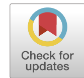
South African Journal of Botany 137 (2021) 52–59

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Wound healing, antimicrobial and antioxidant properties of the leaf and stem bark of *Entada africana* Guill. & Perr.



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ARTICLE INFO

Article History:
Received 27 January 2020
Revised 19 August 2020
Accepted 22 September 2020
Available online 20 October 2020

Edited by AR Ndhlala

Key words:
Entada africana
Wound closure
Anti-bacterial
Dermal excision
Histopathology

ABSTRACT

Entada africana (Mimosaceae) is widely used in African traditional medicine as a wound healing agent. This study investigated the wound healing, antimicrobial and anti-oxidant activities of the leaf and stem bark of *E. africana* in order to scientifically validate its wound healing properties. Methanol extracts of the leaf and stem bark were formulated into creams (5–20%) and tested in the dermal excision wound model in rats. The broth dilution assay was used to determine the antimicrobial activity; DPPH free radical scavenging, ferric reducing power, total antioxidant capacity, total phenolic and total flavonoid content were determined to assess the antioxidant activity. Significant wound healing was demonstrated by topical application of the extract creams. By the 10th day, a total wound surface closure of 98.8%, 95.8%, 96.3% and 73.9% was observed for the 10% stem bark and leaf creams, standard drug and blank cream treated groups respectively. Histopathological examination of the healed tissue sections showed significant collagen production, scanty inflammatory cell infiltrates and re-epithelialization whereas tissues from the blank cream treated group displayed focal areas of abscess and diffused inflammatory cells. The methanol stem bark extract demonstrated antibacterial activity against *S. aureus* and *S. pyogenes* with a minimum inhibitory concentration of 1.56 mg/mL. The stem bark and leaf had antioxidant activity and were found to contain a total phenolic content of 47.25 and 133.7 mg/g Gallic acid equivalent respectively and total flavonoid content of 21.06 and 88.21 mg/g quercetin equivalent respectively. The above results indicate that the leaves and stem bark of *E. africana* have great potential for the treatment of open wounds.

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1. Introduction

A wound is a disruption or breakage in the anatomical or cellular continuity of a living tissue. Wound healing proceeds through physiologically distinct stages of coagulation, inflammation, re-epithelialization and tissue remodeling (collagen deposition)(Fitzmaurice et al., 2011) which when interrupted may result in the formation of non-healing chronic wounds. Treatment of chronic wounds impose immense financial burden on the patient (Kayır et al., 2018) and sometimes end in amputation or disability reducing the quality of life of the patient (Järbrink et al., 2017).

Wound healing plays a vital role in the lives of human beings, hence attempts to optimize the healing process have occurred since time immemorial. In spite of the technological and strategic advances in the field of medicine, wound care is one of the conditions for which many depend on traditional remedies mainly plants for treatment (Lindblad, 2008). Many medicinal plants and herbs are reported as haemostatics and wound healing in folklore medicine and are largely preferred due to their widespread availability and effectiveness (Agyare et al., 2016, Mensah et al., 2006a, Mensah et al., 2006b). Researches focusing on identifying alternative methods to treat and manage acute and non-healing chronic wounds have occupied scientists and clinicians from diverse regions of the world.

For virtually all cultures in both developed and developing countries, people rely on medicinal plants as alternative wound healing agents which are effective, safe and cheap (Nayak et al., 2009). In Ghana, several medicinal plants are reported for their wound healing effects. In most cases, the plant material is crushed or chewed and topically applied as a poultice on the wound and the healing process monitored by assessing the rate of contraction or closure of the open wound (Agyare et al., 2016, Mensah et al., 2006a, Mensah et al., 2006b). Despite the long-term anecdotal usage, the clinical use of most of these plant preparations remains anecdotal without established clinical/scientific efficacy. One such common plant which is

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https://doi.org/10.1016/j.sajb.2020.09.037
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extensively used as a wound healing agent in most African countries is *Entada africana* Guill. & Perr. (Mimosaceae).

E. africana is a small tree that grows in the savannah regions of most Sub-Saharan African countries. In most traditional pharmacopoeias, this plant is specifically indicated for its vulnerary effect. A decoction of the root or stem bark is used to wash wounds and a powder of the same parts is applied topically as a wound healer. The leaves are used as wound dressing to prevent suppuration. The juice from the fresh root or the bark is also used to stop bleeding Burkill (1985). Other uses include as an antidote for food-poisoning, antipyretic, antiseptic, antiulcer, diuretic, for the relief of arthritic and rheumatic pains, bronchitis, cough and for the treatment of infections Kerharo and Adam (1974). Several pharmacological investigations of various parts of the plant have confirmed its anti-inflammatory (Owona et al., 2013), antioxidant (Guissou et al., 2010), antiviral (Galani Tietcheu et al., 2014), hepato-protective (Njayou et al., 2013), antiulcerogenic (Obidike and Emeje, 2011), antibacterial (Mbatchou et al., 2011, Ifemeje et al., 2014, Kwaji et al., 2017), anti-plasmodial and analgesic (Ezenyi et al., 2014) activities. Pharmacognostic standards for its authentication are also published (Baidoo et al., 2019). The roots have been found to possess anti-angiogenic activity (Germanò et al., 2015) and contain polysaccharides and triterpene saponins which have complement fixing and anti-proliferative activities respectively (Diallo et al., 2001, Cioffi et al., 2006). There is however currently no report on the wound healing activity of the plant. The aim of this study was therefore to investigate the wound healing activity of the methanol extract of the leaves and stem bark of the plant and further correlate with its antimicrobial and antioxidant activities.

