

Antimicrobial Screening of *Bridelia ferruginea* Benth (Euphorbiaceae) Stem Bark Extract in Dermatological Formulations

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Abstract

The plant *Bridelia ferruginea* Benth (Euphorbiaceae) has been known for its ethnomedicinal uses, some of which have been justified by scientific studies. Various forms of presentations such as decoction, powders have been used traditionally. None of these have been standardized to assure stability, safety, efficacy, and appropriate dose delivery. The aim of the present work was to develop an antimicrobial dermatological dosage formulation that will be useful in the treatment of various skin diseases. Extraction of the stem bark was carried out with boiled distilled water. The phytochemical constituent of the extract was determined. Thereafter, the extract was formulated into creams using vanishing cream base as a vehicle at concentrations of 2.5, 5.0, 7.5 and 10.0%w/w respectively. The formulation was evaluated for physical characteristics and then tested against selected organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Trichophyton rubrum* and *Aspergillus nige* using Gentamicin and Ketoconazole creams as reference compounds. The results showed the presence of flavonoids, saponin, tannins, anthraquinones, terpenoids and alkaloids as secondary metabolites. The creams gave favourable physical characteristics. The antimicrobial evaluation showed that the cream formulations (2.5, 5.0, 7.5, 10.0 %w/w) had inhibitory effect against organisms used. This inhibition was of increasing order across the concentration range of the formulations tested. The only exception was 2.5 %w/w concentration that did not show inhibitory activity against *Pseudomonas aeruginosa*. Cream formulations of the stem bark extracts of *B. ferruginea* exhibited favourable physical characteristics and promising antimicrobial activities, it could therefore be used in the treatment of certain skin infections.

Keywords: *Bridelia ferruginea*, Creams, Antimicrobial, Organisms, Inhibition.

Introduction

Antimicrobial resistance poses a threat to the effective prevention and treatment of an ever increasing range of infectious diseases. The emergence and spread of new resistance mechanisms globally threatens the ability to treat common infectious diseases, resulting in death and disability of individuals. Resistance to first-line antimicrobial drugs by infections

results in the use of more expensive therapies and a longer duration of illness and treatment, often in hospitals, which increases health care costs as well as the economic burden on families and societies. Hence, there is need for newer and effective anti-infective treatment, without which many standard medical treatments will fail or turn into very high risk procedures¹.

Drug resistant micro-organisms are implicated in skin infections such as carbuncles, folliculitis, impetigo and burn wound sepsis². The issue of resistance of dermatological infections to some medicaments available in the market has sparked up interest in the research of the antimicrobial properties of drugs from natural sources which are active against the major causative organisms of skin infections^{3,4}. One of the ways to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents.

The use of plants for medical purposes dated back to 3000BC^{5,6}. Eighty percent of the world population, majorly Asians and Africans, depend on traditional medicine particularly medicinal plants, for their primary health care needs^{7,8,9}. *Bridelia ferruginea* Benth (Euphorbiaceae) is most often a gnarled shrub growing up to 8 metres tall, and when in suitable condition can grow up to a straggly tree of height 6 - 15 m, up to 1.5 m in girth with a crooked bole branching low^{10,11}. *Bridelia ferruginea* is a common medicinal plant in Nigeria and the commonest *Bridelia* species of the Savannah woodland occurring in other humid Savannah regions of Africa, especially from Guinea to Zaire, Mali and Angola. Its common names in Nigeria include Kirni, Kizni (Hausa), Maren (Fulani), Ora (Iggede), Iralodan (Yoruba), Ola, Egede, Ede (Igbo) KensangeAbia (Boki). The stem bark of the mature tree is dark grey, rough and often marked with scars^{11,12,13}. *Bridelia ferruginea* is use in many parts of Africa traditionally for the treatment of skin diseases and various other ailments¹⁴.

This present study involves the preparation and evaluation of an herbal skin care formulation for antimicrobial action against various selected microorganisms which are associated with localized skin infections. Formulations consist of aqueous extract of *Bridelia ferruginea*

Benth stem bark and this herb has been selected on the basis of a traditional system of use and scientific justification with modern uses.

Materials and Methods.

Methods.

