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Preliminary Investigation of Formulated Herbal Ointment Demonstrates Amelioration of Excision Wound in Experimental Rabbit Model Compared with Silver Sulfadiazine

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Abstract

In light of the use of *Lannea barteri* in the management of diverse illnesses and treatment of wounds in traditional medicine, the current study was conducted to assess the wound-healing efficacy of crude aqueous extract of the stem bark of *Lannea barteri* and its shea butter formulated ointment using an excision wound model in the rabbit. The herbal ointment (5%, w/w) exhibited a significant wound healing activity, showing 99.9% ± 0.3% wound contraction at the end of the experiment (24th day). There was no significant difference ($p > 0.05$) between the 5% (w/w) herbal ointment compared with Silver Sulphadiazine cream (positive control), which showed a 97.8% ± 1.0% rate of excision wound contraction on day 24. Compared with the negative control group (administered with only shea butter), the wound healing activity of the ointment was significant ($p < 0.05$). The current work validates the traditional use of *Lannea barteri* for the treatment of wounds and demonstrates that shea butter usage as a base in formulating an herbal ointment might aid in topical application for wound repair and regeneration, as well as the potential for enhanced wound healing.

Keywords

Excision Wound Model, Wound Healing, *Lannea barteri*, Shea Butter,

Formulation

1. Introduction

Impaired wound therapeutic is a crucial challenge globally, especially for diabetic patients. Harmful substances and microorganisms are protected from the body by the skin. When the skin is damaged, healing must be finely regulated to reinstate the normal function of skin tissue. Wound healing entails the partnership of numerous cell types, comprising keratinocytes, fibroblasts, endothelial cells, and macrophages, which are essential in skin wound repair. Appropriate healing of wounds is necessary for the reinstatement of dislocated anatomical stability and perturbed usable status of the skin [1] [2]. Prolongations of the chronicity of wounds comprise recurrent trauma, deprived oxygenation, as well as undue swelling [3]. Wounding the epidermis results in generating cytokines and growth factors and stimulates the synthesis of extracellular matrix constituents, all of which can control the processes towards keratinocyte migration and propagation, indispensable for re-epithelialization [2] [4]. Fibroblasts principally appear in the wound after the inflammatory phase, then multiply and synthesize fresh extracellular matrix, which is essential for the upkeep of supplementary cell ingrowth [5].

Plants play key functions in the propagation of keratinocytes and fibroblasts [6]. Medicinal plants are the utmost essential protective agents having diverse pharmacological effects, natural compatibility, and minimal side effects [7]. *Lannea barteri* (family Anacardiaceae) is extensively used in West Africa as a traditional medicine [8] [9]. The plant parts, either alone or in combination with other plants, are employed for the treatment of wounds and other illnesses [10] [11] [12]. Pharmacological properties of extracts of the plant such as anti-inflammatory as well as an antioxidant activity have been reported [11] [13] [14].

The kernel of the shea tree (*Vitellaria paradoxa)* is the main source where the oleaginous material, shea butter is obtained [15]. Due to its high nutritious value and low cholesterol levels, it has been designated as good as table oil. Research indicates that shea butter has applications in the food, pharmaceutical and cosmetic industries, and often as a cocoa butter substitute by chocolate manufacturers in addition to usage for margarine and baking purposes [16]. A study [17] indicates that pure natural shea butter could serve as a rich source of natural vitamin A cream and has been shown to be a splendid moisturizer, with a unique skin curative effect. Moreover, it has been affirmed that shea butter has attested to be active against skin-associated conditions such as small skin wounds, skin cracks, rough skin, and avoidance of stretch marks during pregnancy, insect bites, skin allergies such as eczema, dermatitis and many others.

Literature indicates that the wound healing properties of the stem bark of *L. barteri* have not been subjected to *in vivo* studies. We report the *in vivo* wound

healing efficiency of the crude extract alone and the herbal formulation of stem bark aqueous extract incorporated into shea butter in a rabbit wound model.

