

Assessment of Wound Healing Properties of Medicinal Plants: The Case of *Berlinia confusa*

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Abstract

Objectives: *Berlinia confusa* is used as wound healing agent in folklore medicine. However, this has not been scientifically validated and there is dearth of information on its bioactive metabolites. The current research aimed at evaluating the wound healing, anti-oxidant and antimicrobial activities of the stem bark of the plant and track down some of its metabolites. **Methods:** The dermal excision model in Sprague Dawley rats was used for the *in vivo* wound healing activity of the 70% ethanol extract (formulated as 1% and 2% cream). The DPPH radical scavenging and *in-vivo* lipid peroxidation assays were used to assess the antioxidant activity whereas the agar well and broth-dilution assays were used for the antimicrobial activity evaluation. The PrestoBlue cell viability in keratinocytes and acute dermal toxicity assays were used for the safety assessment. **Results:** The 1% ^{w/w} extract showed over 70% wound surface closure from day 13-23 whereas the 2% ^{w/w} exhibited similar effect from day 15-23. The activity of the extract was comparable to 1% silver sulphadiazine (SSD) used as reference agent. The extract (1% ^{w/w}) also significantly reduced malondialdehyde (MDA) levels (455.2 ± 11.91) compared to the negative control group (739.8 ± 44.93) and was also superior to silver sulphadiazine (MDA level 548.4 ± 10.73). The extract again showed a considerable antimicrobial activity against pathogenic microorganisms with MIC's in the range of 5-10 mg/mL. The 2% extract did not show any skin irritation and were mildly toxic ($88.2 \pm 3.76\%$ viability) to keratinocytes. Purification of the extract yielded two (2) major known wound healing metabolites including betulinic acid and catechin. **Conclusion:** *Berlinia confusa* has demonstrated considerable wound healing *in vivo* and also mitigated healing modifying factors such as microbes and reactive oxygen species.

Keywords

wound healing, *Berlinia confusa*, Betulinic acid, Catechin, PrestoBlue, antioxidant, antimicrobial

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Introduction

Humanity has depended on nature for their fundamental needs including food, medicine, fragrances, clothes, and shelter throughout the course of time.¹ A variety of foods, medications, and dietary supplements originating from plants are used to modify the physiological functioning of the body.² Most of the world's healthcare systems still relies greatly on plant-based products and this is particularly evident in underdeveloped countries where over seventy percent of the population reportedly rely on plant-based medicines for their fundamental healthcare.³⁻⁵ In Ghana, the situation is not different, with almost every ethnic group relying on several local medicinal flora for holistic treatment of some common ailments.⁶ In these cultures, medicinal plants are used or commercialised as crude preparations including powders, teas, poultices, tinctures, and other herbal formulations.⁷ They continue to be the primary line of

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therapy in most illness, particularly among rural residents and some city dwellers in Ghana. According to Mintah SO et al,⁶ the availability of therapeutic herbs, their accessibility and affordability dominate the reasons for their preference as first line treatment. Medicinal plants have also contributed to the development of several conventional medicines due to the abundance of structurally diverse metabolites with significant biological activities.

Several molecules isolated from medicinal plants have also been repurposed for use in the prevention, diagnosis, and treatment of illnesses.^{8,9} It is reported that only a tenth of the global plant biodiversity has been explored for their therapeutic potential with the remaining ninety percent still unexploited.^{8,10} Considering this, research efforts need to be scaled up for the discovery of novel therapeutic leads which can be developed into potent drugs to mitigate the threat of emerging diseases.

One condition for which there is extremely limited drug discovery effort is the incidence of wounds. Wounds represent a major health burden (high mortality and morbidity) and drain on healthcare resources in the world including Africa and Ghana and often the psychological sequelae for patients and health impacts are underreported.^{11–13} The sequential and coordinated healing process is often interrupted by factors including foreign body, venous insufficiency, microbial burden, oxidative stress, and other systemic states that result in delay of the timely healing of the lesion, inadequate cosmetic results and a rise in patient morbidity.¹³ Oxidative stress resulting from excessive reactive oxygen species (ROS) production delay the healing process by impairing the function of keratinocyte and dermal fibroblast through proteolysis, degradation of the extracellular matrix and endothelial cell destruction.¹⁴ Microbial infection contributes to the chronicity of wounds by inhibiting endothelial tubule development and disorganised collagen deposition.¹⁵ Supportive innovative strategies that fight oxidative stress and infection and return wounds to normal healing trajectories is thus required. The care for wounds over the years, has seen some significant advancement; incorporating new biomaterials, modifying pH of wound environment and altering fluid balance.¹⁶ The use of pharmaceuticals agents including becapapmin as platelet-derived growth factor and antibiotics as frontline agents, silver dressings as wound decontaminants, human derived factors including collagenase, macrophages and mesenchymal stem cells as enhancers of wound bed appearance, facilitators of wound healing and secretors of pro-generative cytokines, respectively are documented.¹⁶ The use of medicinal honey as anti-infective and pro-healing agent is also known.¹⁶ Due to the cost of some conventional medications, coupled with limited medical professionals with expertise in dermatological conditions especially in underdeveloped countries and under-resourced health facilities, people resort to traditional medicine for the management of wounds.^{17,18} Traditional remedies for skin conditions and herbs that promote wound healing are readily available, affordable, and accessible.^{19,20} Most of these medicinal plants have been used over centuries and are assessed to be safer than

some isolated active compounds.²¹ Ethnopharmacological review have reported several wound healing medicinal plants such as *Aloe vera*, *Clausena anisata*, *Clerodendron splendens*, *Combretum mucronatum*, *Kigelia africana*, *Hillieria latifolia*, *Justicia flava*, *Paullinia pinnata*, *Lannea welwitschii*, *Alchornea cordifolia*, *Balanites aegyptiaca*, *Acacia kirkii* and *Berlinia confusa*.^{6,22,23}

Berlinia confusa is one of the many medicinal plants employed in the management of wounds and other conditions including gastrointestinal (GI) disorders, snake bites and baits for rodents in Ghana and Africa.^{23,24} The antimicrobial activity of the ethyl acetate and hexane extracts of the leaf, root and stem of the plant has been reported by Lasisi AA and Adesomoju AA.²⁴ The anthelmintic activity of the methanol stem bark extract against *Taenia solium* and *Fasciola gigantica* worms as well as cytotoxic effect against brine shrimp larvae have also been reported by Lasisi A and Idowu O.²⁵ The presence of some bioactive constituents including β -sitosterol β -D-glucoside, betulinic acid, 1-O-tetracosanoil-sn-1-glycerol, 1-O-(13-methyltetradecanoil)-sn-glycerol, 1-O-pentadecanoil-sn-glycerol and 1-O-docosanoil-sn-glyceride is documented.^{24,25}

However, there is no scientific evidence to support the use of this plant as a wound healing agent. In continuation of research efforts to scientifically validate the efficacy of medicinal plant remedies, this study is designed to investigate the wound healing potential of *Berlinia confusa* and identify some bioactive constituents.