2. Materials and methods

2.1. Plant collection and authentication

The leaves and stem bark of *E. africana* were collected from Yendi in the Northern Region of Ghana in September, 2017. The plant materials were authenticated by Mr. Clifford Asare of the Herbal Medicine Department, KNUST, Kumasi and a herbarium specimen with the voucher numbers KNUST/HM1/2018/L060 and KNUST/HM1/2018/S011 for the leaf and stem bark respectively were deposited at the Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST herbarium (Baidoo et al., 2019).

2.2. Extraction of plant material

The fresh leaves (1 kg) and stem bark (2 kg) of *E. africana* were cleared of all foreign materials and shade dried at room temperature for two weeks. The dried plant material was coarsely ground into homogenous powder using a mechanical grinder and stored at room temperature until required for use. About 300 g each of the coarsely ground leaves and stem bark powder were separately cold macerated with 700 mL methanol for 72 h. The filtrate was concentrated on a rotary evaporator at 50 °C to obtain a dark brown crude extract of yields, 11.38% $^{w}/_{w}$ and 18.84% $^{w}/_{w}$ for the leaves and stem bark respectively. The extracts were subsequently referred to as ‘EAL’ for the leaves extract and ‘EAS’ for the stem bark extract.

2.3. Phytochemical screening

The powdered plant samples were preliminary screened for the presence of major classes of secondary metabolites according to established methods (Evans, 2009).

2.4. Cream preparation

An emulsifying ointment weighing 200 g was prepared following a standard scaled formula according to the British Pharmacopoeia (British Pharmacopoeia 2009) (Table 1). The emulsifying ointment was used to prepare three different concentrations of aqueous creams for EAL (10,15,20%) and EAS (5,10,15%) according to Table 2.

2.4.1. Evaluation of physical properties of the cream

The colour, grittiness and odour of the prepared creams were studied for four (4) weeks beginning immediately after formulation. The pH of the creams in solution (0.025 M) was determined. The spreading ability of the cream was evaluated according a previously described method (Khan et al., 2013). Briefly, the cream (0.25 g) was placed within a circle of diameter 20 mm pre-marked on a glass plate. Another glass plate was placed on it and a 50 g weight was placed on the upper glass plate for 5 min. The new diameter was measured and the spread-ability of the cream was determined as change in diameters.

2.5. Determination of the wound healing activity

2.5.1. Experimental animals

Forty male Sprague-Dawley rats (90–130 g) were obtained from the Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana. The rats were housed in stainless steel cages ($34 \times 57 \times 40$ cm^3) with wood-shavings as bedding, fed with normal commercial rat feed (GAFCO, Tema, Ghana) and water *ad libitum* and maintained under laboratory conditions (temperature 25 ± 1 °C, relative humidity 60–70%, and 12 h light-dark cycle) in the *vivarium* of the Department of Pharmacology, KNUST. These conditions were maintained throughout the experimental days. All the rats were handled according to the Guidelines for Care and Use of Laboratory Animals (Directive 2010/63/EU; Animal Care and Use Committee, 1998). All protocols used were approved by the Departmental Ethics Committee.

2.5.2. Acute dermal toxicity

To determine the therapeutic dose of the formulated extracts which were not harmful to the animals, the highest concentration of the formulated creams, EAL (20 %) and EAS (15 %) were applied on the shaved back of the rats and observed for 14 days following the OECD guidelines no. 402 (Kokane et al., 2009).

2.5.3. Dermal excision wound model

The dermal excision wound model as described by Dwivedi et al., (2017) was used to evaluate the wound healing properties of the extracts with a few modifications (Dwivedi et al., 2017). Briefly, the experimental animals (40 rats) were anaesthetized by subcutaneous injection with pentobarbitone (40 mg/kg body weight). The dorsal fur was shaved with a razor blade to a circular diameter of about 40 mm. The anticipated area of the wound to be created was outlined on the shaved skin.The shaved surfaces were cleansed with 70 % alcohol and circular wounds of diameter 20 mm (area = 250–350 mm^2) were excised using toothed forceps, surgical blades and pointed scissors. The animals were then grouped into eight with five rats in each group. Wound treatment commenced on the second day after wound

Table 1
Formula for preparing emulsifying ointment

Ingredients	Master Formula/ g	Scale Formula/g (x 0.5)
Emulsifying wax	300	60
White soft paraffin	500	100
Liquid paraffin	200	40

Table 2
Formula for preparing the aqueous cream with the extract

Ingredients (g)	Stem bark (EAS)			Leaves (EAL)		
Emulsifying ointment	21.00	21.00	21.00	21.00	21.00	21.00
Chlorocresol	0.07	0.07	0.07	0.07	0.07	0.07
Purified water	48.93	48.93	48.93	48.93	48.93	48.93
Extract (%)	5.00	10.00	15.00	10.00	15.00	20.00

creation. Groups 1–3 were topically treated with EAL cream at 10, 15 and 20%. Groups 4–6 received EAS cream at 5, 10 and 15%. Group 7 was treated with aqueous cream (blank cream) and the last group treated with 1% silver sulphadiazine cream (positive control). The wounds were dressed with normal saline and treated topically with the extract creams once daily for 21 days.

