



Original Research Article

# Medicinal plants used in wound care: Assessment of wound healing and antimicrobial properties of *Zanthoxylum leprieurii*

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*Zanthoxylum leprieurii* Guill and Perr. (Family Rutaceae) is used in traditional medicine as a diuretic, purgative and in the treatment of wounds, ulcers, pains, arthritis, skin and urinary tract infections, dysentery and intestinal worm infestation. However, no extensive scientific research has been conducted to verify the anti-infective and wound healing properties of the plant. The objective of this study is to investigate the antimicrobial, antioxidant and in vivo wound healing properties of the aqueous-methanol (3:7, v/v) stem bark of extract of *Zanthoxylum leprieurii* (AMZL). Minimum inhibitory concentration (MIC) of aqueous-methanol stem bark extract of *Z. leprieurii* against typed strains of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and clinical strains of *Streptococcus pyogenes* and *Candida albicans* were 20.0, 20.0, 20.0, 40.0, 20.0 and 20.0 mg/mL respectively. The free radical scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) gave an IC<sub>50</sub> of 28.7 µg/mL for the aqueous-methanol stem bark extract of *Z. leprieurii* while the reference antioxidant, α-tocopherol, had IC<sub>50</sub> of 0.79 µg/ml. The excision wound model studies of the 7.5 and 15.0% w/w extract (AMZL) creams with Sprague-Dawley rats showed a significant decrease in wounds treated with 7.5% w/w aqueous cream extract at days 9 ( $p < 0.001$ ) and 11 ( $p < 0.01$ ) compared to the untreated wounds. There was profuse proliferation of fibroblasts with varying degrees of fibrosis in extract treated wound tissues compared with the untreated wound tissues. Fibroblasts and collagen fibers were more present in the extract treated groups compared to untreated wound tissues. Tissues from wounds treated with 7.5% w/w extract cream showed more fibrosis (60 to 70%) than the 15% w/w extract cream (40 to 50%). Preliminary phytochemical screening of the stem bark revealed the presence of alkaloids, flavonoids, carbohydrates and saponins. The above findings may justify the folkloric uses of the plant for the treatment of wounds and microbial infections.

**Key words:** Antimicrobial, antioxidant, wound healing, minimum inhibitory concentration

## INTRODUCTION

Plants and their extracts have been found to have a profound potential in managing and treating wounds not only because they are affordable but also because they are

reported to be safe and hypersensitive reactions rarely occur with the use of these agents (Raina et al., 2008). The presence of diverse life-sustaining constituents in plants

have encouraged scientists to examine these plants with an objective to determine the potential wound healing properties. In Ghana, certain plants have been found to be extremely beneficial in wound healing. Extracts of *Commelina diffusa* and *Spathodea campanulata* have been found to possess wound healing properties (Mensah et al., 2006). A recent study has also shown that methanol extract from the plant *Prosopis africana* has wound healing properties (Ezike et al., 2006). Other plants known to possess wound healing properties in Ghana include *Paullinia pinnata*, *Gossypium arboreum* and *Balanites aegyptiaca* (Annan and Dickson, 2008). We conducted an ethnopharmacological survey among traditional healers and herbalists in Bosomtwe-Atwima-Kwanwoma area in Ghana on medicinal plants used in the treatment of wounds and we identified 104 plants belonging to 46 families. The influence of aqueous and ethanol extracts of the frequently used plants on metabolic activity and proliferation of HaCaT keratinocytes and primary dermal fibroblasts *in vitro* were determined and some of the plants were found to significantly increase the proliferation of the fibroblasts and HaCaT cells. These skin cells are important cells involved in wound healing and repair. Examples of such plants include *Phyllanthus muellerianus*, *Pycnanthus angolense*, *Pupulia lappacea*, *Zanthoxylum leprieurii* etc (Agyare et al., 2009). Wound healing and antimicrobial properties of some these plants including *Zanthoxylum leprieurii* have not been evaluated so far and hence the need to determine its influence on *in vivo* wound healing and treatment of microbial infections.

*Zanthoxylum leprieurii* Guill and Perr. (Family Rutaceae) also known as *Fagara angolensis* Engl. and *Fagara leprieurii* Engl. has wide distribution across tropical Africa. It is called 'nkrangyedua' in Asante-Twi dialect in Ghana. Traditionally, a decoction of its stem bark and root bark is used as diuretic. The stem bark and leaves are used topically to treat wounds, syphilitic sores and leprous ulcers. The bark, when boiled in hot water produces vapour which is inhaled to treat toothache and rheumatic pain. Decoction and poultice of stem bark is used for the treatment of skin and urinary tract infections, dysentery and intestinal worm infestation (Agyare et al., 2009; Adesanya and Sofowora, 1983). It contains essential oils including sesquiterpenoids with E-nerolidol, humuenol and elemol being the major constituents but low amounts of aliphatic and aromatic amides as compared to other *Zanthoxylum* species. Benzophenanthridine and acridone alkaloids are mainly found in the stem bark (Bouba et al., 2010). The essential oils were found to exhibit moderate antibacterial activity (Burkill, 2007).