Materials and Methods

Chemicals and Reagents

All analytical grade solvents (organic); acetone, chloroform (CHCl₃), ethyl acetate (EtOAc), methanol (MeOH), petroleum ether (Pet. ether) and ethanol used in the studies were obtained from Fisher Scientific (Loughborough, UK). Vanillin, anisaldehyde, sulphuric acid, sodium hydroxide, trichloroacetic acid were obtained from BDH laboratory Ltd (Poole, England). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid, were obtained from Sigma Aldrich (St. Louis, MO, USA). Silica gel (63–200 μ m, 70–230 mesh) was acquired from Sigma Aldrich (Spruce Street, St. Louis, MO 63178 USA). Sephadex LH-20 beads were obtained from Amersham Biosciences (Buckinghamshire, UK). Non-calibrated capillary tubes (for TLC analysis) were acquired from Sigma Aldrich (Spruce Street, St. Louis, MO 63178 USA). Precoated aluminium TLC silica gel 60 F254 plates (20 $\times$ 20 cm) used for analytical TLC was obtained from Merck, Darmstadt, Germany. Ciprofloxacin and fluconazole, used as reference drugs were obtained from UNICHEM industries Ltd, Kaneshie-Accra, Ghana.

General Procedures

The absorbance-based assays were measured using the Jenway UV-VIS spectrophotometer (Germany). Heraeus Biofuge

Primo Centrifuge (Hamburg, Germany) was employed in assays that required the use of a centrifuge. The infrared (IR) spectra of compounds were determined by the Perkin Elmer Two FT-IR spectrometer (London, UK) scanned with a resolution power of 4, 24 scans and at a speed of 1 cm^{-1} . The Stuart SMP3 digital melting point apparatus (Bibby Scientific Ltd Stone, Staffordshire, ST15 0SA, UK) was used in determining the melting point of compounds. Extracts were concentrated to small volumes using Buchi (Switzerland) and SEO5 rotary evaporator (Australia). The NMR spectra of isolates were captured using a Bruker AscendTM 500 MHz NMR spectrophotometer (Germany) with tetramethylsilane as an internal reference. Chemical shifts (δ) were represented in parts per million (ppm). The high molecular mass data of isolates was recorded on Waters SYNAPT XS with ACQUITY H-Class PLUS UPLC mass spectrometer (Waters Corporation, Milford, MA, USA) using electrospray ionisation (HR-ESI LC/MS) in positive ion mode. The isolates were prepared in methanol (HPLC grade) and the mass spectra relating to their mass to charge (m/z) recorded. A combination of methods, including mass spectrometry, nuclear magnetic resonance and infrared (IR) and melting point were used to elucidate the structure of the compounds established.

Plant Collection and Authentication

The fresh stem bark of *B. confusa* was collected from Mpraeso in the Eastern Region of Ghana in April 2020 and authenticated by Mr Clifford Asare of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST). Specimen with voucher number KNUST/2020/SB/21 was deposited at the herbarium of the Faculty of Pharmacy and Pharmaceutical sciences, KNUST, Kumasi.

Processing and Extraction of Plant Material

The stem bark of *B. confusa* was washed to clear off all debris under running water. It was then cut into smaller sizes and sun-dried for a period of 96 h. The sample was then pulverised into coarse powder. An amount of 1.8 kg of the powdered stem bark of *B. confusa* was weighed and Soxhlet-extracted for 24 h using 70% ethanol (3.5 L) until the material was exhausted. The filtrate obtained was concentrated under vacuum using the rotary evaporator at 50°C and dried in a desiccator to afford a dried brownish-red extract of 84 g ($4.67\%\text{ w/w}$) labelled as BC.

Chromatographic Separation of Compounds from *B. confusa*

Thirty grams (30 g) of the 70% hydroethanolic extract of the stem bark of *B. confusa* (BC) was subjected to column chromatography using silica and eluted gradiently with 100% petroleum ether, then, increasing the solvent polarity by 10% increments with ethyl acetate. This was followed by 5% increments with

methanol up to 20% methanol in the eluent. On the basis of their TLC profiles the eluates were bulked into three (3) sub-fractions: BCA, BCB and BCD. The rest of the gummy 30 g extract got stuck onto the column. Sub-fraction BCA (450 mg) was column chromatographed on silica eluting with a mobile phase composition of petroleum ether and ethyl acetate, with a linear gradient from 10%-30% ethyl acetate to afford isolate C1 (50 mg) as a white crystalline solid. Sub-fraction BCB (530 mg) was column chromatographed using Sephadex LH-20 eluting isocratically with CHCl_3 : MeOH (50:50) to clean of the pigmented extract. The depigmented fraction (350 mg) was further purified using column chromatography and eluted on silica with petroleum ether and ethyl acetate mixtures, with a linear gradient from 30-50% ethyl acetate in 20 mL aliquots to afford isolate C2 as a light brown crystalline solid (31.8 mg). The optical rotation of isolate C2 was obtained in methanol (MeOH) at a concentration of 0.56 g/mL at 20°C , 589 nm on a Perkin-Elmer Model 341 polarimeter (SpectraLab Scientific Inc., Canada). The specific optical rotation of isolate C2 was determined to be $+26^\circ$. Isolates C1 and C2 were identified as betulinic acid⁴⁹ and (+)-catechin,^{26,50} respectively (Supplementary data; Figure S5 and S6).

Biological Activities of Extracts

Wound Healing Assay. The wound healing activity of the hydroethanolic (70%) extract of the stem bark of *B. confusa* (BC) was evaluated using formulated creams of the extract at concentrations of 1% and 2% with 1% silver sulphadiazine cream as the reference drug. A blank emulsifying cream and normal saline served as the negative controls for the dermal excision wound model deployed in this research.²⁷

Cream Formulation. A topical aqueous cream weighing 100 g each containing 1% and 2% w/w respectively of the 70% ethanol stem bark extract of BC were prepared using a scaled formula for simple ointment according to the British Pharmacopoeia²⁸ (Table S1 and S2). In formulating the respective batches of creams, a separate mixture of emulsifying ointment and another separate mixture of BC and water was prepared. Both mixtures were separately raised to a similar temperature and later added together in a glass beaker and homogenized to obtain an aqueous cream. It was topped up to the required weight using distilled water at the same temperature, stirred and kept cooling to room temperature.

Experimental Animals. Sprague – Dawley rats (8-10 weeks old, 120-160 g) were obtained from the University of Ghana animal house, Legon-Accra, Ghana. They were put in standard aluminium laboratory cages ($34 \times 47 \times 18\text{ cm}$) at a population density of 5 in the Department of Pharmacology's animal house, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. Fine wood shavings served as bedding material and were maintained in a 12-h light and dark cycle,

temperature of 25 ± 2 °C for seven days to get accustomed to the laboratory conditions. They were fed with commercial pellets from Agricare Limited, Kumasi and water *ad libitum* throughout the experiment. All the rats were handled in accordance with the Animal Care and Use Committee's Guidelines for the care and use of laboratory animals²⁹ and subsequently approved by KNUST ethics committee (PCOL/ETH/07022020).

