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ANTIFUNGAL ACTIVITIES OF AN EXTRACT AND CREAM FORMULATION OF *COLA MILLENII* K. SCHUM. IN DERMATOPHYTE-INFECTED WISTAR RATS

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ABSTRACT

Objective: *Cola millenii* K. Schum (Malvaceae) bark have found use in traditional medicine for the treatment of skin infections such as ringworm and scabies. The present study aimed to evaluate the *In vitro* and *In vivo* antifungal potentials of extract and cream formulations of *Cola milleni* K. Schum. bark in dermatomycoses-infected wistar rats. The antifungal activities of methanol extract and formulated cream containing the bark extract of *C. millenii* were investigated *in vitro* and *in vivo* against *Trichophyton interdigitalis*, *T. rubrum*, and *Epidermophyton floccosum.* Susceptibility testing and the minimum inhibitory concentrations (MIC) were determined by the agar diffusion and agar dilution methods, respectively. *In vivo*, male wistar rats infected with *T. rubrum* were treated with the formulated cream and the efficacy assessed using the empirical clinical assessment score. Histopathology using periodic acid schiff (PAS) staining technique and the haematoxylin and eosin (H&E) staining techniques were used to monitor the therapeutic efficacy. *In vitro*, the methanolic bark extract had activity against all three dermatophytes with an MIC between 50- 75 µg/ml. Antimicrobial susceptibilities of the extract and cream formulations showed a dose-dependent response against the dermatophyte's species tested. Treatment of *T. rubrum* infected-rat with methanol extract and the formulated cream induced the clearance of fungal hyphae from the skin tissue and effected wound healing by day 17 (8 days of treatment regime) compare to control groups. These findings suggest that the extract and cream of *Cola millenii* have antifungal activities and are effective against commonly occurring dermatophytes.

KEYWORDS: *Cola millenii*, dermatophytes, antifungal, skin infection, *Cola millenii* cream.

INTRODUCTION

For thousands of year plants all over the world have been used for the treatment of diseases and are now known to contain chemical constituents that could be of therapeutic importance or as precursors for the synthesis of new drugs.[1] Over 50% of modern drugs originated as a natural product, and as such, these natural products play important role in drug development in the pharmaceutical industry.[1]

Dermatophytes' infections are some of the earliest known fungal infections and are very common throughout the world. Although dermatophytosis does not cause mortality, it causes significant morbidity and poses a major public health problem especially in tropical countries due to the hot and humid climate.[2] No race in any geographical location is entirely free from dermatophytosis. Skin, hair, nail, and subcutaneous tissues in human and animals are all susceptible to infection by several organisms, primarily fungi named dermatophytes and cause ringworm (dermatomycoses).[2] While several systemic anti-fungal compounds are available for use in humans, these compounds have significant adverse effects and their usefulness is limited due to their high rates of toxicity.[3] Different treatment methods have been in use for the control of dermatophytes. In general, pharmacological treatment option includes antifungal agents[4-6] but recently the use of some natural plant products has emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented from the late 19th century.[4, 6] They are safe to human than the chemically produced antifungal compounds and are readily available for use by the rural population who are mostly prone to these infections.[4,6] *Cola millenii* K. Schum (Malvaceae) is a deciduous shrub or tree with a low crown of arching branches. The plant usually harvested from the wild is locally used as medicine and as a source of wood.[7] The leaves and bark are reportedly use for the treatment of ringworm, scabies, gonorrhoea, dysentery and ophthalmic disorders.[7] The present study aimed to evaluate the *in vitro* and *in vivo* antifungal potentials of the extract and cream formulations (for topical treatment of dermatomycoses) of *Cola milleni* K. Schum. bark in

dermatomycoses-infected wistar rats comparing activities with commercial antifungal cream.