In the assessment of plant extracts for wound healing properties, *in vitro* and *in vivo* biological assays associated with wound healing have to be employed (Agyare et al., 2013; Houghton et al., 2005). As such, we determined the antioxidant, antimicrobial and *in vivo* wound healing activities of aqueous-methanol stem bark extract of *Z.*

*leprieurii*.

## MATERIALS AND METHODS

### Plant material

Stem bark of *Z. leprieurii* was collected from Krofom, Atwima-Kwanwoma District of Ashanti Region in Ghana in August, 2012 and identified by Dr. Alex Asaase, Department of Botany, University of Ghana, Legon, Ghana and voucher specimen with a number of AA 139 has been kept at the Ghana Herbarium, University of Ghana, Legon, Ghana.

All chemicals and reagents used were purchased from Sigma-Aldrich, Disendorf, Germany unless otherwise stated.

### Preparation of extract

The plant material was air dried between 28 to 30°C, pulverized and extracted with Ultra-Turrax® T25 (IKA, Wilmington, North Carolina, USA) at 4000 rpm using methanol-water (7:3, v/v) for 3 min under ice-cooling, centrifuged and filtered. The filtrate was concentrated under vacuum at 40°C and lyophilized.

### Preliminary phytochemical screening

Preliminary phytochemical constituents screening of the aqueous-methanol extract of stem bark of *Z. leprieurii* (AMZL) was done to determine the main secondary metabolites according to the methods described by Evans (2009) and the total tannin content of the extract was determined according to the method described by Agyare et al. (2012).

### HPLC profile of AMZL extract

The HPLC profile of AMZL extract was determined with Thermo Finnigan HPLC system using Hypersil Gold C18, reversed phase column (150×4.6 mm). The concentration of extracts used was 10 mg/mL. HPLC optimum conditions were injection volume of 10 µL, detection wavelength of 260 nm, mobile phase comprising 0.1% acetic acid and acetonitrile (60:40 v/v, isocratic condition), temperature at 22°C, pump pressure of 28 MPa, flow rate of 1 mL/min and running time of 10 min.

### Determination of antimicrobial activity

#### Test microorganisms

*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NTCC 10073 and clinical isolates of *Streptococcus pyogenes* and *Candida albicans* were used in the determination.

### Agar diffusion method

Determination of antimicrobial activity of aqueous-methanol stem bark extract of *Z. leprieurii* was done according to the method described by Agyare et al. (2012). Extracts were investigated at concentrations of 50.0, 40.0, 20.0 and 10.0% w/v with ciprofloxacin and ketoconazole serving as reference antibacterial and antifungal agents respectively. Sabouraud agar and nutrient agar (Oxoid Limited, UK) media were used for the cultivation of the *C. albicans* and test bacteria respectively. One hundred microliters of  $10^6$  cfu/mL of test organism were seeded into 20 mL nutrient agar and sabouraud agar plates, respectively. In each of these plates, four equidistant wells with diameter of 8.0 mm were bored and the wells were filled with 200  $\mu$ L of the different concentrations of the extracts. Reference drugs were dissolved in dimethyl sulfoxide (DMSO) and pre-incubated for 1 h at room temperature (28 to 30°C) to allow diffusion of the compounds before incubation. The zones of growth inhibition were measured after 24 h incubation at 37°C (for bacterial strains) and 3 days at 30°C for *C. albicans*. The DMSO was used as negative control and it had a zero zone of inhibition against the test organisms.

### Micro-dilution method

Minimum inhibitory concentrations (MIC) of AMZL against the test bacteria were determined according to the modified methods described by Agyare et al. (2012) and Eloff (1998). One hundred microliters of double strength nutrient broth (Oxoid Limited, UK) was introduced into the wells of 96-well plate. Each well was seeded with  $10^6$  cfu/mL of the test organism. Sterile water and extract was added to a total volume of 200  $\mu$ L to obtain the concentrations needed (0.625, 1.25, 2.5, 5.0, 10.0, 20.0 and 40.0 mg/mL) and incubated at 37°C for 24 h. The plates were incubated at 37°C for 24 h. To determine bacterial growth, 10  $\mu$ L of 125 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent was added to each well and incubated at 37°C for 30 min. The experiment was repeated three times. Antifungal activity of the extract was determined according to the guidelines prescribed in the National Committee for Clinical Laboratory Standards (1998). The MIC against *C. albicans* was detected as the minimum concentration of extracts that inhibited the microbial growth after the addition of MTT to the medium and then incubated for 3 days at 30°C to detect the survival of organisms or otherwise. The results are the mean of three replicate experiments.