Acute Dermal Toxicity. Acute dermal toxicity of the formulated creams was evaluated in accordance with OECD No. 402 criteria.³⁰ The rats were administered topical dose (2%) of BC on the shaved dorsal region and monitored for 14 days.

Dermal Excision Wound Model. The wound healing activity of the formulated cream of the plant extract was evaluated by the excision wound model as described by Dwivedi D et al.³¹ The rats were randomly put into 5 groups at a population density of 5 animals, 2 test groups (BC) at concentrations of (1% and 2% creams, one reference control (1% Silver sulphadiazine) and two negative controls (emulsifying cream and normal saline). Pentobarbitone solution (40 mg/kg) was used to anaesthetize the rats and the dorsal fur between the upper part of the front paw and below the neck area shaved with a razor blade. Alcohol (70%) was used to rub the shaved area and an excision wound of diameter 20 mm and depth 5 mm inflicted using toothed forceps and pointed scissors. The wounds were cleaned with normal saline, dressed daily with the test samples and left open. The wound diameter was measured every other day with a ruler for a period of 28 days. The wound area was then calculated using the formula; wound area = $A = 4\pi r^2$, where r is the radius of the wound. The percentage (%) wound closure was deduced from the formula, $WSA (day 1) - WSA (day x) / WSA (day 1) \times 100$, where 'x' represents day of treatment, WSA-wound surface area.

Histopathological Studies. Samples of wound tissues were excised on the 28th day post wounding from the various groups and immediately fixed in 10% buffered formalin (Sigma-Aldrich, United States). In a graded series of ethanol, the fixed tissues were dehydrated after which the paraffin wax-embedded tissues were allowed to solidify and sectioned to 5 mm thickness with a Leica rm2235 rotary microtome (Leica Biosystems, Deer Park, IL 60010 United States). After the paraffin was removed, the tissues were mounted on a transparent glass slide and stained with hematoxylin and eosin (Sigma-Aldrich, St. Louis, USA).³² Microscopic examination of the various tissues was then made using Zeiss Imager Z2 light microscope fitted with an Axiocam camera. (Cambridge, UK) for surface area neovascularization, epithelial layer staining pattern and deposition of collagen.

PrestoBlue Cell Viability Assay

Reagents and Cell Culture. PrestoBlue cell viability reagent (HS) was purchased from Life TechnologiesTM (USA).

Human immortalised keratinocyte (HaCaT) cells were purchased from Cell Line Service (Eppelheim, Germany). These cells were maintained in Dulbecco's modified Eagle medium (DMEM, Gibco, New York, USA) containing 10% fetal calf serum (FCS), supplemented with 1% penicillin-streptomycin (Gibco, USA), and incubated at 37 °C with humidified atmosphere of 5% CO₂. Confluent cells were detached using a trypsin-EDTA solution and counted in a C-Chip DHC-N01 haemocytometer (Cambridge Bioscience, UK).

Cell Viability Assay. The cell viability of the 70% ethanol extract of *B. Confusa* (BC) was performed using the PrestoBlue reagent assay according to the manufacturer's instructions. In brief, HaCaT cells were seeded at 7000 cells/well in a 96 well-plate. The cells were then treated with different concentrations (200-12.5 µg/mL) of BC for 24 h. PrestoBlue reagentTM (HS) (600 µL) was then added directly to the drug-treated cultured cells after 24 h and incubated at 37 °C for 10 min, 1 h and 2 h. The fluorescence was then measured at 560 nm and 590 nm using the SpectraMax M2 microplate reader (Molecular Devices, San Jose, USA). The viability of cells was then deduced and compared with that of the control group.³³

Antioxidant Assays

Lipid Peroxidation. The method described by Heath RL and Packer L.³⁴ was used to determine the degree of MDA formation. In a test tube, 3 mL of the mixture (3 mL 20% trichloroacetic acid with 0.5% thiobarbituric acid) was added to 1 mL of homogenate from experimental animals. The resulting mixture was heated for thirty minutes at 95 °C, allowed to cool immediately, then centrifuged at 5000Xg for ten minutes. The absorbance was then measured at 532 nm and 600 nm. The molar extinction coefficient of MDA-TBA adduct, $155 \text{ mM}^{-1} \text{ cm}^{-1}$, was used to determine the levels of MDA from the equation

$$\text{nmol MDA / mg protein} = \frac{\text{Absorbance}_{532 \text{ nm}} - \text{Absorbance}_{600 \text{ nm}}}{155 \times \text{total protein}} \times 10^6$$

DPPH Free Radical Scavenging Assay. The extracts' ability to scavenge DPPH was measured in accordance with the methodology reported by Govindarajan R.³⁵ In a test tube containing 1 mL of the extract (2000-62.5 g/mL in methanol), 3 mL of DPPH solution (20 mg/L) was added. After incubation at 25 °C for 30 min, the absorbance was measured at 517 nm. The blank was made up of three millilitres (mL) of DPPH solution and one millilitre (mL) of 100% methanol, which was then incubated at 25 °C for 30 min. Gallic acid, as a reference drug was tested at concentrations of 100-0.78 µg/mL. Triplicate test samples were utilized for the analysis. The extract's percentage (%) DPPH scavenging effect (% of control) was calculated as follows:

$$\% \text{ DPPH scavenging effects} = (W_c - W_t) / W_c \times 100$$

where W_c = Absorbance of the control, W_t = Absorbance of the test drug

From the percentage scavenging activity, the concentration (IC_{50}) needed to reduce the absorbance of the original DPPH concentration by 50% was then deduced using GraphPad prism Software.

Antimicrobial Assays

Organisms and media. The microorganisms used for the assay were Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* NCTC 12697), Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *salmonella typhi* ATCC 19430, *Klebsiella pneumonia* NCTC 1368, *Proteus vulgaris* NCTC 4175, *Escherichia coli* ATCC 25922) and *Candida albicans* ATCC 10231. These organisms were obtained from the Department of Microbiology, FPPS, KNUST, Kumasi. The organisms (0.1 mL each) were inoculated into a 10 mL sterilized nutrient broth and incubated at 37 °C for 24 h. A working suspension was prepared by serial dilutions of cultures in sterile normal saline to achieve a suspension of equal turbidity with 0.5 Mc Farland standards. This dilution contained approximately 10^5 CFU/mL. The growth media used for the antimicrobial assays were nutrient agar (Mueller-Hinton agar powder, Oxoid CM0337) and nutrient broth (Sigma S-4681). They were prepared according to the manufacturer's instructions.

