

Evaluation of wound healing activity of the crude extract and solvent fractions of *Rumex nervosus* Vahl (Polygonaceae) leaves in mice

Abel Andualem¹ , Kefyalew Ayalew Getahun² ,
Bahiru Tenaw Goshu³ and Yaschilal Muche Belayneh¹ 

Abstract

Background: In Ethiopian traditional medicine, *Rumex nervosus* Vahl leaves are used to treat wounds. However, despite traditional claims and supportive in vitro findings, no scientific study has been conducted to evaluate the in vivo wound healing activity of *R. nervosus* Vahl leaves.

Aim of the study: To evaluate the wound healing activity of crude extract of the leaves of *R. nervosus* Vahl and its solvent fractions in mice.

Methods: *R. nervosus* Vahl leaves were extracted with 80% methanol, and then the crude extract was fractionated using ethyl acetate, chloroform, and water. Ointments at 5% and 10% strengths were formulated from the crude extract and its fractions. The healing activity of the crude extract was evaluated using linear incision, circular excision, and burn wound models in mice. Additionally, the activity of solvent fractions was assessed using circular excision wounds in mice.

Results: Treatment of wounds with ointments containing 5% and 10% crude extract showed significantly increased wound contraction rate, shorter epithelialization period, and higher skin-breaking strength ($P < 0.05$) compared with the negative control. Both 5% and 10% formulations of the aqueous and ethyl acetate fractions significantly increased wound contraction and decreased the period of epithelialization in the excision wound model ($p < 0.05$), however, the chloroform fraction showed no significant wound healing effect compared with the negative control.

Conclusion: The 80% methanol crude extract as well as the aqueous and ethyl acetate fractions of *R. nervosus* Vahl leaves possess wound healing activity as evidenced by improved wound contraction rate and tensile strength and decreased epithelialization period.

¹Department of Pharmacy, College of Medicine and Health Sciences, Wollo University, Dessie, Ethiopia

²Department of Pharmacology, School of Pharmacy, College of Medicine And Health Sciences University of Gondar, Gondar, Ethiopia

³Department of Anatomy, School of Medicine, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Corresponding author:

Abel Andualem, Department of Pharmacy, College of Medicine and Health Sciences, Wollo University, P.O. Box 1145, Dessie, Ethiopia.
Email: abelandu77@gmail.com



Plain language summary

Rumex nervosus Vahl is used in Ethiopian traditional medicine for wound treatment. The plant exhibits various in vitro antimicrobial, anti-inflammatory, and antioxidant properties that facilitate wound healing processes. The air-dried leaves of *R. nervosus* Vahl were subjected to extraction through maceration using 80% methanol. A portion of the crude extract was fractionated with solvents (chloroform, ethyl acetate, and aqueous) of different polarities. Ointments were formulated in concentrations of 5% and 10%, and their wound healing effects were assessed using excision, incision, and burn wound models in mice. Parameters such as percent of wound contraction, epithelialization period, histological analysis, and tensile strength were measured. Mice were used for evaluating wound healing activity, while rats were employed for acute dermal toxicity tests.

In the excision wound model, both the 5% and 10% crude extract ointments exhibited significant wound healing activity. Among the fractions, the groups treated with ethyl acetate and aqueous fraction ointments displayed significant wound healing activity. In the incision wound model, both 10% and 5% ointments of the crude extract demonstrated significant wound healing effect. Regarding the burn wound model, the percentage of wound contraction significantly increased on most post-wounding days with 10% and 5% ointments.

This in vivo investigation concluded that the crude extract and its aqueous and ethyl acetate fractions possess wound healing activities. These findings validate the traditional use of *R. nervosus* Vahl leaves for wound treatment, suggesting its potential as a source for developing new wound healing agents.

Keywords

Rumex nervosus Vahl, leaves, wound healing, excision wound, incision wound, burn wound

Background

A wound is defined as the disruption or damage to the structural and functional integrity of living tissues. This disturbance leads to an alteration in the typical anatomy and functioning of the skin.^{1–3} Wounds result from a rupture in skin epithelial integrity and can be open or closed. Open wounds, the most common type, involve a break in the skin, exposing underlying tissue to infection.⁴ Wounds can occur as part of a disease process or have an accidental or intentional etiology.^{2,5} Additionally, wounds can be classified as acute or chronic.⁶ Wound healing occurs through a coordinated physiological response of four overlapping but well-defined phases which include hemostasis, inflammation, proliferation, and remodeling.⁷ *R. nervosus* Vahl has been used traditionally in different countries for the treatment of many inflammatory diseases, diarrhea, wounds, typhus, rabies, and skin disorders.⁸ In Ethiopia, *R. nervosus* Vahl is known by several local names, Ambacho or Embwacho⁹ in Amharic and Dhangagoo¹⁰ in Afaan Oromo.

In various regions of Ethiopia such as Gubalafto,¹¹ Tara Gedam,¹² Dega Damot,⁹ Debark Woreda,¹³ Fiche¹⁴ and Kunama,¹⁵ *R. nervosus* Vahl leaves have been traditionally used by local healers to treat wounds through topical application.

A previous in vitro study was conducted with supportive results to the medicinal claim of the plant for wound treatment. The ethanolic extract of *R. nervosus* Vahl and its fractions

showed antioxidant, anti-inflammatory, and antimicrobial activities. Additionally, antimicrobial screening studies showed *R. nervosus* Vahl has anti-bacterial activity against wound infection-causing bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*,¹⁶ and zoonotic bacterial pathogens (*Streptococcus*, *S. aureus*, *L. monocytogenes*, *E. coli*, and *Salmonella*).¹⁷ Similarly, *R. nervosus* Vahl leaves showed antioxidant and anti-inflammatory activity.¹⁶ Thus, this study was conducted to evaluate the wound-healing activity of the leaves of *R. nervosus* Vahl and its solvent fractions in mice.

Materials and methods

Drugs and chemicals used in the study

Drugs, chemicals, and reagents of analytical grade were used. Methanol absolute (AppliChem, Darmstadt, Germany), chloroform (Nice Chemicals Pvt. Ltd, Kochi, Kerala, India), ethyl acetate (Carlo Erba Reagent S.A.S., Val-de-Reuil, France), nitrofurazone skin ointment 0.2% (Shanghai, General Pharmaceutical CO., LTD, Shanghai, China), wool fat (Uni-Chem chemical reagents, Mumbai, Maharashtra, India), hard paraffin (University of Gondar, Pharmaceuticals Laboratory, Gondar, Ethiopia), white soft paraffin (Ethiopian Pharmaceuticals Manufacturing, SH.CO, Addis Ababa, Ethiopia), cecostearyl alcohol (Blulux Laboratories (P) Ltd, Hyderabad, Telangana India), silver sulfadiazine cream USP 1% (Galentic Pharm Pvt. Ltd, Baddi, Himachal

Pradesh, India), ketamine hydrochloride injection (Neon Laboratories Limited, Mumbai, Maharashtra, India), diazepam injection (Gland Pharma Limited, Hyderabad, Telangana, India), halothane inhalation (Piramal Enterprises Limited, Mumbai, Maharashtra, India), distilled water (University of Gondar, Pharmaceuticals Laboratory, Gondar, Ethiopia) and bees wax (Golla Bee Products PLC, Addis Ababa, Ethiopia) were used during the experiment.

