

## A dermal wound healing effect of water extract of the stem bark of *Alafia multiflora* Stapf

David Emery Tsala<sup>1</sup>, Nnanga Nga<sup>2</sup>, Mendimi Nkodo Joseph<sup>3</sup>, Godwe Eric Kalandi<sup>1</sup>, Edmond Jacques<sup>1</sup>, Ze Ze Paul Victor<sup>1</sup>, Dimo Theophile<sup>4\*</sup>, Yueshan Hu<sup>5,6</sup>

<sup>1</sup>Higher Teachers' Training College, University of Maroua, Cameroon.

<sup>2</sup>Faculty of medicine and Pharmaceutical Study, University of Douala, Cameroon.

<sup>3</sup>Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Cameroon.

<sup>4</sup>Faculty of Science, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon.

<sup>5</sup>Avera Institute for Human Genetics, South Dakota, USA.

<sup>6</sup>Department of Psychiatry, University of South Dakota, South Dakota, USA.

\*Corresponding author: dimo59@yahoo.com; Tel: (+237) 77657442;

**Received:** 19 September 2012, **Revised:** 23 October 2012, **Accepted:** 25 October 2012

### Abstract

The effect of the water extract of the bark of *Alafia multiflora* (AM) has been studied on normal and dexamethasone-suppressed wound healing, utilizing excision and incision wound rodent models. Male albino Wistar rats were divided into four groups to receive individual treatment: NaCl 0.9 %; dexamethasone (DX); AM; and DX+AM. The results demonstrated that AM treatment, though resulted in a minor reduce of skin breaking strength (statistically insignificant) in the incision model, significantly reduced the epithelization period, accelerated wound contraction, and decreased erythema (redness) in the excision wound model, when comparing to NaCl treatment. In addition, the photomicrographies of incision wound tissues of rats treated with AM showed complete reepithelization, dense collagen, and blood vessels without macrophages; implicating the wound healing efficacy of AM therapy is probably related to an improved collagen deposition and/or reduced inflammatory reaction. Interestingly, for dexamethasone depressed wound healing, the addition of AM treatment deteriorated both excision and excision wound healing instead of improving, indicating other drug-herb interaction(s) existed besides the previously reported antagonism between dexamethasone and retinoids content of AM. Overall, this study illustrated an excellent potential of AM therapy on dermal wound healing, with a tentative mechanism of action related to improved collagen deposition and reduced inflammatory reaction.

**Keywords:** Wound healing; *Alafia multiflora*; retinoids; collagen; inflammation

### Introduction

The biological mechanism associated with wound healing is complex. Platelet aggregation during haemostasis liberates a number of soluble mediators, which initiate the healing

process. Haemostasis is followed by an early inflammatory phase that is characterized by vasodilatation, increased capillary permeability, complement activation and polymorphonuclear and macrophage migration into the wound within three days. Macrophages are actively phagocytic and secrete regulatory factors that are responsible for proliferation by fibroblasts and endothelial cells (granulation tissue) around the 5<sup>th</sup> day post-injury heralds the “proliferative phase”. Fibroblasts synthesize collagen and ground substance (proteoglycans and fibronectin), which support new cells, and the fragile capillary buds, which appear around this time (angiogenesis). Epithelialization requires the migration of epithelial cells across the granulation tissue, to close the epidermal defect. Collagen synthesis continues for many months after wound closure, but also undergoes continual lysis, so a delicate balance exists between the two processes. This final remodeling phase, accompanied by increasing tensile strength of the wound, and a decreasing cellularity, may continue for up to a year (Bradley *et al.*, 1999). Wound healing is currently a clinical challenge due to inconsistencies encountered in the healing processes and the financial burden (Jones *et al.*, 2007). Therefore, medicinal plants have generated much interest for treatment of skin ailments as they are affordable and purportedly safe from hypersensitive reactions (Raina *et al.*, 2008).