Sample collection and preparation

The stem bark of *Cola millenii* collected from Ijesa-Isu Ekiti, Ekiti State in Nigeria was identified, authenticated at the herbarium of the Department of Botany, University of Ibadan, Nigeria. The plant material was cleaned, air dried at room temperature for 4 to 8 w and then pulverized to coarse powder using a dry electric miller (Moulinex, France). The pulverized plant materials were weighed and subjected to exhaustive Soxhlet extraction with methanol (96%^{V}/v). Extracts were collected and concentrated under reduced pressure using rotary evaporator (Xian Ltd, China) at 45^{o}C. Remaining solvent was removed by desiccation and the dry extract was weighed and stored at 4^{o}C before use. In 20% DMSO, solutions of dried extract to final concentrations of 50 µg/ml, 25 µg/ml, 75 µg/ml, 20 µg/ml were prepared for antimicrobial screening. Lower concentrations in the range 75 µg/ml to 20 µg/ml were also prepared to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the bioactive crude extracts.

Phytochemical Screening

Phytochemical screening was carried out to detect the presence of secondary metabolites using standard methods described by Harborne.[8]

Cream formulation

Aqueous Cream (BP) used as the base was prepared using BPC 1979 methods.[9] Briefly, in a beaker placed on water bath the emulsifying ointment (30%$^{w}/_{w}$) was melted (I) and in another beaker, chlorocresol (0.1% $^{w}/_{w}$) was dissolved in purified water (69.9% $^{w}/_{w}$) with the aid of gentle heat (II). Solution (II) was added to the melted wax (I) while still hot with continuous stirring until it became cold. The methanol extract of the stem bark of *Cola millenii* was used to prepare creams at concentration of 2.5 -10.0%$^{w}/_{w}$ by mixing the required quantity of extract with the aqueous cream BP with continuous stirring until an elegant product was formed. *Cola millenii* cream formulations containing the humectants (glycerine and propylene glycol) at concentrations of 5.0%$^{w}/_{w}$ and 10.0%$^{w}/_{w}$ were prepared by incorporating the appropriate quantity of glycerine or propylene glycol into the cream formulations. Fifteen (15) samples coded A-O were prepared and stored at room temperature (27±2^{o}C) in tightly closed cream jars. Details of the composition of the cream formulations are as presented in Table 3.

IN VITRO STUDIES

Susceptibility testing

The antifungal activities of the dried methanol extract of *Cola millenii* and the formulated cream against clinical fungi isolates were evaluated using the agar well diffusion method.[10,11] Sabouraud's dextrose agar (SDA) in plate was inoculated with 0.1 ml of standardized inoculum (1x10^{7}cfu/ml) of the selected fungi by spreading method. Equidistant wells of 8 mm size were made with sterile cork-borer into agar plates containing the fungi inoculums. A 100 µl of the reconstituted plant extract was carefully introduced into each well of inoculated plates. Antifungal drug-1% tioconazole cream (Neimeth International Pharmaceuticals PLC, Nigeria) and 20% DMSO were used as drug and solvent controls respectively. The plates were left at room temperature for one hour to allow pre-diffusion of the extract into the agar before incubation at 25^{o}C for 3-7 d. The plates were observed periodically during this period for the presence or absence on growth. The diameter (mm) of inhibition zone for susceptible organisms was measured at the end of incubation period.[11]

Determination of minimum inhibitory concentration (MIC)

The MIC of the methanol extract was determined using a modification of standard agar dilution method as previously described.[12] The antimicrobial activities of the extract were tested at various concentrations (20 µg/ml to 75 µg/ml) and compared with those of 1% tioconazole cream, a standard chemotherapeutic agent. The MICs were determined after 3-7 days of incubation at appropriate conditions suitable for fungi growth. The MIC was regarded as the lowest concentration that prevented visible growth from a triplicate experiment.