Collection and Treatment of stem bark of *Bridelia ferruginea* Benth

The plant material was collected from the botanical garden of Pharmacognosy Department, Faculty of Pharmaceutical Sciences, University of Ilorin. It was identified and authenticated at the herbarium section of the Department of Plant Biology, University of Ilorin-Nigeria by a botanist, (Herbarium Voucher number: UILH/001/987). The international plant name index is Euphorbiaceae *Bridelia ferruginea* Niger Fl. [W. J. Hooker]. 511. 1849 [Nov– Dec 1849] (IK)¹². The bark was cleaned and scales removed. The clean bark was cut into pieces and air-dried at room temperature for two weeks, then oven dried at 45°C for 24 hours. The dried bark was pulverized using mortar and pestle, then milled to a fine powder.

Extract Preparation.

According to the method described by Mbah¹², 500g of the ground stem bark was soaked in boiled distilled water overnight and filtered. The filtrate was then freeze dried to obtain the dry extract. The dried extract was stored in the refrigerator until it was needed. The bark extract was subsequently reconstituted in water at appropriate concentrations for the experiment. The concentrations were 2.5, 5.0, 7.5, 10.0 %^{w/w} for the *B. ferruginea* stem bark extract cream formulations.

Preliminary Phytochemical Screening.

The phytochemical composition of the aqueous extract of *B. ferruginea* stem bark was determined using standard procedures previously described by Sofowora^{15,16}. The phytochemical tests for saponins, tannins,

alkaloids, anthraquinones, cardiac glycosides, flavonoids and terpenoids were carried out.

Determination of Organoleptic Properties of the Extract.

The odour, colour and texture of the stem bark extract was determined and recorded.

Preparation of *Bridelia ferruginea* stem bark extract creams.

Stearic acid (I.O.I.Group Malaysia), cetylalcohol (BDH Chemicals Ltd, England) and glyceryl monostearate (Crode Chemicals Ltd, England) constituting oily phase were weighed with

an analytical balance (Ohaus, USA) into a beaker and melted together in a water bath (Fischer Scientific Company, USA) at 70 °C. Glycerin (Oleo-chemmInt, Singapore), potassium hydroxide (Allied Chemicals Ltd, England), methyl and propyl paraben (Clariant, UK) constituting aqueous phase were weighed into a separate beaker and then heated to 75 °C in the water bath. The oily phase was then added to the aqueous phase with continuous stirring until cooled, and the extract at different concentrations as shown in Table 1 was incorporated during cooling. The creams were filled in air tight cream jars and labelled accordingly.

Table 1: *Bridelia ferruginea* Stem Bark Extract Cream Formulations

Ingredients	Quantity of ingredient in each formulation (g)				
	A	B	C	D	E
Stearic acid	13.0	13.0	13.0	13.0	13.0
Glycerin monostearate	1.0	1.0	1.0	1.0	1.0
Cetyl alcohol	1.0	1.0	1.0	1.0	1.0
Glycerin	10.0	10.0	10.0	10.0	10.0
Potassium hydroxide	0.9	0.9	0.9	0.9	0.9
Methyl paraben	0.1	0.1	0.1	0.1	0.1
Propyl paraben	0.05	0.05	0.05	0.05	0.05
<i>B. ferruginea</i> stem bark extract	0.0	2.5	5.0	7.5	10.0
Purified water to	100.0	100.0	100.0	100.0	100.0

Characterization of *B. ferruginea* Stem Bark Extract Creams.

Physical Characterization: The appearance, colour, odour, texture, ease of application, ease of removal, feel on skin, skin irritation, extract-vehicle compatibility, homogeneity and phase separation of the different concentration of creams preparations were observed and recorded.

Evaluation of plant extract release:

The cream was melted, 0.25 mL was measured and poured into a 25 mL volumetric flask, the cream was buffered with phosphate buffer 6 to fill up to 25 mL mark. The volumetric flask was shaken vigorously to mix thoroughly. This procedure was repeated for the different creams formulated. Sterilized nutrient agar was poured into sterile petri dishes and allowed to solidify, the surface of each plate was flooded with 5%w/v ferric chloride solution, and excess solution was

drained and allowed to dry. Cylindrical holes were made on each plate with the aid of number 6 cork borer. 0.5 mL of the different concentrations of buffer and cream sample prepared was poured in the holes. This was done in triplicate and the plates were placed on the bench for 1 hour to allow for proper diffusion. The plates were then incubated at 25°C. The zones of colour changes were measured for each sample after 1, 2, 3, 6, 12 and 24 hours.

