U. E., and Johnson J. R.	246 – 251
Effect of Method of Preparation on some Properties of <i>Hibiscus Sabdariffa</i> Microspheres Eraga S.O., *Arhewoh M.I. and Aiyanyor M.S.	252 – 257
Effect of Artesunate on Disposition Profile of Amodiaquine in Amodiaquine-Artesunate Combination Formulated in Fixed or Separate Doses. Akinleye M.O.*, Phillips I.M, and Adepoju-Bello A.A., Coker H.A.B.	258 – 263
Hepatoprotective Activity of Medicinal Plants - A Review Oyawaluja, A.A.	264 – 285
Corrigendum	286

Antifungal Screening of *Ceiba petandra* and *Lannea kerstingii* Stem Bark Extract Cream Formulations

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ABSTRACT

Aim: Evaluation of the potential application of *Ceiba petandra* and *Lannea kerstingii* creams in the management of topical dermatophytic infections.

Methodology: Antifungal activities of methanolic extract of *Ceiba petandra*, ethyl acetate extract of *Lannea kerstingii* stem barks, their combinations and their cream formulations were studied by the agar well diffusion method. Clinical isolates of *Trichophyton rubrum* and *Trichophyton mentagrophytes* were used as test organisms, while modified Aqueous Cream (BP) was used as the cream base. The extracts were incorporated into modified aqueous cream at concentrations 0.4, 0.8, 1.6, 2.4 and 3.2% w/w. Griseofulvin and ketoconazole cream were used as reference antifungal agents.

Results: Extracts of *Ceiba petandra*, *Lannea kerstingii* and their combinations exhibited significant levels of antifungal activity ($p < 0.05$) against the test dermatophytes. Diameters of inhibition zones ranged from 11.00 ± 0.29 mm to 24.30 ± 0.02 mm for the extracts and 3.00 ± 0.82 mm to 26.33 ± 0.94 mm for the cream formulations, as extract concentrations increased from 0.4 to 3.2% w/w. More inhibitory activities were exerted against *Trichophyton rubrum* compared to *Trichophyton mentagrophytes*.

Conclusion: Stem bark extracts of *Ceiba petandra* and *Lannea kerstingii* and their cream formulations possess useful antifungal activities against some causative agents of dermatophytoses.

Key words: *Ceiba petandra*, *Lannea kerstingii*, stem bark extract, antifungal, creams.

INTRODUCTION

Dermatophytosis is a common contagious disease caused by some fungi, mostly on the topical layers of the skin. The groups of fungi most often implicated are the dermatophytes which invade

keratinized organs of human and animals (hair, nail, horns, feathers, and epidermis). Most of these fungi reside in the soil and are involved in decomposition; however, the dermatophytes can infect living hosts¹. They belong generally to three genera namely *Microsporum*, *Trichophyton* and *Epidermophyton* but most common aetiologic agents are *Trichophyton rubrum* and *Trichophyton mentagrophytes*^{2,3}. The symptoms of dermatophytosis vary, depending on the infecting organism, affected tissues (e.g., skin, hair or nails) and area of the body. In areas of the skin without hair (glabrous), the lesions are usually characterized by inflammation that is most severe at the edges, with erythema, scaling and occasionally blister formation. In haired areas, the hairs become brittle and areas of alopecia may appear⁴.

There is increasing recourse to the use of medicinal plants and local herbs in the management of fungal infections due to ever increasing reports of incidence of resistance and toxicity (particularly allergic and hypersensitivity reactions) with orthodox medicines, and the drive to develop new and novel drugs. Plants have been used as traditional medicine and pharmacopoeia drugs from ancient times. Most of world's population is dependent on plant due to its medicinal value and accessibility⁵. Available literatures show that several plants have potential uses in the treatment of fungal infections. Two of such plants in the study area are *Ceiba petandra* and *Lannea kerstingii*.

