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Accelerated Stability Study and Evaluation of the Wound Healing Activity of the Ointments of *Barteria nigritiana* (Hook. f.) Stem Bark

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ABSTRACT

In traditional African medicine, herbal medicines are at the centre of various interventions in wound management and are known to achieve this through various mechanisms. This study evaluated the wound healing activity of the extract and fractions of *Barteria nigritiana* stem bark in excision and incision wound models. The extract and fractions were formulated in the ointment at 10 and 5% (w/w). The stability of the stressed formulations was evaluated by the Arrhenius accelerated stability method. Only the ointments containing the crude extract and ethyl acetate fraction were degraded by first-order kinetics ($R^2 > 0.9$) with shelf-lives of 24 and 33 days when stressed to 70 ºC. In the excision rat model, the extract, n-hexane, ethyl acetate and butanol fractions caused wound contraction of 8.2-96.9%, 2.1-53.1%, 8.0-100% and 2.6-57.6% respectively compared with blank ointment (2.6-13.9%) and standard control (8.9-100%) from days 4-20 post-wounding. The ointment of the ethyl acetate fraction elicited significantly wound contraction and a decrease in the epithelialization period. The ethyl acetate ointment also caused a significant ($p < 0.05$) increase in ascorbic acid and hydroxyproline content of the wounded tissues. All the extract and fractions ointments elicited a time-dependent decrease in malondialdehyde levels. In the incision wound model, there was a significant increase ($p<0.05$) in the tensile strength of the 10-20 day-old wound when treated with the ointment containing extract and fractions. The study identified the wound healing activity of the herbal ointment formulation of *B. nigritiana* as a validation of its vital role in folk medicine.

Keywords: Accelerated stability, *Barteria nigritiana*, Ethnomedicine, Epithelialization, Wounds.

Introduction

A significant number of the world's population depend on traditional medicine for the treatment of various disease conditions such as sores, wounds, burns, cuts, and lacerations.[1,2] A wound is formed when an injury to the normal structure and function of the skin occurs. It is a bridge in the physical obstruction in a good working tissue and can be managed or cured depending on the cause of the lesion; if it is due to trauma or resulting from a specific pathological condition.[3] Wounds resulting from other disease conditions are difficult to get rid of and can easily turn into chronic ones such as diabetic ulcers.[4] Acute wound heals within a reasonable period while chronic wounds take more than three weeks to heal due to underlying pathological conditions such as diabetic ulcer, infection and metabolic deficiencies of old age with a high chance of reoccurrence.[3]
Wound healing occurs in a well-ordered and timely repair process which is characterized by inflammation, proliferation, and remodeling phases.[5]

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Each phase relies on one another to enable the wound to completely seal giving no lasting evidence of tissue damage and understanding what each state involves is crucial in developing a comprehensive healing plan.[6] It is a stepwise occurrence requiring a biochemical process leading to the repair and regeneration of injured cells. Currently, numerous drugs are available for healing wounds such as antibiotic ointment, antiseptics, corticosteroids, and antioxidants but with many adverse effects which include; kidney failure, Steven Johnson's syndrome, dermatitis, and GIT symptoms.[7] Additionally, plant or plant parts contain phytoconstituents which confer the plants with a wide range of activities including antimicrobial, antioxidant, and anti-inflammatory activities that are the main ingredients in the management and healing of wounds with little or no adverse effect.[8]
Barteria nigritiana (Passifloraceae) is a popular softwood sparsely distributed in Eastern Nigeria and respectively called Ukwuifia, Oko and Idon zakara by the Igbos, Yorubas and Hausas in Nigeria.[9] The scanty volume of information available on this plant has recently elicited interest in them. The plant is useful in ethnomedicine due to its antimicrobial and antioxidant activities.[10] It possesses wounding healing property in folk medicine.[11,12] Traditionally, the crushed leaves are made in paste form and applied to fresh wounds to initiate their healing activity. In some parts of Southeastern Nigeria, the liquid is usually squeezed out from the fresh leaves and applied to fresh wounds for faster healing. These practices have not been validated scientifically. An extensive phytochemical analysis identified the presence of steroids, tannins, alkaloids, oxalate, flavonoids, cyanogenic glycosides, phenol and lipids in abundance with traces of saponins, phlobatanins and terpenoids.[10]

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A standard therapeutic intervention in wound healing involves topical antibiotics for infection control and debridement.[13] These procedures are currently challenged by cytotoxicity, allergic skin reaction, antibiotic resistance and continuous loss of potency as well as a poor uptake by the wound sites.[14,15] In this study, we investigated the wound healing activity of the ointment of the extract and fractions of *B. nigritiana* stem bark to enhance absorption of the bioactive ingredient into the wound area for local action. The rationale for this formulation is that the ointment can help the skin to achieve penetration of the active phytoconstituents and facilitate the release of soluble mediators which are the primary initiators of the wound healing process.

