Bioactive evaluation for wound healing of stem back extracts of *Acacia nilotica* Linn. (Fabaceae)

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**INTRODUCTION**

Wounds are defined as physical, chemical, or thermal injuries that result in an opening or breaking in the integrity of the skin or the disruption of the anatomical and functional integrity of living tissues (Edwin et al., 2008; Meenakshi et al., 2006). Wound healing management through the use of medicinal plants is a common practice in most traditional climes where situations of both acute and chronic wounds are treated with herbal preparation of all kinds. Agyare et al. (2009) and Farzaei et al. (2014), as well as others, have earlier attested to the fact that many plants in the tropical and subtropical regions of the world have been screened for...
their wound-healing activity. In light of this, Boakye et al. (2018) pointed to the need for the screening of a lot of medicinal plants in the search for newer, efficacious, and cost-effective wound healing agents. The rich biodiversity of plant medicines in most of the developing world has endowed them with a great prospect for newer drug agents of natural products. Sofowora (1993) has earlier established that the choice of products for the treatment of wounds varied between regions and cultures.

The wound-healing activities of Acacia nilotica have been investigated by quite a number of researchers. A review of the medicinal activities of A. nilotica (Abduljawad, 2020), revealed the efficacy of adhesive paste from the extract of A. nilotica roots singly and more so in combination with licorice extract when used on 28 patients with recurrent mouth ulcers (Mostafa et al., 2013). In a similar study, Kamil and Abdallah (2018) also found a combination of A. nilotica and Curcuma longa mixture as highly significant with potent wound healing activity. The pod’s aqueous extract of A. nilotica has been found to promote wound healing in rats by ameliorating oxidative stress and suppressing pro-inflammatory cytokines (Kankara et al., 2018).

Acacia is the most significant genus of the family Fabaceae. It was first described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of Acacia worldwide, about two-thirds of them are native to Australia and the rest spread around tropical and subtropical regions of the world (Deshmukh and Bhajipale, 2018). Morphologically, the pods are 7 to 15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, and deeply constricted between the seed giving a necklace appearance; the seeds are 8 to 12 per pod, compressed, ovoid, dark brown shining with hard testa (Iman et al., 2007); the leaves are bipinnate, pinnate 3-10 pairs, 1.3-3.8 cm long, leaflets 10-20 pairs and 2-5 cm long (Bendiwal et al., 1992); the bark is tinge orange or green (orange tree), but older trees have dark, rough bark (Khan et al., 2009). The plant is rich in secondary metabolites of the polyphenolic compounds (tannins, flavonoids, and phenolic acids), essential oil, terpenoids, sterols, saponins, proteins, and alkaloids (Rizwana et al., 2014). Hence, some isolated compounds have shown the presence of condensed tannin, and phlobatannin, and the tannin compounds such as gallic acid, protocatechuic acid, pyrocatechol, (+) catechin (-) epigallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate were also identified in this species (Ali et al., 2012).

It has been estimated that nearly 6 million people suffer from chronic wounds worldwide (Branski et al., 2009). The chronicity could probably be attributed to the inefficiency of conventional drugs or to the resistance of microorganisms to such drugs. Unhealed wounds constantly produce inflammatory mediators that produce pain and swelling at the wound site. Chronic wounds may even lead to multiple organ failures or the death of the patients (Järbrink et al., 2016). Though several conventional drugs are known to increase healing in different kinds of wounds, these drugs are complicated and expensive and their availability is limited (Ukwuani-kwaja et al., 2019). Thus, it is a known fact that Traditional Medicine, being a significant element in cultural patrimony, still remains the main resource for a large majority of people for treating health problems, and this is why the statement by the World Health Organization (WHO) that approximately 80% of the world’s population depends on Traditional Medicine for their health care needs still remains valid. Consequently, it is on the foregoing background issues that the wound healing activity of A. nilotica L. becomes a suitable gap to explore, owing to its numerous cited and documented folklore claims as being used as a wound healing medicine.

