Wound Healing Activity of 80% Methanol Leaf Extract of *Zehneria scabra* (L.f) Sond (Cucurbitaceae) in Mice

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Background: *Zehneria scabra* is one of the folklore plants of Ethiopia frequently used for external wound treatment in humans. Nevertheless, pharmacological investigations have not been performed to substantiate activity of the plant extract in wound healing. Hence, this study attempted to explore the activity of leaf extract of *Z. scabra* in wound healing using a mice model.

Materials and Methods: The air-dried leaf from *Z. scabra* was pulverized and extracted with 80% methanol and prepared with 5% and 10% extract ointments. An acute dermal toxicity study of the extract was conducted in female mice by observing the signs of toxicity. Then 5% and 10% (w/w) ointments of the extract were applied topically to investigate their wound healing activity in incision and excision wound models. Parameters such as wound contraction, period of epithelialization, and tensile strength were determined.

Results: Upon the application of 10% w/w extract ointment, no signs of dermal toxicity were observed in mice. Both 5% and 10% (w/w) extract ointment formulations increased percentage wound contraction and tensile strength, and shortened the epithelialization period.

Conclusion: The findings of this study collectively showed that the leaf extract of *Z. scabra* possessed significant wound healing activity, upholding the folkloric use of the experimental plant.

Keywords: *Zehneria scabra*, wound healing activity, incision model, excision model

Introduction

A wound is characterized as damage of the normal anatomy and function of the skin.¹ This may vary from a minor injury of epithelial tissues of the skin or maybe deeper damage to the subcutaneous tissue and others like blood vessels, muscles, tendons, bones, parenchymal organs, and nerves.¹ Wounds may be caused by pathological changes that start internally or externally from the involved organ. They may have an intentional or accidental etiology or may be the effect of a process of disease.² The physiological reaction to the damaging factor results in bleeding, coagulation of the vessel, complement activation, and inflammatory response.² Wound is one of the most common diseases often having severe complications in relation to health and posing high costs for therapy. To establish the integrity of the damaged tissue, the series of events must be progressed orderly in a well-controlled manner that, otherwise maybe causing physical disability even leading to death.³ Currently available methods of wound management including irrigation, debridement, antibiotics, proteolytic enzymes and tissue grafts are found to be associated with major drawbacks such as invasiveness and are expensive.⁴

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Emergence of resistant strains along with lack, high cost and retarded rate of newly generated antibiotics increase wound related mortality and morbidity.⁵ Globally, the emergence of resistant bacteria strains mostly those wound causing bacteria is a public health crisis. Multi-drug resistant *Pseudomonas* and *Acinetobacter* species, vancomycin resistant *Staphylococcus aureus* (VRSA), and methicillin resistant *Staphylococcus aureus* (MRSA) are some examples.⁶ As a result, infection of a wound is continuing as the most common influencing factor of non-healing wounds existence and remains a significant burden for patients and caregivers alike. Different studies are being made across the world to uncover agents that can facilitate healing and thereby minimize hospitalization costs and save the patient from amputation or other serious complications. However, the holistic management and current medicine for treating chronic wounds is very expensive.⁷ The global advanced wound management market, estimated at \$20 billion, is projected to rise at a combined annual growth rate of around 7% to around \$26 billion.⁸ In England, the National Health Service described the cost incurred for wound treatment as between £2.5 billion and £3.1 billion per year, accounting for 3–4% of the healthcare budget.⁸ Most of the drugs employed in wound treatment are not only costly but also have issues such as hypersensitivity reaction and drug resistance. In addition, among the drugs listed in Western Pharmacopoeia only 1–3% of them are recommended to be used on the skin and wounds; however, approximately one third of herbal medicines are intended for such use.⁹