### Determination of free radical scavenging activity

Antioxidant activity of the extract (AMZL) was determined by the method described by Miller et al. (2000). DPPH in methanol (0.002% w/v) was prepared and 3 mL was added

to each of 1 mL of 6.25, 12.5, 25.0, 50.0 and 100  $\mu$ g/mL of aqueous-methanol extract (AMZL) with similar concentrations of  $\alpha$ -tocopherol as reference antioxidant at different concentrations. The tubes were incubated for 30 min in darkness and absorbance measured at 517 nm. A test tube containing 1.0 mL methanol and 3.0 mL DPPH was used as blank. Antioxidant activity of the extract was expressed in terms of inhibitory concentration ( $IC_{50}$ ).

### Evaluation of wound healing property

#### Experimental animals

Thirty-five males Sprague-Dawley rats with an average weight of 119 g were kept in stainless steel cages and served with normal commercial rat diet (GAFCO Ltd., Tema, Ghana), given water *ad libitum* and maintained under laboratory conditions (temperature of 28 to 30°C, relative humidity 60–70% and normal light-dark cycle). The rats were accustomed in the laboratory for one week before the experiment. This was done to reduce the stress of experimenter handling and conditions. Techniques and methods used in this study were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services Publication no. 83-23, revised 1985). The protocols for the study were approved (PH/WH/0012) by the Department of Pharmacology Ethics Committee Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

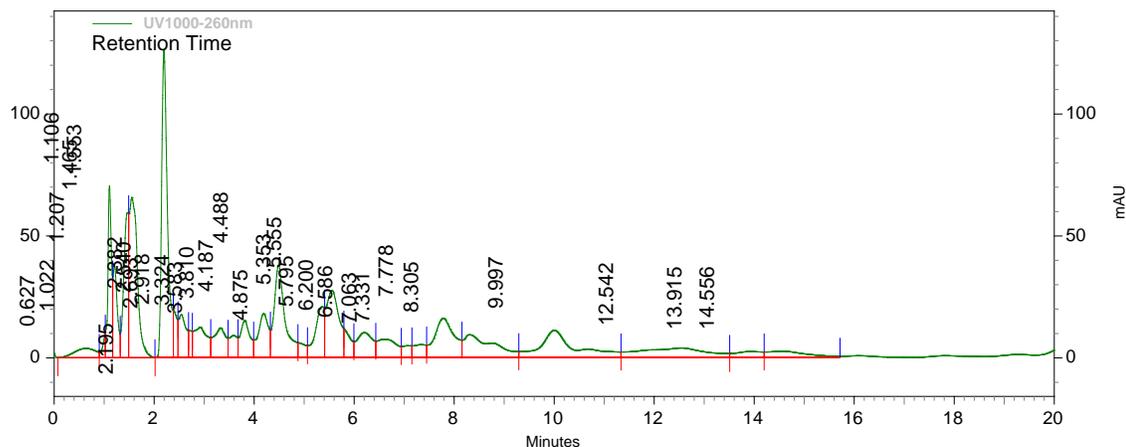
#### Excision wound model

Thirty five Sprague-Dawley rats with average weight of 119 g were anaesthetized with 50 mg/kg ketamine by intramuscular injection. The dorsal furs of the animals were shaved to a circular diameter of about 40 mm with the aid of razor blades and scissors and the anticipated area of the wound was marked on the shaved skin. The areas were cleaned with 70% v/v ethanol before the excision wounds (wound diameter of 40 mm) were created. The wounds were wiped with normal saline and left opened. The animals were divided into five (5) groups of 7 animals each. The first group was left untreated and allowed to go through the normal wound healing process and the second group was topically treated with 1% w/w silver sulphadiazine cream (SS) (Ayrton Drugs, Ghana) as reference drug (Shivhare al., 2010). The third group was treated with cream without extract (vehicle alone). The fourth and fifth groups were treated with 7.5 and 15 % w/w extract aqueous creams respectively and the extracts were prepared according to method prescribed for preparation of aqueous cream in the BP (2007) without the preservative. The aqueous cream of the extracts was prepared freshly daily for the treatment of the wounds. The