Materials and Methods

Experimental animals

Male rabbits weighing 200 – 250 g were used for the skin allergy test. Male Wistar rats weighing 109 – 124 g were used for the excision and incision wound tests. The animals were obtained from the central animal house of the Department of Veterinary Pathology, University of Nigeria, Nsukka. The animals were acclimatized for 7 days in a standard animal house and then exposed to 12 h light and dark cycle at 24 – 25 ºC and humidity of 55%. The animals were allowed free access to water and feed. Before the start of the experiment, the animal ethics committee at the University of Nigeria, Nsukka approved the research protocol involving the use of laboratory animals (approval number FPSRE/UNN/21/0008). All the experiments were carried out according to the International Code of Practices for Care and the use of animals for Scientific purposes (ICPCS).

Plant material

The stem bark of *Barteria nigritiana* was collected in November 2020 from Lejja Nsukka, Nigeria. The plant was authenticated by Mr. Felix Nwafor, a taxonomist at the Department of Pharmacognosy University of Nigeria, Nsukka. A voucher specimen (ID: PCG/UNN/0328) was deposited at the herbarium of the Department for future reference. The stem bark was cut into small sizes, washed with running tap water, and air-dried under shade at 25 ºC for 14 days. The dried stem bark was pulverized to a 1 mm mesh size with a mechanical grinder (Gx160 Delmer 5.5 HP).

Extraction and fractionation

The sample (1 kg) was extracted with methanol (2.5 L) using cold maceration for 48 h. The extract obtained (CME) was evaporated *in-vacuo* to dryness with a rotary evaporator at 40 ºC. The semi-solid extract obtained was further dried completely by exposing it to the atmosphere at 25 ºC for 72 h. A 150 g of CME was re-dissolved in 50 ml of 10%v/v methanol in water, made up to 500 ml, and transferred into a 1000 ml separation funnel. A 500 ml of n-hexane was added to the separation funnel and shook vigorously. This was allowed to stand until separation was observed. The n-hexane phase was run off with care and replaced with a fresh solvent of 500 ml aliquot until the n-hexane phase became clear. The partitioning was subsequently repeated with ethyl acetate (EtOAc) (2 x 500 ml) and n-butanol (2 x 500 ml) in a separating funnel to yield n-hexane (HF), ethyl acetate (EF), n-butanol (BF), and water (WF) fractions. All the fractions were completely evaporated *in-vacuo* to dryness with a rotary evaporator at 40 ºC

Preparation of ointment base

An ointment base was formulated using yellow soft paraffin (50%), yellow beeswax (30%), and olive oil (20%). The preparation was based on the British Pharmaceutical codex.[17] The required quantity of each component base was weighed into a beaker, its content heated over a water bath to 70 ºC, and the beaker allowed to cool to 40 ºC.

Ointment formulation

A weighted quantity of extract or fractions was added to a beaker containing the ointment base at 40 ºC using the fusion method at composition in Table 1. The content was triturated vigorously for 10 min using a tender mixer until a homogenous mixture was obtained. Thereafter, 1.2 g of cetrimide was dissolved in 0.5 ml of water and added to each ointment batch as preservatives. This procedure was also used in all the preparations.

Evaluation of ointment formulation

Skin allergy test

Five (5) rabbits each were used for each batch of ointment and controls. An area equivalent to 1260 mm^2 in an anterior region was surgically shaved. The shaved area was cleaned with water and exposed to drying under normal atmospheric conditions. Each ointment batch (500 mg) was applied with sterile cotton bud over the shaved part once daily for 7 days. The rabbits were observed for signs of skin allergy.[18]

Physical stability of ointment

The organoleptic properties (colour, odour, texture) of each ointment batch were evaluated at various storage time intervals which range from day 1 to day 90 of storage at 29 $\pm$1 ºC. The various organoleptic properties were carried out on alternate days.

Accelerated stability of medicated ointment

Adequate amounts of a medicated ointment containing CME and other fractions were subjected to a pre-calibrated oven set at 30, 50, and 70 ºC and then stored in a suitable air-tight container for 90 days respectively. At a predetermined time interval of 0-90 days, the bulk content of the extract or fraction in the ointment was assayed spectrophotometrically and the course of degradation was subjected to zero, first, and second-order reaction equations.[19] The rate constants (K) determined at each temperature were used to derive a specific rate constant $K_{25ºC}$ using the Arrhenius equation.[19] A plot of the logarithm of the rate constants, k, versus the inverse temperature, 1/T was used for the estimate of the shelf-life (t_{90}) of each formulation.