Plant materials

The fresh leaves of *R. nervosus* Vahl were collected from Tara Gedam located in the Libo Kemkem district, South Gondar Zone of the Amhara Region, northwest Ethiopia in February 2019. The plant was identified and authenticated by a botanist at the Department of Biology, College of Natural and Computational Science, University of Gondar. The plant specimen (voucher number Aba1/2018) has been deposited there for future reference.

Experimental animals

Healthy, adult Swiss albino mice of both sexes (25 \pm 10 g, and 6–8 weeks of age) and adult healthy female Wistar rats weighing 150–200 g and 3–4 months of age were purchased from the Ethiopian Public Health Institute. Mice were used for the evaluation of wound healing activity, and rats were used for acute dermal toxicity tests. Female rats were used for toxicity studies due to their larger size and advanced skin structure, which facilitates the observation of dermal reactions. Conversely, mice were used in wound healing studies due to their ease of handling compared with rats. The animals were housed in cages under standard conditions with 12 h light and dark cycles. They were provided with a standard pellet diet and water ad libitum and acclimatized to the laboratory condition for a week before the start of the experiment. Animals were handled according to international laboratory animal use and care guidelines throughout the experiment.¹⁸ At the end of the experiment, the animals were sacrificed with an overdose of anesthetics.¹⁹

Crude extract preparation

The leaves of *R. nervosus* Vahl were washed under running tap water to remove the surface pollutants and air-dried in the shade at room temperature. The dried leaves of *R. nervosus* Vahl were

coarsely powdered using mortar and pestle. The crude extract was prepared by cold maceration technique. Leaf powder (1000 g) was weighed and soaked in 8000 ml of 80% hydro-methanol, chosen for its high solvating power and rapid evaporation rate compared to other solvents.²⁰ It was macerated for three days in a conical flask with occasional shaking to enhance mixing, improve mass transfer, and increase extraction efficiency. Then, the extract was separated from the residue by using a muslin cloth and further filtered by Whatman filter paper No.1. The residue was re-macerated twice by adding fresh solvent and filtered in the same manner. The collected filtrates were concentrated by a rotary evaporator at 40°C. Then, the concentrated extract was frozen overnight using a deep freezer followed by drying with a lyophilizer at –50°C and vacuum pressure (200mBar). The dried extract was weighed and stored in screw cap vials in a refrigerator at –4°C until used for ointment formulation and solvent fractionation.¹²

Solvent fractionation

Ninety grams of the 80% hydro-methanol crude extract underwent successive extraction with chloroform, ethyl acetate, and aqueous solvents with increasing polarity respectively. The dried crude extract (90 g) was suspended in 500 ml of distilled water and transferred to a separator funnel. Chloroform was added, shaken vigorously and the resulting chloroform fraction was collected. This step was repeated twice. The remaining aqueous portion was then treated with ethyl acetate, with the upper ethyl acetate layer separated and collected. This process was also repeated twice. Finally, chloroform and ethyl acetate fractions were concentrated using a rotary evaporator and dried at 40°C to obtain the respective fractions. The remaining aqueous residue was frozen in a freezer overnight and then freeze-dried with a lyophilizer to obtain an aqueous fraction. All fractions were stored in screw cap vials in a refrigerator at –4°C until used for the formulation of ointments.¹²

Ointment formulation

Medicated ointments of 80% methanol extract as well as chloroform, ethyl acetate, and aqueous fractions and the simple ointment (non-medicated ointment) were freshly prepared on the day of the experiment for each wound model based on British Pharmacopeia.²¹

Table 1. Formula used for the preparation of simple and medicated ointments.

Ingredients	MF	RF
Wool fat	50 g	5 g
Hard paraffin	50 g	5 g
Cecostearyl alcohol	50 g	5 g
White soft paraffin	850 g	85 g
Total	1000 g	100 g

MF, Master formula. RF, Reduced formula.

A hundred grams of simple ointment base was prepared by using the reduced formula from British Pharmacopeia (Table 1). Five grams of hard paraffin and five grams of cecostearyl alcohol were melted in a beaker. 5 g wool fat and 85 g white soft paraffin were melted in another beaker. The mixtures were then combined and stirred until cooled. To prepare the extract ointment, first the extracts were powdered in a mortar and pestle, then 5 g and 10 g of the 80% methanol extract were mixed with 95 g and 90 g simple ointment bases for preparing 5% w/w and 10% w/w crude extract ointment respectively by levigation on the surface of the ointment slab to make an ointment of uniform consistency and smooth texture. In the same manner, the 5% w/w and 10% w/w ointments of each fraction were prepared by incorporating 2.5 g and 5 g of the aqueous, ethyl acetate, and chloroform fraction each into a 47.5 g and 45 g of simple ointment base to get a 50 g medicated ointment of each fraction. Finally, the medicated ointments were transferred to a clean container for topical application during the experiment.²²

Preliminary phytochemical screening

The hydro-methanol extract and its chloroform, ethyl acetate, and aqueous fractions of the leaves of *R. nervosus* Vahl were screened for the presence of secondary metabolites like tannins, alkaloids, phenols, terpenoids, flavonoids, saponins, steroids, and glycosides. The tests were performed according to the standard test procedure.^{23–27}

Acute dermal toxicity study

Acute oral toxicity study was previously done in rats and up to a dose of 7.1 g/kg was not toxic.¹⁶ Acute dermal toxicity test of 80%

methanolic extract of *R. nervosus* Vahl was carried out according to the Organization for Economic Co-operation and Development (OECD) guideline.²⁸ Ten female healthy young adult rats showing normal skin texture were used. Animals were divided into two groups of five animals each for test and control groups. They were housed individually in a cage and acclimatized to the laboratory condition for seven days before the test. Then, the rats were anesthetized with ketamine 80 mg/kg intraperitoneal injection and around 10% of the body surface area fur was shaved from the dorsal area of the trunk 24 h before the study.

First, a sighting study was performed to determine the starting dose by applying 2000 mg/kg of the 10% extract ointment. There was no death or skin irritation observed within 24 h, and then four additional rats from each group were used, and the same dose of the crude extract ointment was applied. The 10% ointment formulation of the extract and the control non-medicated ointment were applied uniformly over the shaved area of test and control rats respectively, and observed for 24 h. During the exposure period, rats were caged individually. At the end of the exposure period, the residual test substance was washed out by distilled water and the animals were observed for the development of any adverse skin reactions daily for 14 days.²⁰ The reactions, defined as erythema and edema, were evaluated and graded according to the OECD 404 grades.²⁹

Grouping and dosing of experimental animals

For the evaluation of crude extract, animals were grouped into four groups for excision, five groups for incision, and another four groups for burn wounds (six animals per group). For the excision wound model, the first group was treated with simple ointment (served as a negative control), the second and third groups were treated with 5% w/w and 10% w/w crude extract ointment, respectively, and the fourth group was treated with nitrofurazone, 0.2% w/w (served as a positive control).³⁰

In the linear incision wound model, Group I–IV were treated similarly to the excision wound model, but animals in Group V were left untreated (served as untreated negative control).³¹ For evaluation of burn wound healing activity, the animals in Group I–III were treated in a similar fashion with the excision wound model, but animals in Group IV were

treated with 1% silver sulfadiazine cream (served as a positive control).³²

To evaluate the activity of the solvent fractions in the circular excision wound model, eight groups of mice (each with six) were used. The excision wound model was selected because it is an ideal method to study physical attributes like wound contraction, epithelization, and scar remodeling.³³ Group I was treated with simple ointment, Group II and III were treated with 5% and 10% of aqueous ointment, respectively, Group IV and V were treated with 5% and 10% of ethyl acetate ointment, respectively, Group VI and VII were treated with 5% and 10% of chloroform ointment, respectively and Group VIII was treated with 0.2% nitrofurazone ointment.