**Table 1.** Preliminary phytochemical screening of aqueous-methanol stem bark extract of *Z. leprieurii*

| Alkaloids | Flavonoids | Tannins | Sterols | Saponin glycosides | Anthracene glycosides |
|-----------|------------|---------|---------|--------------------|-----------------------|
| +         | +          | +       | -       | +                  | -                     |

+: presence of secondary metabolite; -: absence of secondary metabolite



**Figure 1:** Representative HPLC profile of aqueous-methanol stem bark extract of *Z. leprieurii* at  $\lambda$  260 nm.

above concentrations of the extract were selected as the lowest concentrations that exhibited antimicrobial activity (data not provided). Wound treatment commenced on the second day after wound creation. The extract and reference drugs were topically applied to the wounds daily for 11 days. In the course of treatment, scaled photographs of the wound areas were taken (by means of high-resolution Olympus digital camera, Cameron Sino, Hong Kong) alongside a millimeter scale. Wound measurement was done every other day, starting from the first day of wound treatment till the 11th day.

### Histological examination

Wound tissues were taken from the various groups on day 10 after treatment of wounds, to assess the influence of the extracts and reference drugs on skin cells. The cross-sectional full-thickness wound scar of about 5 mm thick sections from each group were collected for the histological evaluation. Samples were fixed in 10% buffered formalin for 24 h, dehydrated with a sequence of ethanol-xylene series, processed and then blocked with paraffin at 40 to 60°C. And trimmed with a microtome at 25  $\mu$  and sectioned into 4  $\mu$  sections. The sections were stained with hematoxylin and eosin stain.

### Statistical analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical

analyses. Data are presented as mean  $\pm$  SEM ( $N=5$ ) and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. \* $p<0.05$ , \*\* $p<0.01$  and \*\*\* $p<0.001$ .

## RESULTS

### Preliminary phytochemical screening

The yield of the aqueous-methanol stem bark extract was 8.4% w/w (related to the dried material). The aqueous-methanol stem bark extract of *Z. leprieurii* (AMZL) were found to contain alkaloids, flavonoids, carbohydrates, saponins and glycosides (Table 1). The total tannin content was 0.98% w/w using pyrogallol ( $\geq 98\%$  HPLC grade) as reference.

### HPLC profile of AMZL extract

The HPLC profile of the extract (AMZL) showed the major peak at 2.19 min accounting for 12.35% of all the components of AMZL and HPLC chromatogram of the extract serves as identification measure for the extract (Figure 1).

### Antimicrobial activity

The aqueous-methanol stem bark extract of *Z. leprieurii* was found to be active against the test organisms with varying

**Table 2:** Mean zone of growth inhibition of aqueous-methanol stem bark extract of *Z. leprieurii* against test organisms

| Extract (%w/w)<br>Organism | Mean zones of growth of inhibition (mm) ± SEM |            |            |      |  | ciprofloxacin<br>(10 µg/ml) | ketoconazole<br>(100 µg/ml) |
|----------------------------|---|------------|------------|------|--|-----------------------------|-----------------------------|
|                            | 50.0  | 30.0       | 20.0       | 10.0 |  |                             |                             |
| <i>S. pyogenes</i>         | 15.0 ± 1.0                                    | 13.3 ± 0.6 | 12.3 ± 0.6 | 0.0  |  | 33.0 ± 1.0                  | nd                          |
| <i>B. subtilis</i>         | 13.7 ± 0.6                                    | 12.3 ± 0.6 | 10.3 ± 0.6 | 0.0  |  | 17.0 ± 1.0                  | nd                          |
| <i>S. aureus</i>           | 14.3 ± 0.6                                    | 11.7 ± 0.6 | 0.0        | 0.0  |  | 25.3 ± 0.6                  | nd                          |
| <i>P. aeruginosa</i>       | 13.0 ± 0.0                                    | 10.0 ± 0.0 | 11.5 ± 0.7 | 0.0  |  | 30.3 ± 1.2                  | nd                          |
| <i>E. coli</i>             | 13.0 ± 1.7                                    | 12.0 ± 1.0 | 0.0        | 0.0  |  | 24.0 ± 0.0                  | nd                          |
| <i>C. albicans</i>         | 12.0 ± 1.0                                    | 0.0        | 0.0        | 0.0  |  | nd                          | 24.7 ± 1.2                  |