*Alafia multiflora* (AM) is a medicinal plant traditionally used in ulcerous wound and sometimes in abdominal pain. Protective effect of the extract used in this work and other extracts on oxidative stress in rats have been previously studied (Dimo *et al.*, 2006; Tsala *et al.*, 2010). Additionally, the water extract was antibacterial on various strains, with Minimum Inhibitory Concentration ranging from 10-40 mg/mL; the antiradical activity of the same extract using 1,1-Diphenyl-2-picryl-hydrazyl method was recorded above 60 % and LD<sub>50</sub> in rats was above 5g/kg (Tsala *et al.*, 2007). Although the bark of AM is traditionally applied to improve wound healing in human subjects in West and Central Africa, the wound healing activity has not been replicated in an experimental animal study yet. Therefore, the present work was designed to study the prohealing potential of the bark of *Alafia multiflora* on rat normal and dexamethasone-suppressed dermal wound healings.

## Methods

### *Animals*

Male albino Wistar rats weighing 160–200 g from the animal house of ENSAI (university of Ngaoundere) were used. Animals were maintained on clean, sterile, polyvinyl cages and normal pellet diet and water *ad libitum*. They were housed under standard experimental conditions of temperature  $23 \pm 2^\circ\text{C}$ , 12 h light/dark cycle. The rats were used for the experiment after three weeks of acclimatization period. The study was conducted in accordance to the NIH guide for the care and use of laboratory animals (NIH Publication No. 80-23; revised 1978).

### *Preparation of the aqueous extract of Alafia multiflora*

Bark of a leafy stem of *Alafia multiflora* was collected in January 2012 (dry season) in the Centre Region of Cameroon, and authenticated at the National Herbarium of Cameroon where a voucher specimen was conserved (reference number 43196/HNC). The bark was dried in the laboratory, at room temperature, powdered using a grinding machine (Straub

Grinding Mill, model 4-E). 200 g of powder was infused in 1 L of hot water (90°C) for 1 hour, followed by filtration (Whatman GF/C, 90 mm Ø) and elimination of the solvent under air-dried oven at 70 °C. The given powder yield 2.5 % of a sticky brown powder of extract.

### ***Quantitative determination of retinoids in the water extract***

Retinoids were determined as vitamin A content, according to the method described by Rutkowski and Grzegorzczak (2007). Briefly, 1 mL of KOH was added to an equal volume of extract. The tube was plugged and shaken vigorously for 1 min, heated in a water bath (60 °C, 20 minutes), and cooled down in cold water. 1 mL of xylene was added to the preparation; this was shaken again and centrifuged at 1500 g for 10 minutes. Absorbance  $A_1$  of the supernatant was measured at 335 nm against xylene. The supernatant was exposed to the UV light for 30 min and the absorbance  $A_2$  measured. The concentration  $C_x$  of vitamin A ( $\mu\text{M}$ ) was calculated, using the formula:  $C_x = (A_1 - A_2) \times 22.23$  where 22.23 is the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette about thickness = 1 cm. The test was performed in triplicate.

### ***Wound evaluation***

#### ***Grouping of animals***

For each model, 20 animals were divided into 4 groups, each model consists of 5 rats as follow: group 1: NaCl 0.9%; group 2: dexamethasone i.m (DX); group 3: *Alafia multiflora* (25 mg) (AM); and group 4: dexamethasone + *Alafia multiflora* (DX+AM). No local or systemic antimicrobial was used.

#### ***Excision wound model***

Animals were anesthezied with ketamin (25 mg/kg) and hairs on the back were removed using an electric clipper. An impression was hence made on the dorsal thoracic region, 1 cm away from the ear. The skin of impressed was excised to the full thickness to obtain a wound of approximately 1 cm<sup>2</sup>. The area of wounds was measured (sq. mm) immediately using a vernier caliper. This was taken as the initial wound area reading. 0.9 % NaCl solution was applied topically to groups 1 and 2, dexamethasone was given *i.m.* and *Alafia multiflora* extract was mixed with 0.9% NaCl solution and applied topically. All the test samples were applied every alternate day, but observations were recorded daily. Wound diameter was recorded in vertical and horizontal planes daily, as well as epithelialization time that indicate the formation of new epithelial tissue to cover the wound. The lesions on each rat were also rated using the following parameters, (1) the presence and type of exudates, (2) erythema, (3) swelling, (4) ulceration and (5) crust formation. The scoring criteria are shown in Table 1 (Masoko *et al.*, 2010).

