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Research article

Evaluation of the wound healing activity of the crude extract of root bark of *Brucea antidysentrica,* the leaves of *Dodonaea angustifolia* and *Rhamnus prinoides* in mice

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ABSTRACT

Background: Wounds are major problems of developing countries that can be managed alternatively using traditional medicinal plants. Since majority of currently available drugs for wound management are expensive and pose problems such as allergy and drug resistance, it is pivotal for the world to have intensified inquiries on the claimed medicinal plants to come up with wound healing chemicals being affordable, effective and safe. Ethiopian traditional healers recruit a wide range of medicinal plants with wound healing activities. Root bark juice of *Brucea antidysentrica*, the leaves of *Rhamnus prinoides* and *Dodonaea angustifolia* are claimed among others in the folklore medicine. Therefore, the aim of this study was to evaluate the in vivo wound healing activities of the root bark juice of *Brucea antidysentrica*, the leaves of *Rhamnus prinoides* and *Dodonaea angustifolia* in mice.

Method: The root bark juice of *Brucea antidysentrica*, the leaves of *Rhamnus prinoides* and *Dodonaea angustifolia* were collected, dried, ground to coarse powders. Then the crude extract was obtained by macerating with 80% methanol. The filtrate was dried, reconstituted in appropriate solvent and the wound healing activity was evaluated using excision and incision wound models.

Results: On the last day of treatment, 80% methanol extracts from the selected medicinal plants showed a significant wound healing activity against control as supported by an increase in % wound contraction and a decrease in Epithelialization period. Ten percent of *Rhamnus prinoides* showed significant wound contraction against the control ($P < 0.05$) on days 2 and 4. But on day 6; except 5% extracts of *Brucea antidysentrica* and *Rhamnus prinoides*; all doses of extracts contracted the wound significantly ($P < 0.05$). Extracts of Dodonea angustifolia (5% & 10%) and 10% of *Rhamnus prinoides* and *Brucea antidysentrica* increases wound contraction rate with increasing significant level on days 8 & 10 ($P < 0.01$) and 12 &14 ($P < 0.001$). Among the extracts, 10% of Dodonea angustifolia showed maximum percent (99.9%; $P < 0.001$) of wound contraction followed by 5% Dodonea angustifolia (99.15%; $P < 0.001$) and 10% *Rhamnus prinoides* (99.00%; $P < 0.001$) on the last day of treatment. In addition; significantly shorter healing time was attained by 5% &10% leaves of Dodonea angustifolia ($P < 0.01$), 10% leaves *Rhamnus prinoides* & root barks *Brucea antidysentrica* ($P < 0.05$). Ten percent of *Rhamnus prinoides* & *Brucea antidysentrica* ($P < 0.05$) and both doses (5% & 10%) of Dodonea angustifolia ($P < 0.01$) significantly increased the tensile strength by 54.10%, 56.58%, 63.04%, and 79.19%, respectively against the control.

Conclusion: The 80% methanol crude extracts of the study plants support the traditional claims for healing of wounds as evidenced by an increase in wound contraction rate and tensile strength, decrease in Epithelialization period.

1. Introduction

Wound which might faced everyone in life time [1] can be described as "a loss or breaking of cellular, anatomical and/or functional connection of living tissues that result in a breach, contravention or interrupting of tissue integrity [2, 3, 4, 5, 6, 7, 8]. These in turn have an important impact on public health care systems and resource expenses [9].

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Based on different criterion; etiology, location, type of injury or presenting symptoms, its depth, and tissue loss or clinical appearance [10]; wounds could be injuries, cuts and bites, diabetic, gastric, and duodenal ulcers [11].

According to the underlying cause of its formation; wound can be open or closed [12] where the blood escapes the body and bleeding is clearly visible or blood escapes the circulatory system but remains in the body respectively [12, 13].

Another parameter to consider for wound classification is its physiology or the time period required to be recovered [14]. Accordingly, the tissue injury caused by cuts or surgical incisions in acute wound is going on through orderly and timely reparative process, results in sustained restoration of anatomic and functional integrity and the healing process has been completed within the predictable time frame [15]. If this orderly and timely reparative process of healing is failed and goes to a state of pathologic inflammation; the wound is considered as chronic [16]. In this type of wound, healing process is delayed, incomplete, and does not proceed in a coordinated manner, resulting in poor anatomic and functional integrity over 3 months [17]. Despite vacant epidemiological profile of chronic wound, identifying and treating its underlying etiology is crucial to successful treatment [18, 19]. Currently about 6 million peoples are estimated to be affected by chronic wounds worldwide [15] and the percentage of the healthcare cost is high [20].

