

Original Research Article

Wound healing activity of methanol root bark extract of *Salacia lehmbachii* in Wistar rats

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Sent for review: 15 August 2025

Revised accepted: 11 January 2026

Abstract

Purpose: To compare wound healing activity of methanolic root bark extract of *Salacia lehmbachii* in Wistar rats, with standard and known wound healing ointments.

Methods: Phytochemical screening of methanol extract was carried out for primary and secondary metabolites. Acute dermal toxicity study was carried out in (6) Wistar rats using “fixed dose” method. Thirty-six rats of mixed sexes were divided into six groups of 6 rats each, namely: Saline, petroleum jelly; 5 % povidone iodine; 10 % solcoseryl, as well as extract (10 %) in either petroleum jelly or saline. Excision wounds were created and treatment for each group was applied topically on the wounds daily for 13 days. Wound healing was assessed by measuring wound size on days 1, 3, 5, 7, 9 and 13.

Results: Preliminary phytochemical analysis of the crude extracts indicated the presence of tannins, phenolic compounds, volatile oil, terpenoids, glycosides, saponins, flavonoids, steroids and alkaloids in varying concentrations. Acute dermal toxicity test revealed no toxicity at a dose of 2, 000 mg/kg of extracts. Extract-treated animals exhibited complete wound healing by the thirteenth day of treatment. Extract-treated animals exhibited 77% reduction in the wound area when compared to control, which was 59 %. Petroleum jelly plus extract showed better activity on the seventh day with 11.20 ± 0.73 % reduction in wound size compared to the reference solcoseryl with 11.80 ± 0.37 % ($p < 0.05$).

Conclusion: Methanol extract of *Salacia lehmbachii* root bark accelerates wound healing process in a dermal wound model, an effect that compares favourably with that observed for standard treatments using Solcoseryl and Povidone iodine. The outcome is indicative of the synergy in activity that exists between petroleum jelly and the extract *Salacia lehmbachii*.

Keyword: *Salacia lehmbachii*, Wound closure, Haemostasis, Inflammation, Proliferation, Remodeling

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Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The use of plants as a major source of traditional and folklore medicine plays important role in global health care services [1]. More than 80% of the World population is known to depend on traditional medicine for the treatment of various ailments, as seen in the management of malaria, wounds, worm infestation and lots more [2].

Plants have formed the basis of traditional medicine systems and Ayurveda that have been in existence for decades; their use by many cultures and climes has been extensively documented [3]. The continuing valuable contributions of nature as a source of potential medicinal agents were reviewed [4]. An analysis of natural products as sources of new drugs over the period 2000-2024 indicated that 40% of new

chemical entities (NCEs) contain pharmacophore derived directly from natural products [4].

The selection of a suitable plant for a pharmacological study is a very important and decisive step in Medicare. This could be achieved through knowledge of traditional usage, analysis of their chemical composition, toxicity, randomized selection, or a combination of several criteria [5]. The most common strategy is careful observation of the use of the plant species in folk medicine in different cultures. *Salacia lehmbachii*, otherwise called *eba-enang* amongst the Efiks and the Ibibios of South - South Nigeria, is a tropical plant belonging to the family *Celastraceae* and Order *Celastrales*. It is one of such plants that have been used over the years and is still in use traditionally in the eastern part of Nigeria in the treatment of many illnesses, including malaria, basically in combination with other root or leaf extracts from *Magnifera indica*, *Psidium guajava* and *Carica papaya* [6]. Its root bark and leaf parts have also been traditionally used in treating various ailments such as infection and inflammatory processes in the tropical regions of West Africa [7].

Healing is a natural phenomenon by which the body itself overcomes the damage to its tissue, but the rate of natural healing without intervention is usually very slow and the chance of microbial infection is high. Intervention in healing process can be accomplished either to shorten the time required for healing or to minimize the undesired consequences. Many countries have rich tradition of plant-based knowledge in healthcare delivery. Several herbs or medicinal plants that proved to be wound healers were identified and formulated for treatment and management of wounds over decades [5].

Wounds heal by primary, secondary and tertiary intention. Healing in acute wounds occurs as a sequence of overlapping processes that requires the coordinated completion of a variety of cellular activities. These activities occur in a coordinated and systematic way that correlates with the appearance of different cell types in the wound at various stages of the healing process. These processes, which are triggered by tissue injury, involve the four overlapping but well-defined phases of haemostasis, inflammation, proliferation and remodeling [8].