Evaluation of wound healing activity

Circular excision wound model. The mice were weighed individually and anesthetized with intraperitoneal 50 mg/kg ketamine and 5 mg/kg diazepam before infliction of the wound. Then, the hair from the dorsothoracic area of the animals was shaved using an electrical hair clipper.³² A circular mark (314mm²) was prepared using a permanent marker. The full thickness of this circular mark was excised carefully

using forceps and scissors to form an excision wound which was left undressed. After recovery from anesthesia, mice were returned to their cage. This was considered as day 0. After 24 h of wound creation, all preparations were applied topically to the wound area once per day until the wound was completely healed. For evaluation of wound healing activity of the aqueous, ethyl acetate, and chloroform fractions, the same wound area was created and ointments were applied as described in the grouping and dosing section.

The mice were observed for wound closure and measurement was done every two post wounding days using a transparent sheet and permanent marker. The evaluated surface area was used to calculate the percentage of wound contraction, taking the initial size of the wound as 100%. The wound healing activities of the crude extract were assessed by the period of epithelialization, percentage of wound contraction, and histological studies while the parameters in the case of solvent fractions were period of epithelialization, and percentage of wound contraction.^{34–36}

The percentage of wound contraction was calculated as follows³⁷;

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

Where n number of days i.e., 2nd, 4th, 6th, etc. until complete wound healing.

The number of days required for the eschar to fall off from the wound surface exclusive of leaving a raw wound behind was taken as the end point of complete epithelialization, and the days required for this were considered a period of epithelialization.³⁸

Linear incision wound model. All mice were anesthetized and shaved as in the excision wound model. Then, a 3 cm long, linear and paravertebral incision was made through the full thickness of the skin on either side of the vertebral column at a distance of 1 cm from the midline. The wound edges were apposed and sutured with non-absorbable surgical thread and a curved needle (no. 11) at intervals of 1 cm. The first four groups were treated similarly to

the groups in the excision wound model, and the fifth was used as untreated control. The treatments were topically applied once daily for 9 days. The sutures were removed on the 8th day of post-wounding. Tensile strength (the force required to open the healing skin) was assessed to measure the extent of healing.³⁹ The tensile strength was measured on day ten (Figure 1) through a continuous water flow technique by considering the gram of water required to break the skin⁴⁰ and the breaking strength of the groups treated with extracts was compared with the standard, simple ointment and untreated control groups. The tensile strength was calculated as follows.²⁰

$$\begin{aligned} &\text{Percent tensile strength (TS) of the extract} \\ &= \frac{\text{TS extract} - \text{TS So}}{\text{TS So}} \times 100 \end{aligned}$$

Percent tensile strength of the reference

$$= \frac{\text{TS reference} - \text{TS So}}{\text{TS So}} \times 100$$

Percent tensile strength of simple ointment

$$= \frac{\text{TS So} - \text{TS Lu}}{\text{TS Lu}} \times 100$$

Where So=simple ointment and Lu=left untreated

Burn wound model. Initially, the mice were divided randomly into four groups of six mice, then anesthetized through intraperitoneal injections of ketamine (50 mg/kg) plus diazepam (5 mg/kg).⁴¹ The hair on the dorsum of each mouse was shaved off to ensure even burn wounding. Then, a molten bees' wax (2gm) at 80°C poured into a metal cylinder with 314mm² circular opening was placed on the back of the animal to inflict a partial thickness burn wound. On solidification of wax after 8 to 10 min, the metal cylinder with wax adhered to the skin was removed and left a markedly circular burn wound (Figure 2). Wound contraction rate and epithelialization period were used as parameters for the evaluation of burn wound healing activity.³²

Animal dissection, tissue collection, and histological processing. For histological studies, a skin specimen from each group in the excision wound model was collected on the 13th day of post-wounding after the mice were sacrificed with an overdose of diazepam and ketamine (four times the anesthetic dose intraperitoneal).⁴² The

examination was blindly done and a vertical midline incision at the wound site from superior to inferior was performed. Samples were fixed in 10% buffered formalin, processed, and blocked with paraffin and then were sectioned into 5-micrometer sections and stained with hematoxylin and eosin (HE) stains, then the slides of the skin tissues were examined by light microscope for epidermal or dermal remodeling.⁴³

Statistical analysis

The data were expressed as mean ± SEM. The result was statistically analyzed using one-way ANOVA followed by post hoc Tukey-tests with SPSS version 23 software, and a P value < 0.05 was considered as statistically significant.

Results

Screening of phytochemicals

The percentage yield of the crude methanol extract was 19%, and the solvent fractionation produced 57.8%, 28.3%, and 13.9% yield of aqueous, ethyl acetate, and chloroform fractions, respectively. Phytochemical screening of the crude extract and solvent fractions showed the presence of different secondary metabolites as shown in Table 2.

Acute dermal toxicity

Topical application of the maximum concentration (10%) ointment formulation of the crude extract using a limit dose of 2000 mg/kg did not

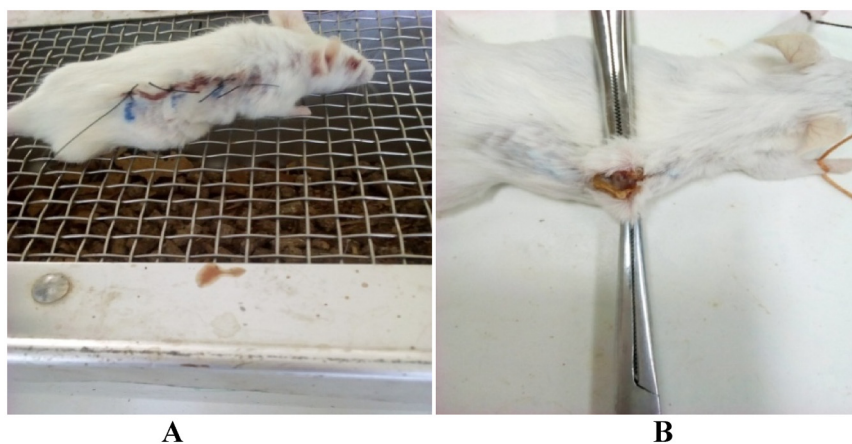


Figure 1. Photograph of incision wound on day 0 (A) and measurement of tensile strength on the 10th day by using water flow technique (B).