2. Materials and Methods

2.1. Collection and Preparation of Plant Material

Well-preserved powdered samples processed from the stem bark of *L. barteri* plant which had previously been validated by a plant taxonomist with a receipt voucher (number: SH 790), was used for this current research work.

2.2. Preparation of Plant Extract

A powdered plant sample (50 g) was submitted to solvent extraction with distilled water (500 ml) at room temperature for 72 h. After filtration, the extract was concentrated in a Rota evaporator (Heidolph Laborota 4001, Heidolph Instruments GMBH, Germany) under reduced pressure and constant temperature of 60˚C. It was subsequently dried to obtain a semi-solid sample and the yield was found to be 12.8% (w/w).

2.3. Preparation of Sterilized Shea Butter

Shea butter (200 g) was weighed into a clean conical flask and placed on a thermostatic hot plate at 120˚C for 2 h. This was to enable all traces of water in the shea butter to evaporate and sterilize from microorganisms. The oil was cooled to room temperature to form a solid material and was stored in a sterile airtight container until use.

2.4. Formulation of *Lannea barteri* Aqueous Extract-Shea Butter Ointment

L. barteri aqueous extract, 1.0 and 2.0 g, was separately mixed with sterilized shea butter (20.0 g) to form 5% (w/w) formulated *L. barteri*-shea butter ointment (LSO-5) and 10% (w/w) formulated *L. barteri*-shea butter ointment (LSO-10) respectively.

The mixtures were warmed separately in the water bath at a constant temperature (40˚C) with continuous stirring for about 30 min to ensure uniformity. The mixtures were then allowed to cool to obtain the respective ointments.

2.5. Quality Control Parameters of Formulation

Quality control of the formulation at varying concentrations was conducted to assess the pH, spreadability, and extrudability. Employing a digital meter, the pH of various formulations was estimated and the measurement was done in triplicate. The herbal ointment, 1 g, was dispersed in 200 ml of distilled water and stored at room temperature for 3 h. The herbal ointment was placed in between the slides under the direction of a certain load and the spreadability was expressed in terms of time in seconds taken by two slides to slip off from ointment. The extrudability of ointment formulations was determined in terms of weight

(g) required to extrude a 0.5 cm ribbon of ointment in 5 s. Physical parameters such as color, odor, smoothness and grittiness of herbal ointment formulations were also noted.

2.6. Preliminary Phytochemical Testing

Preliminary phytochemical screening of *L. barteri* extract previously conducted in our laboratory demonstrated the presence of phytochemicals such as Tannins, Saponins, Polyuronoids, Reducing, Sugars, Terpenoids, Flavonoids and Alkaloids [18].

2.7. Experimental Animals

Healthy rabbits of both sexes (1.50 - 2.00 kg, ages of 18 months) were purchased from the animal breeding laboratories of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, and allowed to acclimatize for one week before the study. They were accommodated separately in cages to avoid fighting and potential wound abrasion amongst each other. The rabbits were provided with water and food pellets *ad libitum* during the research. The animal studies were executed based on the guidelines of the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC) and the National Agricultural Library (USDA) Animal Welfare Act Quick Reference Guides under the strict supervision of the University veterinarian. Animals were grouped into five clusters, groups I to V ($n = 4$).

2.8. Excision Wound Model

The excision wound model described earlier [19] was used to assess the wound healing activity, thus wound contraction and wound closure time. Each group of animals ($n = 4$) was anesthetized with an intravenous dose of ketamine (50 mg/kg·bw) and xylazine (5 mg/kg·bw).

Removal of the back hairs of the rabbits was performed through shaving. A circular wound of approximately (254 - 343 mm^2) was created on the dorsal interscapular region of each animal by excising the skin with sterilized pointed scissors about 4 mm deep by a veterinarian and the wounds were left open.

