Journal of Clinical & Experimental Dermatology Research

Mebude et al., J Clin Exp Dermatol Res 2017, 8:3
DOI: 10.4172/2155-9554.1000379394

Research Article Open Access

Anti-Dermatophytic Potential of Formulated Extract of *Cola nitida* (Vent.) Schott & Endl. (Stem Bark)

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Received date: June 06, 2016; **Accepted date:** May 11, 2017; **Published date:** May 15, 2017

Abstract

The antifungal activities of stem bark ethanolic extract of *Cola nitida* [Vent.) Schott & Endl. against fungi associated with dermatophytes was determined *in vitro* by agar cup diffusion, agar dilution technique, and time kill assay. The result showed that the extract at concentrations ranging between 6 mg/mL to 0.5 mg/mL produced zone of inhibition between 10 ± 0.1 and 25 ± 0.2 mm against the test fungi. The MIC and MFC of the extract ranged from 22.5 to 3120 µg/mL and 45 to 3120 µg/mL respectively. The MICs for *Trichophyton rubrum* and *T. tonsurans* were less than 100 µg/mL and the mechanism of antibiosis indicated that the formulated ethanolic extract was highly fungicidal. Since lower MIC and MFC indicates higher efficacy, the MIC/MFC in this study are within the range of ≤ 100 and ≥ 1000 µg/mL. The highest MIC in this study is 3120 µg/mL and active extract are those having MIC values ≤ 8000 µg/mL. The MIC index values, being less than or equal to 2, indicated the fungicidal attribute of the formulated ethanolic extract and suggested that fungicidal effects be expected on all the tested dermatophytes. In the time kill assay, the percentage log reduction of the viable cell count ranged between 0.35 Log_{10} cfu/mL at 2 h to 0.96 Log_{10} cfu/mL at 4 h for T. tonsurans while it ranged between 0.29 Log_{10} cfu/mL at 2 h to 0.85 Log_{10} cfu/ml. The results showed that the extract exhibited a broad spectrum antifungal activity and justifies the use of *Cola nitida* in folkloric medicine for the treatment of various skin diseases in Nigeria.

Keywords: Dermatophytes; Antifungal; *Cola nitida*; MIC/MFC; *In vitro*

Introduction

Our earliest human ancestors found plants to heal wounds, cure diseases, and ease troubled minds, such that people from all continents have long used hundreds, if not thousands, of indigenous plants, for treatment of various ailments dating back to prehistory [1]. Knowledge about the healing properties or poisonous effects of plants, mineral salts, and herbs accumulated from these earliest times to provide health and predates all other medical treatment [1]. Ethno-medicine refers to the study of traditional medical practice, which is concerned with the cultural interpretation of health, diseases and illness, as well as addressing the healthcare-seeking process and healing practices [2]. The practice of ethno-medicine is a complex multi-disciplinary system constituting the use of plants, spirituality and the natural environment, and has been the source of healing for people for millennia [3]. Research interest and activities in the area of ethno-medicine have increased tremendously in the last decade. Furthermore, since the inception of the discipline, scientific researches in ethno-medicine have made significant contributions to the understanding of traditional subsistence, medical knowledge and practices [3].

Cola is one of the largest genus in the family Sterculiaceae. The species are found mostly in the relatively dry parts of the rain forest, although *Cola milleni* and *Cola gagantea* are widely distributed in wet and dry forest environment [4,5]. Various medicinal and pharmacological values have been observed in species of *Cola*. The seeds referred to as kolanuts are used to treat whooping cough and asthma [6,7]. Odugbemi [8] also reported that the leaves of *Cola milleni* are used in the treatment of ringworm, scabies, gonorrhea, dysentery and opthalmia. Human pathogenic dermatophytes are keratinophilic mould that infects human skin, nails and hair. Three genera (*Trichophyton, Microsporum* and *Epidermophyton*) of these organisms exist, however, their preferred sites for infection varies [9].