This work attempts to highlight and evaluate a herb and medicinal plant, *Salacia lehmbachii* (*S. lehmbachii*), traditionally used for the treatment of inflammatory processes, for its wound healing potential. Though there is paucity of scientific

information in the literature with regard to its biological activities, bio-mimetic synthesis and its therapeutic uses, there are ongoing studies in that respect and this research work on its potential effect on wound healing is one of such.

Salacia lehmbachii is one of the various species of *Salacia* genus. It is a plant of Tropical Africa, the Cameroons, India and Sri Lanka origin with a wide range of purported medicinal properties and values such as antiseptic, antimicrobial, anti-parasitic, anti-inflammatory, anti-mutagenic and nephro-protective properties [7]. While there are limited data to support most of its uses in folk medicine, there is growing scientific evidence that the root and leaf extracts of *S. lehmbachii* possess analgesic, anti-inflammatory, antipyretic, antimicrobial and related pharmacological activities that reflect its traditional medicinal applications in West Africa [7,9].

EXPERIMENTAL

Materials

Solcoseryl ointment was obtained from MedaPharma Switzerland. Normal saline and Povidone-iodine were obtained from Kamel Pharmacy, Calabar. All other reagents and chemicals were of analytical grade and obtained locally from BDH and Sigma-Aldrich, Nigeria.

Plant material

The root bark of *S. lehmbachii* was purchased around June 2023 from a local market in Calabar South Local Government, Cross River State, Nigeria, and was authenticated by Mr. F. I. Apejoye of the Department of Botany, University of Calabar, Cross River State, where a voucher number 688 was given.

Preparation of extract

The root bark was washed, chopped into pieces and oven-dried for about 48 hours at 40 °C in an oven. After complete drying, the entire portion was pulverized into a coarse powder with the help of a locally produced grinding machine and the resultant powder was weighed and stored in an airtight container for extraction. The sample, 5 g, was later wrapped in double-layered Whatman's filter paper into a thimble of the Soxhlet extractor, fitted to a 1,000 ml capacity round-bottom flask containing 500 ml of Petroleum ether. The round-bottom flask was placed in a heating chamber at 60 °C for 12 hours.

The Petroleum ether extracts fats and lipids from the sample, suspending fats and lipids without damaging their essential compositions, so it was used as a tool for making this plant-based extract. The resultant residue (not soluble in Petroleum ether) was dried, repacked and then extracted with methanol. The extract was stored in a dry glass bottle from which solutions were reconstituted with normal saline to obtain suspension of appropriate concentrations. Aliquots of the dried extract were reconstituted and subjected to phytochemical analysis to detect the chemical constituents present in it [10].

Phytochemical screening

Chemical tests were carried out qualitatively on the extract and on the powdered specimens using standard procedures to identify constituents as [10-13] with little modification. It was screened for alkaloids, tannins, saponins, triterpenes, flavonoids, steroids, cardiac glycosides, carbohydrates, fat and proteins.

Test for saponins

300 mg of extract was boiled with 5 ml of distilled water for two minutes, cooled, stirred vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

Test for tannins

To an aliquot of the extract, sodium chloride was added to make to 2% strength. Filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

Test for Triterpenes

300 mg of extract mixed with 5 ml of chloroform was warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

Test for alkaloids

A 300 mg weight of extract was digested with 2 M hydrochloric acid (HCl). Acidic filtrate was mixed with amyl-alcohol at room temperature and examined; the alcoholic layer of a pink color indicated the presence of alkaloids.

Test for flavonoids

The presence of flavonoid was determined using 1 % aluminum chloride solution in methanol,

concentrated HCl, a piece of magnesium, and a potassium hydroxide solution. A yellow colouration indicates the presence of flavonoids. The yellow colouration disappears on standing.

Test for steroids

2 mL of acetic anhydride was added to 0.5 g of the methanol extract with 2 ml sulphuric acid (H_2SO_4). The colour change from violet to blue or green in the sample indicates the presence of steroids.