2.9. Application of Test Samples

For the *in vivo* wound bioassay, each test drug was applied topically to the wounded site immediately after the wound was created with the aid of a surgical blade as follows: Group I—shea butter alone (SB) as negative control; Group II—Silver Sulphadiazine Cream (SS) as positive control; Group III—*L. barteri* aqueous extract alone (LBE); Group IV—5% *L. barteri* aqueous extract-shea butter ointment (LSO-5); Group V—10% *L. barteri* aqueous extract-shea butter ointment (LSO-10). Sterile cotton swab was used to spread topically the test sample evenly to cover the entire wound area. This was done twice daily until the wound was fully cured. Percentage reduction in wounded area was calculated

to represent wound contraction.

2.10. Wound Area and Wound Contraction

The wound was closely monitored and the healing area was calculated using a method described by [20]. Wound areas were measured by tracing the wound on a transparency sheet with a permanent marker and by using millimeter based graph paper on days 0, 4, 8, 12, 16 and 20 and 24 for all groups.

The percentage of wound closure was calculated using Equation (1).

$$\%\ \text{Wound Contraction} = \frac{\text{Initial wound area} - \text{final wound area}}{\text{Initial wound area}} \times 100\% \quad (1)$$

2.11. Statistical Analysis

Results were recorded as mean ± standard deviation. Comparisons between groups were evaluated using two-way ANOVA, followed by Tukey's multiple comparison test, $p < 0.05$ was considered significant. Data were analyzed using GraphPad Prism 8 for Windows (GraphPad Software Inc, San Diego, CA, USA).

3. Results and Discussion

The estimated values of the advancement of wound healing of excision wound model for LSO, LBE, SB, and SS groups are shown in Table 1. There was a

Table 1. Effect of shea butter, *Lannea barteri* extract, formulated herbal ointment and Silver sulfadiazine on excision wound contraction (mm^2).

Group	Topical Treatment	0 day	4th day	8th day	12th day	16th day	20th day	24th day
I	SB (Negative Control)	315.6 ± 3.3	314.0 ± 1.6 (0.5 ± 0.9)	295.2 ± 3.6 (6.4 ± 1.6)	271.2 ± 2.2 (14.0 ± 0.5)	218.4 ± 3.7 (30.8 ± 0.5)	216.3 ± 3.3 (31.5 ± 1.0)	216.0 ± 2.0 (31.5 ± 0.7)
II	SS (Positive Control)	342.6 ± 3.6	245.2 ± 2.5* (28.4 ± 1.3)[a]	136.9 ± 3.8* (60.0 ± 1.8)[a]	65.6 ± 3.2* (80.8 ± 0.8)[a]	52.7 ± 2.8* (84.6 ± 0.9)[a]	16.7 ± 2.78 (95.1 ± 0.7)[a]	7.7 ± 3.5* (97.8 ± 1.0)[a]
III	LBE	254.5 ± 5.9	206.8 ± 5.9*[#] (18.7 ± 3.6)[ab]	158,4 ± 3.1*[#] (37.7 ± 1.0)[ab]	157.7 ± 3.6*[#] (38.0 ± 0.6)[ab]	108.7 ± 3.0*[#] (57.3 ± 0.2)[ab]	56.3 ± 4.1*[#] (77.9 ± 1.1)[ab]	27.5 ± 6.9*[#] (89.1 ± 2.9)[ab]
IV	LSO-5	336.7 ± 4.2	298.4 ± 3.4*[#¥] (11.4 ± 0.4)[abc]	109.0 ± 4.4*[#¥] (67.6 ± 1.5)[abc]	13.0 ± 4.3*[#¥] (96.2 ± 1.2)[abc]	10.6 ± 2.6*[#¥] (96.8 ± 0.7)[abc]	4.8 ± 3.1*[#¥] (98.6 ± 0.9)[abc]	0.5 ± 0.9*[¥] (99.9 ± 0.3)[ac]
V	LSO-10	257.8 ± 4.9	223.9 ± 4.8*[#¥] (13.1 ± 2.7)[abc]	102.9 ± 1.6*[#¥] (60.1 ± .6)[ac]	20.0 ± 4.0*[#¥] (92.2 ± 1.4)[abc]	17.2 ± 3.1*[#¥] (93.3 ± 1.1)[abc]	10.8 ± 2.6*[¥] (95.8 ± 1.0)[ac]	4.6 ± 3.4*[¥] (98.2 + 1.3)[ac]