2.5.4. Wound healing evaluation parameters

2.5.4.1. Wound contraction measurement. The progressive changes in excision wound area was measured in mm^2 every other day starting from the first day of wound treatment by taking the surface diameter with digital calipers until 21 days of treatment and observation.

The wound surface area was calculated as:

wound surface area $(WSA) = 4\pi r^2$; where **r**is the radius of the wound**.**

The percentage wound surface closure was calculated as:

$$\%\ wound\ surface\ closure = \frac{WSA\ (day\ 1) -\ WSA\ [day\ n]}{WSA\ (day\ 1)}\ x\ 100$$

Where '*n*' represents other days of treatment.

2.5.4.2. Histopathological examination. Wound tissue specimens from treated and untreated groups (3 rats from each group) were obtained on day 21 to evaluate the histopathological alterations. The samples were stored in buffered formaldehyde solution (10%) for 24 h and sectioned to obtain a thickness of 5–6 μm. The sections were stained with haematoxylin and eosin on a slide and examined (Talekar et al., 2017). For each specimen, photomicrographs of 640 × 480 pixel resolution were acquired with a light microscope (Leica ICC50 HD) fitted with a digital camera running under imaging analysis program (ImageI, NIH). The intensity of collagen deposition, surface area neovascularization and staining pattern of epithelial layer in treated groups were observed and compared to the control groups (Ukong et al., 2008).

2.6. Antimicrobial activity of extracts

2.6.1. Microorganisms

The microorganisms used for the antimicrobial assay were *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (clinical strain)], *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (NCTC 4175), *Klebsiella pneumonia* (clinical strain) and fungus, *Candida albicans* (clinical strain) obtained from the Pharmaceutics Department, FPPS, KNUST and the clinical strains from the Komfo Anokye Teaching Hospital, Kumasi, Ghana. The organisms (100 μL each) were inoculated into a 10 mL sterilized nutrient broth (or Sabouraud dextrose broth for fungus) and incubated at 37 °C for 24 h (30 °C for 72 h for fungus). On the day of experiment, a working culture suspension was prepared by serial dilution of culture in sterile normal saline to achieve a suspension of equal turbidity with 0.5 Mc Farland standards (10^8 CFU/mL) and subsequently referred to as "standardized culture suspension".

2.6.2. Broth dilution assay

The micro broth dilution assay method as described by Balouri (Balouiri et al., 2016) was used to determine the MIC of EAL and EAS. A concentration range between 0.09 and 12.5 mg/mL of test samples were used. Growth of bacteria was determined using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) as detecting reagent. The MICs of test extracts against *C. albicans* were determined according to the guidelines prescribed in the National Committee for Clinical Laboratory Standards for yeast (Wayne, 2008). Dimethyl sulphoxide (2% DMSO) and sterile water were used as negative controls. Ciprofloxacin and Ketoconazole were used as the reference drugs for the antibacterial and antifungal activity respectively. Each test was carried out in triplicate.

2.7. Antioxidant activity

2.7.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The free radical scavenging activity of the leaf and stem bark extracts (0.78–100 μg/mL) was determined according to an established method (Sharma and Bhat, 2009). Gallic acid (0.78–100 μg/mL) was used as positive control and blank MeOH as negative control. The experiments were performed in triplicate and results presented as the mean of three values. The percentage free radical scavenging activity was calculated according to the following equation:

$$\%\ DPPH\ scavenging\ activity = \frac{Abs\ control -\ Abs\ sample}{Abs\ control}\ x\ 100$$

Where 'Abs sample' and 'Abs control' are absorbance of sample and control respectively.

2.7.2. Total antioxidant capacity (TAC)

A reaction mixture consisting of 1 mL of test extracts (0.78–100 μg/mL) and 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. The absorbance of the mixture was measured at 695 nm after it had cooled to room temperature. A standard curve was prepared using Gallic acid (0.78 – 100 μg/mL) and the TAC of the extracts was extrapolated from this curve. TAC was expressed as Gallic acid equivalent (GAE) in mg/g of dried extract (Apak et al., 2013)

2.7.3. Total phenol content (TPC)

The total phenolic content in the MeOH extracts were determined by Folin-Ciocalteu method with slight modification (McDonald et al., 2001). A reaction mixture of 0.1 mL Folin-Ciocalteu (0.5 N) and 0.5 mL of extract was incubated for 15 min at room temperature followed by addition of 2.5 mL $NaHCO_3$ (2 % $^w/_v$). The mixture was further incubated at room temperature for 90 minutes and absorbance was measured at 760 nm. Gallic acid was used as the reference substance.