Test for terpenoids (Salkowski test)

Five mL of extract was mixed with 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown colouration at the interface formed shows the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test)

Five ml of extract was treated with 2 ml of glacial acetic acid with a drop of ferric chloride solution. This was treated with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a de-oxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form gradually throughout the thin layer.

Animals

Thirty-six Wistar rats of either sex weighing 180 - 200 g were obtained from the animal unit of the Department of Pharmacology, University of Calabar, Nigeria. The animals were divided into six (6) groups containing six (6) rats each. They were caged to acclimatize to conditions of the animal housing facility, including ambient temperature of (29-31 °C) and relative humidity (60 – 70 %) for two weeks. They were fed with standard mash and clean water *ad libitum*.

The Wistar rats of mixed sexes were topically exposed to the extract at a dose of 2,000 mg/kg for fourteen days and were monitored for signs of tremor, convulsion, salivation, diarrhoea, lethargy, sleep, behavioural and clinical abnormalities, gross lesions, body weight changes and coma. Ethical approval was granted by the Directorate of Research and Development (approval no. UC/DR&D/RERB/56). Procedures used were as humane as possible and complied with regulated guidelines for animal care [13].

Acute dermal toxicity – fixed dose procedure

The acute dermal toxicity study was carried out in 6 Wistar rats using the “fixed dose” method of the reported guideline [14]. Extract was applied topically on 2 rats at each dose level of 1,000 and 2,000 mg/kg. The control group had normal saline only and dose level of 1,000 mg/kg was selected on the basis of a sighting study [13]. This is a concentration that would neither produce clear signs of toxicity or mortality nor show evidence of delayed toxicity. Clinical signs and conditions associated with pain, suffering, and impending death were monitored. Each animal, at the commencement of testing, was allocated to cages according to the dose assigned and observed throughout the duration of the study.

Approximately 24 hours before drug testing commenced, fur was removed from the dorsal area of the trunk of the test animals by shaving. Care was taken to avoid abrading the skin to retain its permeability. At least 10% of the body surface area was cleared for the application of the test substance. The weight of the animal was taken into account when deciding on the area to be cleared and on the dimensions of the covering. The test substance was reconstituted in normal saline or petroleum jelly, depending on the group and was topically and uniformly applied over an area that is approximately 10% of the total body surface area [14].

Animals were observed immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours and daily for 14 days. All observations were systematically recorded, with individual records being maintained for each animal. Animals found in moribund condition and animals showing severe pain and enduring signs of severe distress were humanely sacrificed. Signs of toxicity, including changes in skin and fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems; somatomotor activity and behavior patterns were monitored. Attention was also directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

Experimental design (grouping of animals)

Animals were divided into six groups of six (6) rats and their wounds were immediately treated topically as follows:

Group 1: Negative control group; treated with just normal saline

Group 2: Positive control group; treated topically with pure petroleum jelly (100 %)

Group 3: Standard group; treated topically with povidone iodine ointment (5%)

Group 4: Reference group; treated topically with solcoseryl-ointment (10%)

Group 5: Experimental group (1); treated topically with 10% methanol extract in pure petroleum jelly

Group 6: Experimental group (2) treated topically with 10% methanol extract in normal saline (NS).

Induction of wounds

Animals were anesthetized using 1.0 mg of ketamine intramuscularly prior to and during creation of the wounds [15]. The fur on the back surface of the animals was removed by wiping with a suitable depilatory substance (shaving powder) using cotton swabs. A near circular wound of 30 - 45 sq mm was made on the depilatory dorsal thoracic region of the animals by cutting the skin of the animals with forceps and scissors. The wounds were treated immediately and left open.

Treatment protocol

The status and performance of the wounds were assessed and percentage wound closure was determined for each wound on days 1, 3, 5, 7, 9 and 13 post-wound initiation. Topical treatments were continuous on each of those days. The areas of the wound were marked by placing a transparent sheet over the wound, and the recorded areas were measured in sq.mm by using graph paper. The initial wound area (I) was taken as day one. The percentage area reduction (R) was calculated for days 1, 3, 5, 7, 9 and 13 post-treatments [16] using Eq 1.

$$R (\%) = (I - I_n/I)100 \dots\dots\dots (1)$$

Where I_n is the n th day area of the wound.

Statistical analysis

Data are expressed as mean SEM of six observations and statistically assessed by a Two-Way Analysis of Variance (ANOVA) with Tukey's multiple comparison. A probability of $p < 0.05$ was considered significant.