Wound healing studies

Induction of excision wound

The rats were anesthetized with IV ketamine and xylazine (10 mg/ml) to sedate the animals at a dose volume of 50 ml per 100 g body weight. The anterior dorsal side of each rat was drawn up and clipped. The clipped area was sterilized with 70% ethanol and 225 mm^2 and 2 mm depth of circular wound was created with a sterile scalpel razor blade. After the IV injection, the animals were allowed to recover from the anesthetic pains before the application of ointment formulations.[1]

Table 1: Composition of ointment batches

Batches	Extract composition (%w/w)	Ointment base (%w/w)	Cetrimide (%w/w)
A	10.0	88.8	1.2
B	5.0	93.8	1.2
C	5.0	93.8	1.2
D	5.0	93.8	1.2
E	1.0	97.8	1.2
F	0	98.8	1.2

A – D are ointment formulations of CME, HF, EF, and BF respectively; standard (E) and blank (F).

Wound healing evaluation

The rats were randomly divided into six (6) groups of five rats each and kept in a standard separate cage. Ointment formulations (A, B, C, D, E, and F) were applied to cover the surface of each wounded rat once for 20 days. Wounded rats in groups A to D received each of CME, HF, EF, and BF respectively. The rats in groups E and F received gentamycin and blank ointments respectively. The initial wound diameter was measured and monitored to evaluate the rate of healing. The diameter of the wound was the average sum of the vertical and horizontal diameter of the wound area measured every 4 days for 20 days. The period of epithelialization was calculated as the number of days required for falling off of the dead cells without any residual raw wound remaining.[20] The rates of wound contraction were calculated by the formula;

$$\% \text{ wound contraction} = \frac{(initial\ wound\ area - final\ wound\ area)}{initial\ area} \times 100$$

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Lipid peroxidation level
After wound measurement on the specified days, blood from the retro-orbital plexus of the rat eye was withdrawn with a hypodermic syringe and this was carefully introduced into a sterile bottle rinsed with EDTA for the determination of malondialdehyde (MDA).[21] In a test tube, 0.1 ml of plasma separated from rats' blood was added to 0.9 ml of 10% trichloroacetic acid containing 2 ml of 0.67% barbituric acid. The mixture was kept in a boiling water bath for 20 min, centrifuged, and allowed to cool. The absorbance of the supernatant was read at 540 nm and the concentration of malondialdehyde was read and calculated. From the standard graph, a molar extinction coefficient of the chromophores (1.56×10^{-5}/m/cm) and 1,1,3,3-tetraethoxypropane as standard were used.

Determination of ascorbic acid (AA) content
A 0.5 ml of plasma was added to 0.6 ml of ice-cold 10% trichloroacetic acid mixed well and centrifuged for 20 min at 3800 pm. A 0.5 ml of supernatant was added to 0.1 ml of DTC reagent (2-4–dinitrophenyl hydrazine thiourea $CuSO_4$ reagent) and incubated at 37ºC for 3 h.[22] However, 0.5 ml of ice-cold 65% H_2SO_4 was equally added to the reaction mixture and then allowed to stand at room temperature for 30 min. The yellow color developed was read at 510 nm. A standard curve was prepared using various aliquots from the stock solutions of ascorbic acid to obtain the unknown concentration of ascorbic acid.

Determination of L-hydroxyproline (HP) content
Wound granules were first collected from groups of rats treated on the specified days post-wounding, washed in cold saline 0.9% w/v NaCl and lyophilized. The granules were extracted from a 200 mg scab from test and standard batches of wounded rats, then dried in an oven at 60 ºC for 24 h.[23] A 100 mg granule powdered scab was placed in a sealed tube containing 2 ml of 6 N HCl. The scab was hydrolyzed by heating the sealed tube at 110 ºC for 4 h. The hydrolysate obtained was neutralized with 10 N NaOH. To 2 ml of the above solution, 1 ml of the oxidant (1 ml of 7% w/v aqueous chloramines T solution and 4 ml of acetate citrate buffer (pH 6) were added and the solutions were mixed and allowed to stand for 4±1 minutes at 25 – 30ºC. The colour changes were observed by adding 13 ml of the Ehrlich reagent to each solution. The solutions were thoroughly mixed, heated for 25 min at 60 ºC, cooled for 3 min in running tap water, and then transferred to a 50 ml volumetric flask and diluted to the mark with isopropanol. The absorbance of the colour was measured at 550 nm against the blank.