#### ***Incision wound model***

All animals were anaesthetized before wound creation and two paravertebral long incisions were made through the skin at the distance of about 1.5 cm from midline on the depilated back of rat. A 5 cm long abdominal incision was made in shaved area and closed

with interrupted sutures at distance of 1 cm (nylon surgical treat, size 1). On 10<sup>th</sup> day post wounding, animals were sacrificed by cervical dislocation and wound areas from each animal were removed carefully. Stripes of equal size (width) from one side were cut and a line was drawn on either side, 3 mm away from the wound, for breaking strength determination. Histopathological examination was performed on collected wound sites.

### ***Determination of wound breaking strength***

Both ends of each skin stripe were fixed with a pair of steel clip, one clip was allowed hanging on a stand and other clip with a freely suspended polyethylene bag through a string run over the pulley. It was then gradually filled with water from a polyethylene reservoir till the wound stripe was broken at the site of wound. The amount of water required to break the wound was noted and expressed as tensile strength of wound in grams (Kumar et al., 2010).

### ***Histopathological evaluation***

Skin sections were cut and immediately deep in 10% neutral formalin solution to fix the tissues. On the day of analysis, samples were dehydrated with a sequence of ethanol-xylene series of solutions. The inflicted materials were embedded with paraffin at ~60 °C, sectioned (5µm) and stained with hematoxylin and eosine.

### ***Statistical analysis***

The results of statistical analysis were expressed as the mean ± SEM. The results were carried out using SPSS 10.0 and performed by one-way analysis of variance (ANOVA), followed by Duncann's test. The level of significance was set at P<0.05.

## **Results**

### ***Retinoids content***

Retinoids content of the water extract of *Alafia multiflora* as estimated spectrophotometrically was 10.94 µM, given 4.923 µM /g of dried powder.

### ***Excision wound model***

#### ***Wound closure rate and epithelialization time***

In excision wound model, wound contraction was rapid in *Alafia multiflora* group-on day 2 post wounding-, followed by dexamethasone and dexamethasone + extract groups respectively on days 3, 6 and 9 (Figure 1). In the same animals, the extract of *Alafia multiflora* treated animals showed significant reduction in the epithelization period (Table 2) as compared to controls (NaCl 0.9 %). In dexamethasone treated group significant increase in epithelization period was observed when compared to control and extract treated animals. However, there was significant delay in epithelization period in animal receiving both dexamethasone and plant extract (Table 2).

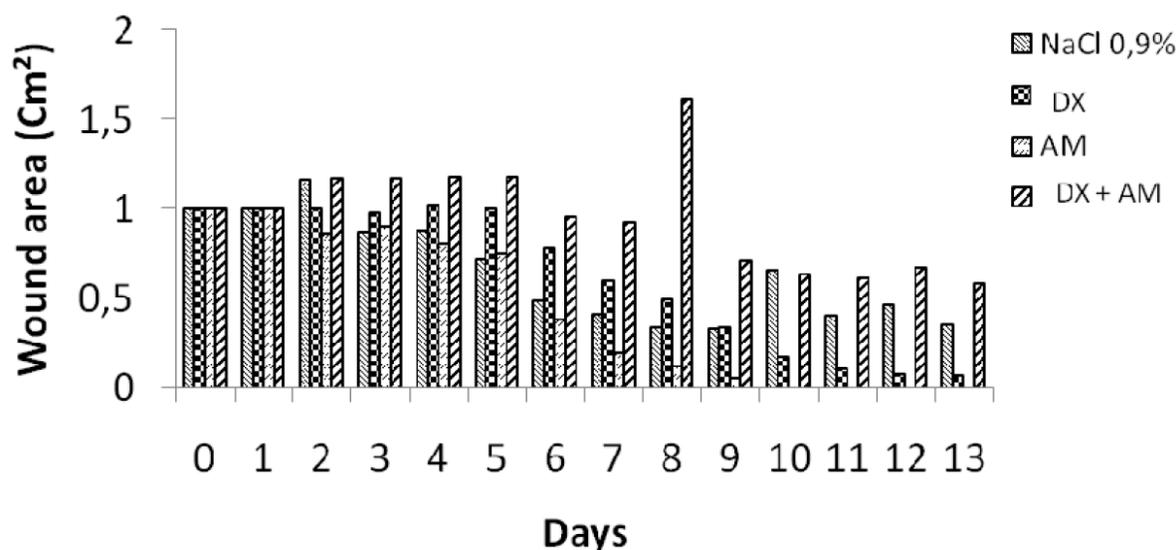


Figure 1. Effect of the water extract of *Alafia multiflora* on wound contraction rate Each value represents mean, n=5. DX: dexaméthasone 0.34 g/kg on day 1, then 0.17 g/kg every two days; AM :*Alafia multiflora* 25 mg; DX+AM = dexaméthasone+*Alafia multiflora*.