Infected individuals hardly go for diagnosis in the case of dermatophytes, especially when they are in hidden regions of the body. This study aimed at addressing the concerns associated with improving public health of the largest organ in the body (skin) by investigating the antifungal potentials of the selected medicinal plant.

Material and Methods

2.1 Collection of plant material

The bark materials of *Cola nitida* were collected in June 2013 from the University of Ibadan, Ibadan, Nigeria. The plant was authenticated in the Department of Botany, and a voucher specimen UIH-22487 was prepared and deposited in the herbarium of the Department of Botany, University of Ibadan.

2.2 Extract preparation

Pulverized plant sample were subjected to Soxhlet extraction with ethanol (distilled). Extracts were filtered, evaporated to dryness in-vacuum, weighed and stored at -4°C until needed.

2.3 Test organisms and preparation of inoculum

The dermatophytes used in these studies were *Microsporum canis, Trichophyton tonsurans, Trichophyton rubrum* and *Epidermophyton*

floccosum. The inocula of the test organisms were prepared using Tryptone Soy Broth (TSB). Colonies picked from 24 h old culture grown on Sabouraud dextrose agar were transferred into TSB. A 0.1 mL of overnight broth was transferred into 9.9 mL of sterile distilled water to give a 10^{-2} dilution of the inoculum.

2.4 Antifungal susceptibility assay by agar diffusion method

The susceptibility of the test fungi to crude ethanolic extract of *C. nitida* bark and ketoconazole (drug control) was determined using the agar cup diffusion technique as described by [10]. Sabouraud dextrose agar plates were carpeted with the fungi. Using sterile Pasteur pipette, 0.2 mL of the 10^{-2} dilution of the organism in TSB was placed on the surface of each SDA plate. This was uniformly spread with the aid of a sterile glass spreader. The inoculated plates were allowed to dry in the incubator at 37°C for 20 mins. A standard cork borer of 8 mm diameter was used to cut equidistant wells in the agar into which was added 100 µL solution of the reconstituted extract at concentrations of 6, 4, 2, 1, 0.5 mg/mL. The plates were allowed to stand for 1 h to allow pre diffusion of extract into agar before incubation at room temperature for 72 h. At the end of the incubation period diameters of inhibition were measured. All assays were carried out in three replicates to ensure accuracy.

2.5 Determination of minimum inhibitory concentration (MIC)

This was carried out using the agar dilution method [11]. Different concentrations of the extract were prepared to final concentration in the range of 1.0 mg/mL to 0.3125 mg/mL. A 2 mL of the extract was mixed with 18 mL of molten Sabouraud dextrose agar and poured into sterile Petri dish allowing the agar to set. The surface of the set agar was allowed to dry before streaking with overnight broth cultures of test organisms. Plates were incubated at 25°C for 48-72 h and observed for the presence or absence of growth. The lowest concentration preventing visible growth was taken as the MIC of the extract.

2.6 Determination of the minimum fungicidal concentration (MFC)

This was determined by a modification of the method of [12]. To a 0.5 mL at different concentrations as used in the MIC assay that showed no visible growth on the agar plate was added 0.5 mL of test organism in tubes. These were incubated at 25°C for 24-48 h. Samples were streaked out from the tubes onto Sabouraud dextrose agar to determine the minimum concentration of the extract required to kill the organism. These concentrations were indicated by failure of the organism to grow on transfer to these media plates. The lowest concentration that prevented fungal growth after days of incubation was recorded as the minimum fungicidal concentration. The entire tests were performed in two replicates to ensure accuracy. Agar plates without extract and another without organism were also incubated to serve as organism and medium control respectively.

2.7. Determination of mechanisms of antibiosis (fungicidal or fungistatic)

The mechanism of antibiosis of the extracts was calculated using the ratio of MFC/MIC or MIC index [13] to elucidate whether the observed antifungal effect was fungicidal or fungistatic. When the ratio of MFC/MIC is ≤ 2.0, the extract is considered fungicidal or otherwise fungistatic. If the ratio is ≥ 16.0, the extract is considered ineffective.