RESULTS

Acute dermal toxicity study

The acute dermal toxicity test, after dermal administration of 2, 000 mg/kg of extract, revealed no toxicity at this dose. The study provides an estimate of the relative toxicity of the test substance by the dermal route of exposure. The animals were found not to exhibit any signs of toxicity or mortality, but they were physically active, consuming food and water in a regular way.

Phytochemical composition

The preliminary phytochemical analysis of the crude extracts indicated the presence of tannins, phenolic compounds, volatile oil and terpenoids, glycosides, saponins, flavonoids, steroids and alkaloids in varying concentrations (Table 1).

Wound status and closure

The effect of *S. lehmbachii* extract and changes due to other regimens on excision wound size in the rats is tabulated in Table 2. Percentage reductions in wound area are shown in Table 3, while the corresponding percentage wound closure is shown in Table 4. Animals treated with *S. lehmbachii* extract showed significant reduction in the wound area ($p < 0.05$) with faster rate of epithelization ($11.20 \pm 0.73 \text{ mm}^2$) when compared with the untreated control group of animals ($28.55 \pm 0.61 \text{ mm}^2$).

Wound healing activity of the root bark extract (both in NS and Petroleum jelly) of this medicinal plant, with complete wound closure, was observed within 13 days. The studies show that all six groups had decreased wound areas from day 1 to day 13. However, on the 9th day post-wounding, NS-treated animals showed delayed wound healing of about 36 %; pure petroleum

jelly-treated rats showed 72 % healing rate, povidone iodine-treated rats showed 100 % healing with total wound closure; high healing rate was also observed in solcoseryl ointment-treated rats with total wound closure on day 13. The analysis of variance showed that there was a significant difference in the percentage wound closure when all the treatment groups were compared with the negative control (normal saline) group ($p < 0.001$).

The group treated with the extract in pure petroleum jelly had about 87 % wound closure in 9 days and achieved a total closure in 13 days as would be expected, similarly, the group treated with extract in normal saline formed a crust on the wound surface on day 5, the crust was subsequently removed by debridement to expose the wound on day 5, then on day 9 about 70% wound closure was achieved with 100% closure on day 13. Tukey's multiple comparison tests showed that all treatment groups were significantly different from each other ($p \leq 0.05$) except for the comparison between the solcoseryl-treated and *S. lehmbachii* extract (10 % in petroleum jelly) groups, where the results of percentage wound closure across treatment duration times were similar, almost superimposable ($p \leq 0.0001$).

Table 1: Phytochemical composition of the Methanol Extract of *Salacia lehmbachii* root bark

Test	Results
Flavonoids	+++
Steroids	++
Saponins	+
Cardiac Glycosides	+
Carbonyl	+
Tanins	++
Alkaloids	+
Anthraquinones	—
Reducing Sugars	+

Table 2: Wound size changes following treatment with *S. lehmbachii* and other known substances