Table 1. Evaluation of erythema and exudate.

| Score | Erythema                 | Exudate              |
|-------|--------------------------|----------------------|
| 0     | No red color at all      | No exudate           |
| 1     | Light red color visible  | Exudate just visible |
| 2     | Clearly red              | Easily visible       |
| 3     | Dark red, not whole area | Substantial quantity |
| 4     | Dark red, widespread     | Large quantity       |

Table 2. Effect of the aqueous extract of *Alafia multiflora* in presence and the absence of dexamethasone in excision and excision wounds.

| Group                                  | NaCl 0.9%    | DX           | AM           | DX+AM         |
|--|--------------|--------------|--------------|---------------|
| Epithelization time (min)              | 13.00±0.00   | 13.80±0.73   | 9.80±0.48*#  | 21.80±0.48*#  |
| Breaking strength (g/Cm <sup>2</sup> ) | 575.03 21.63 | 407.69 18.46 | 467.48 18.34 | 381.48 18.96* |

Each value represents mean ,b SEM, n=5. \*P<0.05: Difference statistically significant compared to NaCl 0.9%; #P<0.05 Difference statistically significant compared to DX. DX: dexamethasone 0.34 g/kg on day 1, then 0.17 g/kg every alternated days; AM: *Alafia multiflora* 25 mg; DX+AM = dexamethasone+*Alafia multiflora*.

### Erythema and exudate

Erythema was significantly reduced in *Alafia multiflora* as well as dexamethasone + extract groups compared to control and dexamethasone groups from days 2 -6 post wounding. Although epithelialization time was markedly delayed in dexamethasone + extract group, erythema was no more visible on day 6 post wounding. Erythema was almost overcome in extract group on day 8 post wounding, and day 12 and day 16 in control group and dexamethasone groups respectively (Table 3). There was less exudate formation in the various lesions; nevertheless, it was observed in NaCl 0.9 % and dexamethasone+extract treated rats until day 2 (Data not shown).

Table 3: Effect of the aqueous extract of *Alafia multiflora* in presence and the absence of dexamethasone on erythema of excision wounds.

| Day    | Erythema                |                         |           |           |
|--------|-------------------------|-------------------------|-----------|-----------|
|        | DM+AM                   | AM                      | DX        | NaCl 0.9% |
| Day 0  | 0.00±0.00               | 0.00±0.00               | 0.00±0.00 | 0.00±0.00 |
| Day 2  | 3.00±0.00 <sup>*#</sup> | 0.50±0.22 <sup>*#</sup> | 1.75±0.19 | 2.00±0.00 |
| Day 4  | 2.00±0.44 <sup>*</sup>  | 0.00±0.00 <sup>#</sup>  | 2.00±0.31 | 0.66±0.18 |
| Day 6  | 0.00±0.00 <sup>*#</sup> | 2.00±0.31               | 1.25±0.19 | 1.33±0.48 |
| Day 8  | 0.00±0.00 <sup>*#</sup> | 0.00±0.00               | 0.50±0.22 | 0.66±0.18 |
| Day 10 | 0.50±0.22 <sup>*#</sup> | 0.00±0.00 <sup>*#</sup> | 0.5±0.38  | 0.66±0.36 |
| Day 12 | 0.50±0.22               | 0.00±0.00               | 0.00±0.00 | 0.33±0.18 |
| Day 14 | 0.00±0.00               | 0.00±0.00               | 0.50±0.22 | 0.00±0.00 |

Each value represents mean  $\pm$  SEM, n=5. \*P<0.05: Difference statistically significant compared to NaCl 0.9%; #P<0.05 Difference statistically significant compared to DX. DX: dexamethasone 0.34 g/kg on day 1, then 0.17 g/kg every alternated days; AM: *Alafia multiflora* 25 mg; DX+AM = dexamethasone+*Alafia multiflora*.