Determination of minimum fungicidal concentration (MFC)

Minimum fungicidal concentration (MFC) of bioactive plant extracts was determined by a modification of the method of Adeniyi *et al*.[13] To a 0.5 mL extract at different concentration 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^{o}C for 48 to 72 h. Samples were streaked onto extract-free Sabouraud's dextrose agar to determine the minimum concentration of the extract required to kill the organisms indicated by failure of the organism to grow on transfer to these media plates. The lowest concentration that prevented the growth of fungal after days of incubation was recorded as the minimum fungicidal concentration (MFC). All tests were performed in triplicates to ensure accuracy. Agar plates without extract and other agar plates without organism and extract were also incubated serving as organism and medium control respectively.[14]

***IN VIVO* STUDIES**

Animal used for *in vivo* antifungal activity

Locally bred male wistar rats age 12-14 weeks weighing 150-200 g obtained from central animal house, University of Ibadan were used in this study. The rats were individually housed in propylene cages at room conditions (temperature of 27±2°C). The animals were feed pellet diet bought from Vita feeds lit, Ibadan, Nigeria and water *ad libidum.*

***In vivo* antifungal assay**

The animals were randomly assigned to treatment groups as presented in Table 5. The hair on the back (dorsoventral view) of each animal was shaved, cleaned and the area to be infected was disinfected with cotton swab saturated with 70% ethyl alcohol before abrasion. On the back of each animal, an area measuring 23-25 mm was cleaned and depilated using pathological scalpel. The fungal inoculum was prepared from 7-d old cultures of *T. rubrum* suspended in sterilized Tryptic soy broth. Following filtration through Whatman No. 1 filter paper to remove hyphal fragments and residual agar. The final suspension was adjusted to 1 x 10^7conidia/ml and 0.2 ml of inoculum was applied on the depilated area of the animals immediately after depilation and left for 8 days. The establishment of an active infection was confirmed on day 4 by isolation of the pathogens from skin scales cultured from infected loci on SDA plates containing antibiotics-penicillin and streptomycin. Infections were also confirmed by visual examination of the animals on days 4-8. In animals with confirmed active infection, treatment commenced on day 8 post infection and continued until day 25 post infection. The groups were treated topically with the various formulated creams of the extract by applying on the shaved surface of the back and the skin was examined daily. The therapeutic effect of formulated cream and the crude extracts was observed and compared with that of 1% tioconazole cream as a standard. The treatments were applied once in day and the infected areas were scored visually for inflammation and scaling. Clinical assessment of inoculated skin area was performed using a lesion scores as presented in Table 1.[15]

Histopathology of the skin of animal post-treatment

Therapeutic efficacy of the cream formulation was assessed by histopathology using periodic acid schiff staining (PAS) to determine the presence of fungal hyphae. In addition, hematoxylin and eosin staining (H&E) was done to monitor the on-going changes within the dermis and epidermis of the skin.

Statistical analysis

All data were replicated and expressed as Mean ± SEM (standard error of the mean). Analysis was performed using Computer software GraphPad Prism 5 (GraphPad software Inc., San Diego, USA) using one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison tests.

RESULTS

Phytochemical screening of the extracts revealed the presence of resins, saponins and alkaloids. The extraction yield was 20.8 g (1.4 % of 1487 g of sample extracted). The anti-fungal activities of *Cola millenii* bark were determined against *Trichophyton interdigitalis*, *T. rubrum* and *Epidermophyton floccosum.* Methanol extract from the bark of *Cola millenii* inhibited the tested fungi as shown in table 2 and fig. 1 (plates A-C). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values are 50 – 75 µg/ml and 75- 200 µg/ml respectively (table 2). The *in vitro* and *in vivo* antifungal activities of cream formulation of *Cola millenii* bark were investigated in *Trichophyton rubrum*- infected rats. Antifungal activities of the formulated cream (table 3) of *Cola millenii* bark against dermatophytes species as presented in table 4 revealed a dose-dependent activity of formulated cream. Post infection results showing the clinical assessments of infected rats before and after applying formulated cream are as presented in table 5. The results revealed the establishment of infection before the commencement of treatment with the formulated cream (fig. 2, plates A-C). After treatment with the formulated cream for sixteen days (16 days), results of H & E staining revealed the thin epidermis, well-developed sub-sebaceous layer, development of hair follicle and shaft. While the result of PAS revealed regeneration of hair follicle, shaft, epidermis and a complete absence of fungal hyphae as shown in fig. 3 (plates E-F).

Table 1: Description of lesion scores for clinical assessment of animals.