Phytomedicines for wound healing are also needed to resolve costs, adverse effects, and antibiotic resistance.⁹ However, scientific confirmation, standardization, and safety assessment of plants used in conventional medicines before they could be recommended for wound healing are needed. The leaves of *Z. scabra* are used traditionally for wound dressing and healing, treatment of helminthic disease, body swelling, and diarrhea.¹⁰ Roots and leaves are also used to manage fever, common cold, skin problems and stomach pain. On top of this, a recent study confirms the ethanol and ethyl acetate shoot extract of *Z. scabra* have antimicrobial activity against the most common bacterial pathogens, ie, *Staphylococcus aureus* and *E. coli*.¹¹ The ethanolic tuber extract was also proved to have potent antifungal properties against five types of fungi including *A. niger*, *A. flavus*, *A. fumigatus*, *M. indicus*, and *C. albicans*. In addition, the 80% methanolic leaves of extract have been proven to have anti-diarrheal and anti-secretory,¹² anti-malarial,¹³ and anti-

inflammatory activities.¹⁴ The present experimental plant is traditionally claimed to have wound healing activity, which needs scientific investigation.

Materials and Methods

Chemicals, Drugs, and Solvents

Methanol (Carlo Erba Reagents, Italy), ketamine hydrochloride (Neon laboratories, India), diazepam (Gland Pharma limited, India), nitrofurazone USP 0.2% ointment (Shanghai General Pharmaceutical co, Ltd, China), wool fat, hard paraffin, white soft paraffin (queens Hygore industrie Plc), and cetostearyl alcohol (Banbury, Oxon, UK) were used. All chemicals and reagents used were of analytical grade.

Plant Material

Fresh leaves of *Z. scabra* were obtained from the College of Health Science, Tikur Anbessa Specialized Hospital compound from February 15–20, 2020. The plant material has been identified and verified by a taxonomist and a voucher specimen (MWBZ-025) was deposited at the National Herbarium of College of Natural and Computational Sciences, Addis Ababa University for future reference.

Experimental Animals

Adult albino mice of both sexes (25–35 g, and 6–8 weeks of age) were obtained from the animal house of School of Pharmacy, College of Health Sciences, Addis Ababa University. The mice were randomly kept in groups of 4 and 5 (n=5) in clean cages with a wire mesh top containing a hygienic bed of sawdust (regularly changed every 3 days) and retained in a well-ventilated room (25±1°C with 55±5% humidity) for excision and incision wound models, respectively. Then the mice were acclimatized with the environment for a week before the initiation of the experiment. All procedures were conducted according to the standard guidelines for the care and use of laboratory animals.¹⁵ The experimental protocol was approved by the Institutional Review Board of School of Pharmacy, College of Health Sciences, Addis Ababa University (ERB/SOP/151/03/2020).

Plant Extraction

The Leaves of *Z. scabra* were dried and pulverized using a mortar and pestle to get a fine powder that could be extracted. A portion of *Z. scabra* leaf powder sample was weighed (1 kg) and macerated in 80% methanol (1:5) for 3

consecutive days at room temperature using a conical flask with occasional shaking. The macerated plant material was then filtered by Whatman filter paper. The residue was re-macerated in 80% methanol for the second time for another 3 days to fully extract the plant material and then filtered. The filtrates were collected and concentrated using a Rota vapor at temperature of 45°C to get rid of the organic solvent. Concentrated extract was then frozen in a deep freezer and dried in a lyophilizer to remove the aqueous content. At last, the dried crude extract was stored in a closed container and kept in a refrigerator for the preparation of ointment formulations.

Percentage Yield of the Crude Extract

The weight of crude extract was measured and the percentage yield was calculated per weight of the sample. The yield (%) of dried extract was determined by Eq. 1.

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100 \quad (1)$$

Formulation of Extract Ointment and Simple Ointment

Simple ointment was formulated using white 170 g white soft paraffin, 10 g hard paraffin, 10 g cetostearyl alcohol, and 10 g wool fat in accordance with the British Pharmacopoeia (BP, 1988a) following the formula.¹⁶ All the constituents of the ointment base were mixed and gently heated, stirring until homogeneous and cold ointment was obtained.