Wound is formed due to physical, chemical, thermal, microbial, or immunological factors [1, 7, 21] and its healing process might be complex, dynamic, and normal biological process [2, 9, 22] which entails efficient management skills [23]. Wound healing process occurs in a persistent & integrated manner [1] and this process involves four temporarily and spatially overlapping phases named as hemostasis, inflammation, proliferation, and remodeling [7, 9, 22, 24, 25]. These phases should be well controlled in order for proper wound healing process [26]. People around the world possess unique knowledge of plant resources [27] where the trends in the use of these resources is on the increase in many countries [28] to meet their primary health care needs [29]. In Ethiopia, the use of traditional medicinal preparation is common [30] due to cultural acceptability, low cost, and inadequate access to modern health facilities [31]. In addition, different types of medicinal plants [3, 9, 32, 33, 34, 35, 36, 37, 38] and others are used to manage wounds. Among these medicinal plants root bark of *Brucea antidysentrica,* leaves of *Rhamnus prinoides,* and *Dodonaea angustifolia* are highly claimed even though their in vivo wound healing activity is not reported: a driving force to conduct this study using both incision and excision wound models.

2. Methods

2.1. Plant materials collection

Leaves of *Rhamnus prinoides* and *Dodonaea angustifolia* and root barks of *Brucea antidysentrica* were collected from East Gojjam Zone, Amhara regional state, Ethiopia and authenticated by a taxonomist at the national herbarium unit of Ethiopian public health institute, Addis Ababa, Ethiopia which then deposited with a voucher specimen (number ZT-002, ZT-003, and ZT-004 respectively).

2.2. Experimental animals

Healthy, adult Swiss albino mice of both sex (25±5g weight and 6–8 weeks of age) were attained from Ethiopian public health institute, Addis Ababa, Ethiopia and they were bred at College of health sciences, university of Gondar. They were kept in cages under standard conditions (25 ± 2 °C, 55 ± 5% relative humidity, and 12 h light and dark cycles) and provided with pellet diet and water ad libitum. Before the actual experiment, they were acclimatized to the laboratory conditions for a week. Handling and care of animals were secured during the experiment as per the international laboratory animal use and care guidelines. At the end of the experiment, they were scarified using high dose of anesthesia [39]. The experiment was conducted following ethical approval by the Ethical Review Committee of Health sciences college, Debre Markos University, for the use of animals for the experiment (ref no. 1354/2018).

2.3. Preparation of experimental plant materials

Using maceration extraction technique, course powdered leaves (300 gm) and root barks (300 gm) of the experimental plant materials were soaked in 80 % (V/V) methanol for at least 3 days in a conical flask separately with frequent agitation and then followed by strain and filtration of each of the mixtures. The marc was re-macerated to increase the yield. The three successive filtrates were combined, concentrated by Rota vapor at 40 °C to eliminate the alcoholic solvent, deep-frozen in a refrigerator at -20 °C, dried using drying oven at 40 °C, and stored in a refrigerator till used for the experimental [5].

2.4. Ointment formulation

Simple ointments of the hydroalcoholic extracts of the experimental plant materials were prepared following the formula (Table 1) described in the British Pharmacopoeia [40].

Different doses (5% and 10% w/w) of medicated ointments from each of the experimental plant materials and simple ointments were formulated. Ingredients of the ointment base were mixed, heated gently with stirring to achieve homogeneity, and then stirring is continued until it cooled [41].

2.5. Grouping and dosing of experimental animals

For the excision model, 8 groups of mice; six animals per group were used. Animals in group 1 & 2 were treated with Nitrofurazone (0.2 %) and simple ointment to serve as a positive & a negative control respectively. Animals from group 3 to 8 were treated with different doses (5% and 10% w/w) of the hydroalcoholic ointments prepared from experimental plant extracts. For the incision model, 9 groups of mice; having six mice per group were used. Here the 9th group was added as compared to the excision model because, in the incision model, one more group which was left untreated was required to compare the tensile strength due to simple ointment. The animals under group 1 to 8 were treated similar to excision model, and the 9th group was left untreated and served as untreated controls.