### ***Incision wound model***

#### ***Wound breaking strength***

In incision wound model *Alafia multiflora* extract did not showed improvement in incision wound breaking strength as compared to animals receiving NaCl 0.9 % (Table 2). There was a reduction in incision wound breaking strength in dexamethasone treated group as compared to NaCl 0.9 % and plant extract treated animals (29.10 % and 12.79 % respectively). Breaking strength was significantly reduced in dexamethasone combined with plant extract treated group (33.65 %), as compared to control and plant extract ( $p<0.05$ ).

#### ***Histopathological evaluation***

Histology of the wound tissue of the control animals showed the presence of macrophages, fibroblastic connective tissue. However, the lesser epithelialization and the development of capillary buds rather than mature blood vessels (Figure 2 A) indicated incomplete healing of the wound in control animals (NaCl 0.9 %). In the sections of dexamethasone treated rats, blood vessels were again present in the form of capillary bud; incomplete epithelialization, macrophages and lesser fibroblasts indicated incomplete healing (Figure 2 B). Skin biopsies of rats treated concomitantly with dexamethasone and plant extract showed deep lesions, with no macrophages below the epithelium layer, even there were enough fibroblasts; only capillary buds were seen and epithelial cells were still migrating across the granulation tissue to close epidermal defect (Figure 2 D). Finally, in plant extract treated animals, increased fibroblasts were observed and photomicrographies showed complete epithelialization, dense collagen, as well as blood vessels without macrophages (Figure 2 C).

### **Discussion**

Wound healing process consists of different phases such as contraction, epithelialization, granulation, collagenation and scar maturation which are concurrent but independent to each other. In this study two different models were used to assess the effect of *Alafia multiflora* extract on various phases. Prohealing action of the extract was demonstrated by an increase in the rate of wound contraction and by a significant reduction of epithelialization ti-