Incision wound model
In the incision wound model, a 4 cm paraventral incision was made by removing a section, full-size skin thickness of rats in each group. The wounds were sutured 1 cm apart with a 2 mm black silk thread and a sterile needle. The rats were anesthetized using 10mg/ml each of ketamine and xylazine before wound creation. An ointment containing extract and fractions was applied topically once daily for 20 days on the wound surface. Groups A-F received CME, HF, EF, BF, gentamycin, and blank ointment formulations respectively. The sutures were removed on the 12th day post wounding and wound tensile strength. The tensile strength (Ts) was measured using continuous constant water flow techniques.[20] The tensile strength is expressed as the minimum weight (in g) of water capable to pull apart the wound edge. The various batches of ointment formulations were compared with the control.

Statistical analysis
All the experimental procedures were carried out in triplicate. The data obtained were analysed using GraphPad Prism v.5 software and the results were expressed as mean ± standard error of the mean (SEM), (n=5), and $p < 0.05$ was considered statistically significant.

Results and Discussion

Extraction of Barteria nigritania stem bark.
Following exhaustive extraction of the stem bark of *Barteria nigritania* by cold maceration method, the total yield of the dried CME was 18% w/w of dried powder. The solvent partitioning of CME yielded 0.5% HF, 2.7% EF, 6.50% BF and 7.29% WF. The yields were calculated with reference to 100 g CME. The major challenges in antibiotics and debridement wound care such as topical allergic reaction, antibiotic resistance and hindered skin permeation can be circumvented through the poly phytotherapeutic approach. Plants have shown great potential in the management of communicable and non-communicable diseases.[24,25] This approach is commonly obtained in the use of plants and their derived products as therapeutic interventions where synergistic antioxidant, skin regeneration and fibroblast growth actions are possible to heal wounds. Therefore, extraction of these phytochemicals is an important aspect of this study. The extraction protocol adopted was based on the ability of methanol to extract both polar and non-polar constituents of plant materials when compared with aqueous hydro alcoholic or other organic solvents.

Physical properties of the ointment
The result of organoleptic properties of ointment formulations showed no remarkable colour changes; batches B and D were dark red while batches A, C, and E were lighter There was consistency in the texture of the formulations. All the formulations showed smooth texture with no solid particles.

Skin allergy test
The evidence of skin allergy during the 7 days of observation of the animal skin such as oedema; redness and erythema were not seen. High-quality ointment shows no visible reaction to a skin allergy test.

Accelerated stability study
The formulated ointments were thermally stressed for 90 days and the periodic analysis was recorded in concentration-time plots of different degradation orders The results indicated that only the CME and EF ointments may have undergone a strictly first-order kinetic degradation. The degradation process showed that the rate of decomposition depended on the concentration of the phytoconstituents of *B. nigritiana*. Other formulations showed mixed reaction orders. The degradation of *B. nigritiana* ointment at 30, 50, and 70 ºC showed that the degradation constant of CME and EF in ointment formulations followed first-order kinetics Figures 1A-C). In the accelerated stability studies of the CME, the first-order rate constants (K) at 30, 50 and 70 ºC were 0.05693, 0.07106, and 0.09338 /day respectively (Table 2).

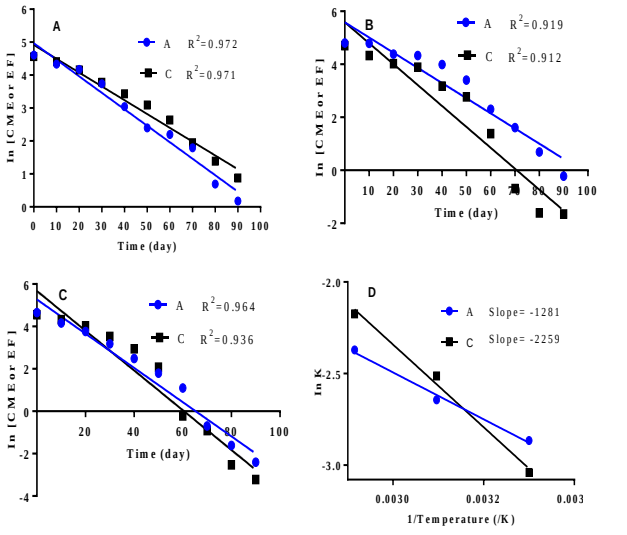

Figure 1: First order degradation kinetics of ointment of CME and EF at 30 °C (A), 50 °C (B), 70 °C (C) and the Arrhenius plot (D). The rate constants of CME and EF ointments were used to obtain Arrhenius plots (Figure 1D). The slopes were used to estimate the activation energies (E_a) of the degradation to obtain the shelf lives (See Table 2)