The extract ointment was prepared in different concentrations, ie, in 5% (w/w) ointment (5 g of crude extract was mixed with 95 g of ointment base B.P) and in 10% (w/w) ointment (10 g of extract was added in 90 g of ointment base B.P).¹⁷ To compare wound healing potential of the extract, nitrofurazone ointment (0.2% w/w NF) was used as a standard drug.

Grouping and Dosing

For the excision model the mice were randomly assigned into four groups (n=5) as follows: Group I mice were treated with a simple ointment base (negative control). Group II and III mice were topically treated with 5% w/w and 10% w/w of extract ointment preparations, respectively; group IV (positive control) mice were treated with 0.2% w/w NF. For incision model the animals were assigned into five groups (n=5) including the untreated group.¹⁸

Acute Dermal Toxicity Study

The acute dermal toxicity test of the crude extract of *Z. scabra* was carried out as per OECD draft guideline number 404.¹⁸ Three female mice having normal skin surface were randomly selected and maintained in a cage individually and acclimatized to the working environment for a week prior to the commencement of the test. Around 10% of the body surface area fur was shaved from the dorsal area of the trunk 24 hours prior to study. 10% w/w of the extract formulation was uniformly applied over the shaved area for 24 hours. Mice were housed individually during the exposure period.

The residual test substance was removed at the end of the exposure period and the mice were observed daily for any adverse skin reactions for 14 days.¹⁸

Wound Healing Models

In the present study to evaluate the wound-healing activity of the leaf extract of *Z. scabra* excision and incision wound models were used.

Excision Wound Model

Animals have been anesthetized with intraperitoneal injection of ketamine (1 mL/kg) and diazepam (1 mL/kg) and the back hair of the animals has been shaved. After this, approximately 300 mm² circular areas were marked and the full thickness of this area was removed by the use of sharp sterilized scissors. Twenty-four hours after wound formation, the preparations were applied topically once daily for each group based on the respective grouping as explained in the grouping and dosing section, to cover the wound surface till complete healing was achieved. The wound closure rate was assessed by tracking the wound on each alternate day post-wounding using transparent paper and a permanent marker. The wound diameters were measured until the wound was fully closed and the areas calculated. The wound was left uncovered in the open environment, after which the wound contraction and the period of epithelization were measured.^{5,19}

Wound contraction measurement: percentage wound contraction was assessed as follows.

$$\text{Percentage Wound Contraction} = \frac{\text{wound sizeday0} - \text{nth days of wound size}}{\text{Wound sizeday0}} \times 100 \quad (2)$$

where n=number of days, ie, 2nd, 4th, 6th, etc., until the wound in the treatment groups healed.

Epithelialization Period Measurement

The removal of dead tissue without any residual raw wound was considered to be the end point of complete epithelialization, and the days required for this were taken to be the period of epithelialization.

Incision Wound Model

Mice were anesthetized and their fur removed as described in the excision wound model. Three centimeters long, linear-paravertebral incisions were made through the full thickness of the skin on either side of the spine, 1 cm from the middle of the spine.

The removed skin was held together and stitched using a chromic catgut (2/0 metric-1/2 Circle) with a curved needle at 1 cm intervals, considered as Day 0. From day 1, the ointments were applied as described in the section above. The ointments were applied once a day for 9 days. The sutures were removed on the 8th day post-wound. The tensile strength was then measured on the 10th day to measure the degree of healing. It was measured by continuous constant flow of water, taking into account the weight of the water needed to break the skin. Wound strength of groups treated with extract ointment was compared with that of the standard, simple ointment and untreated control.⁵ The percentage of tensile strength was calculated as follows;

$$\begin{aligned} & \text{Tensile strength of extract (\%)} \\ & \frac{\text{Tensile strength (extract)} - \text{Tensile strength (simple ointment)}}{\text{Tensile strength (simple ointment)}} \times 100 \quad (3) \end{aligned}$$

$$\begin{aligned} & \text{Tensile strength of reference (\%)} \\ & \frac{\text{Tensile strength (reference)} - \text{Tensile strength (simple ointment)}}{\text{Tensile strength (simple ointment)}} \times 100 \quad (4) \end{aligned}$$

$$\begin{aligned} & \text{Tensile strength simple ointment (\%)} \\ & \frac{\text{Tensile strength (simple ointment)} - \text{Tensile strength (left untreated)}}{\text{Tensile strength (left untreated)}} \times 100 \quad (5) \end{aligned}$$