Ceiba petandra belongs to the family *Malvaceae* and sub-family *Bombacoideae*. Its synonyms are *Bombax pentandrum*, *Ceiba caribaea*, *Eriodendron anfractuosum*, *Bombax ceiba*. It is commonly called Kapok tree or white silk cotton (English), Rimi, Riimaayee (Hausa, Nigeria), Vamber (Tiv, Nigeria), King (Yandang, Nigeria), Ogungun, Araba (Yoruba, Nigeria), Akpu-ota (Igbo, Nigeria)⁶. In Nigerian folk medicine, *Ceiba petandra* is used for the treatment of diabetes and infections. The leaves are used as

laxative, and as infusion for colic in man and in livestock⁷. The leaves are used as curative dressings on sores. Decoction of boiled roots is used to treat oedema. The gum is eaten to relieve stomach upset. In Ivory Coast, it is used to remove foreign matter from the eyes, or as emollient and sedative in Gabon⁸. In many countries in Africa, the bark and the stem are taken as remedies for diarrhoea, localized oedemas, wash sores and to relieve stomach complaints, hernia, blennorrhoea, heart-trouble, asthma, and gargles for gingivitis and sometimes toothache⁷.

Nwachukwu and co-workers reported the antifungal property of water and alcohol extract of *Ceiba petandra*⁹. Results of the study showed that the extract had both fungistatic and fungicidal activity against local and standard strains of *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton rubrum* and *Candida albicans*. The alcohol extract was found to be more effective. The antifungal property of the extract was reported to have been as a result of the presence of saponins and phenols. The phytochemical studies of the methanol extract revealed the presence of saponins, flavonoids, tannins, terpenes, resins and carbohydrates.

Lannea kerstingii belongs to the family Anacardiaceae. It is synonymous to *Lannea barteri*. *Lannea kerstingii* is locally called kondro (Bale, Ivory Coast), tudi (Hausa, Nigeria), kanchimbelli (Sisaala, Ghana), nimbiligh (Tiv, Nigeria)⁷. It is utilized in traditional medicine by various cultures worldwide, although application varies by region¹⁰. In Burkina Faso, a root decoction is taken to cure hernia and a leaf decoction to cure haemorrhoids. The barks are used externally to treat ulcers, sores and leprosy. Macerated roots are used in poultice for the treatment of wounds. Leaves decoction is drunk to cure piles¹¹. Njinga and co-workers reported the presence of tannins, flavonoids, alkaloids, anthraquinones and steroids from phytochemical screening of the plant¹². Another major area of concern in herbal medicine is their formulation into acceptable and readily more usable dosage forms. Therefore the aim of this study is to evaluate the antifungal activities of stem bark extracts of *Ceiba petandra*, *Lannea kerstingii*, their combinations and cream formulations against *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

MATERIALS AND METHODS

Materials

The stem barks of *Ceiba petandra* and *Lannea kerstingii* were collected from Zaria in the month of August, 2015 and authenticated at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria where they

were assigned voucher numbers 1832 and 7059 respectively.

Clinical isolates of *Trichophyton rubrum*, and *Trichophyton mentagrophytes* from the Department of Microbiology University of Ilorin, Nigeria were used. All the organisms were sub-cultured on potato dextrose agar (PDA) plates and incubated for 3 days. Pure cultures obtained were transferred into slants of PDA checked for purity and maintained at a temperature of 4 °C.

Chemicals used include methanol (Guangdong Chemical Reagent, China), ethylacetate (Guangdong Chemical Reagent, China), cetostearyl alcohol (BDH Chemicals Ltd., Poole England), liquid paraffin (BDH Chemicals Ltd., Poole England), polysorbate 80 (BDH Chemicals Ltd., Poole England), white soft paraffin (BDH Chemicals Ltd., Poole England), chlorocresol (BDH Chemicals Ltd., Poole England), potato dextrose agar (Biomalk, India), nutrient agar (Biomalk, India), peptone water (Biomalk, India), 5% w/v ferric chloride solution (BDH chemicals Ltd., Poole England), phosphate buffer 6 (BDH Chemicals Ltd., Poole England), dimethyl sulphur oxide (DMSO) (Guangdong Chemical Reagent, China).

METHODS

Preparation of *Ceiba petandra* and *Lannea kerstingii* extracts

After identification, the stem barks of the two plants were removed and air-dried for three weeks and thereafter pulverized. The obtained materials were sieved to obtain fine powdered mass, which was then stored in polythene bag for further use.