A comparison of the K_1 and $t_{1/2}$ of CME and EF ointments showed that EF (0.1137/day, 6.09 days) degraded more actively at 70 ºC than CME (0.09938/day, 6.97 days) but was more stable than other formulations. This is evidence of uniformity in the degradation pattern of *B. nigritiana* at different controlled temperatures. The implication

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is that the degradation constant (rate constant K below) obtained at these temperatures can be comfortably used in the extrapolation of the stability and shelf life. Apart from the stability issues, a major challenge of topical formulations is the ability to permeate the skin membrane. Formulation of herbal medicines such as ointment has the dual advantage of enhancing the shelf life and permeation of lipid layers. In this study, the ointments were stressed to 70 ℃ and there was a relatively stable ointment considering the content, consistency and colour. The crude ointment was found to maintain a shelf life of 24 days compared with the 33 days of the EF ointment; an indication that both formulations were relatively stable within the wound healing windows of 20 days.

Wound healing activities.

Excision wound model.

The excision wound model (Table 3) revealed that both the extract and fractions of *B. nigritiana* elicited time-dependent wound contraction. The extract caused a significant decrease in the wound areas on days 8-20 of the treatment resulting in 17-96% contraction of wound areas compared with the control. There was no significant difference between the group treated with n-hexane fraction (B) and the control group. However, there was a significant wound contraction in the group treated with EtOAc fraction, similar to the standard compared with the control causing complete wound closures on the 12th day. Epithelialization time varied significantly in all the treatment groups with the lowest days in the CME, EF and standard control groups of 18, 13 and 12 days. The excision wound closure in the rats treated with CME and ET was found to be accelerated compared to the control with different epithelialization periods. All the treatments showed a reduction in open wound area on days 4-20 post-wounding. There was no sign of redness, infection or irritation on the rats' skin throughout the study periods. Topical ointments of crude and EtOAc fraction of *B. nigritiana* were found to cause an accelerated decrease in wound area compared with the control. Both HF and BF did not cause re-epithelialization of the wound throughout the study period. The accelerated wound contraction suggested a possible proliferation of fibroblast, epithelial cell deposition, angiogenesis, proliferation and differentiation of keratinocytes and granulation of the wounds.[26] In wound healing events, collagen formation plays an important role and it is stimulated by fibroblasts. Collagen, a major component of the extracellular matrix, facilitates the formation of granulation tissues which subsequently enhances wound contraction as was observed.[27]

Effect of B. nigritiana ointment on connective tissue and antioxidant parameters

The effects of *B. nigritiana* on the connective tissue (HP and MDA) and antioxidant (AA) parameters of healing wounds are shown in Table 4. Generally, a dose-dependent increase in the HP and AA and a decrease in MDA were observed on the 10th to 20th post-wounding days. Though the extract and all the fractions possessed wound healing activity was more pronounced in the EF than in other fractions. The ascorbic acid level was found to be higher in ointment-containing extract and fractions than in the wounded control group.

Table 2: Chemical kinetics parameters of ointment of *CME*

T	CME ointment			EF ointment		
(ºC)	C_0 (ppm)	C_{90} (ppm)	K (/day)	C_0 (ppm)	C_{90} (ppm)	K (/day)
30	102.3	2.04	0.05693	96.4	4.04	0.04115
50	120.8	0.80	0.07106	110.5	0.20	0.09892
70	105.6	0.09	0.09338	102.4	0.04	0.1137
E_a (kJ/mol)		10.645			18.772	
$K_{25ºC}$ (/day)		0.028448			0.020572	
t_{90} (day)		24.78			33.64	

Shelf life (t90) of the formulations obtained from the Arrhenius plots of Figure 1D