2.8. Formulation of crude extract

Extract was formulated into cream [14] and consists of extract (32 g), emulsifying wax (4.0), oleic acid (2.0), Tri- ethanolamine (2.0), Glycerol (1.0) and water. Briefly the emulsifying wax in a flask was dissolved by placing the flask in temperature-regulated water bath. The extract and tri-ethanolamine were added (part A). The part B made up of Oleic acid, glycerol and water in another flask were melted by placing in water bath. The temperature of both part A and B were regulated using the mercury bulb thermometer at 60°C before being mixed together to form a jelly-like cream.

2.9. Time kill kinetics assay

The rate of kill of susceptible organisms by the ethanol extract was assessed using the modified pour plate technique [12]. Extract was incorporated into 4 mL Tryptone soy broth previously inoculated with the test orgasnism to form test mixture. Sterile Sabouraud dextrose Agar (SDA) was poured in plates and allowed to set firmly. The set SDA plates were inoculated with samples withdrawn from the test mixture after dilution at 0 sec, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min and 24 h intervals. Plates were incubated for 24-36 h at room temperature. Colonies were counted at the end of the incubation period. Plates containing Sabouraud dextrose agar without extract inoculated with test organisms and SDA incorporated with the extracts at the test concentrations without test organisms were included as controls. The values obtained were then used to construct curves to demonstrate changes in colony counts with time as a function of exposure to the test sample. Results were plotted as Log_{10} of observed cfu/mL against time. The percentage reduction and log reduction from initial microbial population for each time point was calculated to express the change (reduction or increase) of the microbial population relative to a starting inoculum. The change was determined as follows:

Percentage reduction (%)=Initial count–Count at x time interval/ Initial count × 100 [15]

The Log reduction was calculated as follows:

Log_{10} reduction=Log_{10} (initial count)–(Log_{10} at x time interval) [16]

3.0 Acute toxicity test

This was conducted according to the OECD guidelines where the limit test dose is 5000 mg/kg. At this concentration, throughout the 14 days observation, there were no changes recorded in the form of morbidity, aparthy, hypo/hyperactivity.

Hematological assessment: Blood samples of formulated cream-treated and control groups were drawn and 0.5 mL of blood was collected in EDTA bottles for hematological parameters.

Hematological profile: The hematological parameters tested include hemoglobin, neutrophils, lymphocytes, eosinophils, basophils, packed cell volume and platelet count.

Histopathological examination: Descriptions of all macroscopic abnormalities of the animals were recorded. Tissues from liver, kidney, and skin of infected, treated and control groups were put in phosphate buffer 4% formaldehyde solution for histological examination.

Results

Identification of various isolates phenotypically was presented in Table 1. The antifungal activity of the ethanolic extract of *Cola nitida* stem bark was evaluated by agar diffusion method against fungi associated with dermatophytes. The degree of the antifungal activity was assayed by agar dilution method to determine the minimum inhibitory concentration (MIC) of the extract. The assays showed that the fungi exhibited varied susceptibilities to the extract at different concentrations used. The diameter of zones of inhibition obtained from 20 mg/mL of ketoconazole ranged between 18 ± 0.2–28 ± 0.1 mm as shown in Table 1. At the highest concentration of the extract (6 mg/mL), *Trichophyton tonsurans* had the widest zone of inhibition (30 ± 0.2 mm) while *Trichophyton rubrum* had the least zone of inhibition (14 ± 0.1 mm). The fungi were not susceptible to 10% ethanol used in the control assay (Table 2). *Trichophyton rubrum* and *Trichophyton tonsurans* had MIC<100 µg/mL, while the other test organisms had MIC>1000 µg/mL as shown in Table 3. The effect of single dose of the extract on hematological parameters is presented in Table 4 while Table 5 presents the changes in behavioural patterns of Wistar rat using various parameters.