2.6. Determination of wound healing activity

2.6.1. Excision wound model

As per the excision wound model, wound site was made by anesthetized the experimental animals. The surgical processes were done under sterile conditions by utilizing ketamine (80 mg/kg) plus diazepam (5 mg/kg i. p) and a full-thickness of circular excision wound measuring 314 mm^2 and 2mm depth was prepared on the shaved dorsal thoracic region. The dose of ketamine and diazepam was selected at the indicated range due to the limited cardiovascular effects including minimal hypotension and better anesthetics effect during the actual experiment. Then both medicated and simple ointments were applied topically on daily bases as described under grouping and dosing section. Wounding day was considering as day 0. Wound closure to the wounded area and its period of epithelialization were observed at 2, 4, 6, 8, 10, 12, and 14th days of post wound [42]. The percentage of wound contraction effect of the extracts was calculated (Taking the initial size of wound i.e. 314mm^2 as 100 %) using equation [1] as follow [43, 44].

$$\%\ \text{Wound contraction} = \frac{\text{Wound area on day0} - \text{Wound area on day } n \ \text{X } 100}{\text{Wound area on day0}} \quad (1)$$

2.6.2. Linear incision wound model

After preparation of the mice in the same fashion to excision wound model, their dorsal fur was thieved and a 3 cm long longitudinal para-vertebral incision wound was created and sutured 1 cm apart using a surgical thread (no. 000) & curved needle (no. 11). To achieve better wound closure, the thread was tightened continuously on both wound edges. After 24 h of wound creation (on 1st day), a topical formulation of simple ointment, extract ointments, and standard drug were applied daily on wounded animals according to the grouping and dosing section for 9 days. The sutures were removed on day 8 post-incision and tensile strength was measured and calculated (Eqs. (2), (3), and (4)) on the 10th post-wounding day using the continuous water flow technique [45] as shown below [46] in Figure 1.

$$\text{Tensile strength (TS) of extract } (\%) = \frac{\text{TS extract} - \text{TS vehicle}}{\text{TS vehicle}} x100 \quad (2)$$

$$\text{Tensile strength (TS) of reference } (\%) = \frac{\text{TS reference} - \text{Ts vehicle}}{\text{TS vehicle}} x100 \quad (3)$$

$$\text{Tensile strength (TS) of simple ointment } (\%) = \frac{\text{TS simple ointment} - \text{Ts L.U}}{\text{TS L.U.}} x100 \quad (4)$$

where L.U = left untreated groups.

2.7. Phytochemical screening

The hydroalcoholic extracts of the leaves of *Rhamnus prinoides* and *Dodonaea angustifolia,* and root barks of *Brucea antidysentrica* were screened for the presence of secondary metabolites like alkaloids, saponins, flavonoids, terpenoids, phenols, and tannins according to standard tests described [47, 48, 49, 50]. The specific tests for the phytochemicals were done as follows.

2.7.1. Test for alkaloids (Wagner's test)

Ten mg of each of the crude extracts were dissolved in 1ml of distilling water. With this solution three drops of Wagner's reagent was added. The presence of alkaloids was confirmed by the formation of a reddish-brown colored solution.

2.7.2. Tannins test (Lead acetate and ferric chloride test)

For the lead acetate test, 0.1 gm of each of the extracts was dissolved in 2ml of distilling water. Then 1ml of each of the solutions was taken and 0.5 ml of 1% lead acetate was added to it. Formation of yellowish precipitate was observed for the presence of tannins. For the ferric chloride test, 0.5 ml of 5% ferric chloride solution was added to the same solution used for the lead acetate test, and the development of dark bluish or black color was observed for the presence of tannins.

2.7.3. Test for Triterpenoid

The dry crude plant extracts of each of the preparations (10mg) were dissolved in 2ml chloroform and then 1 mL acetic anhydride was added to each of the solutions. Then 1mL concentrated sulphuric acid was

Table 1. The formula used for the preparation of the ointment.

Ingredients	MF	RF
Wool fat	50 g	10 g
Hard paraffin	50 g	10 g
Cecostearyl alcohol	50 g	10 g
White soft paraffin	850 g	170 g
	1000 g	200 g

MF, Master formula; RF, reduced formula.

A B C

Figure 1. Incised mice (A) and continuous water flow method for determination of tensile strength (B &C).