nd = not determined, SEM = Standard error mean, diameter of well = 8 mm

**Table 3:** Minimum inhibitory concentrations (MIC) of aqueous-methanol stem bark extract of *Z. leprieurii*

| Organism             | Extract (mg/mL) | Ciprofloxacin (µg/mL) | Ketoconazole (µg/mL) |
|----------------------|-----------------|-----------------------|----------------------|
| <i>S. pyogenes</i>   | 20.0            | 0.10                  | nd                   |
| <i>B. subtilis</i>   | 20.0            | 0.10                  | nd                   |
| <i>S. aureus</i>     | 20.0            | 0.25                  | nd                   |
| <i>P. aeruginosa</i> | 20.0            | 0.25                  | nd                   |
| <i>E. coli</i>       | 40.0            | 0.13                  | nd                   |
| <i>C. albicans</i>   | 20.0            | nd                    | 5.0                  |

nd: not determined

means zones of growth inhibition at higher concentrations from 20.0 to 50.0% w/w (Table 2). The MIC ranges of *Z. leprieurii* aqueous-methanol extract against *B. subtilis*, *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *E. coli* and *C. albicans* were 20.0, 20.0, 20.0, 20.0, 40.0 and 20.0 mg/mL respectively (Table 3).

### Free radical scavenging activity

The extracts exhibited low antioxidant activities compared to the reference antioxidant,  $\alpha$ -tocopherol with IC<sub>50</sub> of 28.7 µg/mL and 0.79 µg/mL for AMZL and  $\alpha$ -tocopherol respectively.

### Rate of wound closure

Both 7.5 and 15.0 %w/w AMZL increased the rate of wound closure compared with treated groups on days 9 ( $p < 0.05$ ) and 11 ( $p < 0.01$ ) post treatment (Table 4; Figures 2 and 3).

### Histological investigations

There was profuse proliferation of fibroblasts with varying degrees of fibrosis. Fibroblast cells and collagen fibers were more present in the reference and extract treated groups as compared to untreated control. Tissues from wounds treated with 7.5% w/w extract cream showed more fibrosis (60 to 70%) than the 15% w/w extract cream with 40 to 50% fibrosis with very little granulation tissue and inflammatory cells. The tissues left untreated showed dense

fibrosis together with giant cell foreign bodies. The positive control, silver sulphadiazine (1% w/w) showed 70 to 80% fibrosis (Figure 4).

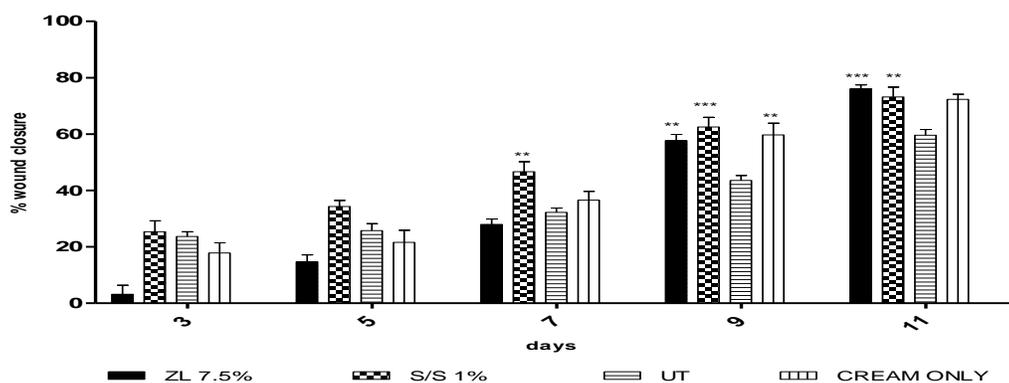
## DISCUSSION

The wound healing activity exhibited by most plants is due to the synergistic or additive actions of their constituents. The stem bark of *Z. leprieurii* was found to contain alkaloids, flavonoids, carbohydrates, saponins and glycosides. The presence of tannins and flavonoids present in the plant is very significant as these secondary metabolites have been found to act as free radical scavengers (Marja et al., 1999). They exert their antioxidant property by increasing the activity of catalase and glutathione peroxidase, which detoxify hydrogen peroxide by converting it to oxygen and water (Rahman, 2007). Aside from their antioxidant properties, they have also been known to promote wound healing. Flavonoids present in the plant may increase the viability of collagen fibrils by causing an increase in the strength of collagen fibers. This reduces cell damage by promoting DNA synthesis (Panda and Tripathy, 2009). Flavonoids have been found to exhibit antimicrobial activity and therefore may significantly help to prevent or reduce wound infections (Owoyele et al., 2008). Presence of tannins in the plant may cause an increase in wound contraction and increase the rate of epithelialization due to their astringent and antimicrobial property (Panda and Tripathy, 2009). Alkaloids present in