Organisms	Culture Characteristics	Microscopy	Catalase Test	Urease Test	Suspected Organism
G01	White golden yellow border with fluffy center	Thin walls of about 3-6 cells with rounded ends	Negative	Positive	*Microsporum canis*
H10	Yellow/colorless folds	Thin smooth walls that are irregular in shape	Positive	Positive	*Trichophyton tonsurans*
H08	Yellow to colorless edges	Walls are smooth and irregular in shape	Positive	Positive	*T.tonsurans*
G03	Whitish raised borders	2-8 cells on parallel sides	Positive	Negative	*T. rubrum*
H11	Raised golden yellow border with whitish center	Numerous thin walls with 3-5 cells at the edges	Positive	Positive	*M. canis*
H12	Seemingly colorless flat edges	Thin smooth and irregular shape	Positive	Positive	*T. tonsurans*
H09	Yellow, colorless folds	Rare, thin smooth walls with irregular shape	Positive	Positive	*T. tonsurans*
G04	Whitish, shinning raised edges	Numerous cells appearing on parallel sides	Positive	Positive	T. rubrum
H02	Whitish center with shiny yellow edges	Many thin wall with moderate cells	Positive	Positive	*Microsporum sps.*
V08	Slow growing, brownish surface and a raised center	Smooth, thin walled and club shaped	Negative	Positive	*E. floccosum*
V06	Raised center, slow growing and a khaki like surface	Smooth thin walled and club shaped	Positive	Positive	*T. rubrum*
F16	Whitish raised edges	Numerous cells parallel to each other	Positive	Positive	*T. rubrum*
H20	Yellowish, raised edges	Irregular shaped walls	Positive	Positive	*T. tonsurans*
H04	Golden yellow and a raised fold at the edge	Smooth wall with irregular shape	Positive	Negative	*T. tonsurans*
H14	White fluffy center with golden yellow border	Many spiny thin wall with 3-6 cells with rounded ends	Positive	Positive	*M. canis*
H18	Yellow to colorless folds	Rare thin smooth walls with irregular shapes	Positive	Positive	*T. tonsurans*
V13	Whitish brown color	Numerous cells present	Positive	Positive	*T. rubrum*

Table 1: Identification of various isolates phenotypically.

Orgs	6 mg/mL	4 mg/mL	2 mg/mL	1 mg/mL	0.5 mg/mL	Ketoconazole 20 µg/mL
V06	22 ± 0.1	18 ± 0.1	15 ± 0.2	15 ± 0.2	15 ± 0.2	22 ± 0.1
H09	30 ± 0.2	22 ± 0.2	20 ± 0.2	18 ± 0.2	16 ± 0.1	24 ± 0.2

H10	20 ± 0.1	18 ± 0.1	16 ± 0.2	15 ± 0.1	15 ± 0.1	22 ± 0.1
V13	18 ± 0.2	16 ± 0.2	15 ± 0.2	15 ± 0.2	14 ± 0.1	20 ± 0.1
H11	18 ± 0.2	18 ± 0.2	17 ± 0.2	16 ± 0.2	12 ± 0.1	20 ± 0.2
G04	22 ± 0.2	20 ± 0.2	18 ± 0.2	16 ± 0.1	12 ± 0.1	28 ± 0.2
H20	16 ± 0.2	15 ± 0.1	14 ± 0.1	13 ± 0.1	13 ± 0.2	20 ± 0.2
F16	23 ± 0.2	20 ± 0.2	20 ± 0.2	-	-	25 ± 0.1
H14	21 ± 0.2	20 ± 0.2	20 ± 0.2	18 ± 0.2	16 ± 0.2	22 ± 0.1
G03	18 ± 0.2	18 ± 0.2	17 ± 0.2	16 ± 0.2	14 ± 0.1	20 ± 0.2
V08	16 ± 0.2	14 ± 0.1	12 ± 0.1	10 ± 0.1	-	20 ± 0.1
G01	19 ± 0.2	15 ± 0.1	13 ± 0.1	10 ± 0.1	10 ± 0.1	20 ± 0.2
H08	16 ± 0.2	16 ± 0.2	12 ± 0.1	12 ± 0.1	10 ± 0.1	18 ± 0.2
H02	18 ± 0.2	15 ± 0.1	13 ± 0.1	12 ± 0.1	12 ± 0.1	18 ± 0.2
H04	15 ± 0.1	10 ± 0.1	10 ± 0.1	10 ± 0.1	10 ± 0.1	20 ± 0.1
H12	20 ± 0.2	16 ± 0.1	12 ± 0.1	10 ± 0.1	10 ± 0.1	20 ± 0.2