Table 2. Effect of topical application of hydroalcoholic extracts of experimental medicinal plants on percent wound contraction and epithelization time of an excision wound in mice.

Groups	Wound area (mm^2) ± SEM (% Contraction)							EP (in days)
	2	4	6	8	10	12	14	
SO (control)	227.81 ± 14.19 (27.45)	177.88 ± 15.27 (43.35)	146.05 ± 21.96 (53.49)	103.35 ± 17.71 (67.09)	64.61 ± 15.64 (79.43)	42.39 ± 8.03 (86.50)	31.72 ± 9.24 (89.90)	17.80 ± 1.07
5% w/w DA	145.62 ± 26.70 (53.63)	116.38 ± 24.53 (62.94)	73.83 ± 14.75*1 (76.49)	48.87 ± 10.20*2 (84.44)	21.00 ± 6.36*2 (93.31)	7.07 ± 2.51*3 (97.75)	2.67 ± 1.30*3 **(99.15)**	14.00 ± 0.63*2
10% w/w DA	140.91 ± 24.07 (55.13)	111.83 ± 22.41 (64.39)	70.69 ± 9.99*1 (77.49)	41.45 ± 5.88*2 (86.80)	16.02 ± 3.65*2 (94.90)	3.46 ± 1.58*3 (98.90)	0.32 ± 0.19*3 **(99.90)**	13.60 ± 0.68*2
5% w/w RP	148.88 ± 15.39 (52.59)	113.55 ± 15.68 (63.84)	85.76 ± 14.72 (72.69)	59.19 ± 8.17 (81.15)	36.74 ± 4.07 (88.30)	18.21 ± 1.41*2 (94.20)	5.65 ± 0.38*3 (98.20)	15.20 ± 0.37
10% w/w RP	137.57 ± 18.07*1 (56.19)	97.11 ± 13.96*1 (69.08)	68.93 ± 9.29*1 (78.05)	42.90 ± 6.75*2 (86.34)	26.61 ± 7.28*2 (91.53)	13.50 ± 4.90*3 (95.70)	3.14 ± 1.43*3 **(99.00)**	14.20 ± 0.74*1
5% w/w BA	153.75 ± 18.98 (51.04)	116.14 ± 11.42 (63.01)	106.81 ± 23.94 (65.98)	59.19 ± 8.17 (81.15)	37.72 ± 3.66 (87.99)	21.67 ± 2.98*1 (93.10)	7.22 ± 0.46*3 (97.70)	15.80 ± 0.20
10% w/w BA	138.83 ± 23.03 (55.79)	103.43 ± 18.29 (67.06)	72.26 ± 9.92*1 (76.99)	42.90 ± 6.75*2 (86.34)	26.14 ± 6.36*2 (91.68)	14.13 ± 4.49*3 (95.50)	4.16 ± 1.98*3 (98.68)	14.40 ± 0.68*1
0.2%NF	127.68 ± 11.57*1 (59.34)	91.26 ± 11.30*1 (70.94)	68.14 ± 10.53*1 (78.30)	43.69 ± 8.44*3 (86.09)	10.99 ± 4.76*3 (96.50)	3.14 ± 2.43*3 (99.00)	0.00 ± 0*3 (100.00)	12 ± 0.707*3,$^{+}$2,@1

Values are expressed as mean ± SEM (n = 6 animals in each group) and analyzed by one-way ANOVA followed by tuckey post hoc test; numbers from 2-14 indicate the day on which contraction rate measurement was taken; EP = epithelization period; SO = simple ointment (control) base; DA = *Dodonaea angustifolia*; RP = *Rhamnus prinoides*; BA = *Brucea antidysentrica*, NF = nitrofurazone ointment, *: compared against the control, + = against 5% *Brucea antidysentrica*, @ = against 5%*Rhamnus prinoides*, 1p < 0.05; 2p < 0.01; 3p < 0.001.

added to the solution. Formation of reddish-violet color shows the presence of Triterpenoid.

2.7.4. Test for flavonoids (Alkaline reagent or NaOH test)

The crude extracts (0.3 g) of each of the preparations were dissolved in 2ml of distill water. To these, three drops of 20% sodium hydroxide solution were added. An intense yellow color was formed which turned to colorless with the addition of three drops of 20 % hydrochloric acid which indicated the presence of flavonoids in each of the extracts. Besides, a lead acetate test was performed. To the same solution used above 3 drops of 10% lead acetate was added and the formation of yellow precipitate was observed for the presence of flavonoids.