Agar Well Diffusion Assay. Sterilized petri dishes containing about 20 mL nutrient agar each were inoculated with microorganisms (0.1 mL) and allowed to solidify. Four equidistant holes were aseptically created using a sterile cork borer of 8.75 mm in diameter. Two-thirds (2/3) of the holes were filled with the stem bark extract (20, 10, 5 mg/mL) and the standard drugs; ciprofloxacin (1 mg/mL) and fluconazole (1 mg/mL) for bacteria and fungus respectively. The petri dishes were allowed to stand for about 30 min to allow adequate diffusion to take place after which they were incubated at 37 °C for 24 h.³⁶ The clear area formed around each well/hole was measured; the measurement made from the center of the hole to the circumference of the clear zone. The diameter of the well (cork borer) was then subtracted from the measured area to determine the zone of inhibition.

Broth Dilution Assay. The broth microdilution assay using the 96-well plate was used to determine the MIC of the hydroethanolic stem bark extract of the plant (0.3125-20 mg/mL), with ciprofloxacin (0.0031-1.0 mg/mL) and fluconazole (0.0031-1.0 mg/mL) as controls according to Balouiri M et al.,³⁷ with slight modifications. The wells of the microplate were filled with 125 μ L of double strength broth media, (25 μ L) of microbial inoculums, (100 μ L) of the stem bark extracts and sterile water at varying volumes. The plates were incubated at 37 °C for 24 h. Sterile water was used as the negative control. Each test was carried out in triplicate. 20 μ L MTT (1% w/v) was added to each well after the 24-h incubation period and allowed to stand for 15 min. A blue colour indicates

microbial growth while a brownish yellow colour shows no growth.

Statistical Analysis

The experimental results for the DPPH scavenging and cell viability assays were recorded as the mean $\pm$ standard deviation (SD). Results were recorded as the mean $\pm$ standard error of the mean (SEM) for the dermal excision and lipid peroxidation assays. Significant differences between the treatment groups and the negative control were determined by the One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. $P \leq 0.05$ was considered statistically significant. GraphPad for Windows version 6 (GraphPad Prism Software, San Diego, USA) was used for the analyses.

Results

Acute Dermal Toxicity

In the acute dermal toxicity tests, the highest dose (2% w/w) of *B. confusa* stem bark creams did not result in skin irritation, physical changes, or any other form of harmful effect on the skin.

Dermal Excision Wound Healing Activity

There was a progressive reduction in wound area for all tested concentrations of the plant extract over the period of study, however, this reduction was insignificant ($P > .05$) in comparison to the normal saline (control) treated group. BC (1% w/w) showed over 70% wound surface closure from day 13-23 while BC (2% w/w) exhibited similar effect from day 15-23. This observation was comparable to the effect exhibited by 1% SSD. However, total healing (100% wound contraction) was recorded on day 19 for SSD while this effect was realized on day 23 for both concentrations of the plant extract. The order of wound healing activity was as follows: 1% SSD > BC (1% w/w) > BC (2% w/w). These effects were, however, markedly lower in the blank cream and normal saline treated groups (Figure 1; Table S3).

Histopathological Studies

The 1% cream of *B. confusa* extract showed considerable healing ability with appreciable scar formation with evident hyperplastic epithelium. The underlying thin/thick papillary and reticular dermis showed areas of collagen formation with diffused blood vessels in the stroma. Moderate inflammatory cell infiltration with atrophic skin appendages (hair follicles, sebaceous glands) were also observed (Figure 2A). The highest concentration (2% cream) of *B. confusa* extract showed moderately good healing ability with thin non-keratinised squamous epithelial proliferation. The underlying dermis showed mild acute inflammatory cell infiltration and appreciable angiogenesis with morphologically skin appendages in the stroma (Figure 2B). 1%

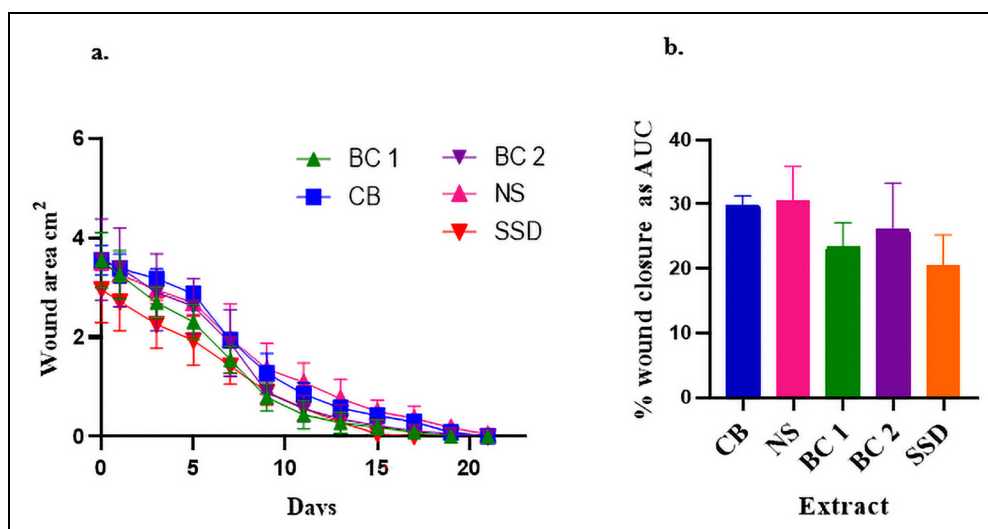


Figure 1. The effect of BC (1 & 2%) cream, silver sulphadiazine (1% SSD), cream base CB) and normal saline (NS) on the (a). wound surface area and (b). % wound area expressed as AUC in the dermal excision wound model in rats. Each value is expressed as mean $\pm$ SEM ($n = 5$); no significant differences between treatments and control; BC1/BC2 – 1% and 2% *B. confusa* cream; SSD – 1% Silver sulphadiazine, CB-Blank cream, NS-Normal saline.

SSD treated wound tissues showed evidence of hyperplastic epithelium, granulation tissue formation, mild infiltration of inflammatory cells, angiogenesis and moderate collagen activity (Figure 2C). Cream base treated tissues showed mild healing ability with persistent inflammation and mild collagen deposition at wound edges (Figure 2D). Normal saline/untreated wound tissues showed persistent inflammation and moderate collagen deposition as well as morphologically skin appendages in the dermis (Figure 2E).

Lipid Peroxidation and DPPH Scavenging Effects

In the lipid peroxidation assay, the normal saline-treated group (control) exhibited a significant increase in MDA levels (739.8 ± 44.93) compared to the extract/drug treated groups. *B. confusa* (BC-1) extract also significantly ($F_{(8, 18)} = 6.047$) reduced MDA levels (455.2 ± 11.91) compared to the control group. Silver sulphadiazine (SSD) treated group showed a decreased MDA level (548.4 ± 10.73), however, this reduction was not significant ($F_{(8, 18)} = 6.047$, $P = 0.0775$) compared to the control group (Table S4). The 70% ethanolic extract of *B. confusa* and the reference drug, gallic acid showed varying DPPH scavenging activity with an IC_{50} of 12.28 ± 0.58 and 2.06 ± 0.12 μ g/mL, respectively. The order of decreasing activity depicted by IC_{50} is gallic acid > 70% ethanol *B. confusa* (Table S4).