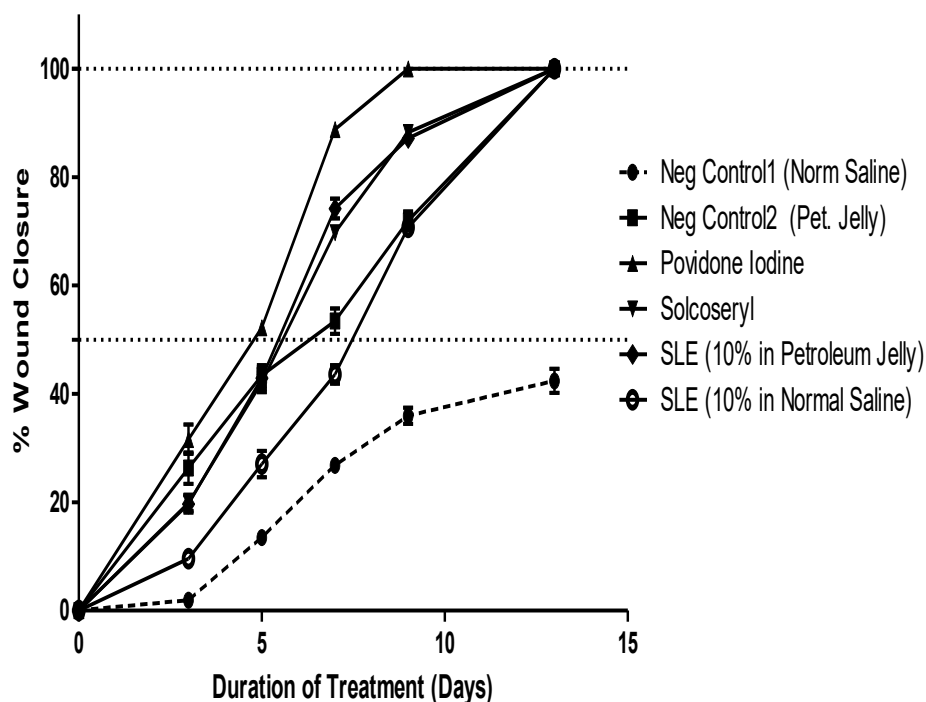
Group	Days/mm ²					
	1	3	5	7	9	13
Normal Saline	39.00±0.58	38.25±0.63	33.75±0.75	28.55±0.61	25.00±0.91	22.50±1.19
Petroleum Jelly	42.10±1.58	30.90±0.42*	23.75±0.48†	19.50±0.29†	11.80±0.48	0.00±0.00†
Povidone Iodine	39.75±1.55	27.25±1.89†	19.00±0.91†	4.50±0.65†	0.00±0.00†	0.00±0.00†
Solcoseryl	39.00±0.84	31.20±0.37†	22.60±1.08†	11.80±0.37†	4.60±0.24†	0.00±0.00†
SLE (10% in Pet. Jelly)	43.60±1.69†	35.00±1.41*	24.80±0.73†	11.20±0.73†	5.60±0.40†	0.00±0.00†
SLE (10% in N S)	37.60±0.24	34.00±0.45**	27.40±0.75†#	21.20±0.58†	11.00±0.32†	0.00±0.00†

Table 3: Percentage reduction in wound size (area) following treatment with *Salacia lehmbachii* and other known substances

Group	Days/%					
	1	3	5	7	9	13
Normal Saline	100.00±0.00	98.09±1.20	86.51±0.78	73.19±0.74	64.05±1.47	57.60±2.23
Petroleum Jelly	100.00±0.00	73.71±2.89 [†]	56.60±1.90 [†]	46.57±2.35 [†]	28.05±1.68 [†]	0.00±0.00 [†]
Povidone Iodine	100.00±0.00	68.37±2.70 [†]	47.76±0.84 [†]	11.19±1.21 [†]	0.00±0.00 [†]	0.00±0.00 [†]
Solcoseryl	100.00±0.00	80.11±1.49 [†]	57.86±1.84 [†]	30.25±0.58 [†]	11.78±0.49 [†]	0.00±0.00 [†]
SLE (10% in Pet. Jelly)	100.00±0.00 [†]	80.30±1.59 [†]	57.08±2.00 [†]	25.80±1.84 [†]	12.86±0.87 [†]	0.00±0.00 [†]
SLE (10% in N S)	100.00±0.00	90.44±1.33 [†]	72.93±2.43 [†]	56.40±1.68 [†]	29.27±0.98 [†]	0.00±0.00 [†]

Table 4: Percentage wound closure following treatment with *Salacia lehmbachii* and other known substances

Group	Days/ (%)					
	1	3	5	7	9	13
Normal Saline	0.0±0.00	1.9±1.20	13.5±0.78	26.8±0.74	36.0±1.47	42.4±2.23
Petroleum Jelly	0.0±0.00	26.3±2.89 [†]	43.4±1.90 [†]	53.4±2.35 [†]	71.9±1.68 [†]	100.0±0.00 [†]
Povidone Iodine	0.0±0.00	31.6±2.70 [†]	52.2±0.84 [†]	88.8±1.21 [†]	100.0±0.00 [†]	100.0±0.00 [†]
Solcoseryl	0.0±0.00	19.9±1.49 [†]	42.1±1.84 [†]	69.8±0.58 [†]	88.2±0.49 [†]	100.0±0.00 [†]
SLE (10% in Pet. Jelly)	0.0±0.00	19.7±1.59 [†]	42.9±2.00 [†]	74.2±1.84 [†]	87.1±0.87 [†]	100.0±0.00 [†]
SLE (10% in N S)	0.0±0.00	9.6±1.33 [†]	27.1±2.43 [†]	43.6±1.68 [†]	70.7±0.98 [†]	100.0±0.00 [†]