Assessment of Antimicrobial effect of *B. ferruginea* Stem Bark Extract Creams

Collection of Isolates:

Identified forms of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* and *Trichophyton rubrum* from the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Ilorin were used. The bacterial organisms were sub-cultured on Nutrient Agar (NA) and the fungal organisms on Saboraud Dextrose Agar (SDA) plates and incubated for 24 hours and 3 days respectively. Pure cultures obtained were transferred into slants of NA and SDA, checked for purity and maintained at a temperature of 4 °C.

Inoculation of Agar Plates:

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 25°C for fungal organisms and 37°C for bacterial strains respectively. The obtained microorganisms in the broth were standardized using McFarland (Number 1) test to obtain a population density of 10^6 cfu/ mL. Petri-dishes containing saboraud dextrose agar were streaked with the suspension of the fungi strains namely *Trichophyton rubrum*, *Candida albicans* and *Aspergillus niger* using sterile cotton swabs. Petri-dishes containing nutrient agar were streaked with the suspension

(10^6 cfu/mL) of the bacterial strains namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Incorporation of the cream into the agar plates:

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. The appropriately labelled holes were then filled with different concentrations (0, 2.5, 5, 7.5, 10 %^{w/w}) of the cream preparations and controls with a sterile scapel. The plates were allowed to stand on the laboratory bench for 1hour to allow for diffusion of the creams into the medium. The plates were subsequently incubated. At the end of the incubation period, the plates were observed for clear zones of inhibition of growth. Ketoconazole and Gentamicin creams were used as positive controls and formulation without the plant extract serve as the negative control. All experiments were carried out in triplicate.

Statistical Analysis:

One-way ANOVA was used to determine if there was a significant difference in the mean zones of inhibition of the different concentrations of *B. ferruginea* stem bark extract creams. Also, Student t-Test (unpaired, one-tailed) was used to establish if there was a significant difference in the mean zones of inhibition of the highest concentration of the *B. ferruginea* stem bark extract cream (10.0%^{w/w}) and the positive controls (Ketoconazole Cream, Gentamicin cream).

Results and Discussion:

The result of the phytochemical screening of the aqueous stem bark extract revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, free anthraquinones, and absence of cardiac glycosides and combined anthraquinones as shown in (Table 2).

Table 2: Phytochemicals present in *Bridelia ferruginea* stem bark extract

Metabolite	Test	Extract
Alkaloid	Drangendorff's	+
	Wagner's	+
	Meyer's	+
Flavonoid	Shinoda's test	+
	Lead acetate	+
Tannins	FeCl ₃	+
Saponins	Frothing	+
	Emulsifying	+
Anthraquinone	Combined (Modified Borntrager's Test)	-
	Free (Borntrager's Test)	-
Cardiac glycoside	Keller-Kiliani	-
	Kedde	-
Terpenoids	Lieberman-burchard	+

Key: (-) Absence of component; (+) Presence of component

This is in agreement with previous investigations by Mbah^{12, 13, 17}. The absence of some phytochemicals might be due to some factors which have been known to influence the quantity and composition of bioactive compounds present in plants such as the part of the plant used, solvent used for extraction, extraction procedure, geographic and climatic conditions, the genotype and growth phase of the plant^{8,17}. The presence of some of these phytoconstituents may be

responsible for the antimicrobial properties of the bark extract¹⁸.

Organoleptic evaluation of the dried extract revealed a brown extract with a faint odour. The physical characteristics of the different concentrations of the *B. ferruginea* stem bark extract creams such as the appearance, colour, odour, feel on skin, texture, ease of application and removal, skin irritation are shown in Table 3.

Table 3: Physical characterization of cream formulations.