Score	Description
0	No visible lesion
1	Few erythematous lesions
2	Well defined vesicles
3	Large areas of marked redness, incrustation, scaling
4	Mycotic foci well developed in addition to score 3 lesions

Table 2: Susceptibility of dermatophytes to methanol extract of *Cola millenii* bark. Diameter of zone of inhibition (mm) ± SEM.

Dermatophyte species	Extract Concentration(µg/ml)			MIC (µg/ml)	MFC (µg/ml)	TIO (1%)	DMSO (20%)
	100	75	50				
T. rubrum	24±0.2*	16±0.2*	14±0.2*	50*	75*	36±0.2*	-
T. interdigitale	26±0.2*	18±0.2*	-	75*	75*	35±0.2*	-
E. floccosum	22±0.2*	16±0.2*	-	75*	200*	40±0.2*	-

* = Average result of three experiments; (-) = Not Sensitive (i.e. Resistant); (TIO) = 1% Tioconazole cream; (DMSO) = 20% Dimethylsulphoxide.

Table 3: The details of the ingredients used for *Cola millenii*cream formulations.

Formulation code	Ingredients Used in percentage $^{w}/_{w}$			
	Aqueous cream BP	*C. millenii* extract	Glycerin	Propylene glycol
A	97.5	2.5	0.0	0.0
B	95.0	5.0	0.0	0.0
C	90.0	10.0	0.0	0.0
D	92.5	2.5	5.0	0.0
E	90.0	5.0	5.0	0.0
F	85.0	10.0	5.0	0.0
G	92.5	2.5	0.0	5.0
H	90.0	5.0	0.0	5.0
I	85.0	10.0	0.0	5.0
J	87.5	2.5	10.0	0.0
K	85.0	5.0	10.0	0.0
L	80.0	10.0	10.0	0.0
M	87.5	2.5	0.0	10.0
N	85.0	5.0	0.0	10.0
O	80.0	10.0	0.0	10.0
P	100.0	0.0	0.0	0.0
Q	0.0	100.0	0.0	0.0

Table 4: Susceptibility of dermatophytes to cream formulations of *Colamillenii*bark.
Diameter of zone of inhibition (mm) ± SEM

Dermatophyte / Cream formulation*	*T. rubrum*	*T. interdigitalis*	*E. floccosum*
A	09±0.20	09±0.20	10±0.20
B	10±0.20	12±0.20	10±0.20
C	14±0.25	13±0.22	12±0.20
D	10±0.20	10±0.22	10±0.20
E	10±0.20	10±0.22	10±0.20
F	13±0.22	13±0.22	14±0.25
G	10±0.20	10±0.22	10±0.20
H	12±0.20	12±0.20	12±0.20
I	13±0.22	13±0.22	12±0.20
J	10±0.22	11±0.20	11±0.20
K	12±0.20	12±0.20	14±0.25
L	14±0.25	14±0.25	14±0.25
M	10±0.20	10±0.20	10±0.20
N	12±0.22	10±0.20	12±0.20
O	14±0.25	10±0.20	12±0.20
P	08±0.02	08±0.02	08±0.02
Q	24±0.25	26±0.25	22±0.25
R	36±0.2	35±0.2	40±0.2

*Cream formulation details in Table 3above

Table 5: The clinical assessments of infected rats before and after applying Treatment *invivo* (Lesion score ± SEM).