**Figure 1:** Percentage wound closure following treatment with *Salacia lehmbachii* and other known substances. Pet. Jelly – Petroleum jelly

DISCUSSION

The study was carried out to confirm the traditional and folkloric wound healing activity of *Salacia lehmbachii*. Differential healing pattern with complete wound closure was observed in treated rats within 13 days. The studies reveal that all the groups showed decreased wound areas from day to day. However, in the normal saline-treated group (Group-1), the animals

showed a delayed healing pattern seen as an increase in the number of days to close the dermal lesion and characterized by inadequate matrix synthesis, poor re-epithelialization and infection or formation of greenish-yellow substance (Slough) on the wound surface. The healing process in this group was unaided and depended entirely on the inherent immunity of the animals and nature's complex process of restoring cellular structures and tissue layers in

wounds to their normal state. Group-2 treated animals showed moderate healing rate in variance with that of other treated groups. The Povidone-iodine group showed maximum percentage of healing as early as the 9th day. All the groups were statistically significant and comparable with control as can be seen in Figure 2, healing in all the groups were time dependent without the formation of scar tissue.

Wound breaks the epithelial integrity of the skin tissue; the breakage may impair or stop the skin's functions at the site of effect. The study reveals that methanolic extract of *Salacia lehmbachii* has good wound healing properties and is statistically significant.