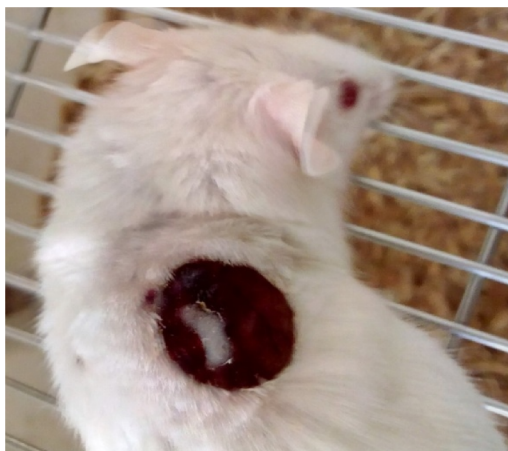


Figure 2. Circular burn wound on day 0.

show any sign of edema and erythema in rats. Additionally, no signs of toxicity and no mortality were observed during the 14 days of observation.

Wound healing activity of the crude extract on excision wound

Contraction of wound and period of epithelization. As shown in Table 3, treatment with 10% crude extract and nitrofurazone 0.2% ointment significantly enhanced wound contraction by the second day compared to the control group. Mice treated with 5% crude extract ointment exhibited significant wound contraction from the fourth day onward. Both 5% and 10% plant extract ointments, along with nitrofurazone 0.2% ointment, significantly facilitated wound contraction from the sixth to the sixteenth day compared to the control group. There was no significant difference in wound healing activity between the standard drugs, 10%, and 5% crude extracts. Animals treated with 5%, 10% (w/w) extract, and nitrofurazone ointments showed a significant reduction in epithelization period compared against the negative control (23.39%, 33.91%, and 27.96%, respectively, $p < 0.05$).

Histopathological Analysis. Granulation tissue of healed wounds in standard and 10% w/w extract treated groups showed more organized collagen fiber and fewer concentrations of inflammatory cells compared with the negative control group. Negative control groups had fewer collagen fibers, and more inflammatory cells, and thus showed delayed wound healing processes (Figure 3).

Wound healing activity of the crude extract on incision wound

Tensile strength. As shown in Table 4, both 5% and 10% ointment preparations of crude extract significantly increased tensile strength compared to negative controls ($p < 0.001$). The 10% extract group exhibited the highest percentage of tensile strength among all treatments.

Wound healing activity of the crude extract on burn wound

Contraction of wound and period of epithelization. The 10% (w/w) extract ointment-treated group showed significant ($p < 0.01$) wound contraction starting from the 2nd day to 18th day, whereas the 5% (w/w) extract showed significant ($p < 0.05$) wound contraction effect starting from the 4th day to 18th day in comparison with the negative control group (Table 5). A higher rate of wound closure was observed with the standard drug (100% at day 18) followed by 10% (w/w) ointment (99.34% at day 18). Wound healing with complete wound closure was observed in standard drug and 10% (w/w) extract ointment treated groups within 18 and 19 days, respectively. However, the period of wound closure in the negative control group was beyond 19 days.

The burn wound healing effects of 5% and 10% ointments were demonstrated by faster epithelialization. Both ointments significantly reduced epithelialization time compared to the negative control ($p < 0.05$ for 5% and $p < 0.01$ for 10%). The reduction in epithelialization time was dose-dependent. The average epithelialization periods were 17.83 days for the 5% extract, 16.5 days for the 10% extract, 16.33 days for the standard drug, and 20.17 days in the negative control group (Figure 4).

Wound healing activity of solvent fractions

Contraction of wound and period of epithelization. In the excision wound model, both aqueous and ethyl acetate fraction ointments showed wound healing activity. The 5% aqueous fraction ointment significantly improved wound contraction on most post-wounding days ($p < 0.01$). The 10% aqueous fraction ointment showed significant wound contraction from day 2 onwards ($p < 0.01$), highly significant from days 6 to 10 ($p < 0.001$), surpassing the positive control's effect except on day 12. Similarly, the 5% ethyl acetate fraction ointment significantly enhanced wound contraction from day 4 ($p < 0.05$), becoming

Table 2. Phytochemical screening of the crude extract and solvent fractions of *R. nervosus* Vahl leaves.

Metabolites	80%methanol extract	Ethyl acetate fraction	Chloroform fraction	Aqueous fraction
Tannins	+	+	–	+
Alkaloids	–			
Saponins	+	+	–	+
Flavonoids	+	+	–	+
Terpenoids	+	+	–	+
Phenols	+	+	+	+
Steroids	+	–	+	–
Glycosides	+	+	–	+

(+ = Present, - = absent).

Table 3. Effect of topical application of 80% methanolic crude extract of the leaves of *R. nervosus* Vahl on wound contraction of excision wound model.

Group	Wound area (mm ²) ± SEM (% contraction)								EP (days) (% reduction)
	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	
SO	286.98 ± 6.58 (8.61)	247.92 ± 7.47 (21.05)	212.50 ± 10.80 (32.32)	164.06 ± 8.83 (47.75)	125.52 ± 8.56 (60.03)	86.98 ± 9.18 (72.23)	47.40 ± 5.38 (84.90)	25.53 ± 5.44 (91.86)	19.67 ± 0.67
NFO	258.86 ± 8.25* (17.56)	213.54 ± 6.78* (31.99)	130.73 ± 4.8*** (58.36)	82.81 ± 6.83*** (73.62)	43.23 ± 8.48*** (86.23)	14.59 ± 6.54*** (95.35)	3.13 ± 2.13*** (99)	0.00 ± 0.00*** (100)	15.00 ± 0.73* (27.96%)
5% MLE	274.48 ± 5.95 (12.59)	219.27 ± 6.96* (30.17)	143.75 ± 4.77*** (54.22)	83.81 ± 6.97*** (73.31)	47.40 ± 9.14*** (84.90)	21.88 ± 6.35*** (93.03)	8.34 ± 4.39*** (97.34)	3.65 ± 3.06*** (98.84)	13.00 ± 0.63* (23.39%)
10% MLE	257.81 ± 7.11* (17.89)	209.90 ± 7.41** (33.15)	130.21 ± 7.68*** (58.53)	72.83 ± 8.07*** (76.81)	33.34 ± 9.54*** (89.38)	10.42 ± 6.78*** (96.68)	1.57 ± 1.07*** (99.5)	0.00 ± 0.00*** (100)	14.17 ± 0.54* (33.91%)

Each value denotes the mean ± SEM (n = 6 mice in each group) and is analyzed by one-way ANOVA followed by post hoc Tukey test; * p < 0.05, **p < 0.01, ***p < 0.001 when compared with the negative control group. SO = simple ointment base; MLE = methanol leaf extract; NFO = nitrofurazone ointment; EP = epithelialization period.

highly significant from day 10 to 14 (p < 0.001) compared to the negative control. The 10% ethyl acetate fraction ointment also significantly improved wound contraction on most days (p < 0.01) compared to the negative control. However, ointments of the chloroform fraction did not show significant wound healing activity compared to the negative control. There was no

statistically significant difference in the wound contractions between the three fraction ointments as depicted in Table 6.