Values are mean ± SD; $n = 3$; values in ( ) are % rate of excision wound contraction; *Significantly different ($p < 0.05$) compared to SB group (negative control) in respective day; [#]significantly different ($p < 0.05$) compared to SS group (positive control) in respective day; [¥]Significantly different ($p < 0.05$) compared to LBE group in respective day; [a]( ) significantly different ($p < 0.05$) compared to SB group (negative control) in respective day; [b]( ) significantly different ($p < 0.05$) compared to SS group (positive control) in respective day; [c]( ) significantly different ($p < 0.05$) compared to LBE group in respective day; SB = shea butter only; SS = Silver sulfadiazine; LBE = *Lannea barteri* aqueous extract only; LSO-5 = 5% (w/w) formulated *Lannea barteri*-shea butter ointment; LSO-10 = 10% (w/w) formulated *Lannea barteri*-shea butter ointment.

significant reduction ($p < 0.05$) in all groups from the 4th day to the 12th day compared to the negative control group (SB), which saw a reduction of 315.6 ± 3.3 to 271.2 ± 2.2 mm^2. The wound area for SB remained fairly the same from the 16th to the 24th day.

The percentage rate of wound contraction from the 4th to the 12th day was in the order LSO-5 (11.4% to 96.2%) > LSO-10 (13.1% to 92.2%) > SS (28.4% to 80.8%). The percentage rate of wound contraction in groups LSO-5, LSO-10, and SS increased from a range of 84.6% to 96.8% on the 16th day to a range of 97.8% to 99.9% on the 24th day, with LSO-5 showing 99.9% contraction. In the same period, LBE increased from 57.3% to 89.1%, while SB exhibited a % wound contraction of 30.8% to 31.5%.

It was also observed that apart from day 4, LSO-5 exhibited the highest percentage excision wound contraction rate on all days compared to the other treatments including the standard drug SS, indicating that LSO-5 could offer the best treatment among the rest (Figure 1).

Delayed contraction rate of the circular excised wound was exhibited in control rabbits (SB) from day 0 to day 4 compared with the standard drug silver