Extraction of *Lannea kerstingii* powdered bark was carried out as described by Njinga *et al*¹². A hundred (100) g of the stem bark of *Lannea kerstingii* was extracted exhaustively using sequential solvent extraction. While 140 g of the powdered stem bark of *Ceiba petandra* was extracted with methanol after defatting with petroleum ether for 24 hours. The methanolic extract was concentrated using rotary evaporator at 40 °C and 85 rpm (Buchi Rotary Evaporator, Germany). The concentrate was stored in the refrigerator prior to analysis. Test fractions of the plant extracts were prepared in 30% DMSO to obtain concentrations of 100, 200, 400, 600 and 800 mg/mL for the individual plants and a combination of both at 1: 3 ratio of *Ceiba petandra* to *Lannea kerstingii*.

Antifungal activity of extracts on test isolates

A loopful of the test organism was taken from their respective agar slants and sub-cultured into test tubes containing peptone water. The test tubes were incubated for 48 hours at 30 °C. The obtained

microorganisms in the broth were standardized using MacFarland test to obtain a population density of 10^6 cfu/ mL. Disposable petri-dishes containing potato-dextrose agar were streaked with the suspension (10^6 cfu/mL) of the fungi strains namely *Trichophyton rubrum* and *Trycophyton mentagrophytes* using sterile cotton swabs. The antifungal activities of 100, 200, 400, 600 and 800 mg/mL concentration of *Ceiba petandra* and *Lannea kerstingii* and a combination of the two extracts were determined using agar well diffusion method as described by Russell and Furr¹³ with slight modification. Five cylindrical holes were bored into the agar medium using a sterile number 6 cork borer. The holes were sealed with molten agar

and filled with 100 μ L of 100, 200, 400, 600 and 800 mg/mL concentration of the extracts.

Formulation of creams

Aqueous cream base (BP) in which the sodium lauryl sulphate (SLS) was substituted with polysorbate 80, was used for the formulation of the herbal cream product. Replacement of SLS was due to its reported allergic, hypersensitivity and keratolytic effects. The modified aqueous cream was used to prepare 25g of oil in water (O/W) emulsion based cream of concentrations 0.4, 0.8, 1.6, 2.4 and 3.2% w/w; with each plant extract and a combination of the two extracts at 1:3 proportion incorporated (Table 1).

Table 1: Composition of the 25 g modified aqueous cream incorporated with the stem bark extract of *Ceiba petandra*, *Lannea kerstingii* and a combination of the two extracts

Formulations	Ingredients	Quantity of ingredient in each formulation			
		A	B	C	D
	Liquid paraffin (g)	1.50	1.50	1.50	1.50
	White soft paraffin (g)	3.75	3.75	3.75	3.75
	Cetostearyl alcohol (g)	2.00	2.00	2.00	2.00
	Polysorbate 80 (g)	0.25	0.25	0.25	0.25
	Chlorocresol (g)	0.10	0.10	0.10	0.10
	<i>Lannea kerstingii</i> stem bark extract (mL)	1.00	0.00	0.75	0.0
	<i>Ceiba petandra</i> stem bark extract (mL)	0.00	1.00	0.25	0.00
	Purified Water (mL)	16.40	16.40	15.40	17.40
	Total weight (g)	25.00	25.00	25.00	25.00

KEY:

A - cream formulation of bark extract of *Lannea kerstingii*,

B - cream formulation of the stem bark extract of *Ceiba petandra*,

C - cream formulation of the stem bark extract of *Ceiba petandra* and *Lannea kerstingii*,

D - cream formulation without extracts (negative control)

Microbiological evaluation

The antifungal activity of the formulated creams was screened by agar well diffusion method¹⁴ in petri dishes containing potato dextrose agar medium. The plates were inoculated as described in the test for antifungal activity of extracts. Five cylindrical holes were bored into the agar medium using a sterile number 6 cork borer. The holes were sealed with 2 drops of molten agar and then filled with 0.5 g of different concentrations (0.4, 0.8, 1.6, 2.4 and 3.2% w/w) of cream formulations A, B and C with a sterile scapel. The plates were allowed to stand on the laboratory bench for 1 hour to allow for diffusion of the creams into the medium. The plates were subsequently incubated at 27 °C for 7 days and