Table 2: Antimicrobial activities of formulated ethanolic extract of *Cola nitida* bark. Diameter (mm) of zone of inhibition SEM.

	MIC (µg/mL)	MFC (µg/mL)	DIFF(Ratio of MIC and MFC
VO6	3120	3120	1
H09	45	45	1
H10	45	90	2
V13	3120	3120	1
H11	3120	3120	1
G04	3120	3120	1
H20	3120	3120	1
F16	3120	3120	1
H14	3120	3120	1
G03	45	45	1
V08	3120	3120	1
G01	3120	3120	1
H08	45	90	2
H02	3120	3120	1
H04	3120	3120	1
H12	3120	3120	1

Table 3: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of formulated ethanolic extract of *Cola nitida* bark (n=2).

Parameters	Control	Extract	Normal range
Hemoglobin gm/dl	13.8	14.1	12.2-14.8

Neutrophils (%)	0	1	0-2
Lymphocytes (%)	79	83	76-98

Eosinophils (%)	0	2	0-3
Basophils (%)	0	0	0-2
Hematocrit%	39	39	36-40

Table 4: Effect of single dose (0.5 g) of extract on hematological parameters.

Parameter	Control	Extract
Skin and fur	Normal	Normal
Eyes	Normal	Normal
Behavioral pattern	Normal	Normal
Salivation	Normal	Normal
Sleep	Normal	Normal
Diarrhea	None	None
Coma	None	None

Table 5: Changes in behavioural/physical patterns of Wistar rat using various parameters.

The result of the kinetic kill assay is presented in Figure 1. Changes in the Log_{10} cfu/mL of the test organism indicated that the extract exhibited a significant fungicidal activity evident in the rate of reduction in the viable count of microorganism in relation to time. At 2 h contact time with extract, the viable count of the organism reduced significantly and at 4 h, the reduction is clearly evident. At 4 h contact time, the reduction in the viable count ranged from 0.90-0.96Log_{10} cfu/mL while at 24 h contact time, there was total (100%) kill of the test organism.

In this study, Figure 1 represents the time kill assay of dermatophytes whose MIC value were lesser than 100 µg/mL and this was corroborated by Rios and Recio 2005 that MIC greater than 1000 µg/m of crude extract or 100 µg/mL for isolated compounds should be avoided; and proposed that activity would be very interesting in MICs of 100 µg/mL for crude extract and 10 µg/mL for isolated compounds respectively. The *in vivo* study in this research was drawn from the result of the MIC/MBC as four of the dermatophytes tested had MIC that was termed interesting [17]. The animals for the *in vivo* studies were divided into 3 groups viz: infected/test group, treatment group and the control group. The animals in the test group were infected with an overnight broth culture of the dermatophytes to be assessed after making an abrasion at the back of the healthy male Wistar rat. This was followed by daily assessment for inflammation, reddening and other features typical of dermatophytes infection. The symptoms started appearing 7-day post infection and all features characteristic of a dermatophytes infection was seen after day 14. One Wistar rat for each group was assessed histologically for the effect of the dermatophytes infection and the results are as shown in Figures 2-5.