Statistical Analysis

Data analysis was performed using the Social Science Statistical Software (SPSS) version 25 for Windows (SPSS Inc, Chicago, IL). The results were expressed as mean±standard mean error (SEM) for each group. Statistical differences between groups were analyzed through a one-way

variance analysis (ANOVA) using Tukey as a post-hoc test. Statistical significance was determined at $p < 0.05$.

Results

Yield of the Crude Extract

From a total of 800 g dried and powdered leaves of *Z. scabra* macerated with 80% methanol for 72 hours twice, 50 g of dried crude extract was obtained. Hence, the percentage (% w/w) yield of *Z. scabra* leaves extract was found to be 6.25%.

Acute Dermal Toxicity

After 24 hours of application of 10% formulation of the extract, there was no dermal toxicity (inflammation, irritation, or redness) observed. There were no also signs and symptoms as well as mortality manifested when the animals were monitored for 48 hours and for 14 consecutive days of cage side observation.

Wound Healing (Excision Model)

Wound Contraction

Starting from treatment day-4 to the end day-16 a significant reduction in the size of the excised wound was recorded (Table 1, Figure 1). The 5% and 10% (w/w) ZS ointments, and 0.2% NF ointment treated mice produced significant ($p < 0.05$) wound area reduction starting from day 4, and highly significant ($p < 0.01$) differences were seen from day-10 onward in comparison with the simple ointment treated group. There was no significant difference in activity between 5% (w/w) and 10% (w/w) extracts. But, a higher rate of wound closure was observed with 10% (w/w) ointment. 10% (w/w) ZS extract showed comparable efficacy with standard drug after day-12. The maximum wound contraction rate was seen on the 14th and 16th day which was 96.6% and 99.3%, respectively (Figure 2). Highly significant ($p < 0.01$) wound contraction was also obtained in the 0.2% (w/w) NF ointment treated group from day-4 to day-10 onward as compared to the simple ointment treated group. The maximum rate of wound contraction for 0.2% (w/w) NF ointment was seen on the 12th, 14th, and 16th day, which was 96.6%, 99.3%, and 99.99%, respectively.

Period of Epithelization

0.2% (w/w) NF was shown as the fastest period of epithelialization as compared to simple ointment, 5% and 10% (w/w) ZS extract ointments, which was 11 ± 0.24 days (Table 2). There was no significant difference between 5% and

10% (w/w) ZS ointments in the epithelialization period. However, both 5% and 10% (w/w) ZS extract ointments elicited a significant ($p<0.05$) shortening of the epithelialization period as compared to the simple ointment group.

Incision Wound Model

Wound Breaking Strength (Tensile Strength)

On the incision wound model, the extract ointments applied topically were found to be effective in increasing the tensile strength of the healing wound (Table 3). Both 5% and 10% extract ointments produced significantly ($p<0.05$) higher tensile strength of the healing wound in comparison with the simple ointment and left untreated groups. Treatment with the standard agent 0.2% (w/w) NF also exhibited significantly ($p<0.01$) increased tensile strength compared to both of these groups. The highest percent tensile strength (72%) was shown in the 0.2% (w/w) NF treated group.