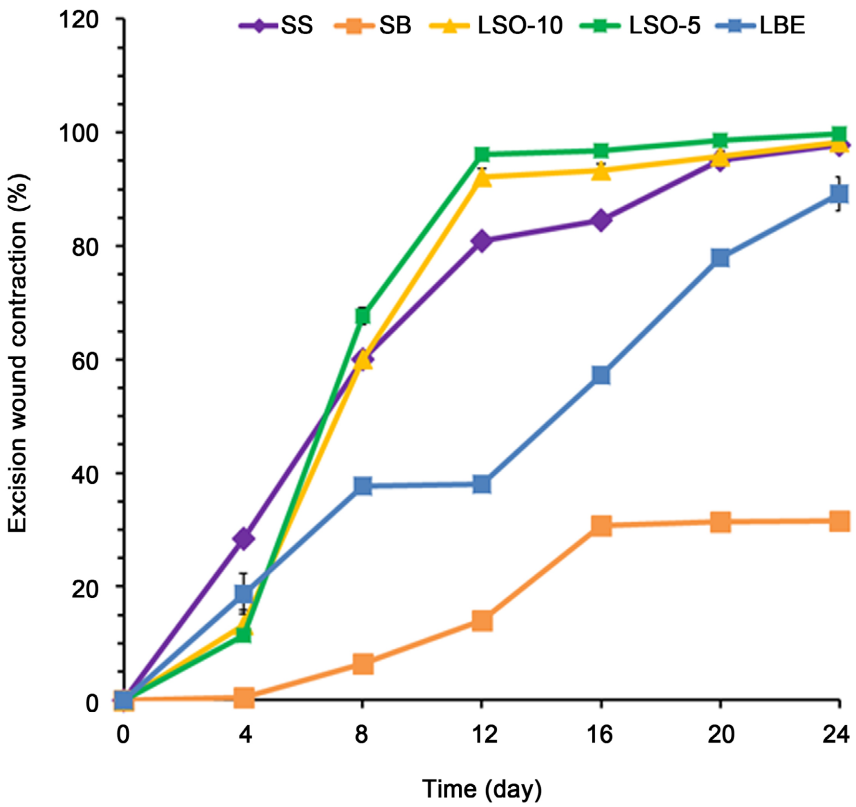

Figure 1. Percentage rate of excision wound contraction. SB = Group I, treated with shea butter only (negative control); SS = Group II, treated with Silver sulfadiazine (positive control); LBE = Group III, treated with *Lannea barteri* aqueous extract only; LSO-5 = Group IV, treated with 5% (w/w) formulated *Lannea barteri*-shea butter ointment; LSO-10 = Group V, treated with 10% (w/w) formulated *Lannea barteri*-shea butter ointment.

sulfadiazine treated animal (SS) as well as the ointment formulation-treated animals (LSO) (Figure 1). The results support the positive influence of herbal ointment in wound healing. Both the test and standard drug treated animals showed a significant reduction in wound size as compared with control group animals (Figure 2). There was no noticeable conduct of discomfort in any of the animals during the period of the experiment, since none of the following was observed: loss of weight, immobility, failure to groom, abnormal posture, licking or biting of wound area was absent.

Curative, an intricate process is commenced as a result of a reaction to an injury and reinstates the function and integrity of impaired tissues [21]. Donkor *et al*. [18] reported that the aqueous fraction of *L. barteri* exhibited a higher antibacterial activity against some selected wound infectious pathogens with MIC of 6.25 mg/ml compared to its ethanol extract. We aimed at evaluating the wound healing potential of aqueous fraction of the raw extract and the extract formulated with shea butter *in vivo*. For the first time, the findings of this study revealed the enhancement of the wound contraction rate in rabbits treated with an ointment containing shea butter and *L. barteri* aqueous extract. The outcome highlights a novel potential application of the traditional pharmaceutical plant. The herbal formulation passed all the quality control parameters such as pH, spreadability-time in seconds, extrudability, color, odour, smoothness, and grittiness. Herbal ointment formed was consistent and pleasantly applicable to the skin. It is worth noting that research into the shelf-life of the ointment, which is an