2.7.4. Total flavonoid content (TFC)

The aluminum chloride colorimetry method was used to determine the total amount of flavonoids in the crude extracts with slight modification (Chang et al., 2002). A reaction mixture of 0.5 mL test extract, 0.3 mL of 5% $NaNO_2$, and 0.3 mL of 10% $AlCl_3$ was incubated at room temperature for 30 min. 2 mL of 1 mol/L NaOH solution was then added and absorbance was measured at 415 nm. Quercetin was used as positive control. A standard curve was prepared using quercetin and the TFC of the extracts extrapolated from this curve. TFC was expressed as Quercetin equivalent (QCE) in mg/g of dried extract.

2.7.5. Ferric reducing antioxidant power (FRAP)

The reducing antioxidant power of the crude extracts was determined according to a method described by Rabeta and Faraniza (Rabeta and Faraniza, 2013) with slight modification. A reaction mixture of 1 mL MeOH extracts, 2.5 mL sodium phosphate buffer and

2.5 mL potassium ferricyanide (1% $^{w}/_{v}$) was incubated at 50 °C for 20 min. Trichloroacetic acid (10% $^{w}/_{v}$) (2.5 mL) was added to the mixture. The supernatant was obtained and thoroughly mixed with 2.5 mL distilled water and 0.5 mL Ferric chloride (0.1% $^{w}/_{v}$). Absorbance of the mixture was measured at 700 nm. A standard curve was prepared using Gallic acid and the FRAP of the extracts was extrapolated from this curve. FRAP was expressed as GAE in mg/g of dried extract.

2.8. Data analysis

Results are displayed as mean ± SEM with analysis performed using one-way analysis of variance (ANOVA) with Dunnet's Multiple Comparisons Test *post hoc.* $P < 0.05$ was considered statistically significant. The analyses were carried out with GraphPad for Windows version 6 (GraphPad Prism Software, San Diego, USA).

3. Results

3.1. Preliminary phytochemical screening

Both leaf and stem bark were found to contain tannins, saponins, coumarins, phytosterols, flavonoids and terpenoids. Alkaloids were not detected. The results are consistent with previous reports of plant species collected from various locations (Hassan et al., 2018, Njayou et al., 2013, 2015).

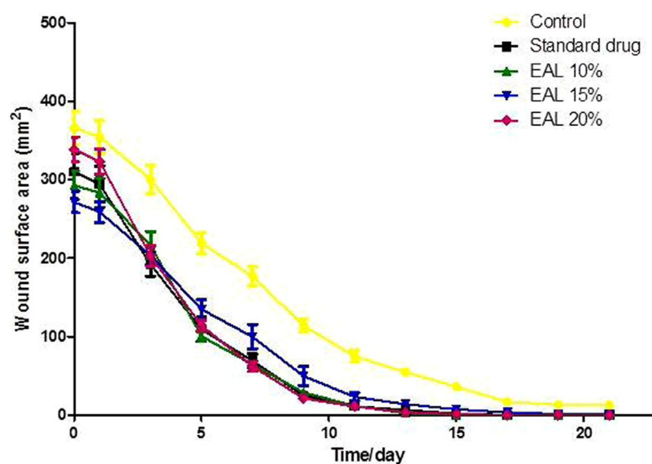
3.2. Physical properties of formulated creams

All the creams formulated from the test extracts maintained their original colour, odour and grittiness over a four (4) week period of observation. The spread-ability of creams prepared with extracts however decreased as the concentration of the extract increased. The pH values of the creams were fairly constant with a deviation of ± 0.40 from the initial reading in the first week (Table 3).

3.3. Wound healing activity

The preliminary acute dermal toxicity studies of the cream prepared from extract revealed no signs of skin irritation, physical changes or toxicity to the skin at the highest dose tested.

The extract formulated creams (EAL and EAS) and the standard drug, 1% silver sulphadiazine significantly ($P < 0.001$) reduced wound surface area compared to the blank cream treated group (Fig. 1a, b). The progressive changes in the wound surface area measured every other day post-surgery in all treatment groups are shown in Figs. 1a, b and Fig. 2 and Table 4. The activity of extracts was not dose dependent. Significant reduction of wound area for all treated groups began from day 10 to 21 with the highest rate of wound closure occurring between days 14 and 19. By day 10 post surgery, more than 90% wound healing was recorded in the extracts and 1% silver sulphadiazine treated groups, whereas the blank cream treated group had about 70% wound-surface closure (Table 4). Almost total healing