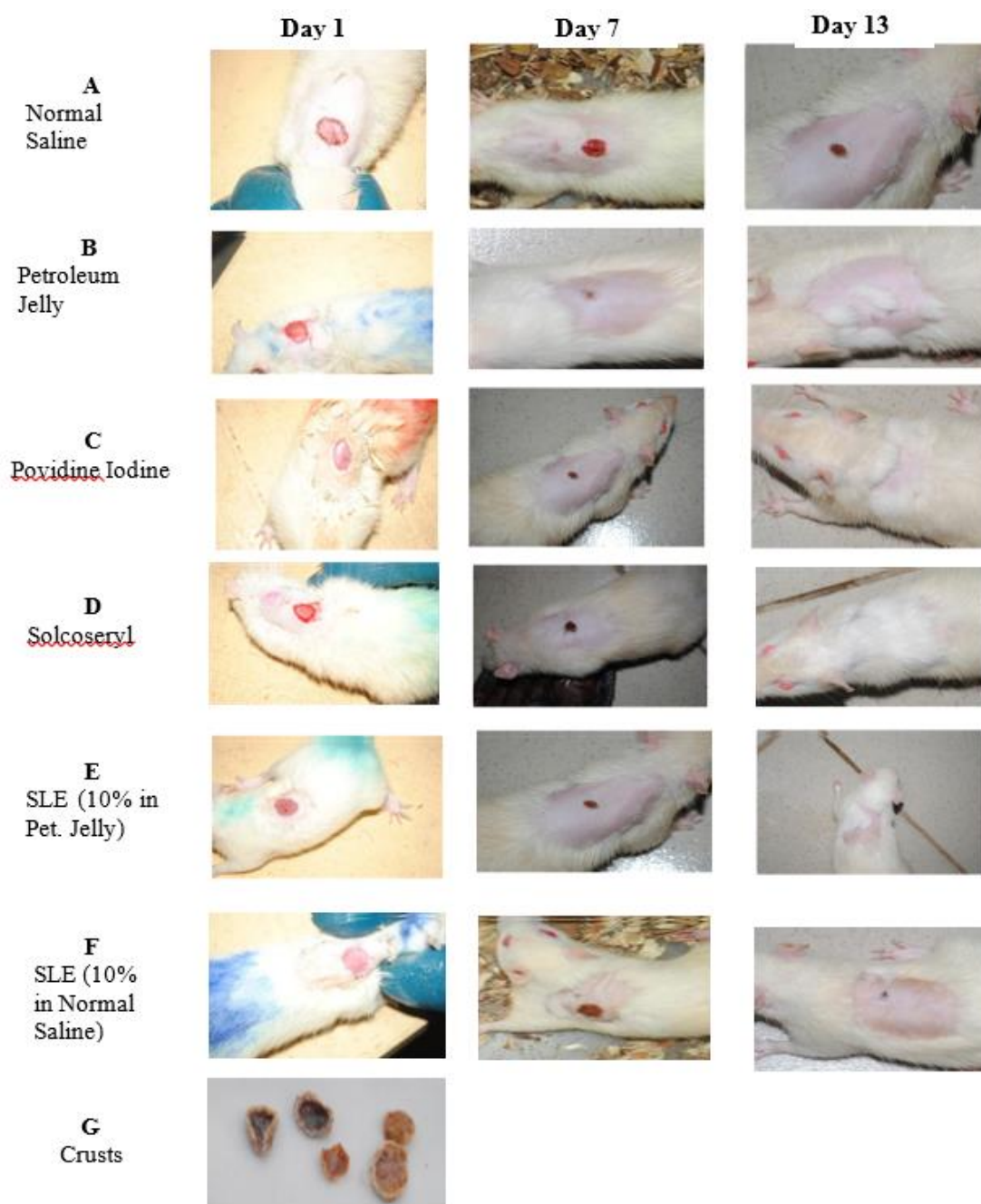


Figure 2: Photographs of the wound status during treatments with *Salacia lehmbachii* and other regimens. The row panels show treatment regimens and the column panel shows days after wound initiation. The regimens were applied daily to rats for the entire duration of the treatment. The crusts in panel G were removed on day 5 from rats treated with a 10% solution of *Salacia lehmbachii* (SLE) in normal saline

The ability of this plant extract to heal wounds could be favorably compared with that of known wound healing formulations. Povidone iodine is a known antimicrobial agent, while solcoseryl is a natural wound healing agent by enhancing the formation of granulation tissue (neogenesis). The healing in the various groups may show some complexities; in the extract groups, one can assume the mechanism that reconstruction of the damaged tissues required the coordinated action of a large number of biochemical healing agents like saponins, tannins, triterpenes, alkaloids, flavonoids, steroids, terpenoids and cardiac glycosides in the extracts [17], this is not to ignore the effects of the presence or absence of contaminating toxins in the wounds, wound infection resulting from the impaired immunity and exposure or poor hygiene which are most commonly encountered clinically important impediments to wound healing.

Infection on the wound surface was seen as sloughs or greenish-yellow formation in the normal saline group; the injured skin that was potentially vulnerable to invasive microbial infections of all kinds might have subsequently developed wound sepsis before complete epithelial repairs occurred. Injury becomes infected because the wound area is an ideal medium for the multiplication of the infecting organism [18]. These were not found in groups treated with herbal extract and in the group treated with povidone-iodine used in wounds as antimicrobial agent; thus, it is assumed that the extract effectively inhibited microbial growth and enhanced epithelial growth to accelerate wound healing. The enhanced wound healing potency of this extract may also be attributed to the antioxidant effect of flavonoids and the property of other phytoconstituents present in the extract [19].

Antioxidants help to control wound oxidative stress, thereby accelerating wound healing. The quicker process of wound healing could be a function of either the individual or the synergistic effects of these bioactive molecules. Flavonoids, which are present in *S. lehmbachii*, are known to enhance wound healing in general by increasing collagen viability and strength, improving circulation, protecting cells from oxidative damage, and promoting DNA synthesis [20].

Flavonoids are said to reduce lipid peroxidation by preventing or slowing the onset of cell necrosis and by improving vascularity. Any drug that inhibits lipid peroxidation is also known to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing cell

damage and promoting the DNA synthesis [21]. Studies have also revealed that flavonoids promote the wound healing process by their astringent and antimicrobial properties, which appear to be responsible for the increased rate of epithelialization in wound healing [22]. Flavonoids, being potent antioxidant do, have free radical-scavenging effect, promoting the level of antioxidant enzymes in granuloma tissue [23]. Tannins are the main components of many plant extracts and they act as free radical scavengers [24]. Saponins' healing property is linked to their antioxidant and antimicrobial activities, which appear to be responsible for wound contraction and elevated rate of epithelialization. The results of various studies performed so far are significant for different parameters in wound healing activity and showed that the usage of herbal extracts obviously accelerates wound healing. Overall, the percentage wound contraction assessment showed that the healing process of the wounded tissues in groups five and six treated with *S. lehmbachii* in 10% petroleum jelly and normal saline, respectively, was comparably close to the reference and standard groups treated with povidone iodine and solcoseryl. Delayed healing was observed in negative control (normal saline) group.