Properties	Cream Concentrations (% ^{w/w})				
	0	2.5	5.0	7.5	10.0
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth
Colour	White	Light brown	Brown	Dark brown	Chocolate brown
Odour	Odourless	Agreeable	Agreeable	Agreeable	Agreeable
Texture	Smooth and soft	Smooth and soft	Smooth and soft	Smooth and soft	Smooth and soft
Ease of application	Easy to apply on rubbing	Easy to apply on rubbing	Easy to apply on rubbing	Easy to apply on rubbing	Easy to apply on rubbing
Ease of removal	Easy to remove with water	Easy to remove with water	Easy to remove with water	Easy to remove with water	Easy to remove with water
Skin irritation	Non irritant	Non irritant	Non irritant	Non irritant	Non irritant

The extract had a fine texture, brown colour with an agreeable odour. These properties may be a contributing factor to the widespread use of *B. ferruginea* ethnomedicinally. The *B. ferruginea* stem bark extract creams appeared from light brown to chocolate brown, increase in colour intensity was with increased extract concentration in cream formulation. The creams had a smooth appearance and were uniformly mixed except for formulation with the highest concentration of extract (10 %^{w/w}) which had a gritty appearance. All the creams were easy to apply and easily removed with water. All creams formulated were non-irritating when applied to the skin, with a soft and smooth feel. The colour of creams were appealing,

hence colourants might not be necessary when producing on a large scale. The creams also had an agreeable odour but might require addition of fragrance on large scale production to improve aesthetic and customer acceptance. Extract-vehicle compatibility was observed in all the formulated creams made with the *B. ferruginea* extracts as there was no separation after five weeks of formulation. The antimicrobial activity displayed by the *B. ferruginea* stem bark extract creams may be due to either or combination of the phytochemical constituents of the stem bark. The formulation was observed to have a concentration dependent activity as shown in Table 4.

Table 4: Mean Inhibition zones (mm) of the *B.ferruginea* stem bark extract creams:

Cream concentrations (% ^{w/w})	<i>Aspergillus niger</i>	<i>Trichophyton Rubrum</i>	<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
2.5	12.68 ± 0.47	13.00 ± 0.00	11.36 ± 0.47	13.33 ± 0.94	.00
5.0	14.00 ± 0.82	14.3 ± 0.47	12.00 ± 0.00	14.68 ± 0.47	12.00 ± 0.82
7.5	14.33 ± 0.47	14.6 ± 0.47	12.33 ± 0.47	16.33 ± 0.47	13.33 ± 0.47
10.0	24.00 ± 1.41	24.3 ± 0.94	19.60 ± 0.47	21.68 ± 0.47	21.33 ± 1.25
Negative control	0.00	0.00	0.00	0.00	0.00
Positive control	28.00 ± 0.82	25.0 ± 0.82	25.00 ± 1.41	27.00 ± 2.83	24.33 ± 1.25

Negative control – Cream without extract incorporated 0 % w/w

Positive controls –Ketoconazole Cream(For fungi), Gentamicin Cream(For Bacteria)

. These phytochemicals are biologically active compounds^{8, 9, 3} and have a broad range of biological activities. Phytochemicals such as tannins exhibit antioxidant, antimicrobial and antiviral

effects¹⁹, saponins have anti-inflammatory effects²⁰, haemolytic activity, and cholesterol binding properties and alkaloids have been reported to exert analgesic, antispasmodic and antibacterial activities²¹.

Assessment of the release of the active phytochemical from the cream formulations is indicated in Figure 1 below.