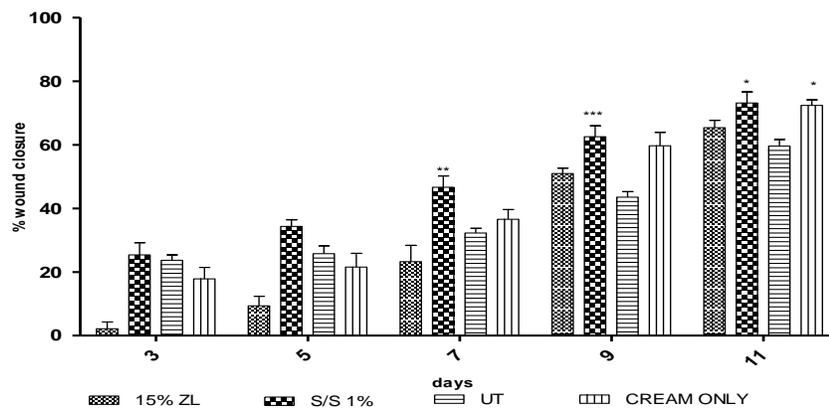
**Table 4:** Summary of wound closures for selected time points

| DAY | UT ± SEM     | S/S ± SEM      | Cream only ± SEM | 7.5% w/w ±SEM  | 15% w/w ±SEM |
|-----|--------------|----------------|------------------|----------------|--------------|
| 1   | 27.00 ± 1.10 | 25.80 ± 0.97   | 30.20 ± 2.22     | 24.20 ± 0.66   | 25.40 ± 1.36 |
| 3   | 20.54 ± 0.46 | 19.60 ± 0.87   | 24.60 ± 1.33     | 24.20 ± 1.16*  | 25.40 ± 1.36 |
| 5   | 19.94 ± 0.37 | 17.32 ± 0.85   | 23.60 ± 1.88     | 20.60 ± 0.40   | 23.20 ± 1.46 |
| 7   | 18.28 ± 0.76 | 14.00 ± 0.80   | 19.00 ± 1.23     | 17.40 ± 0.40   | 19.40 ± 1.33 |
| 9   | 15.26 ± 0.83 | 9.88 ± 0.91*** | 11.80 ± 0.37     | 10.20 ± 0.37** | 12.40 ± 0.51 |
| 11  | 10.84 ± 0.41 | 7.08 ± 0.90*   | 8.20 ± 0.20      | 5.80 ± 0.37**  | 8.70 ± 0.44  |