Animal Group /Treatment Day	I	II	III	IV	V	VI	VII	VIII	IX
Infected days 1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	1±0.50	0	1±0.20	0	1±025	1±0.25	0
5	1±0.50	1±0.20	2±0.40	1±0.20	2±0.20	0	2±0.20	2±0.20	1±0.20
6	2±0.20	2±0.20	3±0.25	2±0.20	2±0.25	1±0.20	2±0.25	2±0.50	1±0.20
7	2±0.25	2±0.20	3±0.20	3±0.20	3±0.25	2±0.20	3±0.20	3±0.20	2±0.20
8	3±0.20	3±0.02	3±0.02	3±0.29	3±0.29	2±0.20	3±0.25	3±0.25	2±0.25
Treatment days									
9 (PTD1)	3±0.25	3±0.25	3±0.25	3±0.25	3±0.25	2±0.25	3±0.25	3±0.25	2±0.20
10 (PTD2)	3±0.25	3±0.25	3±0.20	3±0.25	3±0.23	2±0.22	3±0.25	3±0.25	2±0.24
11(PTD3)	3±0.25	3±0.20	3±0.20	2±0.20	3±0.20	2±0.25	2±0.25	3±0.25	3±0.20
12(PTD4)	2±0.25	2±0.25	2±0.25	2±0.25	2±0.20	1±0.25	1±0.25	2±0.25	3±0.22
13(PTD4)	2±0.25	1±0.20	2±0.25	1±0.25	1±0.25	1±0.20	0	2±0.50	2±0.25
14(PTD5)	2±0.25	1±0.20	1±0.25	1±0.25	1±0.25	1±0.20	0	2±0.25	2±0.24
15(PTD6)	1±0.20	1±0.20	1±0.25	0	0	0	0	2±0.25	2±0.20
16(PTD7)	1±0.20	0	0	0	0	0	0	2±0.20	2±0.22
17(PTD8)	0	0	0	0	0	0	0	2±0.02	2±0.25
18 (PTD9)	0	0	0	0	0	0	0	2±0.02	2±0.20
19(PTD10)	0	0	0	0	0	0	0	2±0.02	2±0.20
20(PTD11)	0	0	0	0	0	0	0	2±0.02	2±0.22
21(PTD12)	0	0	0	0	0	0	0	2±0.02	2±0.22
22(PTD13)	0	0	0	0	0	0	0	2±0.00	2±0.20
23(PTD14)	0	0	0	0	0	0	0	2±0.00	2±0.20
24(PTD15)	0	0	0	0	0	0	0	2±0.00	2±0.20
25(PTD16)	0	0	0	0	0	0	0	2±0.00	2±0.20

* = Average result of experimental animals in each group; PTD= Post treatment day; I =5% *Cola milenii* cream; II= 150µg/mL *Cola millenii* extract; III=10% *Cola millenii* cream containing glycerine; IV = 75 µg/mL *Cola millenii* extract; V = 10% *Cola millenii* cream containing propylene glycol; VI = 10% *Cola millenii* cream; VII = 1% Tioconazole cream; VIII = No treatment; IX= Base (Aqueous cream British Pharmacopedia

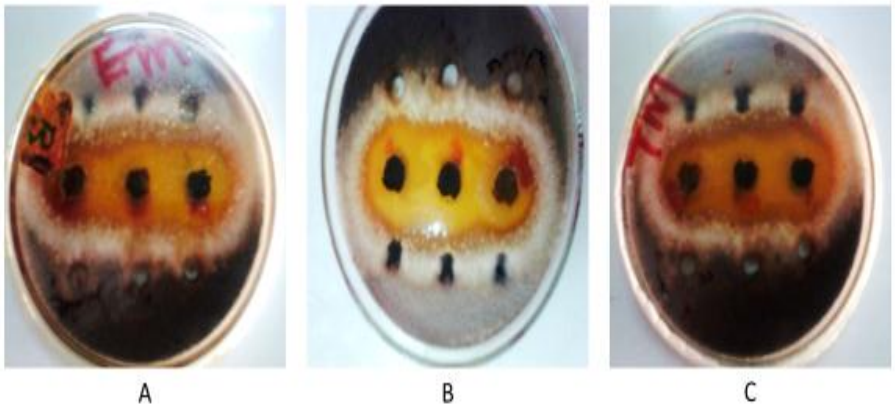

Figure 1

Figure 1. Agar plates diffusion method. Cork borer was used to bole a hole on Sabourraud's dextrose agar (SDA) and 20 uL of the text extract of the back of *Cola millenii* dissolved in DMSO were added per well and the plates incubated. The above Pictures/results showed the millimeter (mm) zones of inhibition of on (A). *Epidermophyton floccosum.* (B) *Trichophyton interdigitale.* (C) *Trichophyton rubrum*