Table 3: Effect of *B. nigritiana* ointments on excision model of Wistar rats

Treatment	Wound area (mm^2) /wound closure (%)/Time (days)						EP
	0	4	8	12	16	20	(days)
A	226.2 ± 0.2	207.6 ± 0.1	186.9 ± 0.7	98.3 ± 0.1	49.6 ± 0.3	6.9 ± 0.7	18.6
	-	8.22	17.37*	56.54*	78.07*	96.95*	
B	225.5 ± 0.7	220.7 ± 0.3	201.4 ± 0.1	195.8 ± 0.6	140.1 ± 0.1	105.7 ± 0.6	>25
	-	2.13	10.69	13.17	37.87*	53.13*	
C	228.1 ± 0.5	209.7 ± 0.3	127.4 ± 0.6	3.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	13.6
	-	8.07	44.15*	98.60*	100*	100*	
D	223.8 ± 0.4	217.9 ± 0.4	196.6 ± 0.4	169.4 ± 0.5	101.9 ± 0.3	94.7 ± 0.5	20.1
	-	2.64	12.15	24.31*	54.47	57.69*	
E	228.2 ± 0.9	207.9 ± 0.1	103.7 ± 0.3	1.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	12.8
	-	8.90	54.56*	99.43*	100*	100*	
F	225.8 ± 0.6	219.9 ± 0.1	212.8 ± 0.2	218.9 ± 0.3	205.9 ± 0.4	194.2 ± 0.0	>30
	-	2.61	5.76	3.06	8.81	13.99	

A – D are ointment formulations of CME, HF, EF, and BF respectively; standard (E) and blank (F); EP= epithelization time; data represent mean ± SEM (n=5); *p < 0.05 compared to group F (post-hoc Dunnett's test)

Additionally, a decrease in the lipid peroxy group was *observed showing an antioxidant effect.* The effect of *B. nigitiana* on MDA showed that the ointment containing extract and fractions elicited decreased MDA content compared to the control with an increased level of MDAs as shown in (Table 4). The assay of the HP content of the excision wounds was carried out to measure the collagen deposition in the tissue, as HP is a non-proteinogenic amino acid mainly found in collagen fibers and holds a constant mass of about

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13.4% in collagen, therefore its measurement can be used as an indicator of collagen content.[28] Collagen is known to be a significant structural protein in the body, is crucial in the connective tissues by providing durability and proline is rarely emerging in other proteins.[29] The HP levels were measured in the lesions on days 10, 15, and 20 of post-wounding and the results reaffirm the accelerated wound closure which represents the level of HP in CME and EF-treated groups at all-time points. Based on the results, the increased wound healing by the EF suggests an increase in the healing process by the virtue of its anti-inflammatory, re-epithelialization, induction of fibroblast proliferation, and synthesis of collagen. An increase in ascorbic acid content increases hydroxyproline content and this invariably increases wound healing because ascorbic acid is an inhibitor of lipid peroxidation.[25] Malondialdehyde content (MDA) evaluation showed a decreased lipid peroxidation activity with time and this suggests an enhanced antioxidant defense mechanism to prevent the formation of excessive free radicals.

Incision wound model

The results of incisional wound healing showed that the tensile strength of rats treated with EF ointment was significantly higher than the control rats (Figure 2). There was a statistically significant difference ($p < 0.001$) between the breaking strength of incisional wounds treated with the standard drug (541 g), EF (544 g) and CME (451 g) compared with the placebo (334 g) control rats. The influence of *B. nigritiana* on the collagen contents was supported by the tensile strength of the incision wound assessment on the post-wounding days. Tensile strength of incision showed enhanced collagen biosynthesis which a previous study had attributed to a strong intermolecular cross-linking of collagen with proteoglycan or glycoprotein of the incision wound.[30] It should be noted that the study did not identify the phytochemical constituent(s) of *B. nigritiana* responsible for the recorded wound healing activity. However, synergistic effects of steroids, tannins, alkaloids, oxalate, flavonoids, cyanogenic glycosides, phenol and lipids previously identified from the plant could be responsible for the activity.[10]

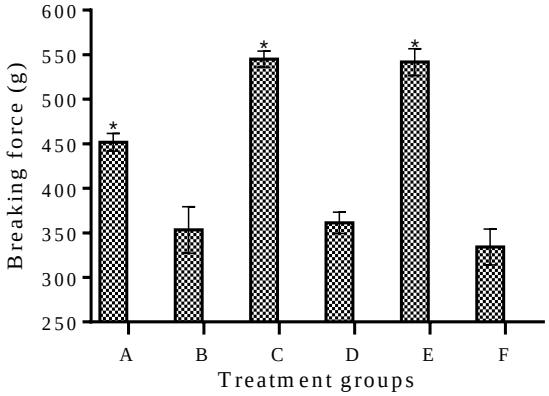

Figure 2: Effect of *B. nigritiana* on incision wound model. Data represent mean ± SEM (n=5); *$p < 0.05$ compared to group A (post-hoc Dunnett's test