observed for the zone of inhibition of growth each day. The zones were measured with a vernier caliper and the result recorded in millimeters. The screening was done in triplicates. **Statistical analysis** The results are expressed as mean values $\pm$ standard deviation. Statistical comparison was carried out by one way analysis of variance (ANOVA) followed by Dunnett's comparison test. The difference between the means of zones of inhibitions of the different formulations and control was evaluated using paired t-test. The statistical analysis was carried out with Graph pad prism version 5.04 software. Significant differences were set at *p* values lower than 0.05 and were used to either accept or not to accept the following Null and Alternate Hypotheses: **Null Hypotheses** H_{01} : There

is no difference in the mean diameter of the inhibition zones of formulation A, B, C as compared to control against *Trichophyton rubrum*.H₀₂: There is no difference in the mean diameter of the inhibition zones of formulation A, B, C as compared to control against *Trichophyton mentagrophytes*. **Alternate Hypotheses** H_{A1}: There is a difference in the mean diameter of the inhibition zones of formulation A, B, C as compared to control against *Trichophyton rubrum*.H_{A2}: There is a difference in the mean diameter of the inhibition zones of formulation A, B, C as compared to control against *Trichophyton mentagrophytes*.

RESULTS

The results of the agar diffusion test, to determine the susceptibility of different concentrations of the extracts on the two fungal species are presented in Table 2. It showed that in general, antifungal activity (measured by the diameters of zones of inhibitions) of the extracts of *Lannea kerstingii* against *T. rubrum* and *T. mentagrophytes* was slightly greater than that effected by *Ceiba petandra*. The combination of the two extracts were neither additive nor synergistic; however, activity was slightly higher than that elicited

by *C. petandra* but very comparable to that of *L. kerstingii* alone. A 30: 70 combination of the two extracts exerted same level of inhibitory activity as that of *L. kerstingii* alone.

It was also observed that at low concentrations, activity was low, increasing with increasing concentrations and at higher concentrations, level of antifungal activity was comparable to that of the reference antifungal agent (Griseofulvin).

When incorporated into the modified Aqueous Cream base, pronounced antifungal activity was exerted against the two fungal species by each of the plant extract only at concentrations of 2.4% w/w and above (Table 3). There was also better activity by creams containing *C. petandra* extracts compared with those containing *L. kerstingii* extracts, which is in contrast to the observation made with the extracts of the suspensions alone (i.e., not incorporated into any formulation). Moreover, the creams containing the combination of the two extracts did not exhibit any inhibitory activity against one of the fungal isolates (i.e., antagonistic effect elicited). Also, the level of inhibition against the test fungal isolates by the cream formulations were generally lower than those exerted by the suspensions of the extracts.

Table 2: Comparative levels of inhibition of crude extracts of *L. kerstingii* and *C. petandra* bark extracts on *T. rubrum* and *T. mentagrophytes* isolates

Isolate/Extract	Diameters of Zones of Inhibition at different concentrations				
	100 mg/m	200 mg/mL	400 mg/mL	600 mg/mL	800 mg/mL
<i>T. rubrum</i>					
<i>L. kerstingii</i>	13.02 ± 0.21	17.37 ± 0.07	19.19 ± 0.38	21.04 ± 0.02	22.11 ± 0.27
<i>C. petandra</i>	11.03 ± 0.15	12.33 ± 0.12	16.87 ± 0.32	18.21 ± 0.18	21.43 ± 0.34
Extract combination	0.0	13.40 ± 0.06	12.33 ± 0.11	20.67 ± 0.21	22.43 ± 0.22
Griseofulvin					20.00 ± 0.17
<i>T. mentagrophytes</i>					
<i>L. kerstingii</i>	0.0	16.83 ± 0.19	18.29 ± 0.15	23.10 ± 0.23	24.09 ± 0.28
<i>C. petandra</i>	0.0	11.0 ± 0.07	11.29 ± 0.12	16.55 ± 0.14	18.29 ± 0.28
Extract combination	0.0	11.35 ± 0.14	17.22 ± 0.26	21.05 ± 0.19	24.30 ± 0.02
Griseofulvin					25.00 ± 0.38

Table 3: Comparative levels of inhibition of Aqueous Cream formulations containing extracts of *L. kerstingii* and *C. petandra* bark extracts on *T. rubrum* and *T. mentagrophytes* Isolates