Effect of Plant Extract (BC) on Cell Viability in Keratinocytes (HaCaT)

Human keratinocytes (HaCaT) were treated with various concentrations (200–12.5 μ g/mL) of plant extract (BC). After a 10 min incubation period, Extract BC exhibited mild toxicity,

recording 88.2 ± 3.76 percentage viability at the highest concentration (Figure 3). Similar toxicity trends were observed after 1 and 2 h incubation (Figure 3).

Antimicrobial Activity

Agar Well Diffusion and Broth-Dilution Assays. The hydroethanolic extract of *B. confusa* (BC) exhibited varying inhibition/antimicrobial activity against the selected microbial strains (Table S5). The extract of BC at the highest concentration (20 mg/mL) demonstrated varying inhibition against all microorganisms tested with mean zone inhibitions in the range of 8.0–16.5 mm. At the lowest concentration (5 mg/mL), there was inhibition of all organisms except *E. faecalis* (Table S5). The MIC of BC against the microorganisms tested was in the range of (5–10) mg/mL (Table 1).

Discussion

Herbal medicines play a significant role in the provision of primary healthcare in impoverished nations on a global scale. Providing scientific evidence for their application would not only encourage or improve their potential acceptance by the public healthcare systems but also guarantee consumer safety. Therefore, to encourage evidence-based practice, the current study assessed the wound healing activity of *Berlinia confusa* extract and identified some of its components.

Wound Healing Activity and Toxicity of *B. confusa*

In the dermal excision wound healing activity, the 1% cream of *B. confusa* demonstrated a superior activity than the 2% cream as

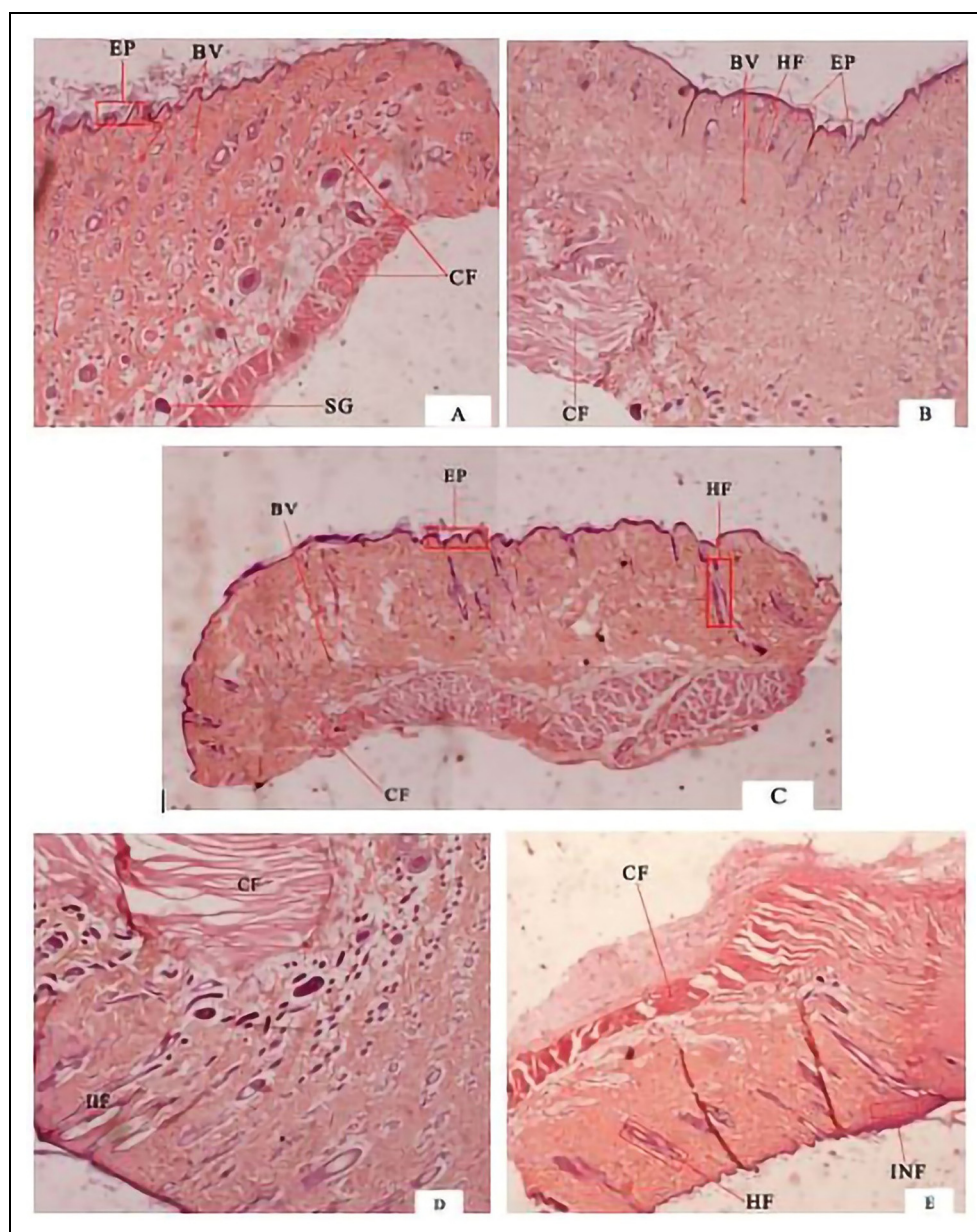


Figure 2. Photomicrograph of histopathological sections of wound tissues of rats (stained with haematoxylin and eosin, $\times 40$ magnification). Histopathological sections of wound tissues treated with 1% cream (A); 2% cream (B); 1% silver sulphadiazine (C); cream base (D); Key: EP-Epithelium, BV-Blood vessel, CF-Collagen fibre, SG-Sebaceous gland, HF-Hair follicle, INF-Inflammatory cell infiltration.

evidenced by the percentage contraction within the period of the experiment (Figure 1). This was supported by histopathological findings where scanty cell infiltrates, thin epithelialization, excellent collagen formation and atrophic skin appendages as well as high density blood capillaries, which are important markers for wound healing were observed in tissues from the wound bed of the 1% cream treated animals (Figure 2A). These cells were present in moderation in scar tissues from the 2% cream treated animals. The activity shown by the reference drug, silver sulphadiazine, was comparable to that of the 1% BC containing cream with similar

histopathological profile. This study has shown for the first time that *B. confusa* shows wound healing activity as suggested by folklore medicine and lower concentrations could be explored further to accelerate the wound healing process. Purification of the extract afforded betulinic acid and catechin as constituents of the stem bark (Supplementary data). The body of evidence suggests that these compounds could have contributed to the wound healing properties. For example, Li J³⁸ reported that betulinic acid increases the longevity of skin flaps with random patterns by stimulating angiogenesis, inhibiting apoptosis, and reducing oxidative stress. Cho SA³⁹ also