Table 4: Effect of *B. nigritiana on* connective tissue and antioxidant parameters

Treatment/ day	HP (µg/100 mg protein)			MDA (nMol/mg)			AA (g/dl)
	10	15	20	10	15	20	
A	0.45 ± 0.10	0.47 ± 0.23	0.50 ± 0.12	4.5 ± 0.2	3.3 ± 0.1	2.5 ± 0.4	6.8 ± 0.3
B	0.12 ± 0.02	0.11 ± 0.04	0.20 ± 0.04	9.2 ± 1.1	9.3 ± 1.2	8.8 ± 0.9	3.1 ± 0.1
C	0.59 ± 0.13	0.78 ± 0.03	0.83 ± 0.16	1.1 ± 0.4	0.9 ± 0.1	0.8 ± 0.2	9.6 ± 0.5
D	0.22 ± 0.09	0.23 ± 0.08	0.30 ± 0.04	6.7 ± 0.2	6.8 ± 0.2	5.5 ± 0.3	6.4 ± 0.2
E	0.48 ± 0.03	0.56 ± 0.03	0.74 ± 0.11	1.8 ± 0.2	1.2 ± 0.4	0.9 ± 0.1	5.9 ± 0.1
F	0.09 ± 0.01	0.10 ± 0.03	0.10 ± 0.04	9.5 ± 0.2	9.4 ± 0.4	8.4 ± 1.1	3.6 ± 0.3

Data represent mean ± SEM (n=5); *$p < 0.05$ compared to group A (post-hoc Dunnett's test)

Conclusion

The wound healing potential of *B. nigritiana* in the excision and incision models is reported for the first time and has further validated the folkloric use of the plant in the management of wounds. The increased hydroxyproline and ascorbic acid and decreased lipid peroxidation postulate anti-oxidation and anti-inflammation mechanisms. The study represents a starting point for the discovery of a new class of phytochemicals as an alternative to topical antibiotics and debridement commonly used in wound care and management.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Nnadi CO, Udeani TKC, Ugwu LO. Wound-Healing and antimicrobial properties of dichloromethane fraction of *Dialium guineense* (Wild) fruit coat. Res Pharm Sci. 2016; 11(3): 219-226.
2. Erawati T, Fitriani RD, Hariyadi DM. Topical antimicrobial microparticle-based polymetric materials for nurn wound infection. Trop J Nat Prod Res. 2021; 5(10): 1694-1702.
3. Olsson M, Järbrink K, Divakar U, Bajpai R, Upton Z, Schmidtchen A, Car J. The humanistic and economic burden of chronic wounds: A systematic review. Wound Repair Regen. 2019; 27(1): 114-125.
4. Kathawala MH, Ng WL, Liu D, Naing MW, Yeong WY, Spiller KL, Van Dyke M, Ng KW. Healing of chronic wounds: an update of recent developments and future