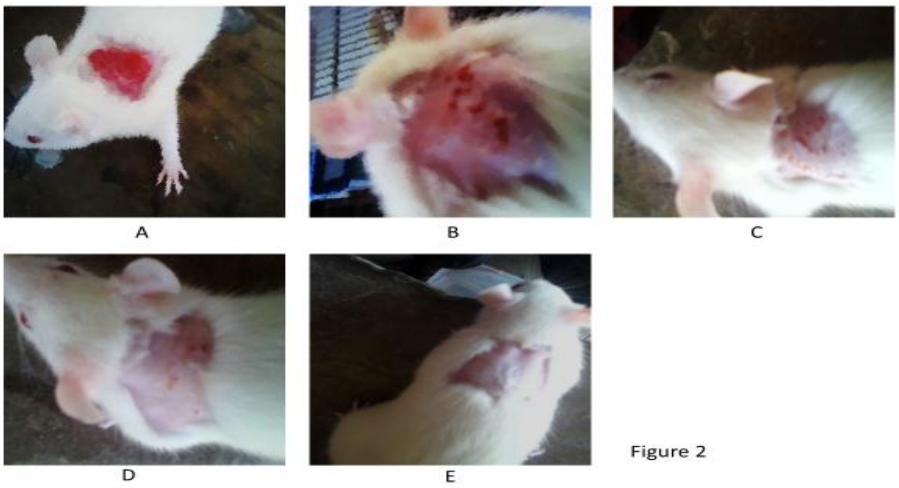

Figure 2: Pictures of animals infected with *T. rubrum* and treated with *Cola millenii* cream formulations. A. Freshly depilated animal skin showing the focus of abrasion before infection. B. Depilated skin showing the development of infection on day 4 post infection period. C. Depilated areas of infected skin foci showing the development of visible vesicles and rashes formation on day 7 before commencement of treatment. D. Recovery of depilated areas of infected skin with disappearance of wound, erythema, vesicles and rashes on day 13 (4-day post treatment with formulated cream of *Colla millenii* bark. E. Recovered depilated skin foci showing the appearance of fur on infected skin foci on day 15 (6-day post treatment).

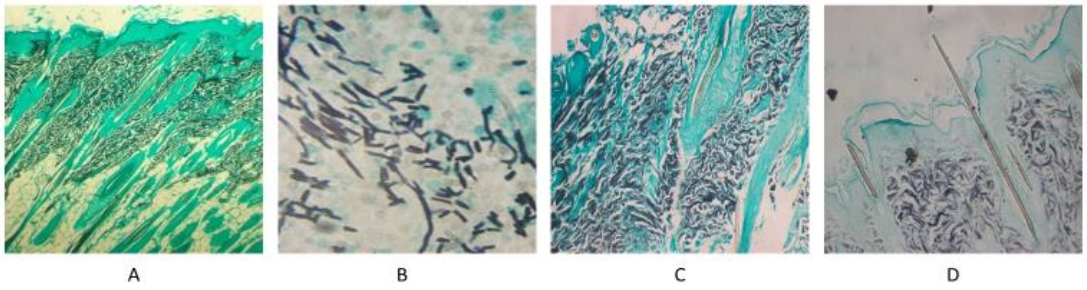

Fig.3. Histopathology PAS staining of the skin sections of experimental animals showing view before and after treatment with formulated cream of *Cola millenii*. A. Normal-looking skin showing hair follicle, sub-cutaneous fat deposit and prominent epidermis. B. Result of periodic acid staining (PAS)revealed the presence of fungal hyphae on the skin of albino rat before treatment. C. Result of Periodic Acid Schiff revealing the clearing of the hyphae and regeneration of hair follicle and shaft, recovery of epidermis and the cells of the epidermis appeared ballooned on the fourth (4) day of treatment with the formulated cream of the bark of *Cola millenii*. D. The result of Periodic Acid Schiff (PAS) revealing the regeneration of the hair follicle, shaft, epidermis and a complete absence of fungal hyphae after treatment with the formulated cream.