The treatment of the formulated extract of *Cola nitida* bark was shown in Figure 2. After 14 days of treatment with formulated cream one animal from each group was also taken for histological assessment to ascertain the level of clearance of the infection and the results are as shown in Figures 3-5. Figure 3 shows the macroscopic abnormalities of the tissue of the infected skin and the regeneration of the sebaceous gland formally atrophied due to the dermatophytes infection. Figure 4 shows the macroscopic abnormalities in the liver of the infected Wistar rat and the mild diffuse vacuolar regeneration of the liver cells after treatment with *Cola nitida* formulation while Figure 5 shows the macroscopic abnormalities of the tissue of the kidney of the infected Wistar rat and the renal cortical regeneration after treatment with the formulated extract of *Cola nitida*.

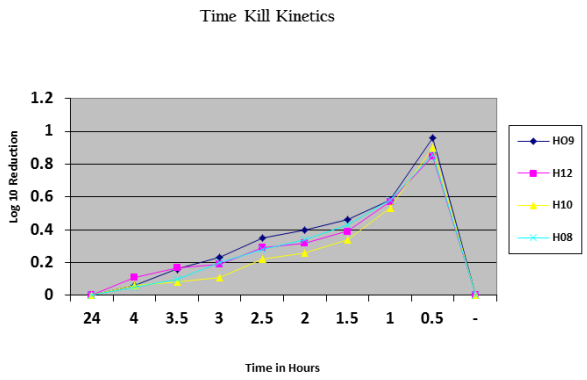

Figure 1: Time-kill assay of *C. nitida* on susceptible microorganisms showing the rate of reduction of viable population at various exposure time. Results are expressed as log10 cfu/mL against time.

Figure 2: Treatment of Wistar rat with formulated extract of *Cola nitida* bark.

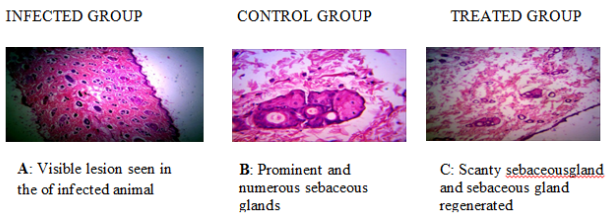

Figure 3: The macroscopic abnormalities of the tissue of the infected skin (A) and the regeneration of the sebaceous gland (C) formally atrophied due to the dermatophytes infection are shown.

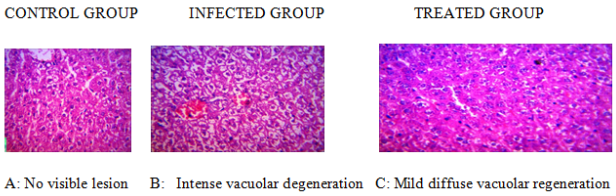

Figure 4: The macroscopic abnormalities in the liver of the infected Wistar rat (B) and the mild diffuse vacuolar regeneration of the liver cells after treatment with *Cola nitida* formulation (C).

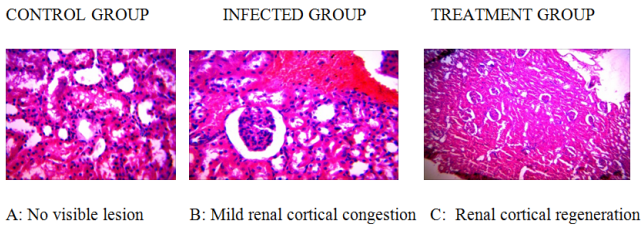

Figure 5: The macroscopic abnormalities of the tissue of the kidney of the infected Wistar rat (B) and the renal cortical regeneration after treatment with the formulated extract of Cola nitida (C).

Discussion

The components of ethno-medicine have long been ignored by many bio-medical practitioners for various reasons. For example, the chemical composition, dosage and toxicity of the plants used in ethno-medicine are not clearly defined [3]. However, it is interesting to note that the ethno-medicinal use of plants is one of the most successful criteria used by the Pharmaceutical industry in finding new therapeutic agents for the various fields of biomedicine [18]. Today, about 80% of the world's population relies predominantly on plants and plant extracts for healthcare [19]. In addition, of the top 150 priority drugs used in the United States of America, 57% contain at least one major active compound currently or once derived from plants [20].