MATERIALS AND METHODS

Plant collection and preparation

Fresh barks of A. nilotica were collected from Sokoto North Local Government Area of Sokoto State, Nigeria around 5 pm in April 2021 with the help of a traditional healer. Taxonomic identification and authentication were confirmed by Malam Musa Magaji of the Herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Usman Danfodiyo University, Sokoto. A plant sample voucher number PCG/UDUS/Faba/0008 was identified for the procured sample and referenced. The collected barks were air dried for two weeks and later pulverized using mortar and pestle into coarse form. The powder was stored in an air-tight container until needed.

Methanolic crude extraction

Two hundred and sixty grams of powdered stem bark samples of A. nilotica were macerated in 1200 mL methanol (absolute) for 48 h with frequent agitation every 4 h.

This was followed by filtration and the filtrate obtained was concentrated in an oven at 40°C for some 36 h to obtain a dark brown residue. This was labeled as CME and kept for wound healing evaluation.

Qualitative phytochemical test

A wet phytochemical (2° metabolite) test was carried out on CME according to standard methods outlined in Harborne (1983, 1992), to confirm those found in published literature.

Wound healing assay

Preparation of the CME ointment of A. nilotica stem bark

The CME A. nilotica stem bark was mixed with soft white melted paraffin and made into graded concentration doses of 2.5, 5,
and 10% ointment-based were prepared based on BP (British Pharmacopoeia, 2009).

Experimental animals

Healthy Wister albino rats of both sexes weighing 120 to 160 g were used in this study. The rats were obtained from the Animal Research Centre (ARC) of Ahmadu Bello University (ABU) Zaria, Nigeria. The rats were housed under standard laboratory conditions and allowed to acclimatize before the commencement of the study. All animals were treated in accordance with the “Principle of Laboratory Animal Care” (NIH Publication No. 85-23, revised 1985). Ethical approval was duly sought from the Health Research Ethics Committee (HREC) of the Usmanu Danfodiyo University, Sokoto-Nigeria, and a UDUS HREC reference was issued as PTAC/ES/CAF/OT/44-22; dated 10/09/2021.

Experimental protocol (grouping and dosing)

The animals were grouped into five (I-V) groups; each consisting of five animals (n=5) as follows:

- **Group I**: Wounded rats without treatment (Negative Control),
- **Group II**: Wounded rats treated with 5% w/w povidone iodine ointment (Standard Control),
- **Group III**: Test group treated with 2.5% w/w CME ointments-base,
- **Group IV**: Test group treated with 5% w/w CME ointments-base,
- **Group V**: Test group treated with 10% w/w CME ointments-base.

Acute dermal toxicity studies

This was carried out to determine the therapeutic dose of the CME formulated. Thus, the acute dermal toxicity testing of CME was performed by applying the CME ointment with the highest concentration of 10% (w/w) on the shaved back of the rat, following the OECD Guidelines No. 402.

Excision wound model assay

The method of Sumitra and Nidhi (2013) was adopted with slight modifications. The rats were anesthetized by administering 2% lidocaine (4 mg/kg s.c). The back of all the rats was shaved using a clipper and the exposed skin was scrubbed with methylated spirit. One circular full-thickness wound of 1.5 cm diameter was created at the dorsal area using a sterile sharp circular rod (mimicking biopsy punch) and the excised skin was removed with surgical scissors. Hemostasis was achieved by staining the wound with cotton wool soaked in normal saline. This was considered day zero. Thus, beginning from the first day, the rats were treated daily with the graded doses as outlined in the grouping earlier. Images of the wounds were captured appropriately and selectively. Wound areas measured at the time of wounding (day 0) and on days 5, 10, and 15 post-wounding were taken and recorded. The rate of healing was calculated and expressed as the percentage of wound contraction on days 5, 10, and 15. The percentage of wound contraction was calculated as follows:

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\text{% Wound Contraction} = \frac{\text{wound area on day 0} - \text{wound area on day } n \times 100}{\text{Wound area on day 0}}
\]

where n = the days when the measurement was taken.