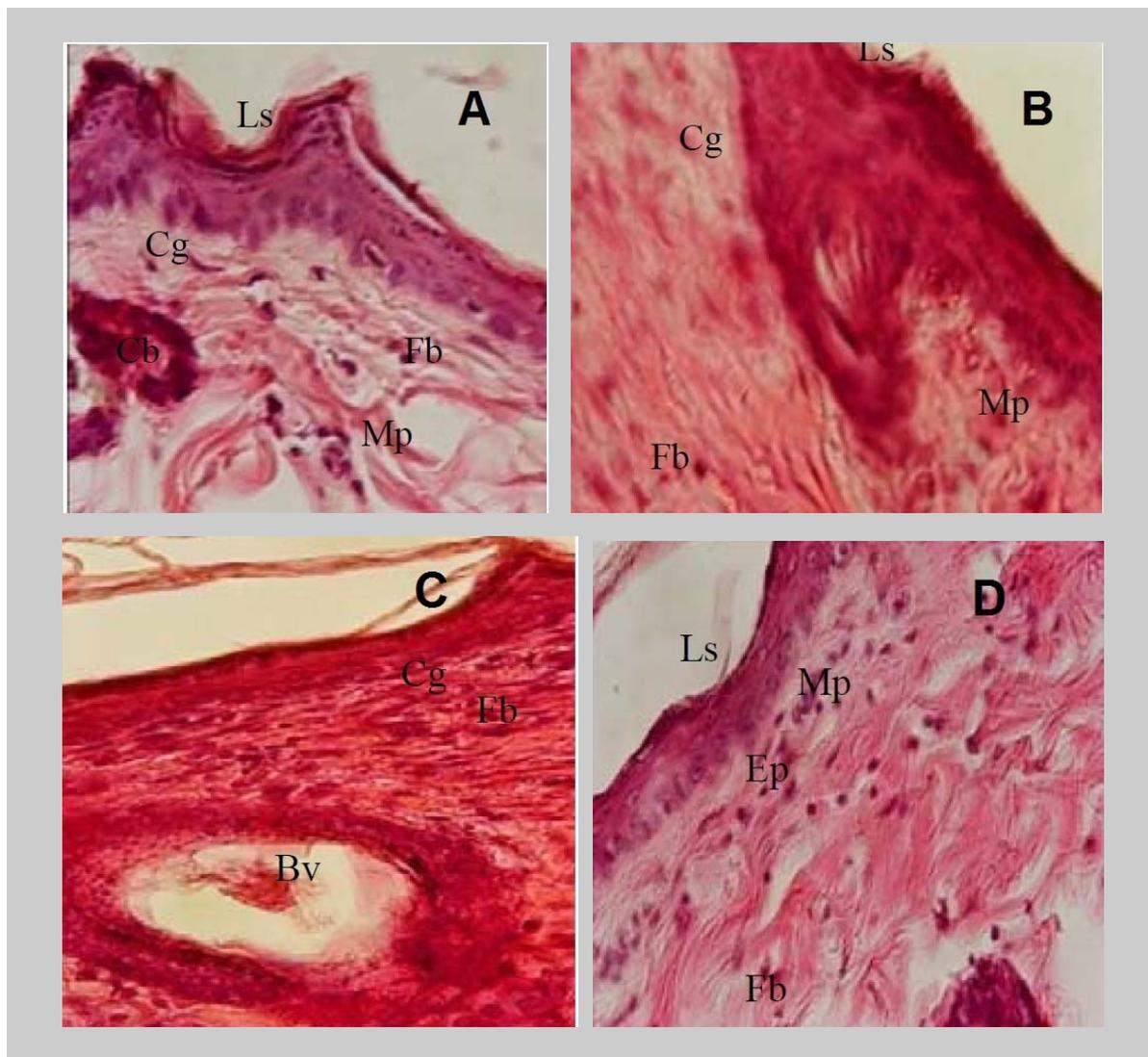


Figure 2. Histological section of wound treated with NaCl 0.9% (A), dexamethasone (B), *Alafia multiflora* (C), and dexamethasone + *Alafia multiflora* (D) (HE stain, x 400). Bv: Blood vessel; Cb: capillary bud; Cg: collagen; Ep: epithelial cells; Fb: fibroblast; Mp: macrophage.

me. Significant increase ( $P < 0.05$ ) in tensile strength compared to dexamethasone was observed, which were further supported by histopathological analysis, indicating improved collagen maturation. In normal tissues, strength, integrity and structure are provided by collagen. If too much collagen is deposited in the wound site, normal anatomical structure is lost, function is compromised and fibrosis occurs. Conversely, if an insufficient amount of collagen is deposited, the wound is weak and may dehiscence (Diegelman and Evans, 2004). Hence, bark extract of *Alafia multiflora* can probably restore skin structure and function.

The exact mechanism involved in the extract effect was not studied; nevertheless, the reduction in epithelization time may be related to an anti-inflammatory effect of the extract during wound repair. This was demonstrated by the absence of macrophages in tissue sections of *Alafia multiflora* extract treated rats 10 days after wounding, reduction in erythema and wound contraction 48h after injury. In fact, erythema (redness) is a sign that the immune

system is active and the healing process has begun (Masoko *et al.*, 2009). By 48-72 hours after injury, fixed tissue monocytes become activated to become wound macrophages which are perhaps the most essential inflammatory cells involved in the normal healing response and inhibition of macrophage function will delay the healing response. Afterwards, the role of the extract on inflammatory phase was supported by the presence of macrophages in the skin slices of control and dexamethasone treated animals 10 days after injury, because wound macrophages is a marker that the inflammatory phase is nearing at end and that the proliferative phase is beginning (Diegelman and Evans, 2004). This was confirmed by the presence of capillary buds, a marker of angiogenesis, in all the groups, except the one receiving the plant extract alone. Conversely, the absence of macrophages in extract treated animals and the presence of canalized capillaries are a marker that the proliferative phase was completed.

Vitamin A supplementation has been reported to improve wound healing through increasing collagen cross-linking and then breaking strength (Seifter *et al.*, 1975). The efficacy of AM on wound healing may be partially attributed to the retinoids, as proved by the vitamin A content of AM extract in this study. Many research studies including ours (Tsala, 2009) have illustrated the strong antagonism between steroids and retinoids (Wicke *et al.*, 2000). Interestingly, in this study we observed that the AM+DX treatment significantly deteriorated the wound healing rather than improving when compared with DX treatment alone, indicating complex drug-herb interaction(s) existed and/or other effective compound(s) responsible for the wound healing effectiveness of AM regimen.