In this study, the formulated ethanolic extract of *Cola nitida* demonstrated significant inhibitory and fungicidal effects against all the test fungal isolates. The differences in the values of MIC and MFC suggested a selective antifungal activity of the extract, while the almost competitive fungicidal activities of the formulated extract with synthetic drug can be partly attributed to the documented antimicrobial activities of some of the components used in the formulation of the cream [21,22]. Since lower MIC and MBC indicates higher efficacy [23], the MIC/MBC in this study are within the range of ≤ 100 and ≥ 1000 µg/mL. The highest MIC in this study is 3120 µg/mL and [24] defined active extract as those having MIC values ≤ 8000 µg/mL. The MIC index value being ≤ 2, indicated the fungicidal attribute of the formulated ethanolic extract and suggested that fungicidal effects be expected on all the tested dermatophytes [25]. Where MIC=MBC, the fungicidal potential with a broad spectrum and great therapeutic potential of the plant is indicated.

The kill kinetic study shows a dose-dependent decline of the surviving population at 2 × MIC compared with the slower rate of decline in the ½ × MIC and 1 × MIC. The significant reduction in the cell count between 2 to 4 h shows that extract was highly fungicidal owing to the fact that fungal colonies were almost wiped out after incubation at 4h. The decline in population was however slower incubating the fungi and extract at ½ × MIC. The degree of the antifungal activity of the formulated extract may be accounted for by the presence of flavonoids as indicated in the phytochemical screening of *Cola nitida* [26]. Since *in vitro* test do not necessarily confirm that plant extracts are effective medicine or a suitable candidate for drug development, this study further performed an *in vivo* analysis of the formulated plant extract on Wistar rat. The hematological parameters monitored during the course of the study reveal that hemoglobin, neutrophils, lymphocytes, eosinophils, basophils, packed cell volume and the platelet count were within the normal range for Wistar rat administered extract and the control group. Tissues from skin, liver and the kidney of the Wistar rat were assessed during infection and after treatment histologically. It was observed that in the skin after treatment, the sebaceous gland that has become atrophied during infection regenerated the liver cells from intense vacuolar degeneration during infection mildly regenerated while the kidney cell in the treatment group had its renal cortical regenerated (Figures 3-5).

Since investigating the pharmacological potential of plant extract could bring about the designing of less expensive therapeutically active agent to be used in less privileged region [27], understanding the chemical nature and isolating the active principle(s) in *Cola nitida* is ongoing which will provide an opportunity to synthesize new and effective antifungal cream in combating dermatophytes infections.

Conclusion

The use of *Cola nitida* in the treatment of whooping cough, asthma, scabies, and dysentery has been well documented while few or none of its antifungal potentials has been. This study shows the great potential of *Cola nitida* in the treatment of dermatophytes and further research into isolating the active components is ongoing at the Department of Pharmaceutical Microbiology, University of Ibadan. From this study, we conclude that formulated *Cola nitida* stem bark showed broader spectrum of activity and equally justifies the use of *Cola nitida* in folkloric medicine for treating ringworm infections in Nigeria.

Acknowledgement

The authors appreciate the senate of the University of Ibadan that approved the grant in conducting this research SRG/FP/2010/1C to BAA.