Statistical analysis

All data obtained were expressed as means ± standard deviation of the mean. Comparisons between groups were performed by one-way analysis of variance (ANOVA) using SPSS statistical package software. Differences were considered significant if the p-value is less than or equal to 0.05.

RESULTS AND DISCUSSION

Qualitative phytochemical test

The stem bark CME of *A. nilotica* reveals the presence of tannins, saponins, glycosides, alkaloids, and terpenoids (Table 1). The much likely presence of phenolic compounds here is of great significance in terms of the wound-healing potential of *A. nilotica*. For instance, it has been noted that tannins act by iron deprivation, hydrogen bonding, or specific interaction with proteins such as enzymes, cell envelopes, and complex formation with polysaccharides (Dharmananda, 2003).

A tannin molecule (palas tannin) isolated from Butea Gum had been reported to possess wound-healing properties (Sanjay et al., 2018). More so, the saponins were reported to possess antibacterial properties. Their mode of action was attributed to their ability to cause leakage of proteins and certain enzymes from bacterial cells (Selvi et al., 2011). Consequently, these compounds have been reported to be biologically active (Scalbert, 1991, Maikai and Kobo, 2009) and hence may contribute singly or synergistically as antibacterial, antifungal, and wound healing agents/enhancers in the total wound healing activity exhibited. Medicinal plants heal the wound healing process by promoting blood clotting, fighting against infection, and accelerating wound healing. It can be stated that plants and chemical agents obtained from plants improve treatment and manage wound healing (Raina, 2008). Medicinal plants show wound healing effects by different mechanisms, such as modulation in wound healing, decreasing bacterial count, improving collagen deposition, increasing fibroblasts and fibrocytes, etc. (Rodriquez et al., 2018).

Acute dermal toxicity

There was no change in general behavior or appearance, loss in body weight, or any other skin irritation. The toxicity study showed no mortality up to the maximum selected dose of 10% (w/w) and all throughout the experiment.

Excision wound healing effect

All the test samples of CME exhibited significant wound
healing properties when compared with the negative control group, distilled water (DW) (Table 1). However, the standard drug showed better activity compared to the treatment groups with CME of A. nilotica stem bark at 2.5 and 5%, while the 10% group on the other hand exhibited good effect, showing an almost complete re-epithelization of wound closure (after the 10 and 15th day post wounding), which is comparably similar to the effect of 5% povidone-iodine (PI) treatment group on same days. Thus, both Table 1 and Figure 1 show summarily that the effect of 2.5, 5, and 10% CME treatments had comparable wound contraction to PI at p< 0.05.

Wound healing appearance (rate of wound contraction)

There was a moist healing environment maintained without infection in spite of the wound exposure to the atmosphere; however, as the wound contraction progresses, a hard scaly mass became embedded at the surface due to complete re-epithelization, especially towards the 15th-day post-wounding of the treated groups with both the CME and PI (Plate 1). Thus, the appearance of the wound sites indicates that progressive improvement (significantly, p<0.05) in the percentage of wound contraction occurred in the treated excised wounds compared to the untreated control (DW) group all through the study period (Plate 1: Figure 1). On the 15th-day post wounding, wound contraction (WC) of comparable significant increase (P<0.05) with the PIPC was conspicuously observed in the treated animals with either 2.5%, 5%, or 10% CME, which corresponds to a WC of 93.3, 94.7 or 100%, respectively (Table 1).