Most studies of the wound healing process have been undertaken on acute wounds, usually in experimental animals. How closely the healing of a chronic wound follows the healing pattern of an acute wound is not clear (Bradley *et al.*, 1999). The present study strongly confirmed the traditional use of the bark of *Alafia multiflora* as ulcerous wound healing agent. However, to understand fully the process of wound healing by *Alafia multiflora* extract, it is essential to study anti-inflammatory activity of *Alafia multiflora* extract in acute, subacute and chronic models, and understand the basic cell biology, immunology and biochemistry involved in the processes of inflammation and collagen metabolism, and how these pathways are regulated.

The water extract of the bark of *Alafia multiflora* demonstrated wound healing effect by accelerating wound closure and epithelialization *in vivo*. This effect may be due the plant action on the inflammatory phase of the healing process and may be attributed at least partly on its improvement of collagen deposition. Results obtained provided additional data for the use of the bark of *Alafia multiflora* as wound healing agent. However, more data are needed to determine the exact chemical compound that is responsible for the biological activity of the extract.

### Acknowledgement

Histology was performed in the Laboratory of Animal Physiology of the University of Yaounde I (Cameroon), and part of the exploited literature was provided by Prof Kobus Eloff, University of Pretoria (South Africa).

## Conflict of interest

There is no conflict of interest associated with the authors of this paper.

## References

- Bradley M, Cullum N, Nelson EA, Petticrew M, Sheldon T, and Torgerson D. (1999). Systematic reviews of wound care management (2): Dressings and topical agents used in the healing of chronic wounds. *Health Technology Assessment*, 3 (17), 143p
- Diegelmann RF and Evans MC. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. *Frontiers in Bioscience*, 9, 283-289
- Dimo T, Tsala DE, Dzeufiet DPD, Penlap BV, Njifutie N. (2006). Effects of *Alafia multiflora* Stapf on lipid peroxidation and antioxidant enzyme status in carbon tetrachloride-treated rats. *Pharmacologyonline*, 2, 76-89.
- Jones KR, Fenni K, and Lenihan A. (2007). Evidence-Based Management of Chronic Wounds. *Advance in skin & wound care*, 20 (11), 592-600
- Kumar N., Prakash D., and Kumar P. (2010). Wound healing activity of *Solanum xanthocarpum* Schrad. And Wendl. fruit. *Indian Journal of Natural Product and resources*, 1 (4), 470-475
- Masoko P, Picard J, Eloff JN, Picard J. (2010). The use of a rat model to evaluate the in vivo toxicity and wound healing activity of selected *Combretum* and *Terminalia* (Combretaceae) species extracts. *Onderstepoort Journal of Veterinary Research*, 77(1), Art. #2, 7 p. DOI: 10.4102/ojvr.v77i1.2
- Raina R, Parwez S, Verma PK, Pankaj NK. (2008). Medicinal plants and their role in wound healing. *Online Veterinary J*, 3, 21
- Rutkowski M, and Grzegorzcyk K, 2007. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. *Acta Sci. Pol., Technol. Aliment*, 6(3), 17-28
- Seifter E, Crowley LV, Rettura G, Nakao K, Gruber C, Kan D, Levenson SM.(1975). Influence of vitamin A on wound healing in rats with femoral fracture. *Ann Surg*. 1975. 181(6):836-41.
- Tsala DE, 2009. Activités antibactérienne, antioxydante et cicatrisante des extraits d'*Alafia multiflora*. Thèse de Doctorat, Université de Yaounde I
- Tsala DE, Dimo T, Ngondi I, Nnanga N., Penlap BV, Boda M, Njifutie N. (2007). Screening of *Alafia multiflora* for antibacterial, antiradical activity and LD<sub>50</sub> investigation. *International Journal of Pharmacology*, 3 (4), 327-333
- Tsala DE, Penlab BV, Nnanga N, Mendimi NJ, Kouamouo J, Dimo T. (2010). Protective activity of the stem barks methanol extract of *Alafia multiflora* against carbon tetrachloride-induced hepatotoxicity in rats. *International Journal of Pharmaceutical Sciences Review and Research*, 3 (2), 157-163.
- Wicke C, Halliday B, Allen D, Roche NS, Scheuenstuhl H, Spencer MM, Roberts AB, Hunt TK.(2000). Effects of steroids and retinoids on wound healing. *Arch Surg*. 135(11):1265-70.