References

1. United states department of Agriculture (2005) Forest service and medicinal botany. USDA.
2. Krippner S (2003) Models of Ethnomedicinal Healing. Paper Presented at the Ethnomedicine Conferences, Munich.
3. Lowe HA, Payne Jackson, SM. Beckstrom Sternberg, Duke JA (2000) Jamaica's Ethnomedicine: Its potential in the healthcare system. Canoe Press; University of the West Indies, Jamaica, 170.
4. Kuoame C, Sacande M (2006) Cola nitida (Vent.) Schott & Endl. Seed Leaflet P :111.
5. Olorode O (1984) Taxonomy of West African Flowering Plants. Longman Group, United Kingdom, p. 180.
6. Jayeola OC (2001) Preliminary studies on the use of kolanuts (Cola nitida) for soft drink production. J Food Technol Afr 6: 25–26.
7. Kim K (2001) Encyclopedia of Alternative Medicine (2nd ed). USA, 22: 203–204.
8. Odugbemi T (2006) Outlines and Pictures of Medicinal plants from Nigeria (2nd ed). University of Lagos Press, Nigeria.
9. Brillowska-Dąbrowska A, Saunte DM, Arendrup MC (2007) Five hour diagnosis of dermatophytes nail infections with specific detection of Trichophyton rubrum. J Clin Microbiol 45: 12000.
10. Adeniyi BA, Odelola HA, Oso BA (1996) Antimicrobial potential of Diopyros mespiliformis. Africa J Med Sa 25: 179-184.
11. Lajubutu BA, Pinney RJ, Robert MF, Odelola HA, Oso BA, et al. (1995) Antimicrobial activity of diosquinone and plumbaginfrom D.mespiliformis (Hostch) (Ebenaceae). Phytother Res 9: 346-350.
12. Adeniyi BA, Odufowora RO (2000) In-Vitro antimicrobial properties of Aspilla africana(Compositae). Afr J Biomed Res (3): 167-170.
13. Shanmughapriya SA, Manila A, Sujith S, Selvin J, Kiran GS, et al. (2008) Antimicrobial activities of seaweed extracts against multi resistant pathogens. Ann Microbiol 58: 535-541.
14. Audu-Peter JD, Olorunfemi PO, Njoku N (2006) Antimicrobial and pharmaceutical properties of Khaya senegalensis seed oil. J Pharm Biores 3: 19- 24.
15. ASTM E2315-03 (2003) Standard guide for assessment of antimicrobial activity using a time kill procedure. ASTM International, West Conshokocken, PA.
16. Oladosu P, Isu NR, Ibrahim K, Okolo P, Oladepo DK, et al. (2013) Time kill kinetics antibacterial study of Acacia nilotica. Afr J Microbiol Res 7: 5248-5252.
17. Rios JL, Recio MC (2005) Medicinal plants and antimicrobial activity. J Ethnopharmacol 100: 80-84.
18. Cox PA, Balick MJ (1994) The ethnobotanical approach to drug discovery. Sci Am 270: 82-87.
19. Setzer MC, Werka JS, Irvine AK, Jackes BR, Setzer WN, et al. (2006) Biological activity of rainforest plant extracts from far north Queensland, Australia. In: Biologically active natural products for the 21st century. Research Signpost, India.
20. Grifo F, Rosenthal J (1997) Biodiversity and Human health. Island press, USA.
21. Dilika F, Bremner PD, Meyer JJ (2000) Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. Fitoterapia 71: 450-452.
22. Desbois AP, Smith VJ (2010) Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol 85: 1629-1642.
23. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev Clinical microbiol Rev 12: 564-582
24. Fabry W, Okemo PO, Ansorg R (1998) Antibacterial activity of East African medicinal plants. J Ethnopharmacol 60: 79-84.
25. Mims CA, Playfair JHL, Roitt LM, Wakelin D, Williams R (1993) Antimicrobials and Chemotherapy in Medical Microbiology review. CA Mims, Ed, vol 35, Mosby Europe, London,UK pp. 1-34.
26. Adeniyi BA, Mebude, OO, Lawal TO, Nwanekwu KE (2016) In-vitro Antifungal Activities of Cola nitida Schott & Endl (Sterculiaceae) against Five Candida species and Four Dermatophytes. Br Microbiol Res J 14:2.
27. Gatsing D, Nkeugouapi CFN, Nkah BFN, Kuiate JR, Tchouanguep FM, et al. (2010) Antibacteria activity, bioavailability and acute toxicity evaluation of the lear extract of Alchornea cordifolia (Euphorbiaceae). Int J pharmacol 6: 173-182.