Table 1. Effect of CME of A. nilotica on Excision Wounds in Rats.

| Treatment      | Day 5       | Day 10      | Day 15      |
|---------------|-------------|-------------|-------------|
|               | Mean ± SD   | P-Value     | Mean ± SD   | P-Value     | Mean ± SD   | P-Value     |
| Distilled WaterNC | 10.0±3.8    | 0.115       | 26.7±7.7    | 0.000*      | 63.4±3.9    | 0.000*      |
| CME 2.5%      | 22.7±16.7   | 0.792+      | 48.0±11.9   | 0.038*      | 93.3±6.7    | 0.108*      |
| CME 5%        | 25.3±15.9   | 0.945+      | 60.0±13.3   | 0.705+      | 94.7±5.6    | 0.237+      |
| CME 10%       | 22.7±9.0    | 0.792+      | **82.7±6.0**| 0.083+      | **100.0±0.0**| 1.000+      |
| Povidone-iodinePC 5% | 30.0±11.5    | -           | 66.7±7.7    | -           | 100.0±0.0    | -           |

NC = Negative control; PC = Positive control; a = P-values for one-way ANOVA post hoc test at p < 0.05. *Significantly lower wound contraction with the PC; +Significantly comparable wound contraction with the PC.

Source: Analysis of results (data obtained) using the software 'IBM SPSS Statistics 26'.

Histological analysis

The histological findings of epidermal regeneration, granulation tissue thickness, and angiogenesis are shown in Plate 2. In all the test concentrations (2.5, 5, and 10%) of A. nilotica CME treated groups, there was moderate to complete re-epithelisation with improved epidermal regeneration; hence, the microscopic images showed thin keratin layer, moderately hyperplastic stratified squamous layer and hyperplastic dermis layer with glands, fibroblasts, and inflammatory cells. All of these are noted repair patterns with the best results at 2.5 and 10%.

However, in the controls, the treated group with DW exhibited little dermal or epidermal organization with a significant reduction in granulation tissue, which explains the tissue images seen as being distorted with a thin keratin layer, thick stratified squamous layer with hyperplasia, and the dermis also hyperplastic showing fibroblasts, inflammatory cells with scanty glands. While in the standard treated group with PI, a well-organized healing pattern is showcased through a thin keratin layer, with also a thin epidermis stratified squamous layer. Consequently, as observed in Figure 1, it can be said in the affirmation that in terms of granulation tissue thickness, the 10% CME treatment of A. nilotica stem back compared favourably with the standard control group at 5% PI based on increased epithelisation, angiogenesis, and collagen fibre formation. Wound healing has earlier been attributed to a coordinated process completed via four “highly coordinated and overlapping” phases viz hemostasis, inflammation, proliferation, and tissue remodeling which must occur in proper sequence and time frame for successful wound healing (Abood et al., 2015). Thus, the findings herein have corroborated those of Kankara et al. (2017) and Kamil and Abdullah (2018) on wound healing properties of A. nilotica, which has been established with a wealth of scientific data.

Conclusion

This study demonstrated that topical application of a
Figure 1. Wound healing rate of CME of *A. nilotica* stem bark at days 5, 10, and 15th. 
Source: Analysis of results (data obtained) using the software 'IBM SPSS Statistics 26'.

Plate 1. Select images of wound healing pattern (wound contraction) from each group. 
Source: Analysis of results (data obtained) using the software 'IBM SPSS Statistics 26'.

[Image of bar chart showing wound healing rate at different days for different treatment groups.]

The test group treated with 2.5% w/w *A. nilotica* ointment.

Test group treated with 5% w/w *A. nilotica* ointment.

Test group treated with 10% *A. nilotica* ointment.

Standard control (treated with 5% w/w povidone iodine ointment).

Negative control (No treatment).

methanolic extract of *A. nilotica* stem bark hastens wound healing and thus validates its folkloric use for wound healing. This finding has thus become significant owing to the report that wounds represent a major global health
challenge, which puts much economic, financial, and social stress on health institutions, caregivers, patients, and their families (Benbow, 2011). The study also agrees closely with literature findings that A. nilotica aside from its wound-healing properties, has both antibacterial and antifungal activity considering that there was no incidence of wound infection noticeable in the inflicted wounds on rats during the study. In addition, the study also revealed that A. nilotica stem bark is a potential source of pharmacologically active phyto-agents worthy of further exploration.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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