Discussion

In Ethiopia, medicinal plants have been used traditionally for several years as topical and internal preparations to promote wound repairs. They have a great potential for wound healing by promoting the speed of wound healing with lower pain, discomfort, and scarring of the patient.²⁰ The leaf of *Z. scabra* was traditionally claimed to be used for wound dressing and healing, and reducing body swelling.²¹ Therefore, in the present study we scientifically explored these traditional claims. The results of this study conclusively showed that the hydroalcoholic extract of *Z. scabra* significantly promotes wound healing in the

excision wound model compared to the simple ointment treated group. In addition, the wound healing effect of 10% (w/w) extract ointment was comparable with 0.2% w/w NF after day-12. On the 16th day of treatment, significant healing with a percentage wound closure of 99.99% was observed in the 0.2% w/w NF treatment group while it was 98% and 99.3% in the 5% and 10% (w/w) ZS treatment groups, respectively. Wound contraction, which contributes to wound closure, is expressed as a decrement in the percentage of the first wound size.²² During healing, contraction plays a crucial role as it decreases the dimension of the wound and hence shortens the healing time. Moreover, contraction reduces the extracellular matrix amount needed to repair the defect and helps re-epithelization by reducing the distance traveled by migrating keratinocytes.²³ Another parameter used in this study was the epithelialization period (time period at which epithelial reorganization takes place). The standard drug, 0.2% (w/w) NF, exhibited the fastest period of epithelialization as compared to simple ointment, 5% (w/w), and 10% (w/w) ZS extract ointments which was 12 ± 0.29 days. Both strengths of the extract ointment produced a significant ($p<0.05$) difference of the epithelialization period as compared to the simple ointment group.

Increased rate of wound contraction and decrease in epithelialization period in mice treated with the extract may be attributed to the presence of phytoconstituents such as unsaturated sterols, triterpenoids, alkaloids, flavonoids, saponins, tannins, and phenolic compounds which are known to promote the wound healing process.²⁴ Flavonoids reduce the formation of

Table 1 Effect of 80% Methanol Leaf Extract of *Z. scabra* on Wound Contraction in Mice

Wound Area in mm ² on Post-Wounding Day								
Groups	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16
Simple ointment	289.51 ±6.12	243.35±6.73	191.86±2.41	182.44 ±14.10	136.9±4.32	88.71±6.87	55.58 ±3.27	30.30 ±2.04
5% (w/w) ZS	290.45 ±16.96	235.28±11.05 ^{a*}	180.41±7.41 ^{a*}	142.71 ±16.40 ^{a*}	64.21±6.3 ^{a**}	47.89±2.4 ^{a**}	28.26 ±0.0 ^{a**}	6.48 ±0.82 ^{a**}
10% (w/w) ZS	266.59 ±14.85	227.17±8.41 ^{a*}	158.41±4.55 ^{a*}	88.70±69 ^{a**}	52.91±2.67 ^{a**}	22.34 ±1.50 ^{a**}	5.36 ±1.34 ^{a**}	1.2 ±0.31 ^{a**}
0.2% (w/w) NF	266.9 ±17.28	158.41 ±4.55 ^{a**b**c*}	113.67 ±8.43 ^{a**b**c*}	75.52 ±2.98 ^{a**b*}	30.61 ±3.57 ^{a**b**c*}	10.36 ±1.4 ^{a**b*}	2.2 ±0.58 ^{a**b*}	0.1 ±0.19 ^{a**b*}

Notes: Data are expressed as Mean ± SEM; (n=5 animals in each group) and analyzed by one-way ANOVA followed by Tukey post-hoc test; numbers from 2–16 indicate the day on which contraction rate measurement was taken, ^acompared with simple ointment, ^bcompared with 5% extract, ^ccompared with 10% extract. * $p<0.05$, ** $p<0.01$.
Abbreviations: NF, nitrofurazone; ZS, *Zehneria scabra*.

Post-wounding
Days

Treatment Groups

Figure 1 Wound contraction progress in simple ointment, 5% w/w and 10% (w/w) ZS extract ointments, and 0.2% w/w NF treated groups across post-wounding days in excision model.