As depicted in Figure 5, animals treated with the 10% w/w and 5% w/w ointments of each fraction and the standard drug had a shorter period of epithelialization compared to the control (simple ointment base). However, the

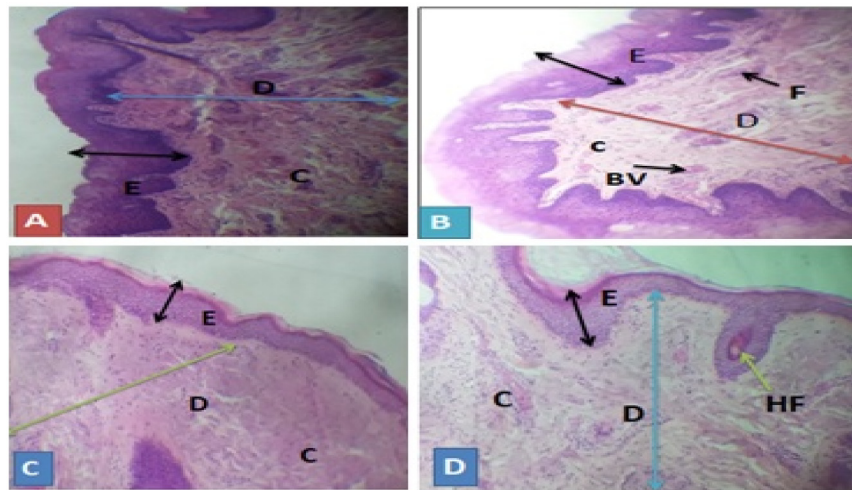


Figure 3. Photomicrograph of hematoxylin and eosin staining of tissues from excision wound model in mice. (A) Mice treated with negative control (HE $\times 40$). (B) Mice were treated with the standard treatment of nitrofurazone (HE $\times 40$). (C) Mice treated with 5% methanolic leaf extract ointment (HE $\times 40$). (D) Mice treated with 10% methanolic leaf extract ointment (HE $\times 40$) C=Collagen fiber, D=Dermis, E=Epidermis, HF=Hair follicle, BV=Blood vessels, F=Fibrocytes.

Table 4. Effect of topical application of 80% methanolic crude extract ointment of *R. nervosus* Vahl leaves on tensile strength in mice incision wound.

Group	Tensile strength in gram	% Tensile strength
Untreated control	169.87 ± 11.45	-
Simple ointment	186.38 ± 10.63	9.72
5% MLE w/w extract	$269.18 \pm 8.67a^*b^*$	44.43
10% MLE w/w extract	$302.98 \pm 7.23a^*b^*$	62.56
0.2% Nitrofurazone ointment	$298.76 \pm 6.08a^*b^*$	60.30

Note: Values are expressed as mean \pm SEM (n=6 mice in each group) and analyzed by one-way ANOVA followed by post hoc Tukey test; a, compared to the untreated group, b, compared to the simple ointment treated group; *p < 0.001.

epithelialization period showed by the chloroform fraction ointments was not statistically significant when compared to the negative control. Groups treated with the standard drug and 10% aqueous fraction ointments showed a faster rate of epithelialization ($p < 0.001$) compared to the negative control. In addition, mice treated with 5% and 10% ethyl acetate fraction and 5% aqueous fraction ointments showed shorter ($p < 0.01$) epithelization periods as compared to the control group. There was no significant difference between the treatment doses of the three fractions as well as with the standard drug. Animals treated with the standard drug and 10% w/w aqueous fraction ointment showed a faster rate of epithelialization (16.17 and 16.00 days respectively).

Discussion

Wound healing is a complex and dynamic process of tissue repair which initially involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules and finally the formation of a scar. Drugs that influence one phase of the wound healing process, may not necessarily affect the other phase.⁴⁴ Therefore, the development of an ideal wound healing agent by targeting the different phases of the wound healing process is still a challenge to medical scientists. The ideal agent should meet the criteria such as rapid contraction of the wound area resulting in fast healing, reduction of the wound epithelization period, and achievement of improved tensile strength.⁴⁵

Table 5. Percentage of wound contraction of partial-thickness burn wounds treated with extracts of *R. nervosus* Vahl leaves.

Wound area (mm ²) ± SEM (% contraction)										
Group	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	
SO	293.75 ± 7.22 (6.45)	277.08 ± 9.22 (11.76)	250.00 ± 11.06 (20.38)	212.50 ± 11.06 (32.33)	172.92 ± 12.98 (44.93)	116.15 ± 10.53 (63.01)	70.21 ± 7.54 (77.64)	35.94 ± 3.93 (88.55)	17.71 ± 4.47 (94.35)	
5% MLE	274.48 ± 3.65 (12.59)	233.88 ± 7.75** (25.52)	202.08 ± 8.01** (35.64)	159.38 ± 7.86** (49.24)	124.48 ± 10.95* (60.37)	77.08 ± 10.16* (75.45)	42.19 ± 5.46* (86.56)	14.59 ± 4.68* (95.35)	5.21 ± 3.39* (98.34)	
10% MLE	265.63 ± 4.77** (15.40)	234.38 ± 4.77** (25.36)	184.38 ± 7.35*** (41.28)	138.54 ± 9.05*** (55.87)	100.00 ± 8.07** (68.15)	65.62 ± 8.46** (79.12)	29.69 ± 6.54** (90.55)	6.77 ± 5.00** (97.84)	2.08 ± 2.08** (99.34)	
1% SSD	261.46 ± 5.69** (16.73)	220.75 ± 8.60*** (29.69)	183.33 ± 9.90*** (41.62)	136.46 ± 11.79*** (56.69)	96.88 ± 10.88*** (69.14)	59.79 ± 9.92*** (80.96)	26.05 ± 7.77** (91.70)	5.21 ± 3.39*** (98.34)	0.00 ± 0.00*** (100)	

Note: Values are expressed as mean ± SEM (n = 6 animals in each group) and analyzed by one-way ANOVA followed by Tukey post hoc test; SO = simple ointment base; MLE = methanol leaf extract; SSD = silver sulfadiazine cream; * p < 0.05, **p < 0.01, ***p < 0.001 when compared with the negative control group.

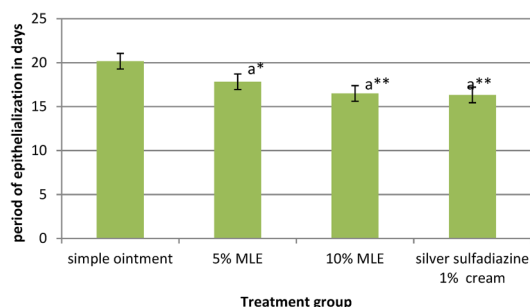


Figure 4. The effect of the crude extract on the period of epithelialization on burn model in mice. Note: values are expressed as mean \pm SEM ($n = 6$ mice in each group) and analyzed by one-way ANOVA followed by post hoc Tukey test; a, compared to the negative control; * $p < 0.05$, ** $p < 0.01$.