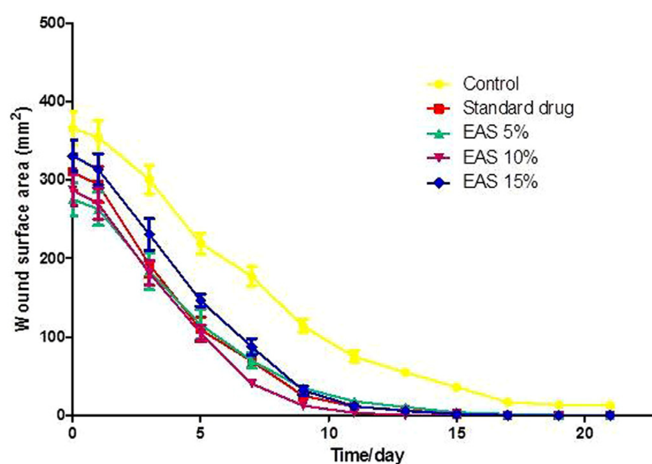


Fig. 1. Time course curve of the wound surface area of extract, standard and vehicle treated wounds over 21 day period; a) EAL- *E. africana* leaf cream; Standard- 1% silver sulphadiazine. b) Standard- 1% silver sulphadiazine, EAS – E. africana stem bark cream.

(99.87%) was observed for the treatment group that received 10% EAS by day 13 and this was statistically significant ($P < 0.001$) from healing percentages in the other treated groups (Fig. 1b, Table 4). EAS 5% and EAL 10% had relatively similar wound reduction activity as 1% silver sulphadiazine. Throughout the experiment, the percentage rate of wound closure in the blank-treated group was significantly lower than those of extract and standard drug-treated groups (Table 4).

3.3.1. Histopathological examination

After 21 days of treatment, tissue sections of rats that received 10% EAL cream showed an appreciable healing process with

Table 3
Summary of the physical properties of the formulated creams

Cream	Conc.	Odour	Colour	Grittiness	pH Value	Spread-ability/cm
EAL	10%	Characteristic	Yellowish-green	++	4.38 – 5.01	1.83 ± 0.21
	15%	Characteristic	Greenish-brown	+++	4.88 – 5.10	1.23 ± 0.12
	20%	Characteristic	Dark green	+	5.04 – 5.20	0.93 ± 0.25
EAS	5%	Characteristic	Light brown	+++	4.01 – 4.26	1.2 ± 0.18
	10%	Characteristic	Brown	+++	4.00 – 3.92	1.07 ± 0.15
	15%	Characteristic	Dark brown	++	3.93 – 3.70	0.63 ± 0.15
Blank		Characteristic	White	+++	5.10 – 5.31	2.21 ± 0.17

Keys: + (smooth), **++** (moderately smooth), **+++** (very smooth)

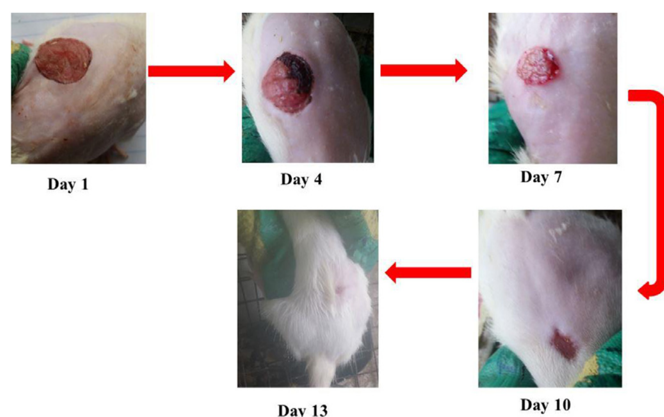


Fig. 2. Photographic representation of wound repair at different time intervals in excision wound model in rats treated with 10% EAS.

significant tensile strength and good scar formation. Tissue sections showed thinned epithelium, marked fibrosis, morphologically atrophic skin appendages (hair follicle, sweat gland, sebaceous gland) and few acute inflammatory cell infiltrates (Fig. 3A). Moderate hyperplastic epithelium and acute inflammatory cell infiltrates were observed in tissues sections of rats treated with 15% EAL. The stroma appeared oedematous and showed areas of necrosis and mild to moderate fibrous tissue signifying an insignificant healing process (Fig. 3B). The underlying epithelium of the animals treated with 20 % EAL displayed epithelial hyperplasia and significant hyperacanthosis. There was evidence of stromal angiogenesis and oedema formation (Fig. 3C).

Tissue sections of rats treated with 5 % EAS showed moderate hyperplasia of the epithelium, diffused areas of necrotic debris and acute inflammatory cell infiltrates with oedematous stroma. Underlying skin appendages were morphologically intact and blood vessels were considerably congested (Fig. 3D). Tissue sections of rats treated with 10 % EAS showed significantly thinned epithelium with mild hyperacanthotic cells, significant fibrosis and presence of collagen. There were scanty inflammatory cell infiltrates and atrophic blood vessels in the fibro-connective tissues. Underlying skin appendages appeared atrophic (Fig. 3E). Diffused inflammatory cell infiltrates, moderate vascular proliferation, mild fibrosis and collagenization were present in the tissue sections treated with 15 % EAS cream (Fig. 3F).

Treatment with 1% sliver sulphadiazine resulted in significant wound healing observed as a thinned epithelium, significant fibrosis and collagen formation with thinning of skin appendages. Acute inflammatory cell infiltrate with focal areas of residual abscesses were observed (Fig. 3G). The section of wound tissue from rats treated with the blank cream displayed a tissue with diffused pus which extended into the dermal muscles signifying poor healing process (Fig. 3H).