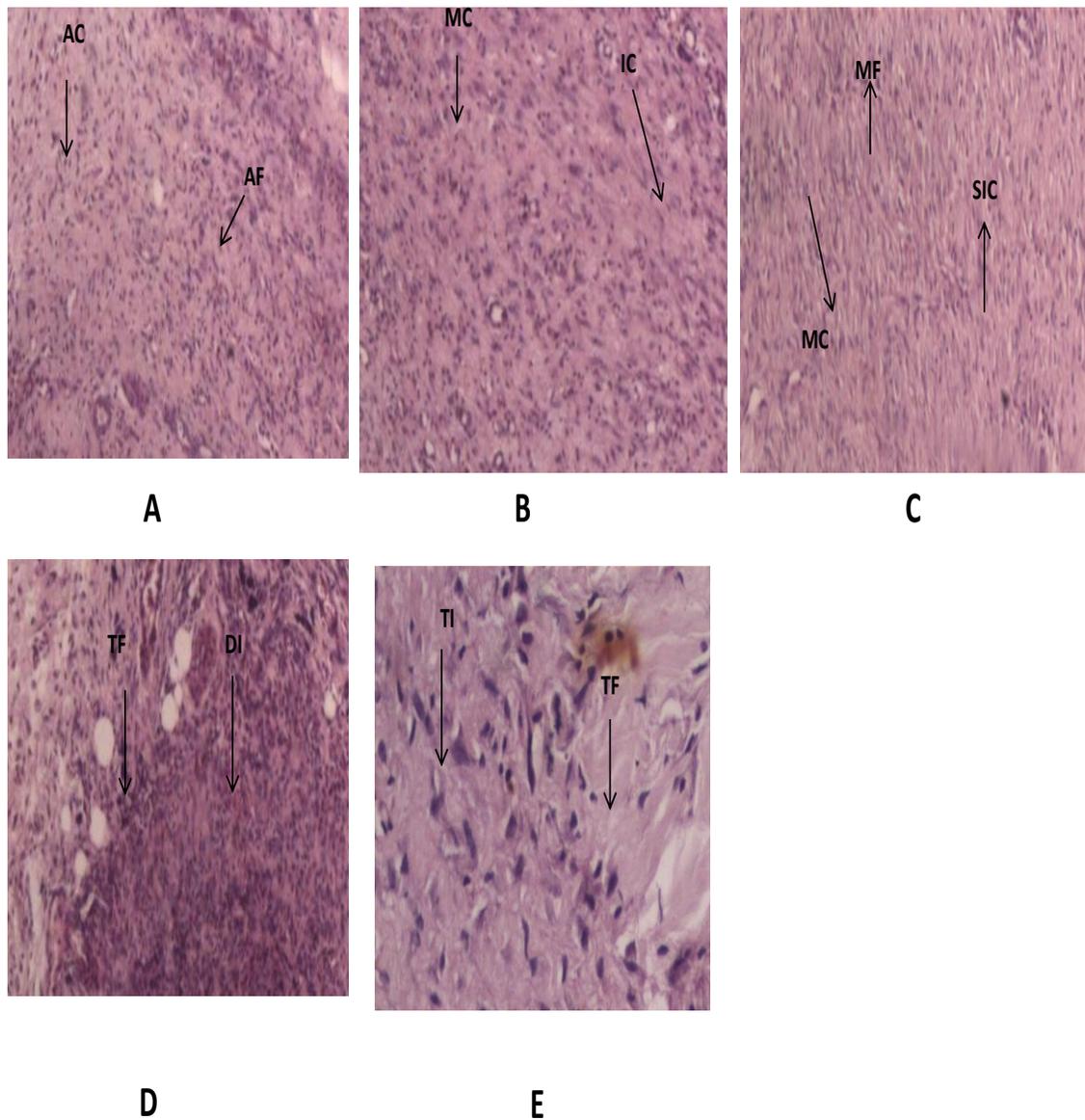
Key: SEM - Standard Error Mean, UT - Untreated wounds, S/S - Silver sulphadiazine 1% w/w, \*p<0.05, \*\*p< 0.01 and \*\*\*p<0.001 compared to untreated. Values are mean wound area (mm<sup>2</sup>) ± SEM for untreated wounds and wounds treated with 1%w/w silver sulphadiazine, 7.5, 15% w/w of *Z. lepreurii* extract creams and cream only. N=5 rats per group. Data analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.



**Figure 2:** Effect of 7.5% w/w aqueous-methanol stem bark extract of *Z. lepreurii* on wound closure. Values are mean diameter of wounds expressed as mean ± SEM (N=5). \*p<0.05, \*\*p<0.01, and \*\*\*p < 0.001. 1% w/w silver sulphadiazine was used as positive control. ZL: *Z. lepreurii* stem bark aqueous-methanol extract, UT - untreated wounds, S/S - 1% w/w silver sulphadiazine.



**Figure 3:** Effect of 15% w/w aqueous-methanol stem bark extract of *Z. lepreurii* on wound closure. Values are mean diameter of wounds expressed as mean ± SEM (N=5). \*p<0.05, \*\*p<0.01, and \*\*\*p < 0.001. 1% w/w silver sulphadiazine was used as positive control. ZL: *Z. lepreurii* stem bark aqueous-methanol extract, UT - untreated wounds, S/S - 1% w/w silver sulphadiazine.



**Figure 4:** Histological examination of wound tissues. Representative images stained with hematoxylin and eosin stain after daily treatment with 7.5% w/w and 15% w/w creams of aqueous-methanol stem bark extract of *Z. leprieurii*. (A): 7.5% w/w extract, (B): 15% w/w extract, (C): cream only, (D): 1% w/w silver sulphadiazine and (E): untreated wound tissues. DF: dense fibrosis; AF: active fibrosis; MC: moderate collagenation; DI: dense inflammation; MF: mild fibrosis; SIC: scanty inflammatory cells. Magnification X600.

the plant may also increase cell proliferation and thus increase the rate of wound healing (Barbakadze et al., 2009).