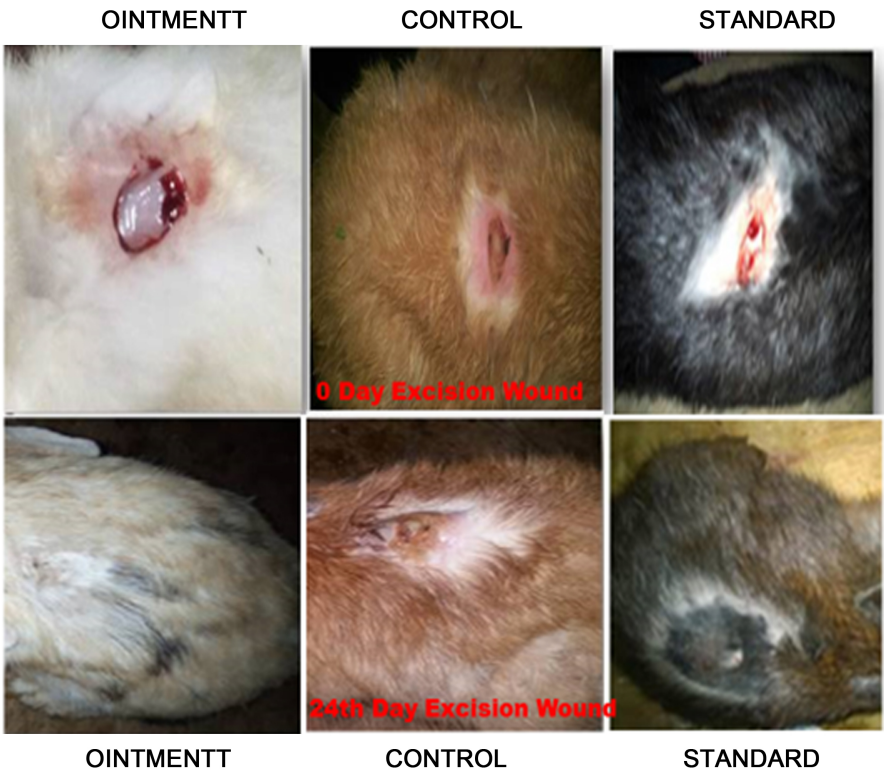

Figure 2. Healing pattern in excision wound at day 0 and day 24.

important quality control parameter, is presently ongoing in our laboratory.

The ointment preparation of the therapeutic plant might accomplish wound maintenance restoration. Thus, the extract was formulated as an ointment for the excision wound experiments. Although the acute toxicity test was not performed, an acceptable safety limit of the ointment would need to be determined before the drug could be recommended for possible use for clinical purposes. The lower dose (5% w/w) of the ointment significantly accelerated wound repair ($p < 0.05$) after comparing it with the results acquired for the control groups. In detail, the 5% w/w ointment exhibited an enhanced therapeutic effect with respect to the positive control in the excision tests. The outcome of the results demonstrated the effectual wound curative potential of a low dose of *L. barteri* crude extract-shea butter ointment per excision tests. Nonetheless, an increment dose of 10% w/w of the plant extract in the ointment was advantageous as presented in the results obtained regarding the ointment treated test models. An earlier report in our laboratory concerning an antibacterial study [18], the efficient dosage of both aqueous and ethanol extracts displayed a similar trend to this current result. Correspondingly, it could be inferred that the wound healing activity of the formulated ointment might likewise be linked to its antimicrobial activity that occurs at a specific dosage interval.

The antibacterial and wound healing activities might be due to the existence of alkaloids, flavonoids, and steroids as reported by [18]. Increased rates of wound contraction, epithelialization, and prevention of secondary bacterial infections that would have complicated and delayed wound healing are said to be attributable to these metabolites which play a critical role in the wound healing process. Tannins being phenolic compounds, typically act as an astringent and are found in a variety of plant products used in wound healing. The astringent property is responsible for wound contraction and accelerates the rate of epithelialization at the granulation formation and scar remodeling phases [22].

The primordial and therapeutic application of *L. barteri* established its innocuous character and specified the potential development in the pharmaceutical arena, and hence it is believed to have a high potential medicinal value. Hitherto, the substantial constituents of the wound healing activity and the action of mechanisms were undetermined. Subsequently, this study would focus on the analysis of the healing marker and *in vivo* dose-effect relationship. The acute dermal toxicity of the crude extract of the plant and the formulated ointment would be carried out to find the limited dose that is safe.

4. Conclusion

The wound healing activity of ointment of *L. barteri* leaf extract was revealed. Wound contraction together with the restored tissue supports the experiential wound healing. The results of the study demonstrate the usage of shea butter as a base in formulating an herbal ointment of the plant could enhance wound healing.

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Authors' Contributions

AMD, AWYKS conceived and designed the study; AWYKS collected the plant samples and performed the experiments; AMD and MND performed statistical/data analysis; AMD, MND, RM and AWYKS contributed equally in drafting and writing the paper. All authors read and ratified the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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