It was observed that wounds treated with *S. lehmbachii* in normal saline formed a thick film or crust a few days after initiation of treatment. The crust is believed to have occurred due to the inability of the SLE to retain fluid to keep the wound surface wet. It could be a build-up of fibrils, vitronectin, fibronectin, thrombospondin and other extracellular matrix that would have formed clots at the excision of wounds. Extract in petroleum jelly formed no such crust during the healing process; this was thus attributed to the wetness of the wound surface by the jelly, a property that enhanced better healing in this group than the extract in normal saline.

Debridement of the wound and reapplication of the extract allowed for better healing in that group (Extract in Normal saline); the wound surface at this time is believed to have less microbial activity as more of the *S. lehmbachii* extract would have gotten into the wound surface to improve epithelialization, seen as collagen deposits and granulation tissue formation. It may thus be said that *S. lehmbachii* has the potential to satisfy requirements of an ideal dressing material in that it provides an environment at the surface of the wound in which healing takes place at the maximum rate consistent with the formation of granulation tissue. The fact that the plant extract in normal saline left prominent crust

formation at the wound sites in the early phase of healing, then removing the crust and reapplying the extract showed enhanced progression in wound healing, indicates that the extract, if applied often and correctly, will accelerate granulation tissue formation and improve healing. The study provides a rationale for the use of *S. Lehmbachii* extract preparations in the traditional system of medicine to improve granulation of wound bed and promote healing. This effect may be explained by several mechanisms, such as wetting the wound to encourage epithelial cell growth, chelating free radicals and reactive oxygen species to increase the formation of new cells and fibroblasts, thus stimulating the contraction and narrowing of the wound area.

Comparatively, groups were seen to exhibit a close pattern of healing in time and space, the solcoseryl group and that of *S lehmbachii* in petroluem jelly had similar rate of wound closure, which is attributed to the synergy between the jelly and extract constituents. Treatment was effective and comparable among the extract groups, reference group and the standard group. The standard and the reference groups had to be separated in this work because povidone iodine is a known antimicrobial agent, while solcoseyil is a natural wound healing agent by enhancing the formation of granulation tissue (neogenesis); they therefore promote wound healing process differently.

Group interaction and synergy were observed in group 5 treated with extract in petroleum jelly and group 2 treated with petroleum jelly alone. In this experiment, interaction accounts for 6.03% of the total variance and presented a graph with horizontal slope. There were interactions within formulations as seen more in that of *S lehmbachii* in petroleum jelly than *S lehmbachii* in Normal saline. Interaction was found to account for 6.03% of the total variance and the P value is <0.0001. If there is no interaction overall, there is a less than 0.01% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered extremely significant. Furthermore, in analyzing the time effect, it was found to account for 77.65% of the total variance (after adjusting for matching) and the P value is <0.0001. If time has no effect overall, there is a less than 0.01chance of randomly observing an effect this big (or bigger) in an experiment of this size; therefore, time is considered extremely significant because the day-to-day wound closure was a standard to measure healing amongst groups and the time effect was very significant with p-value < 0.0001 in the overall treatment groups.

CONCLUSION

This study demonstrates that wound healing activity of methanolic extract of *Salacia lehmbachii* root bark is effective and functional. The result may be attributed to the phytoconstituents present in it, presenting their individual or cumulative effects that enhance wound healing and provide scientific evidence to the ethnomedicinal use of this plant. Wound healing is a biological process that begins with trauma and ends with scar formation. The goals of wound care include reducing risk factors that inhibit wound healing, enhancing the healing process and lowering the incidence of wound infections. *Salacia lehmbachii* root bark is potent in wound healing and can provide leads that may be therapeutically beneficial in wound healing.

DECLARATIONS

Acknowledgement/Funding

The authors are grateful for the support received from the Staff of the animal house, Department of Pharmacology, University of Calabar.

Declaration of interest

The authors declare that there is no conflict of interest.

Credit author statement

Grace Akpanika: Conceptualization and Methodology, Software, Validation and oversight functions.

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