The healing ability of the test extract was as follows: 10% EAS (Very Good) > 5% EAS (Moderately Good) >10% EAL (Good) 15% EAL (Poor) = 15% EAS (Poor) = 20% EAL (Poor). The histopathological findings of all animals are summarized in Table 5.

3.4. Antimicrobial activity

The MeOH extracts, EAL and EAS exhibited varying degrees of antimicrobial activity against both Gram positive and negative bacteria as well as *C. albicans*. The minimum inhibitory concentration of EAS ranged from 1.56 to 12.5 mg/mL towards all test organisms. The highest activity was exhibited by EAS against *S. pyogenes* and *S. aureus*. EAL showed inhibition only towards *S. aureus* and *C. albicans* at MICs of 3.13 and 12.5 respectively (Table 6). *P. aeruginosa* and *K. pneumonia* were however not susceptible to the highest concentration of EAL (20%).

3.5. Antioxidant activity

Both leaf and stem bark extracts of *E. africana* demonstrated significant dose dependent radical scavenging activity in the DPPH free radical scavenging assay. The stem bark showed a better radical scavenging activity than the leaf and also had a higher phenolic content, flavonoid content and total antioxidant capacity (Table 7).

4. Discussion

Wound healing plays a vital role in the lives of human beings, hence attempts to optimize the healing process have occurred since time immemorial. In spite of the technological and strategic advances in the field of medicine, wound care is one of the conditions for which many depend on traditional remedies mainly plants for treatment (Lindblad, 2008). Many medicinal plants and herbs are reported as haemostatics and wound healing in folklore medicine and are largely preferred due to their widespread availability and effectiveness (Mensah et al., 2006b). In this study, the wound healing, antimicrobial and antioxidant properties of *E. africana*, an indigenous plant widely known for its wound healing activity were investigated.

From the results obtained, both leaf and stem bark extracts significantly increased the rate of wound contraction, re-epithelialization and formation of granulation tissue which are all evidence of marked treatment response compared to the negative control group. There was no production of exudate and foul odour for all treated groups, implying absence of infection. The overall results suggested that for both leaf and stem bark extracts there was no significant increase in wound contraction at higher concentrations (15−20 %). However there was significant difference in the rate of wound contraction between the extract treated and untreated groups. Tannins, coumarins, flavonoids and triterpenoids which were identified in the plant samples may be responsible for the observed wound contraction and increased rate of re-epithelialization as these are reported to have

Table 4
Percentage wound surface closure

Treatment Group	Percentage (%) wound closure						
	Day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 21
EAL 10 %	26.41±3.64	77.37±4.93	95.87±1.18	98.16±0.67	99.87±024	100.00	100.00
EAL 15 %	25.11±6.11	63.82±8.67	91.61±3.47	95.06±2.39	98.79±0.85	99.51±0.46	99.66±0.39
EAL 20 %	40.12±4.28	81.60±3.39	96.60±1.86	98.75±0.89	99.96±0.05	100.00	100.00
EAS 5 %	34.17±6.14	74.83±5.78	93.47±3.54	96.08±2.56	99.37±0.91	99.75±0.41	99.83±0.35
EAS 10 %	36.79±7.27	85.95±1.71	98.86±0.59	99.87±0.16	100.00	100.00	100.00
EAS 15 %	30.49±6.65	73.52±5.14	96.97±1.81	98.40±1.18	99.90±0.19	99.99±0.03	100.00
SS 1%	37.94±5.31	76.75±8.12	96.38±0.32	97.88±0.67	99.94±0.11	100.0.00	100.00
Blank cream	17.92±1.14	51.43±6.02	73.90±6.40	79.56±4.31	84.25±1.35	86.24±1.11	88.47±0.96

Each value is expressed as mean ± SEM (n=5); EAL − *E. africana* leaf cream, EAS − *E. africana* stem bark cream; SS- silver sulphadiazine