The aqueous-methanol stem bark extract of *Z.*

*leprieurii* exhibited moderate activity against all the test organisms. However, greater activity was observed with *S. pyogenes* since larger zones of inhibition were observed at higher concentrations of 20.0, 30.0 and 50.0% w/v as

compared to the other test organisms. Low concentrations of the extract did not show activity against *C. albicans* except the highest concentration (50% w/v) with mean zone of inhibition of  $12.0 \pm 1.0$  mm. Comparing activities of the reference drugs (ciprofloxacin, 10 µg/mL and ketoconazole, 100 µg/mL), the antimicrobial activity of the extract was relatively lower. A number of organisms have been found to infect wounds and some of them including *P. aeruginosa*, *S. aureus* and *E. coli* may lead to chronic or unhealed wounds (Odimegwu et al., 2008). Thus the plant extract may contribute to wound healing by inhibiting these organisms that may be present in wounds.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of active extract at which no microbial growth was observed. The MIC of 20 mg/mL was recorded for all the test organisms including *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *B. subtilis* and *C. albicans* with the exception of *E. coli* which had MIC of 40 mg/mL. According to Fabry et al. (1998), extracts exhibiting activity where MIC values are below 8 mg/mL possess very effective antimicrobial activity. From the results, the extract exhibited weak antimicrobial activity.

The  $IC_{50}$  of aqueous-methanol stem extract of *Z. leprieurii* was 28.7 µg/mL whereas that of  $\alpha$ -tocopherol was 0.79 µg/mL. The relatively moderate antioxidant property of the extract may contribute the wound healing property by mopping up excessive production of free radicals such as superoxide during wound healing process and the activity may be due to the presence of polyphenols including tannins and flavonoids present in the extract (Marja et al., 1999).

The wound size decreased significantly in the treated group with 7.5% w/w extract cream on day 3 ( $p < 0.05$ ), day 9 ( $p < 0.01$ ) and day 11 ( $p < 0.01$ ) compared to the untreated wounds. For the percentage wound closure, significant increase in wound closure was observed on days 9 and 11 (Table 4; Figures 3 and 4). The percentage wound closure on day 9 was 57.8 for the treated rats and that for the untreated was 43.6%. On day 11, rate of wound closure for the rats treated with 7.5% w/w cream was 76.0% and that of untreated wounds was 59.6% (Figures 3 and 4). The results indicate that better wound healing activity was obtained for wounds treated with 7.5% w/w extract cream. The increased wound contraction for rats treated with 7.5% w/w extract cream may be due to increased myofibroblast activity which results in an increment in the reduction of tissue defect by centripetal movement of the surrounding skin (Hinz, 2007).

The influence of 15% w/w *Z. leprieurii* extract cream on rate of wound contraction was not significant compared to the untreated wounds. Comparing the rate of wound contraction by 7.5% w/w to the 15% w/w extract cream, the 7.5% w/w extract cream appeared to produce more effective wound healing activity with wound diameters being less than that of the 15% w/w extract cream as the days progressed. The most probable reason for this may be

due to cytotoxicity in skin tissues of wounds treated with 15% w/w extract cream.

Wound tissues treated with 7.5% w/w extract cream showed better fibrosis than the untreated, with very little granulation tissue and inflammatory cells. In fibrosis, myofibroblasts persist in the tissue and are responsible for increased matrix synthesis and for contraction of the tissue. Therefore presence of fibrosis may indicate good wound healing activity (Darby and Hewitson et al., 2007). The presence of fibrosis is also an indication of possible good collagen formation which will strengthen the tissues. Though healing was taking place, lots of red blood cells were present indicating re-vascularization and thus possible prolongation of the inflammatory phase of the wound healing process. More granulation were observed in wound tissues treated with 7.5% w/w extract cream compared with the untreated. Thus, from the studies, the animals treated with 7.5% w/w extract cream exhibited better wound healing activity compared to the untreated. From the above biological activities of stem bark of *Z. leprieurii*, its wound healing property may be due to its moderate antimicrobial, antioxidant actions and influence on proliferation and differentiation of skin cells involved in wound healing process. There is a need to perform bioactivity guided isolation and characterization of compounds present in stem bark of the plant responsible for the above biological activities.

## Conclusion

The aqueous-methanol stem bark extract of *Z. leprieurii* possesses exhibited moderate antimicrobial and antioxidant properties. There was significant improvement in the rate of wound closure of wounds treated with 7.5% w/w of *Z. leprieurii* compared to the untreated wounds.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

We acknowledged the technical assistance provided by Mr. Thomas Ansah, Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in the animal work and Nana Yaw Atefah for the collection of the plant material.

## Authors' contributions

Corresponding Author (CA) was responsible for the conception, design, coordination and implementation of the work. He also supervised the antimicrobial, antioxidant and wound healing work. EK performed phytochemical and HPLC work. IYK contributed to the antimicrobial,

antioxidant and excision wound model works. PPSO worked on the histological studies of the wound tissues. CA coordinated the preparation of the manuscript and all authors read and approved the final manuscript.

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