Conc. of Extract (% w/w)	<i>Ceiba petandra</i>		<i>Lannea kerstingii</i>		Combination of extracts	
	<i>T. rubrum</i>	<i>T. menta-</i>	<i>T. rubrum</i>	<i>T. menta-</i>	<i>T. rubrum</i>	<i>T. menta-</i>
		<i>grophytes</i>	<i>grophytes</i>	<i>grophytes</i>		<i>grophytes</i>
0.4	-	-	-	-	-	-
0.8	-	-	-	-	-	-
1.6	3.00 ± 0.82	6.33 ± 0.47	-	-	-	-
2.4	19.33 ± 1.70	21.67 ± 1.25	16.00 ± 1.63	15.33 ± 0.47	13.67 ± 1.70	-
3.2	26.33 ± 0.94	23.67 ± 0.47	19.67 ± 0.94	16.00 ± 0.62	15.67 ± 0.94	-

Statistical analysis of antifungal activity of the extracts and cream formulations

Results of t-test analysis of the diameters of zones of inhibition showed p-values of 0.0226 for *Trichophyton rubrum* and 0.003 for *Trichophyton mentagrophytes* for formulations A, B, and C compared with the control data, which are less than 0.05. This implies that the respective null hypotheses were rejected in favour of the alternate hypotheses in the two cases.

DISCUSSION

Dermatophytosis which occurs frequently in children and is often associated in adults with immune deficiency conditions such as diabetes usually present unpleasant sights and difficulties in its management. A host of available orthodox drugs are becoming ineffective and hence the search for alternatives, particularly among herbal remedies has become necessary and important. While many herbs and medicinal plants have been found useful in management of various disease conditions including fungal infections, their application and acceptability has been hampered largely by their presentation forms. *Ceiba petandra* and *Lannea kerstingii* are known for their medicinal properties in healing wounds and treatment of skin infections¹⁵. In the present work, extracts were obtained from these plants and used in the formulation of a dosage form that can be used in the treatment of dermatophytosis. The stem bark of the plants was selected on the basis of data obtained from the literature for the treatment of skin disorders.

In fungal topical infections, the most readily acceptable form of application is the cream, due to its obvious advantages such as ease of spread, wettability, non-staining and ready release of its active ingredients. Moreover, drugs formulated as creams more effectively interact and readily penetrate biological membrane. This therefore, underlines the choice of this dosage form for formulation of the extracts of these two plants.

The pronounced level of antifungal activities displayed by the two plants is in agreement with earlier works of Adebayo-Tayo *et al.*¹⁶ and Njinga *et al.*¹². The higher antifungal activity of extract of *Ceiba petandra* as compared to that of *Lannea kerstingii* is probably due to differences in their compositions and other intrinsic properties. The observed general increased inhibitory activity against the test fungal organisms is not unexpected as increase in concentration is always expected to result in higher amount of bioactive compounds and therefore greater level of interactions at the molecular levels.

The level of inhibition was much reduced following incorporation of the extracts into the cream. This might probably be due to the fact that creams by their nature, are multiphase systems, comprising aqueous, oil and emulgent phases and some of the bioactive compounds would have been trapped within the latter two phases in the cream. The resultant effect is a reduction in the concentration of the extract in the aqueous phase that is normally responsible for antimicrobial activity. Generally, for a drug formulated in a semi-solid preparation to be effective, it must be able to diffuse out of the base. The reduction in the zone of inhibition of the cream may be attributed to the partitioning of the extract between the aqueous and organic components of the cream since its solubility in the aqueous and organic environment will be different. Besides, lipophylic drug substances formulated with non-aqueous base often have difficulty in migrating out of the base to exert its action, whereas lipophobic substances will not present with such difficulty and vice-versa. It is however, interesting that the cream formulation containing the combination of the extracts was not only less effective, it had no inhibition on one of the fungal organisms. This may be attributed to possible incompatibility of the extracts with the cream base used.

CONCLUSION

The in vitro antifungal activity exerted by the stem bark extracts of *Lannea kerstingii* and *Ceiba petandra* were also demonstrated in the cream formulations produced in this study, but with reduced efficacy. Cream formulations containing extracts from only one of these plants are more promising rather than the combinations. In general, this study showed that *Lannea kerstingii* and *Ceiba petandra* extracts can be formulated into creams for potential use in treating infections caused by *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

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