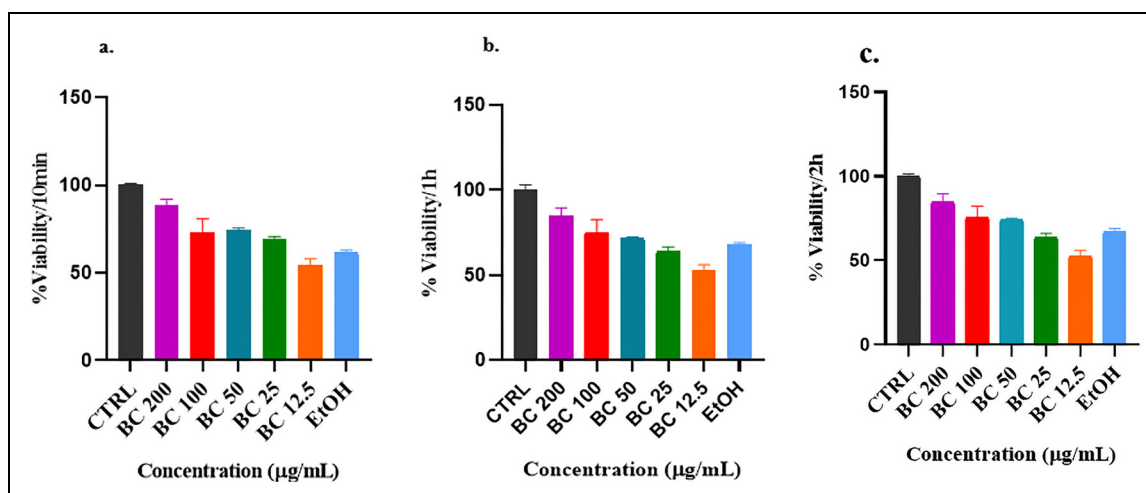


Figure 3. Effects of various concentrations of extract (BC) on cell viability in human keratinocytes (HaCaT) after (a). 10 min (b). 1 h and (c). (2 h). The percentage cell viability was calculated as the percentage of control (non-treatment). Values are plotted as Mean $\pm$ SEM.

Table 1. Minimum Inhibitory Concentration (MIC) of the Hydroethanolic Extract of *B. confusa*.

Microorganisms	MIC (mg/mL)	
	<i>B. confusa</i>	Reference drug
<i>Klebsiella pneumoniae</i>	10	0.25
<i>Enterococcus faecalis</i>	10	0.25
<i>Proteus vulgaris</i>	5	0.25
<i>Salmonella typhi</i>	5	0.5
<i>Escherichia coli</i>	10	0.25
<i>Pseudomonas aeruginosa</i>	10	0.25
<i>Staphylococcus aureus</i>	10	0.25
* <i>Candida albicans</i>	10	1.0*

Reference drug: Ciprofloxacin, * Fluconazole.

reported that betulinic acid enhances angiogenesis and re-epithelialization of wound tissues by stimulating the synthesis of collagen. The stimulatory activity of betulinic acid on the synthesis of collagen in human fibroblasts has also been reported.⁴⁰ Catechin has been reported to enhance wound healing by stabilizing type 1 collagen, the main structural protein of skin, bone and tendon.⁴¹ Again, it has been reported to demonstrate improved anti-inflammatory activity in human primary acute and chronic wound derived fibroblasts via decreasing the expression of TNF- α and TGF- β .⁴² Although, these metabolites were not tested for healing, the evidence so far points to their possible contribution to the wound healing activity of *B. confusa*.

There were no observable skin irritations, physical changes or any other deleterious effect following the administration of creams from extract of *B. confusa* in the acute dermal toxicity studies. This signifies that the tested concentration of the cream extract was safe under the laboratory conditions. At the cellular level, however, extracts from *B. confusa* showed mild toxicity to keratinocytes (80% viability) as shown in the Presto blue cell viability studies (Figure 3). Against this

background, it will be interesting to elucidate the actual mechanism of wound healing exhibited by *B. confusa* using *in vitro* models.

Antimicrobial and Antioxidant Activities

Despite the weak wound healing activity observed for the crude extract, there was no pus or bad odour from the wound during the period of treatment. This is suggestive of possible microbial decontamination and healing. Among the many intrinsic and extrinsic variables that might hinder wound healing, infection is the most common and, potentially, the most detrimental.⁴³ Microbial contamination of wounds delays the healing process leading to chronic wounds. The 70% ethanol extract of *B. confusa* showed broad spectrum anti-infective activity against the tested microbial strains (Table 1; Table S5). The antimicrobial activity of *B. confusa* is corroborated by reports of Lasisi and Adesomoju.²⁴ Wound dressing with *B. confusa* could therefore decontaminate and accelerate the healing process.

Reactive oxygen species (ROS) have also been implicated in delayed healing via endothelial cell destruction and disruption in timely reepithelialization of the wound.¹⁴ In the present study, lipid peroxidation products such as malondialdehyde accumulated in the stressed control animals. Saline-treated animals relative to the *B. confusa* treated rats presented with significantly higher levels of MDA, a positive indicator of oxidative stress. The levels of MDA were markedly reduced in all test groups (Table S4). In the DPPH antioxidant activity, *B. confusa* extract demonstrated a considerable radical scavenging activity (Table S4). This is an indication that possible damaging processes such as lipid peroxidation and free radical production were inhibited by *B. confusa* extract. Thus, the antioxidant capacity of this plant extract may play a key role in mitigating the damaging effects of excess free radicals and enhance the wound healing process. The isolated compounds may have played a

critical role in the antioxidant activities of the extract. Xie W et al⁴⁴ in validating the wound healing activity of betulinic acid, demonstrated that the compound reduces oxidative stress via the upregulation of endothelial nitric oxide synthase activity, which may have protective effects on blood vessels. Additionally, it activates the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway, which in turn activates antioxidant genes like glutathione peroxidase and superoxide dismutase 2.⁴⁵ Its ability to reduce inflammation by inhibiting the nuclear factor κ B (NF κ B) signalling pathway has also been reported.^{46,47} Due to their well-known anti-inflammatory, angiogenesis, re-epithelialization, and antioxidant actions, flavonoids have been shown in numerous studies to have wound-healing capabilities and this has been attributed to the hydroxylation effect on their basic structure.⁴⁶ Catechin has been reported to demonstrate improved anti-inflammatory activity in human primary acute and chronic wound derived fibroblasts via decreasing the expression of TNF- α and TGF- β .⁴⁸ Its anti-inflammatory and antioxidant activities have also been documented.⁴⁴ Thus, the contribution of these compounds to the biological activities of *Berlinia confusa* is not in doubt. Future studies could explore the molecular wound healing mechanism and compounds in the polar gummy fraction.