In the excision wound model of this study, both 5% and 10% ointments of the plant extract showed significant wound contraction compared to the control group. Although there was no statistically significant difference observed in wound healing activity between the standard drug and the 10% w/w or 5% w/w crude extract ointments, interestingly the 10% w/w extract ointment exhibited a better observable effect in terms of wound contraction and epithelialization compared to the standard drug and the 5% w/w extract ointment. Previous in vitro study reported that methanolic extract of the leaves of *R. nervosus* Vahl has antibacterial activities against common wound-infecting bacteria such as *Staphylococcus aureus* and *Escherichia coli*,¹⁷ and this antimicrobial activity may partly contribute to the wound healing effect of the plant through eradication of the colonizing organisms from wound area and allowing a suitable environment for the wound healing process. In addition, the period of epithelialization for the excision wound model was significantly reduced in mice treated with 5% w/w extract, 10% w/w extract, and nitrofurazone ointments compared to the negative control. The occurrence of enhanced epithelialization and wound contraction could be due to the ability of *R. nervosus* Vahl leaf extract to enhance collagen synthesis as evidenced by the Histopathological findings. Moreover, the reduced epithelialization period could be due to rapid wound contraction as contraction shortens the distance for migrating keratinocytes.⁴⁶

The wound healing activity of the crude extract was evidenced by increased tensile strength in incision wounds of mice. Tensile strength indicates how much the repaired tissue resists opening under tension and may indicate

the quality of the repaired tissue.⁴⁷ The increased tensile strength can be due to the stimulation of collagen synthesis and its maturation, angiogenesis, and stabilization of fibers.^{48,49} According to a previous phytochemical study, *R. nervosus* Vahl is a rich source of vitamin A.⁵⁰ Vitamin A promotes the growth and differentiation of epithelial cells and aids in collagen synthesis, pivotal for enhancing the strength and integrity of newly formed tissue. Collagen provides structural support to the wound site, thus contributing to its tensile strength. Furthermore, vitamin A plays a role in forming new blood vessels (angiogenesis) during the proliferative phase of wound healing ensuring an adequate blood supply to the wound site and promoting tissue repair.⁵¹

The mechanisms of plant-derived compounds in the treatment of burn wounds include anti-inflammatory, antioxidant, antimicrobial, collagen synthesis stimulation, cell proliferative, and angiogenic actions.⁵² As described earlier, previous studies indicated that the leaves of this experimental plant have antimicrobial, anti-inflammatory, and antioxidant activities which might contribute to the burn wound healing effect. In addition, burn injury partially suppresses the immune system. Therefore, the use of antioxidants may have a positive effect on the regeneration of the damaged skin tissue and other burn wound complications.⁵³

In the excision wound model, ointments prepared from aqueous and ethyl acetate fractions enhanced wound contraction. Increased contraction rate accelerates wound healing by reducing wound area, and reduced wound area facilitates re-epithelialization by shortening the distance keratinocytes must migrate.⁵⁴ Additionally, the epithelialization time was significantly reduced in animals treated with aqueous as well as ethyl acetate fraction ointments. Wound re-epithelialization occurs due to the proliferation and migration of epithelial cells across the wound bed.⁵⁵ Therefore, the shorter epithelialization time in the aqueous and ethyl acetate fraction could be due to the facilitated proliferation of epithelial cells. Unlike the aqueous and ethyl acetate fraction ointments, the chloroform fraction did not show a significant wound healing activity. This could be due to the absence of wound healing phytochemicals such as tannins, terpenoids, flavonoids, saponins and glycosides in the chloroform fraction as evidenced by the preliminary phytochemical analysis of this study. Tannins improve wound healing by facilitating tissue regeneration through their antioxidant activity,⁵⁶ and the wound-healing effect of

Table 6. Topical effect of solvent fractions of *R. nervosus* Vahl leaves on percentage of wound contraction.

Group	Wound area (mm ²), after wounding days \pm SEM (% contraction)									
	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16		
Simple ointment	297.92 \pm 3.92 (5.12)	268.75 \pm 5.10 (14.41)	237.54 \pm 3.67 (24.35)	195.32 \pm 2.88 (37.80)	132.29 \pm 3.49 (57.87)	100.52 \pm 3.46 (67.99)	63.02 \pm 3.37 (79.93)	26.56 \pm 2.77 (91.54)		
Nitrofurazone 0.2%	276.04 \pm 5.21* (12.09)	244.27 \pm 3.06* (22.21)	205.73 \pm 3.65*** (34.48)	163.02 \pm 3.06** (48.08)	101.04 \pm 4.95*** (67.82)	71.40 \pm 3.68*** (77.26)	33.86 \pm 5.00** (89.21)	4.69 \pm 2.99** (98.51)		
EAF 5% w/w	287.50 \pm 1.08 (8.43)	246.36 \pm 2.60* (21.54)	215.63 \pm 4.77* (31.32)	169.31 \pm 4.44* (46.07)	105.73 \pm 3.82** (66.32)	73.44 \pm 4.40** (76.61)	37.50 \pm 4.78** (88.06)	8.34 \pm 4.39* (97.34)		
EAF10% w/w	275.00 \pm 3.61** (12.42)	242.18 \pm 4.40** (22.87)	211.46 \pm 4.39** (32.65)	169.19 \pm 3.48* (46.11)	105.72 \pm 4.45** (66.33)	74.48 \pm 3.46** (76.28)	36.46 \pm 4.39** (88.38)	7.82 \pm 3.20* (97.50)		
AQF 5% w/w	277.08 \pm 4.75* (11.75)	242.18 \pm 4.4** (22.87)	206.77 \pm 3.65*** (34.15)	167.71 \pm 3.49** (46.59)	106.77 \pm 3.65** (65.99)	74.48 \pm 3.65** (76.28)	34.90 \pm 5.95** (88.86)	7.29 \pm 4.09* (97.67)		
AQF10% w/w	273.33 \pm 3.91** (12.95)	239.58 \pm 6.18** (23.70)	202.61 \pm 4.59*** (35.47)	162.50 \pm 4.03*** (48.25)	99.48 \pm 5.26*** (68.32)	72.40 \pm 5.73** (76.94)	31.77 \pm 3.82** (89.88)	4.17 \pm 2.08** (98.67)		
CHF 5% w/w	288.54 \pm 4.39 (8.10)	255.73 \pm 4.73 (18.56)	220.84 \pm 3.76 (29.67)	178.04 \pm 7.37 (43.29)	114.06 \pm 4.40 (63.67)	86.98 \pm 3.46 (72.30)	50.52 \pm 3.65 (83.91)	19.79 \pm 3.76 (93.69)		
CHF10% w/w	286.98 \pm 4.45 (8.60)	252.08 \pm 5.27 (19.72)	220.31 \pm 5.34 (29.84)	174.48 \pm 7.92 (44.43)	113.02 \pm 4.30 (64.00)	83.86 \pm 5.26 (73.29)	47.92 \pm 5.91 (84.74)	18.22 \pm 4.74 (94.19)		

Note: values are expressed as mean \pm SEM (n = 6 mice in each group) and analyzed by one-way ANOVA followed by post hoc Tukey test; * p < 0.05, **p < 0.01, ***p < 0.001 when compared with the negative control; AQF = Aqueous fraction, EAF = Ethyl acetate fraction, CHF = Chloroform fraction.