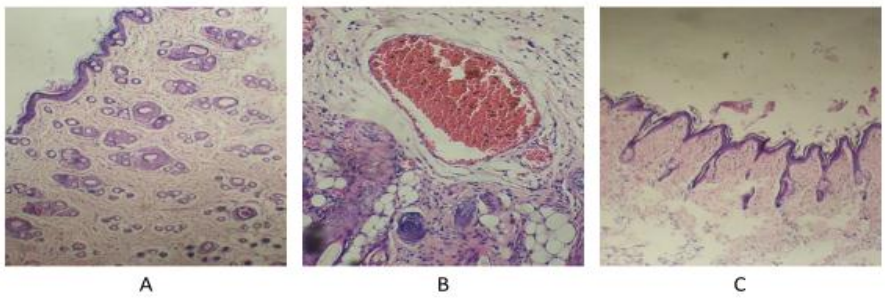

Fig.4. Histopathology H&E staining of the skin sections of experimental animals showing view before and after treatment with formulated cream of *Cola millenii*. A. Normal-looking skin showing hair follicle, sub-cutaneous fat deposit and prominent epidermis. (B). Result of Hematoxylin and Eosin (H & E) showing peri-glandular

necrosis and cellular infiltration, congested subcutaneous blood vesicles epidermal atrophy, exocytosis, perivascular dermatitis, appearance of dark spot infiltrated cell before treatment. C. The result of Hematoxylin and Eosin staining (H & E) revealing the thin epidermis and well developed sub-sebaceous layer, development of hair follicle and shaft after treatment with the formulated cream.

DISCUSSIONS

In search for new antidermatophytic agents with a lower toxicity, the antifungal activities of a methanol extract of *Cola millenii* stem and bark were investigated in some common dermatophytes. Phytochemical screening of the extracts revealed the presence of resins, saponins and alkaloids. The extraction yield was 20.8 g (1.4 % of 1487 g of sample extracted). The anti-fungal activities of *Cola millenii* bark, seed and leaf were determined against *Trichophyton interdigitalis*, *T. rubrum*, and *Epidermophyton floccosum.* The preliminary screening results showed that methanol extracts from the leaves and seed of *Cola millenii* had no activity (data not shown) and as such were not further studied. Only the methanol extract from the bark of *Cola millenii* exhibited promising results *in vitro* against the tested fungi and thus was chosen for further studies, which included determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) as well as *In vivo* studies. The activity demonstrated by methanol extract of *Cola millenii* bark could be due to the presence of saponins, alkaloids and resin. These phytochemical compounds have been reported to possess varying antifungal potentials against many fungi species.[16-18] Zhang and colleagues in 2005[19] reported the antifungal activity of eight (8) steroidal saponins isolated from *Tribulus terrestris* while Lanzotti *et al*[20] reported the presence of antifungal saponins in the bulbs of white onion (*Allium cepa* L). The major mechanism of antifungal activity of saponins is apparently due to their ability to complex with sterols in fungal membranes and to cause loss of membrane integrity although the precise mechanism is not fully understood.[21] Many research works have reported the antifungal property of alkaloids.[22,23] The mechanism of action of alkaloids is attributed to their ability to intercalate with DNA.[22,23]