Limitations of the Study

Although the study demonstrated the wound healing activity of *B. confusa*, its mechanism of action was not evaluated. The isolated compounds were also not assessed for wound healing actions. Again, the amount of each constituent in the active extract was not determined. Additionally, within the limitation of resource constraints, phytoconstituents from the polar gummy fraction of the extract were not explored. The mechanism of wound healing activity of the extract and compounds should be considered in future collaborative research. Unlocking the compounds in the polar gummy fraction could also prove vital in the quest to discover novel wound healing agents.

Conclusion

This study has demonstrated that the hydro-ethanolic extract of *B. confusa* possesses considerable wound healing activity *in vivo* and also mitigated healing modifying factors such as microbes and reactive oxygen species. Phytochemical investigation of the extract of *B. confusa* resulted in the isolation and characterisation of betulinic acid and catechin and these have shown to partly contribute to the wound healing activity of the plant extract. The present studies have also shown that topical application creams containing extracts of *B. confusa* does not have any untoward effect on the skin.

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Conceptualization: SA, IKA, EE, CH. Data curation: SA, IKA, CH, JG, OD, AED. Formal analysis: SA, IKA, EE, CH, JG, OD, AED. Funding acquisition: SA, IKA, CH. Investigation: SA, IKA, EE, CH, JG, OD, EAK. Methodology: SA, IKA, EE, CH, JG, OD, AED, EAK. Project administration: SA, IKA, EE, CH, JG, OD. Resources: SA, IKA, EE, CH, JG, OD, EAK. Supervision: IKA, CH, EE, JG. Validation: SA, IKA, EE, CH, JG, OD, AED, EAK. Writing – original draft: SA, IKA, EE, EAK. Writing – review and editing: SA, IKA, EE, CH, JG, OD, AED, EAK.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable

Data Availability

Data included in article/supplementary material/referenced in article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

This study was approved by the KNUST ethics committee of experimental Animals, Kumasi, Ghana

Ethical Considerations

Protocols for animal experiments were approved by the Animal Experimental Ethics Committee of the KNUST (Approval no. PCOL/ETH/07022020) on November 10, 2020, in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Statement of Human and Animal Rights

All the rats were handled in accordance with the Animal Care and Use Committee's Guidelines for the care and use of laboratory animals and subsequently approved by KNUST ethics committee with number (PCOL/ETH/07022020).

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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Supplemental Material

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References

- Dar RA, Shahnawaz M, Qazi PH. General overview of medicinal plants: a review. *J Phytopharmacol*. 2017;6(6):349-351. doi:10.31254/phyto.2017.6608.
- Sen T, Samanta SK. Medicinal plants, human health and biodiversity: a broad review. *Adv Biochem Eng Biotechnol*. 2015;147:59-110. doi:10.1007/10_2014_273.
- WHO. *WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems*. World Health Organization; 2004.
- Tyavambiza C, Dube P, Goboza M, Meyer S, Madiehe AM, Meyer M. Wound healing activities and potential of selected African medicinal plants and their synthesized biogenic nanoparticles. *Plants (Basel)*. 2021;10(12):2635. Published 2021 Nov 30. doi:10.3390/plants10122635
- Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules*. 2016;21(5):559. doi:10.3390/molecules21050559.
- Mintah SO, Archer MA, Asafo-Agyei T, et al. Medicinal plant use in Ghana: advancement and challenges. *Am J Plant Sci*. 2022;13(3):316-358. doi:10.4236/ajps.2022.133020.
- Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. *Life Sci*. 2005;78(5):431-441. doi:10.1016/j.lfs.2005.09.012.
- Priya S, Satheshkumar PK. Natural products from plants: recent developments in phytochemicals, phytopharmaceuticals, and plant-based nutraceuticals as anticancer agents. In: Prakash B, ed. *Functional and Preservative Properties of Phytochemicals*, 1st ed. Academic Press; 2020:145-163. doi:10.1016/b978-0-12-818593-3.00005-1
- Adjei S, Amponsah IK, Bekoe SO, et al. Fruits of *Vitex doniana* sweet: toxicity profile, anti-inflammatory and antioxidant activities, and quantification of one of its bioactive constituents oleanolic acid. *Heliyon*. 2021;7(9):e07910. doi:10.1016/j.heliyon.2021.e07910.
- Dias DA, Urban S, Roessner UA. Historical overview of natural products in drug discovery. *Metabolites*. 2012;16(2):303-336. doi:10.3390/metabo2020303.
- Janssen H, Janssen I, Cooper P, et al. Antimicrobial-Resistant Bacteria in infected wounds, Ghana, 2014¹. *Emerg Infect Dis*. 2018;24(5):916-919. doi:10.3201/eid2405.171506.
- Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensel A. An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *J Ethnopharmacol*. 2009;125(3):393-403. doi:10.1016/j.jep.2009.07.024.
- Singh S, Young A, McNaught C-E. The physiology of wound healing. *Surgery (Oxford)*. 2017;35(9):473-477. doi:10.1016/j.mpsur.2017.06.004.
- Wlaschek M, Scharffetter-Kochanek K. Oxidative stress in chronic venous leg ulcers. *Wound Repair and Regen*. 2005;13(5):452-461. doi:10.1111/j.1067-1927.2005.00065.x.
- Wynn MO. The impact of infection on the four stages of acute wound healing: an overview. *Wounds*. 2021;17(2):26-32.
- Patenall BL, Carter KA, Ramsey MR. Kick-starting wound healing: a review of pro-healing drugs. *Int J Mol Sci*. 2024;25(2):1304. doi.org/10.3390/ijms25021304.
- Mahé A, Faye O, N'Diaye HT, et al. Definition of an algorithm for the management of common skin diseases at primary health care level in sub-Saharan Africa. *Trans R Soc Trop Med Hyg*. 2005;99(1):39-47. doi:10.1016/j.trstmh.2004.03.008.
- Builders PF, Builders MI. Wound care: traditional African medicine approach. In: Fonseca CJV, ed. *Worldwide Wound Healing - Innovation in Natural and Conventional Methods*. InTech; BoD—Books on Demand; 2016:1-26. doi:10.5772/65521.
- Jeevan Ram A, Bhakshu LM, Venkata Raju RR. In vitro antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. *J Ethnopharmacol*. 2004;90(2-3):353-357. doi:10.1016/j.jep.2003.10.013.
- Njoroge GN, Bussmann RW. Ethnotherapeutic management of skin diseases among the Kikuyus of Central Kenya. *J Ethnopharmacol*. 2007;111(2):303-307. doi:10.1016/j.jep.2006.11.025.
- Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect*. 2001;109(suppl1):69-75. <https://doi.org/10.1289/ehp.01109s16>
- Agyare C, Boakye YD, Bekoe EO, Hensel A, Dapaah SO, Appiah T. Review: African medicinal plants with wound healing properties. *J Ethnopharmacol*. 2016;177:85-100. doi:10.1016/j.jep.2015.11.008.
- Koné WM, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in north Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *J Ethnopharmacol*. 2004;93(1):43-49. doi:10.1016/j.jep.2004.03.006.
- Lasisi AA, Adesomoju AA. New monoacylglycerol and antimicrobial constituents from the stem barks of *Berlinia confusa*. *J Herbs, Spices Med Plants*. 2012;18(2):111-131. doi:10.1080/10496475.2011.645269.
- Lasisi A, Idowu O. In vitro anthelmintic and cytotoxic activities of extracts from the stem barks of *Berlinia confusa* (C. Hoyle) and identification of its active constituents. *J Saudi Chem Soc*. 2014;18(6):939-944. <https://doi.org/10.1016/j.jscs.2011.11.016>.
- Seto R, Nakamura H, Nanjo F, Hara Y. Preparation of epimers of tea catechins by heat treatment. *Biosci Biotechnol Biochem*. 1997;61(9):1434-1439. DOI:10.1271/bbb.61.1434.
- Boakye YD, Agyare C, Ayande GP, Titiloye N, Asiamah EA, Danquah KO. Assessment of wound-healing properties of