Figure 2 Percentage of wound area contraction effects of simple ointment, 5% w/w and 10% w/w ZS extract ointments, and 0.2% w/w NF treatments in mice in excision model.

Abbreviations: SO, simple ointment; ZS, *Zehneria scabra*; NF, nitrofurazone.

Table 2 Effect of Topical Application of 80% Methanol Leaf Extract of *Z. scabra* on Period of Epithelialization (Number of Days) Post-Wound Creation in Mice

Groups	Period of Epithelization
Simple ointment	21.8±0.37
5% (w/w) ZS	15.59±0.31 ^{a*}
10% (w/w) ZS	14.50±0.30 ^{a*}
0.2% (w/w) NF	12±0.29 ^{a*}

Notes: Data are expressed as Mean ± SEM; (n=5 animals in each group) and analyzed by one-way ANOVA followed by Tukey post-hoc test, ^acompared with simple ointment, *p< 0.05.

Abbreviations: NF, nitrofurazone; ZS, *Zehneria scabra*.

inflammatory metabolites by inhibiting both cyclooxygenase and lipoxygenase activities.²⁵ Besides, flavonoids prevent neutrophil degranulation, which is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells which imparts to the wound healing activity of the extract.²⁶ The astringent and antimicrobial properties of these phytochemicals might also be responsible for wound contraction and increased epithelialization rates.²⁷ Tannins are seen as active detoxifiers and inhibit bacterial growth.²⁸ In vitro study on the leaf of methanolic extract of *Z. scabra* revealed activity against common wound pathogens such as *S. aureus* and *P. aeruginosa* which could support findings of this study.²⁹

Further efficacy of *Z. scabra* extract in wound healing was demonstrated by tensile strength in incision wounds. The tensile strength of both 5% and 10% (w/w) extract ointments treated groups was significantly higher ($p<0.01$) compared to simple ointment treated and untreated groups. Tensile strength indicates how much the tissue being repaired resists tension and can indicate the quality of the tissue being repaired. The tensile strength of a wound depends mainly on an increase in the concentration of collagen and stabilization of fibers.³⁰ A study revealed that phytochemical constituents identified in *Z. scabra* plant were directly responsible for antioxidant, antimicrobial, and antifungal activities through different mechanisms.²⁷ For example, tannins promote wound healing by chelating free radicals and reactive oxygen species, reducing proteins due to their astringent effect, encouraging wound contraction, and increasing the formation of capillary vessels and fibroblasts.³¹

Table 3 Effect of the Ointment of 80% Methanol Leaf Extract of *Z. scabra* on Tensile Strength in Mice

Groups	Wound Breaking Strength (g)	% Tensile Strength
Left untreated	231±1.76	–
Simple ointment	236±1.87	2%
5% (w/w) ZS	381±0.92 ^{a*c*}	62%
10% (w/w) ZS	390±1.12 ^{a*c*}	65%
0.2% (w/w) NF	407±1.87 ^{a*b*c*}	72%

Notes: Data are expressed as Mean ± SEM; (n=5) and analyzed by one way ANOVA followed by Tukey post-hoc test; ^acompared with simple ointment, ^bcompared with 5% extract, ^ccompared with left untreated, *p< 0.05, **p< 0.01.

Abbreviations: NF, nitrofurazone; ZS, *Zehneria scabra*.

On the other hand, flavonoids are responsible for reducing lipid peroxidation by preventing or slowing down cell necrosis and improving vascularity, which increases the viability of collagen fibrils by increasing circulation, preventing cell damage and promoting DNA synthesis.^{32,33}

In conclusion, the different phases of wound repair; wound contraction, epithelialization, and tensile strength were improved by ointments prepared from 80% methanol leaf extract of *Z. scabra* as compared to the simple ointment treated group. The findings of this study therefore support the traditional claims of the plant for wound treatment.

Abbreviations

NF, Nitrofurazone; ZS, *Zehneria scabra*.

Disclosure

The authors declare no competing interests.

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