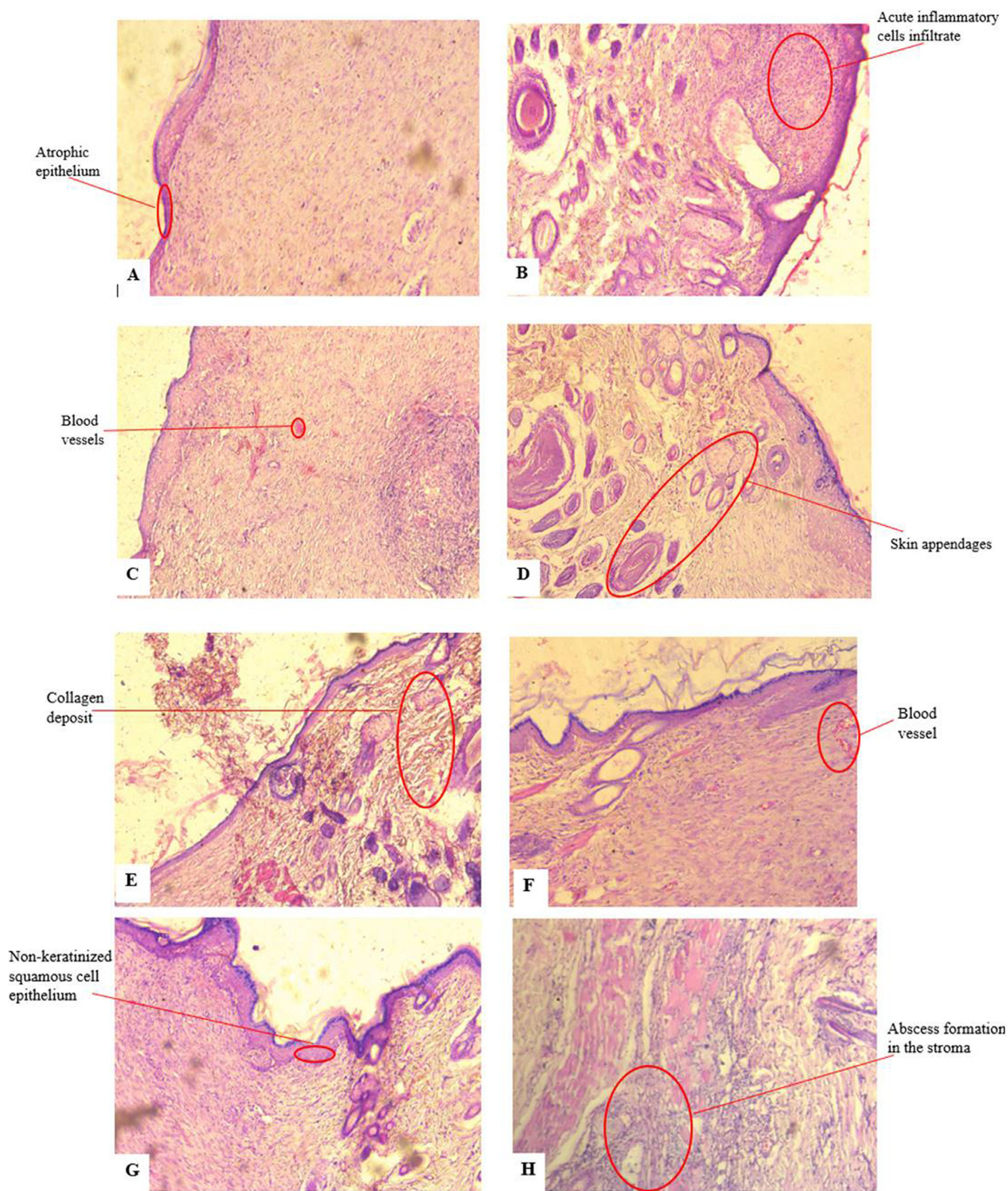


Fig. 3. Photomicrograph of histopathological sections of wound tissue of rats (stained with haematoxylin and eosin, at × 40 magnification). Fig. 3A-C: histopathological section of group with EAL 10, 15, 20% respectively; Fig. 3D-F: histopathological section of group treated with EAS 5, 10, 15% respectively; Fig. 3G: histopathological section of group treated with 1% silver sulphadiazine; Fig. 3H: histopathological section of group treated with blank cream.

Table 5
Summary of histopathological findings

Drug	Concentration (%)	Epitheliali-zation	Neo-vascularization	Inflammatory cells infiltrate	Collagen formation	Fibrosis	Abccess / oedema	Healing ability
EAL	10	Thin	Absent	Few	+	Diffused	Absent	Good
	15	Thick	Present	Moderate – Severe	_	Moderate	Present	Poor
	20	Thin	Present	Severe	_	Mild	Present	Poor
EAS	5	Thick	Present	Few	+	Mild	Present	Moderately good
	10	Very thin	Present	Very few	+++	Significant	Absent	Very good
	15	Thick	Absent	Severe	+	Mild	Present	Poor
SS	1	Thin	Absent	Few	++	Significant	Present	Good
Blank cream		Very thick	Present	Very severe	_	Diffused	Present	Very poor

Key; Collagen formation: **+** (Good); **++** (Very Good); **+++** (Excellent); - (Absent)

Table 6
Minimum inhibitory concentration (MIC) determined in the broth dilution assay

Extract	Minimum inhibitory concentration, (MIC; mg/mL)Test organisms						
	S. aureus(ATCC 25923)	*S. pyogenes*(CS)	*E. coli* (ATCC 25922)	*P. aeruginosa* (ATCC 27853)	*P. vulgaris*(NCTC 4175)	*K. pneumonia*(CS)	*C. albicans*(CS)
EAL	3.13	> 12.5	> 12.5	> 12.5	> 12.5	> 12.5	12.5
EAS	1.56	1.56	3.13	6.25	3.13	6.25	3.13
Standard	0.025	0.025	0.05	0.075	0.05	0.075	0.025*