possibilities. Tissue Engineering Part B: Reviews. 2019; 25(5): 429-444.
5. Lima TD and Passos MF. Skin wounds, the healing process, and hydrogel-based wound dressings: a short review. J Biomat Sci Polymer Ed. 2021; 32(14): 1910-1925.
6. Zuhriddinovich SK, Anatolevich AA, Raufjanovich KM, Anvarovna NZ. Current aspects in the treatment of burn wounds. Вопросы науки и образования. 2020; 13(97): 93-97.
7. Bowers S and Franco E. Chronic wounds: evaluation and management. Am Family Phys. 2020; 101(3): 159-166.
8. Sharifi S, Hajipour MJ, Gould L, Mahmoudi M. Nanomedicine in healing chronic wounds: opportunities and challenges. Mol Pharm. 2020; 18(2): 550-575.
9. Mezzonato-Pires AC, Sousa HC, Mendonça CB, Gonçalves-Esteves V. Pollen morphology of Barteria and Paropsia: implications for the systematics of Passifloraceae sensu stricto. Palynol. 2019; 43(3): 373-382.
10. Ezeonu CS and Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. New J Sci. 2016; 2016, 5601327.
11. Aworinde DO and Erinoso SM. Ethnobotanical investigation of indigenous plants used in the management of some infant illnesses in Ibadan, South-Western Nigeria. Afr J Trad Compl Altern Med. 2015; 12(1): 9-16.
12. Djiéto-Lordon C, Dejean A, Gibernau M, Hossaert-McKey M, McKey D. Symbiotic mutualism with a community of opportunistic ants: protection, competition, and ant occupancy of the myrmecophyte *Barteria nigritana* (Passifloraceae). Acta Oecologica. 2004; 26(2): 109-116.
13. Bowler PG. Antibiotic resistance and biofilm tolerance: a combined threat in the treatment of chronic infections. J Wound Care. 2018; 27(5): 273-277.
14. Nuutila K, Grolman J, Yang L, Broomhead M, Lipsitz S, Onderdonk A, Mooney D, Eriksson E. Immediate treatment of burn wounds with high concentrations of topical antibiotics in an alginate hydrogel using a platform wound device. Adv Wound Care. 2020; 9(2): 48-60.
15. Atkin L. Understanding methods of wound debridement. Br J Nursing. 2014; 23(sup12): S10-S15.
16. Nnadi CO, Onah CM, Ugwu CL, Obonga WO. Wound Healing Potential of Combined Extracts of Stem Bark and Leaves of *Sphenocentrum jollyanum*: A Classical Factorial Design Model Approach in *Biologically Active Natural Products from Asia and Africa: A Selection of Topics*, Anna Capasso (Ed.). Bentham Science Publishers. Netherlands, 2021. 106-118 p.
17. Feldmann EG. The British Pharmaceutical Codex 1968.
18. Oppenheimer J, Nelson HS. Skin testing. Annals Allergy, Asthma Immunol. 2006; 96(2): S6-S12.
19. Obonga WO, Nnadi CO, Okafor SN, Omeje EO, Igbinnaduwa P, Okoli CO, Osadebe PO. Anti-inflammatory activity and accelerated stability studies of crude extract syrup of *Cannabis sativa*. Trop J Pharm Res. 2019; 18(2): 295-301.
20. Ghosh D, Mondal S, Ramakrishna K. A topical ointment formulation containing leaves extract of Aegialitis rotundifolia Roxb., accelerates excision, incision and burn wound healing in rats. Wound Med. 2019; 26(1): 100168.
21. Altavilla D, Saitta A, Cucinotta D, Galeano M, Deodato B, Colonna M, Torre V, Russo G, Sardella A, Urna G, Campo GM. Inhibition of lipid peroxidation restores impaired vascular endothelial growth factor expression and stimulates wound healing and angiogenesis in the genetically diabetic mouse. Diabetes. 2001; 50(3): 667-674.
22. Moores J. Vitamin C: A wound healing perspective. Brit J Comm Nursing. 2013; 18(Sup12): S6-S11.
23. Rezvanian M, Ng SF, Alavi T, Ahmad W. *In-vivo* evaluation of Alginate-Pectin hydrogel film loaded with Simvastatin for diabetic wound healing in Streptozotocin-induced diabetic rats. Int J Biol Macromol. 2021; 171(2): 308-319.
24. Nnadi CO, Okorie HN, Nwodo NJ. Evaluation of *In Vitro* Antiprotozoal and Cytotoxic Activities of Selected Medicinal Plants used in Nigerian Folk Medicine. Trop J Nat Prod Res. 2021; 5(4): 609-612.
25. Onoja SO, Nnadi CO, Udem SC, Anaga AO. Potential antidiabetic and antioxidant activities of a heliangolide sesquiterpene lactone isolated from *Helianthus annuus* L. leaves. Acta Pharm. 2020; 70(2): 215-216.
26. Hao Y, Zhao W, Zhang H, Zheng W, Zhou Q. Carboxymethyl chitosan-based hydrogels containing fibroblast growth factors for triggering diabetic wound healing. Carbohydrate Polymers, 2022; 287(6): 119336.
27. Chen K, Sivaraj D, Davitt MF, Leeolou MC, Henn D, Steele SR, Gurtner GC. Pullulan-Collagen hydrogel wound dressing promotes dermal remodelling and wound healing compared to commercially available collagen dressings. Wound Repair Regen. 2011; 30(3): 397-408.
28. Ali A, Garg P, Goyal R, Khan A, Negi P, Li X, Kulshrestha S. An efficient wound healing hydrogel based on a hydroalcoholic extract of *Moringa oleifera* seeds. South Afr J Bot. 2022: 145(3): 192-198.
29. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Allah Verdi A, Mofidian SMA, Lame Rad B. Induction of diabetes by streptozotocin in rats. Indian J Clin Biochem. 2017; 22(2): 60–64.
30. Ayoka TO, Nwachukwu N, Ene AC, Igwe CU, Ogara AL, Nnadi CO. *In-Vitro* Antioxidant Activity and Acute Toxicity of the Alkaloidal Constituents of *Zanthoxylum zanthoxyloides* Leaves. Trop J Nat Prod Res. 2022; 6(2): 276-280.