- medicinal plants: the case of *Phyllanthus muellerianus*. *Front Pharmacol*. 2018;21(9):945. Doi:10.3389/fphar.2018.00945.
28. *British Pharmacopoeia*, Vol. 2. Her Majesty Stationary Office; 2009.
 29. European Commission. Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union*. 2010;50:33-79.
 30. OECD. Test No. 402: *Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing; 2017. <https://doi.org/10.1787/9789264070585-en>.
 31. Dwivedi D, Dwivedi M, Malviya S, Singh V. Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. *J Tradit Complement Med*. 2017;7(1):79-85. doi: 10.1016/j.jtcme.2015.12.002.
 32. Talekar YP, Apte KG, Paygude SV, Tondare PR, Parab PB. Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays. *J Ayurveda Integr Med*. 2017;8(2):73-81. doi: 10.1016/j.jaim.2016.11.007.
 33. ThermoFisher Scientific Protocol. PrestoBlue™ Cell Viability Reagent, 2021 [2021-12-14]. <https://www.thermofisher.com/pl/en/home/references/protocols/cell-and-tissue-analysis/protocols/prestoblue-cell-viability-reagent-for-microplates-protocol.html>
 34. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*. 1968;125(1):189-198. doi:10.1016/0003-9861(68)90654-1.
 35. Govindarajan R, Vijayakumar M, Shirwaikar ANNIE, Rawat AKS, Mehrotra S, Pushpangadan P. Antioxidant activity of *Desmodium gangeticum* and its phenolics in arthritic rats. *Acta Pharm*. 2006;56(4):489. Accessed June 10, 2004. <https://pubmed.ncbi.nlm.nih.gov/19839141/#>.
 36. Nalawade TM, Bhat KG, Sogi S. Antimicrobial activity of endodontic medicaments and vehicles using Agar well diffusion method on facultative and obligate anaerobes. *Int J Clin Pediatr Dent*. 2016;9(4):335-341. doi:10.5005/jp-journals-10005-1388.
 37. Balouiri M, Sadiki M, Ibensouda SK. Methods for in vitro evaluating antimicrobial activity: a review. *J Pharma Anal*. 2016;6(2):71-79. doi: 10.1016/j.jpha.2015.11.005.
 38. Li J, Bao G, ALyafei E, et al. Betulinic acid enhances the viability of random-pattern skin flaps by activating autophagy. *Front Pharmacol*. 2019;10:1017. Published 2019 Sep 13. doi:10.3389/fphar.2019.01017
 39. Cho SH, Gottlieb K, Santhanam U. Cosmetic compositions containing betulinic acid. Chesebrough Ponds USA Inc. 1996; U.S. Patent 5,529,769.
 40. Drag-Zalesińska M, Rembiałkowska N, Borska S. A new betulin derivative stimulates the synthesis of collagen in human fibroblasts stronger than its precursor. *In Vivo*. 2019;33(4):1087-1093. doi:10.21873/in vivo.11577.
 41. Madhan B, Subramanian V, Rao JR, Nair BU, Ramasami T. Stabilization of collagen using plant polyphenol: role of catechin. *Int J Biol Macromol*. 2005;37(1-2):47-53. doi: 10.1016/j.ijbiomac.2005.08.005.
 42. Monika P, Chandrababha MN, Murthy KNC. Catechin, epicatechin, curcumin, garlic, pomegranate peel and neem extracts of Indian origin showed enhanced anti-inflammatory potential in human primary acute and chronic wound derived fibroblasts by decreasing TGF- β and TNF- α expression. *BMC Complement Med Ther*. 2023;23(1):181. doi:10.1186/s12906-023-03993-y.
 43. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. *Adv Ther*. 2017;34(3):599-610. doi:10.1007/s12325-017-0478-y.
 44. Xie W, Hu W, Huang Z, et al. Betulinic acid accelerates diabetic wound healing by modulating hyperglycemia-induced oxidative stress, inflammation and glucose intolerance. *Burns Trauma*. 2022;10:tkac007. doi:10.1093/burnst/tkac007.
 45. Ghiulai R, Roșca OJ, Antal DS, et al. Tetracyclic and pentacyclic triterpenes with high therapeutic efficiency in wound healing approaches. *Molecules*. 2020;25(23):5557. doi:10.3390/molecules25235557.
 46. Metelmann HR, Brandner J, Schumann H, Bross F, Hoffmann M, Podmelle F. Accelerating the aesthetic benefit of wound healing by triterpene. *J Craniomaxillofac Surg*. 2012;40(5):e150-e154. doi: 10.1016/j.jcms.2011.07.020.
 47. Zulkefli N, Che Zahari CNM, Sayuti NH, et al. Flavonoids as potential wound-healing molecules: emphasis on pathways perspective. *Int J Mol Sci*. 2023;24(5):4607. doi:10.3390/ijms24054607.
 48. Chaniad P, Tewtrakul S, Sudsai T, Langyanai S, Kaewdana K. Anti-inflammatory, wound healing and antioxidant potential of compounds from *Dioscorea bulbifera* L. bulbils. *PLoS One*. 2020;15(12):e0243632. doi:10.1371/journal.pone.0243632.
 49. Noviany N, Osman H. Structure elucidation of betulinic acid from *Sesbania grandiflora* root. *J Phys: Conf Ser*. 2021;1751(2021):012090. doi:10.1088/1742-6596/1751/1/012090.
 50. Abdulah R, Suradji EW, Subarnas A, et al. Catechin isolated from *Garcinia celebica* leaves inhibit *Plasmodium falciparum* growth through the induction of oxidative stress. *Pharmacogn Mag*. 2017;13(Suppl 2):S301-S305. doi:10.4103/pm.pm_571_16