The *in vitro* and *in vivo* antifungal activities of cream formulation of *Cola millenii* bark was investigated in *Trichophyton rubrum*- infected rats. Antifungal activities of the formulated cream of *Cola millenii* bark against dermatophytes species revealed a dose-dependent activity of formulated cream and showed that the crude extracts (at 100 µg/mL) compared well with the drug (1% tioconazole cream) control. These activities are attributable to the presence of the phytochemical compounds present in the sample. Post infection results showing the clinical assessments of infected rats before applying formulated extract *In vivo* revealed the establishment of infection before the commencement of treatment with the formulated extracts. Histopathological examination of the skin of *T. rubrum*-infected rats by PAS staining as well as H&E staining revealed the presence of fungal hyphae, ballooned epidermis, diffuse dermatitis, peri-glandular necrosis and cellular infiltration. Also observed were congested sub-cutaneous blood vesicles, epidermal atrophy (decreased thickness of epidermis), exocytosis (usually refers to lymphocytes, and implies a benign process), perivascular dermatitis, and appearance of dark spot infiltrated cell on the infected skin before treatment. Results of PAS revealed the clearing of the fungal hyphae and regeneration of the hair follicle and shaft, recovery of epidermis and the cells of the epidermis appeared ballooned on the fourth (4) day of treatment with the formulated cream of the bark of *Cola millenii.* After treatment with the formulated cream for sixteen days (16 days), results of H & E revealed the thin epidermis, well-developed sub-sebaceous layer, development of hair follicle and shaft. The result of PAS revealed the regeneration of the hair follicle, shaft, epidermis and a complete absence of fungal hyphae. It was observed that there was little difference in the clinical assessment response of the rats to the formulated extracts at different percentage ($^{w}/_{w}$ %) when compared with drug control (1% tioconazole), but the standard cream was first to respond to the treatment on the third day post-infection period. All animals in other groups showed varying degree of treatment response. Presence of rashes and visible lesion were still noticed in the group of animals used as negative control (i.e. infected but not treated; infected and treated with base) until day twenty-five (day 25) after infection period. The presence of rashes and visible lesion in these groups further strengthen the curative anti-fungal potential of the formulated cream of *Cola millenii* in the experimental groups. Subsequent analysis of the infected skin foci at different stages of treatment regime revealed the clearance of the hyphae from the skin tissue and wound healing in response to the treatment. The treated animals showed the appearance of fur on the infected skin foci from day 16 of treatment. Our finding agrees with the report of Njateng et al[24] who reported the activity of extracts from the bark of *Polyscias fulva* (Hiern) in *Trichophyton mentagrophytes*-induced dermatophytosis in a guinea pigs' model. Additionally, Mousavi and Kazemi[25] reported that of *Myrtus communis* L. and *Cinnamomum zeylanicum* Blume extracts significantly cured *T. mentagrophytes* and *M. canis* infection on days 9 and 13 as well as 9 and 11days respectively in male wistar rats.[25] Giwa *et al*[26] also reported the antifungal activity of *Cola millenii*. In dermatophytes infections as was observed in this study, inflammatory responses are usually characterized by relative degree of redness (erythema) and scaling at the edges of the lesions, and/or occasionally, blister formations. These are often accompanied by mild irritation, although the irritation could be more severe with some types of fungal infection.[27] However, the observed erythema quickly decreased with the administration of the formulated extract and the decrease

was dose-dependent. The observed erythema did not persist in the rats and suggested that the plant extract is safe at the test doses. It was observed that the constituents of the bases used for formulation of the cream did not interfere with the activity of the extract and thus suggests that use of these bases as vehicles for administering this extract is safe.

CONCLUSION

In this study, we investigated the *in vitro* antifungal effects of extracts of *Cola millenii* leaf, bark and seed as well as the *in vivo* anti-dermatophytes effects of both the crude and formulated cream to confirm their ethnopharmacological claims in the treatment of ringworm and scabies. The bark of *Cola millenii* was the most active against the selected clinical dermatophytes used in this study. The *in vivo* study revealed the ability of the formulated cream to clear fungal hyphae from the skin tissue and thus effect wound healing as response to treatment in *T. rubrum*-infected rat. These findings suggest good potentials for the use of *Cola millenii* compounds as anti-fungal agents. Our further studies will focus on isolation and characterization of active pure compounds with both *in vitro* and *in vivo* activities.

List of abbreviation

H&E (Hematoxylin and Eosin), **PAS (**Periodic Acid Schiff)**, DMSO, SDA (**Sabourraud's dextrose agar)**, MIC** (minimum inhibitory concentration)**, MFC** (minimum fungicidal concentration)**, BPC** (British pharmacopoeia compendium)**, ANOVA** (Analysis of variance)**, SEM** (Standard error measurement)**, DNA (**Deoxyribonucleic acid)

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Authors Contribution

The research was conceived and designed by BAA. TSA was a M.Sc. student who carried out the research supervised by BAA and co-supervised by TOL. TOA and OAO assisted with cream formulation. All authors participated in the manuscript writing.

Conflict of Interest: The authors reported no conflict of interest.

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