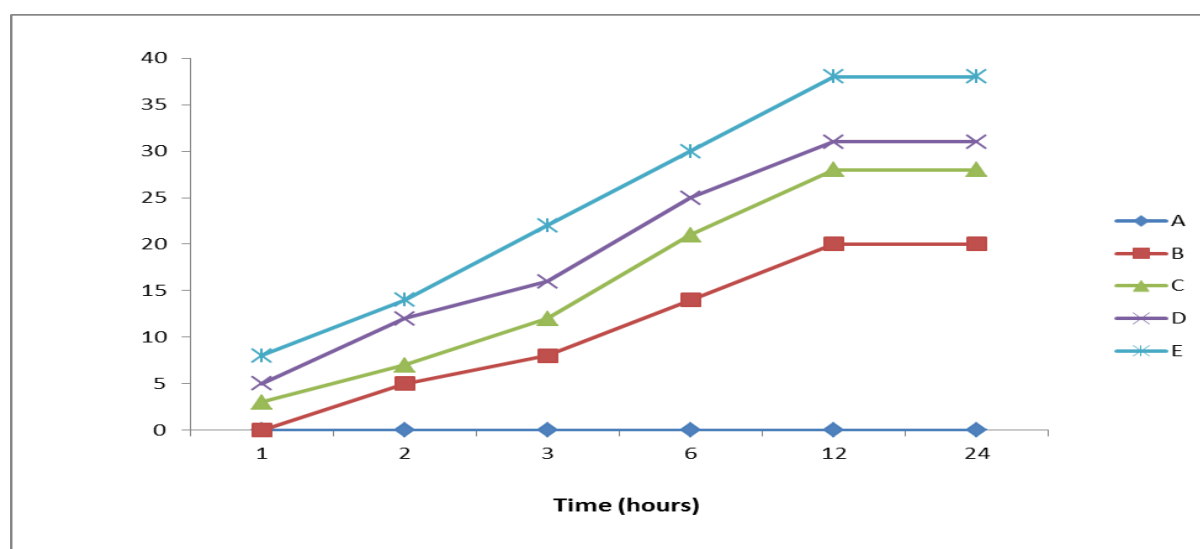


Figure 1: Graph of diameter of zone of colour change against time for the cream formulations at 37°C

Key: A – 0 % w/w B – 2.5 % w/w C – 5.0 % w/w D – 7.5 % w/w E – 10.0 % w/w

The zone of diameter of colour change of agar increased with time and maximum was observed after 24 hours for all the samples at 37°C. All the cream formulations with extracts incorporated showed release. The rate of release of phytochemicals from the cream base was observed to be time dependent. The nature of the cream base and temperature could also affect diffusion, rate of release and absorption of the active constituents. The antimicrobial properties of the *B. ferruginea* stem bark extract have been established by earlier workers^{11, 22}. The antimicrobial activity of the *B. ferruginea* stem bark extract creams is presented in Table 3. As can be seen, the *B. ferruginea* stem bark cream formulations (2.5, 5.0,

7.5, 10.0 % w/w) had inhibitory effect against organisms used. This inhibition was of increasing order across the concentration range of the formulations tested. The 2.5 % w/w cream had no inhibitory effect against *Pseudomonas aeruginosa*, a prototypical multidrug resistant pathogen which is recognized for its ubiquity and intrinsically advanced antibiotic resistance mechanisms. The lack of inhibitory effect of the 2.5 % w/w *B. ferruginea* stem bark extract cream against this organism may be due to the low concentration of the extract in the cream or as a result of the nature of the organism itself and its multidrug resistance mechanisms. However, the *B. ferruginea* stem bark extract creams with higher

concentrations of the extract inhibited the growth of the *Pseudomonas aeruginosa* ($P = 0.001$) for comparison of mean zone of inhibitions of cream formulations which ascertain that there is a significant difference ($P < 0.05$) between the inhibitory activities of the different concentrations of *B. ferruginea* stem bark extract creams. The inhibitory activity at 10 % w/w concentration was almost comparable with that of reference compounds (Ketoconazole cream and Gentamicin cream). The inhibition of the growth of the organisms in this study is probably attributed to disruption of architecture of the microorganism's cell membrane or cell wall, leading to changes in membrane permeability, and resulting in cell destruction^{23, 24}. It could also be due to penetration of phytochemicals into bacterial cells which promote the coagulation of their content¹⁸. Tannins and alkaloids, secondary metabolites which are present in the *B. ferruginea* stem bark extract, have been established to have antimicrobial effects^{19, 21, 18, 21}. Moreover, *B. ferruginea* stem bark has also been reported to possess antioxidant activity and anti-inflammatory activity²⁵. These activities are advantageous to the use of the cream formulations since as an antioxidant, there would be prevention of oxidative degradation and formation of free radicals which could damage cells, also the anti-inflammatory activity is desirable to take care of the inflammation which is commonly present in skin infections. Hence, the cream formulations made from the *B. ferruginea* stem bark extract potentially have antibacterial, antifungal, anti-inflammatory and antioxidant activities. This can prove to be highly cost effective.

Conclusion

All formulated creams showed potentials for use in the treatment of skin infections. The results of the study thus indicate that *B. ferruginea* stem bark extract can be

formulated into creams for topical application in treating infections caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Trichophyton rubrum* and *Candida albicans*. Further work can be carried out to isolate the bioactive components of *B. ferruginea* stem bark extract and formulate these pure bioactive constituents of the extracts with potentially greater activities as the crude extract has proven to be effective against the micro-organisms studied.

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