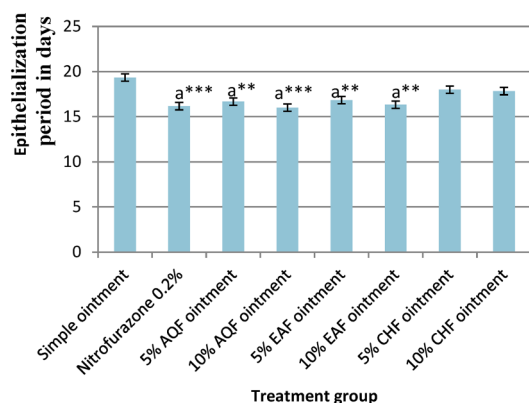


Figure 5. The effect of solvent fractions on the period of epithelialization. Note: Values are expressed as mean \pm SEM ($n = 6$ mice in each group) and analyzed by one-way ANOVA followed by post hoc Tukey test; a, compared to the negative control, ** $p < 0.01$, *** $p < 0.001$.

flavonoids is related to their antibacterial and antioxidant activities.⁵⁷

The wound healing activity of phenolic compounds is due to their antioxidant, anti-inflammatory, and antimicrobial activities.⁵⁸ Similarly, terpenoids inhibit inflammatory mediators by inhibiting phospholipase A2 in the metabolism of arachidonic acid,⁵⁹ and saponins have membrane stabilizing and anti-inflammatory activity.⁶⁰ Glycosides have antimicrobial, anti-inflammatory, antitumor, and antioxidant actions.⁶¹

Conclusion

The crude 80% methanol extract of *R. nervosus* Vahl leaves and its ethyl acetate and aqueous fractions showed in vivo wound healing activities. However, the chloroform fraction failed to demonstrate any significant wound healing activity suggesting that the more polar constituents of the extract contain the biologically active secondary metabolites with wound healing activity.

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Authors' contributions

All authors made substantial contributions to conception and design, analysis and interpretation of data; took part in drafting the article and revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval and consent to participate

The experiment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals,⁴¹ and the study has been approved by Department of Pharmacology, College of Medicine and Health sciences, University of Gondar with a reference number of SOP4/106/11.

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ORCID iDs

Abel Andualem <https://orcid.org/0000-0002-7727-3299>

Kefyalew Ayalew Getahun <https://orcid.org/0000-0001-7317-2995>

Yaschilal Muche Belayneh <https://orcid.org/0000-0001-5564-7176>

References

- Demilew W, Adinew GM and Asrade S. Evaluation of the wound healing activity of the crude extract of leaves of *Acanthus polystachyus* Delile (Acanthaceae). *Evid Based Complement Alternat Med* 2018; 2018: 2047896.
- Farahpour M and Habibi M. Evaluation of the wound healing activity of an ethanolic extract of Ceylon cinnamon in mice. *Vet Med (Praha)* 2012; 57: 53–57.
- Nagar HK, Srivastava AK, Srivastava R, et al. Pharmacological investigation of the wound healing activity of *Cestrum nocturnum* (L.) ointment in Wistar albino rats. *J Pharm (Cairo)* 2016; 2016: 9249040.
- Agyare C, Boakye YD, Bekoe EO, et al. African medicinal plants with wound healing properties. *J Ethnopharmacol* 2016; 177: 85–100.
- Solanki Y and Jain S. Wound healing activity of *Clitoria ternatea* L. in experimental animal models. *Pharmacologia* 2012; 3: 160–168.
- Velnar T, Bailey T and Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 2009; 37: 1528–1542.
- Guo S and DiPietro LA. Factors affecting wound healing. *J Dent Res* 2010; 89: 219–229.
- Qaid MM, Al-Mufarrej SI, Azzam MM, et al. Anti-coccidial effect of *Rumex nervosus* leaf powder on broiler chickens infected with *Eimeria tenella* oocyst. *Vet World* 2021; 11: 167.
- Wubetu M, Abula T and Dejen G. Ethnopharmacologic survey of medicinal plants used to treat human diseases by traditional medical practitioners in Dega Damot district, Amhara, north-western Ethiopia. *BMC Res Notes* 2017; 10: 157.