Standard drugs: ciprofloxacin; *ketoconazole; EAL- *E. africana* leaf extract; EAS- *E. africana* stem bark extract; CS- clinical strain

Table 7
Antioxidant activities of E. africana leaf and stem bark

Extract	Antioxidant activity				
	DPPH (IC_{50}/ μg/mL)	TAC (mg/g GAE)	TPC (mg/g GAE)	TFC (mg/g QCE)	FRAP (mg/g GAE)
EAL	92.25 ± 9.87	288.1 ± 41.30	47.25 ± 7.53	21.06 ± 4.22	292.3 ± 54.8
EAS	18.63 ± 2.11	718.8 ± 47.29	133.7 ± 19.98	88.21 ± 9.03	206.6 ± 15.3
Gallic acid	3.64 ± 0.43				

EAL- *E. africana* MeOH leaf extract; EAS- *E. africana* MeOH stem bark extract

astringent, antioxidant and antimicrobial activities (Dash and Murthy, 2011).

Studies have confirmed that the different phases of wound healing require a delicate balance between oxidative stress and antioxidants (Fitzmaurice et al., 2011). While the normal physiology of wound healing relies on low levels of reactive oxygen species (ROS), an excess exposure to ROS leads to impaired wound healing and formation of chronic wounds. Antioxidants are thus postulated to help control wound oxidative stress and accelerate the wound healing process (Fitzmaurice et al., 2011). In the antioxidant studies of *E. africana*, both leaf and stem bark extracts demonstrated significant antioxidant activity and radical scavenging effects attributable to the high phenolic and flavonoid content. This is consistent with previous studies on the antioxidant activity of various solvent fractions of the stem bark and leaves of *E. africana* (Njayou et al., 2013, 2015, Tibiri et al., 2007, Tibiri et al., 2010). Positive correlation has been demonstrated between phenolic content, antioxidant activity and wound healing (Agyare et al., 2013, Mireku et al., 2014) suggesting that the antioxidant property of *E. africana* may contribute greatly to the observed wound healing effect.

Most wounds are easily colonized by microorganisms such as *S. pyogenes, S. aureus* and *P. aeruginosa* whose activity induces a cascade of inflammatory responses that prolong the inflammatory phase, degrade growth factors, delay epithelialization and prolong the entire healing process (Edwards and Harding, 2004). The topical application of a wound healing agent with considerable antimicrobial activity is therefore an efficient therapy method for destroying microbial populations because of the availability of the active agents at the wound site. In the broth dilution assay, the MeOH extract of the stem bark of *E. africana* demonstrated moderate broad spectrum antimicrobial activity against all test organisms with the highest activity against *S. pyogenes* and *S. aureus* at MIC of 1.56 mg/mL. According to Fabry and his colleagues (Fabry et al., 1998), an MIC below 8 mg/mL for crude extracts signifies moderate antimicrobial activity. The presence of polyphenols in the extract may contribute to the antimicrobial activity (Daglia, 2012). In previous studies, the stem bark of *E. africana* showed inhibitory effects against *B. subtilis* and *S. typhi* (Ifemeje et al., 2014, Mbatchou et al., 2011). Various solvent fractions also demonstrated antibacterial activity against *S. aureus, E. coli* and *E. faecalis* (Kwaji et al., 2017).

In other studies, the stem bark of *E. africana* demonstrated very potent anti-inflammatory activity through the suppression of nitric oxide synthase and pro-inflammatory cytokine (TNF-α, IL-6 and IL-1β) gene expression (Owona et al., 2013) . Thus the anti-inflammatory activity of this plant may also contribute to its wound healing effects. The previously reported antiulcerogenic effect of the leaf extract of *E. africana* against indomethacin-induced gastric ulcer (Obidike and Emeje, 2011) complements the current results of wound healing effect of the stem bark. This is expected as both leaf and stem bark are found to contain similar phytoconsituents (Baidoo et al., 2019, Mbatchou et al., 2011). From the roots of *E. africana*, polysaccharides mainly pectins displaying a core skeleton of rhamnogalacturonan type I and arabinogalactan-protein type II polymers with complement fixing activity were identified (Diallo et al., 2001). Plant polysaccharides have been shown to promote wound healing by their immune-modulatory properties and ability to stimulate the proliferation of dermal fibroblasts and keratinocytes (Pereira et al., 2016, Tabandeh et al., 2014). The presence of polysaccharides in the stem bark may also be a contributing factor to its wound healing property. In further studies, we consider the feasibility of obtaining a polysaccharide and polyphenolic rich formulation from *E. africana* for further studies of its wound healing effects.

5. Conclusion

This study has given scientific credence to the folkloric use of the leaves and stem bark of *Entada africana* as wound healing agents. Though the specific wound healing compounds and mechanisms of action are yet to be established, the current results clearly provide the first evidence that topical application of *E. africana* on skin lesions accelerates wound healing.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors are grateful to the technicians of Pharmacology and Pharmaceutics Departments, Mr. Clement (Komfo Anokye Teaching Hospital, Kumasi, Ghana) and Dr. Daniel Sia (Veterinary Medicine Department, KNUST) for their assistance and support.

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