10. Wondimu T, Asfaw Z and Kelbessa E. Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi zone, Ethiopia. *J Ethnopharmacol* 2007; 112: 152–161.
11. Chekole G. Ethnobotanical study of medicinal plants used against human ailments in Gubalafto District, northern Ethiopia. *J Ethnobiol Ethnomed* 2017; 13: 55.
12. Fentahun S, Makonnen E, Awas T, et al. In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei*-infected mice. *BMC Complement Altern Med* 2017; 17: 1.
13. Abebe E. Ethnobotanical Study on Medicinal Plants Used by Local Communities in Debark Wereda, North Gondar Zone, Amhara Regional State, Ethiopia [MSc thesis]. Addis Ababa University, Addis Ababa, Ethiopia. 2011.
14. d'Avigdor E, Wohlmuth H, Asfaw Z, et al. The current status of knowledge of herbal medicine and medicinal plants in Fiche, Ethiopia. *J Ethnobiol Ethnomed* 2014; 10: 38.
15. Gidey M, Beyene T, Signorini MA, et al. Traditional medicinal plants used by Kunama ethnic group in northern Ethiopia. *J Med Plants Res* 2015; 9: 494–509.
16. Al-Sunafi S. *Pharmacognostical Study of Rumex nervosus Vahl. Family (Polygonaceae) growing in Yemen*. Cairo, Egypt: Cairo University Theses, 2016.
17. Abebe E and Mekonnen N. In vitro antibacterial activity of *Rumex nervosus*, *Plantago lanceolata*, *Solanum incanum*, and *Lepidium sativum* against selected bacterial pathogens of humans and animals. *Ethiop Vet J* 2016; 20: 119–131.
18. National Research Council. *Guide for the Care and Use of Laboratory Animals*. Bethesda, Maryland: Natl Acad Press, 2010.
19. Rigalli A and Di Loreto V. *Experimental Surgical Models in the Laboratory Rat*. Boca Raton, Florida: CRC Press, 2016.
20. Mulisa E, Asres K and Engidawork E. Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* J. (Polygonaceae) in mice. *BMC Complement Altern Med* 2015; 15: 341.
21. British Pharmacopoeia Commission. *British Pharmacopoeia 2009*. London: Pharm Press, 2011.
22. Deshmukh PT, Fernandes J, Atul A, et al. Wound healing activity of *Calotropis gigantea* root bark in rats. *J Ethnopharmacol* 2009; 125: 178–181.
23. Yadav R and Agarwala M. Phytochemical analysis of some medicinal plants. *J Phytol* 2011; 3: 10–14.
24. Hossain MA, Al-Raqmi KA, Al-Mijizy ZH, et al. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pac J Trop Biomed* 2013; 3: 705–710.
25. Ismail AM, Mohamed EA, Marghany MR, et al. Preliminary phytochemical screening, plant growth inhibition, and antimicrobial activity studies of *Faidherbia albida* legume extracts. *J Saudi Soc Agric Sci* 2016; 15: 112–117.
26. Khanam Z, Wen CS and Bhat IUH. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). *J King Saud Univ Sci* 2015; 27: 23–30.
27. Pandey S. Preliminary phytochemical screening and in vitro antibacterial activity of *Bauhinia variegata* Linn. Against human pathogens. *Asian Pac J Trop Dis* 2015; 5: 123–129.
28. OECD. *OECD 402 guideline for the testing of chemicals: Acute Dermal Toxicity: Fixed Dose Procedure*. Paris, France: OECD, 2002.
29. OECD. *OECD 404 guideline for the testing of chemicals: Acute Dermal Irritation/Corrosion*. Paris, France: OECD, 2002.
30. Beshir K. Evaluation of wound healing activity of 70% ethanol leaf extract of *Becium grandiflorum* Lam. (Lamiaceae) in mice [MSc thesis]. Addis Ababa University, Addis Ababa, Ethiopia. 2017.
31. Kuru P. Beneficial effects of dietary fiber on metabolic syndrome, obesity, and type 2 diabetes: current knowledge and future perspectives. *Food Sci Hum Wellness* 2014; 3: 89–95.
32. Thakur R, Jain N, Pathak R, et al. Practices in wound healing studies of plants. *Evid Based Complement Alternat Med* 2011; 2011: 1–17.
33. Rawat S, Singh R, Thakur P, et al. Wound healing agents from medicinal plants: a review. *Asian Pac J Trop Biomed* 2012; 2: S1910–7.
34. Malviya N and Jain S. Wound healing activity of aqueous extract of *Radix paeoniae* root. *Acta Pol Pharm* 2009; 66: 543–547.
35. Kokane DD, More RY, Kale MB, et al. Evaluation of wound healing activity of root of *Mimosa pudica*. *J Ethnopharmacol* 2009; 124: 311–315.
36. Wang JP, Ruan JL, Cai YL, et al. In vitro and in vivo evaluation of the wound healing properties of *Siegesbeckia pubescens*. *J Ethnopharmacol* 2011; 134: 1033–1038.
37. Zeng Q, Xie H, Song H, et al. In vivo wound healing activity of *Abrus cantoniensis* extract. *Evid Based Complement Alternat Med* 2016; 2016: 1–7.
38. Pawar RS, Chaurasiya PK, Rajak H, et al. Wound healing activity of *Sida cordifolia* Linn. in rats. *Indian J Pharmacol* 2013; 45: 474.
39. Jha R, Garud N and Nema R. Excision and incision wound healing activity of flower head alcoholic extract of *Sphaeranthus indicus* Linn. in albino rats. *Glob J Pharmacol* 2009; 3: 32–37.
40. Fikru A, Makonnen E, Egale T, et al. Evaluation of in vivo wound healing activity of methanol extract of *Achyranthes aspera* L. *J Ethnopharmacol* 2012; 143: 469–474.
41. Hiyaama Y, Marshall AH, Kraft R, et al. Effects of metformin on burn-induced hepatic endoplasmic reticulum stress in male rats. *Mol Med* 2013; 19: 1.
42. Al-Henhena N, Mahmood A, Al-Magrami A, et al. Histological study of wound healing potential by ethanol leaf extract of *Strobilanthes crispus* in rats. *J Med Plants Res* 2011; 5: 3660–3666.
43. Hegazy R and Hegazy A. Hegazy's simplified method of tissue processing (consuming less time and chemicals). *Ann Int Med Den Res* 2015; 1: 57–61.
44. Paschapur MS, Patil M, Kumar R, et al. Evaluation of aqueous extract of leaves of *Ocimum kilimandscharicum* on wound healing activity in albino Wistar rats. *Int J PharmTech Res* 2009; 3: 544–550.
45. Biswas T, Auddy B, Bhattacharya N, et al. Wound healing activity of human placental extracts in rats. *Acta Pharmacol Sin* 2001; 22: 1113–1116.
46. Yadav KS, Yadav NP, Rawat B, et al. An assessment of wound healing potential of *Argyrea speciosa* leaves. *Sci World J* 2014; 2014: 1–6.
47. Shivhare Y, Singour PK, Patil U, et al. Wound healing potential of methanolic extract of *Trichosanthes dioica* Roxb. (fruits) in rats. *J Ethnopharmacol* 2010; 127: 614–619.
48. Prasad V, Jain V, Girish D, et al. Wound-healing property of *Momordica charantia* L. fruit powder. *J Herb Pharmacother* 2006; 6: 105–115.
49. Samanta R, Pattnaik AK, Pradhan KK, et al. Wound healing activity of *silibinin* in mice. *Pharmacogn Res* 2016; 8: 298.
50. Al-Sunafi S. *Pharmacognostical study of Rumex nervosus Vahl. family (Polygonaceae) growing in Yemen*. Cairo Univ, 2016.
51. Polcz ME and Barbul A. The role of vitamin A in wound healing. *Nutr Clin Pract* 2019; 34: 695–700.
52. Bahramsoltani R, Farzaei MH and Rahimi R. Medicinal plants and their natural components as future drugs for the treatment of burn wounds: an integrative review. *Arch Dermatol Res* 2014; 306: 601–617.
53. Bahramsoltani R, Farzaei MH, Abdolghaffari AH, et al. Evaluation of phytochemicals, antioxidant and burn wound healing activities of *Cucurbita moschata* Duchesne fruit peel. *Iran J Basic Med Sci* 2017; 20: 798.

54. Strodbeck F. Physiology of wound healing. *Newborn Infant Nurs Rev* 2001; 1: 43–52.
55. Nagori BP and Solanki R. Role of medicinal plants in wound healing. *Res J Med Plant* 2011; 5: 392–405.
56. Agyare C, Bempah SB, Boakye YD, et al. Evaluation of anti-microbial and wound healing potential of *Justicia flava* and *Lannea welwitschii*. *Evid Based Complement Alternat Med* 2013; 2013: 1–10.
57. Nayak B and Pereira LMP. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement Altern Med* 2006; 6: 41.
58. Gautam M, Purohit V, Agarwal M, et al. In vivo healing potential of *Aegle marmelos* in excision, incision, and dead space wound models. *Sci World J* 2014; 2014: 1–9.
59. Ullah HA, Zaman S, Juhara F, et al. Evaluation of antinociceptive, in vivo and in vitro anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. *BMC Complement Altern Med* 2014; 14: 346.
60. Krishnaiah D, Devi T, Bono A, et al. Studies on phytochemical constituents of six Malaysian medicinal plants. *J Med Plants Res* 2009; 3: 67–72.
61. Ling SK, Tanaka T and Kouno I. Effects of iridoids on lipoxygenase and hyaluronidase activities and their activation by β -glucosidase in the presence of amino acids. *Biol Pharm Bull* 2003; 26: 352–356.

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