2.7.5. Test for saponins (Foam test)

About 0.3g of each of the crude extracts was taken and dissolved in 20 ml of distill water. After vigorous shaking the formation of persistent foam observed for 30 min was taken as an indication for the presence of saponins. But root bark of *Brucea antidysentrica* did not confirm its presence.

2.7.6. Test for phenols (Ferric chloride test)

Ten mg of each of the extracts was dissolved in 1ml of water. Half ml of 5% ferric chloride the solution was added to it and the development of deep blue or black color was taken as an indicator for the presence of phenols.

2.7.7. Test for steroids (Liebermann-Burchard test)

About one-half gram (0.5 g) of each of the crude extracts was dissolved in 0.5mL dichloromethane to produce a dilute solution. To this solution 0.5 mL of acetic anhydride was added, followed by three drops of concentrated sulphuric acid. Formation of a blue-green coloration indicated the presence of steroids.

2.7.8. Test for glycoside (Glycoside tests)

A small amount of the extracts (0.1g) were dissolved in 1 ml of distill water and then three drops of 20% sodium hydroxide solution was added and the formation of yellow color confirms the presence of glycosides.

2.8. Acute oral toxicity study

Since the skin of the mice was either incised or excised and in doing so, their internal organs were easily accessible to the medicated ointments and if it is toxic they may be died. Therefore, it is essential to check the oral toxicities of the extracts before doing the actual experiment. Five healthy Swiss albino female mice were involved and the test was performed by administering hydroalcoholic solutions from each of the extracts according to the Organisation for Economic Co-operation and Development (OECD) 425:2008 guideline [51]. Occurrence or absence of parameters indicating toxicity such as lacrimation, hair erection, and loss of motor/or feeding activities, and mortality as well as weight reduction were observed for fourteen days.

2.8.1. Statistical analysis

The experimental result was expressed as mean ± SEM (standard error of the mean). The results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Post Hoc Tukey tests with Statistical Package for the Social Sciences (SPSS) version 20 where $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Yields of extraction

From 300 gm leaves of *Dodonaea angustifolia and Rhamnus prinoides* and roots of *Brucea. Antidysentrica* macerated with 80% methanol;

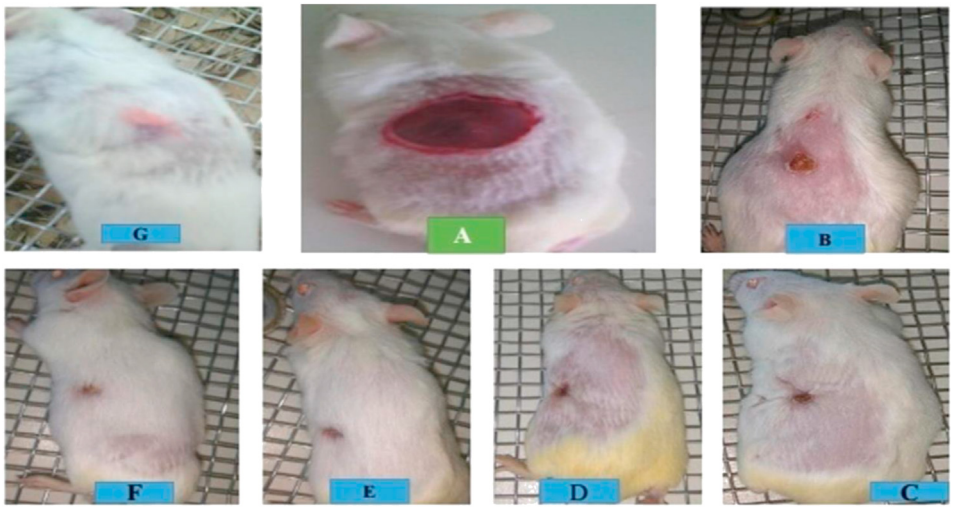

Figure 2. Excision wound immediately after wounding and healing progress after application of the hydroalcoholic medicated extracts: A = Immediately after excision; B=Simple ointment, C = 10% w/w *Brucea antidysentrica*; D = 5% w/w *Dodonaea angustifolia*; E = 10% w/w *Rhamnus Prinoides*; F = 10% w/w *Dodonaea angustifolia*; G = Standard (0.2% Nitrofurazone ointment).

Table 3. Effect of topical application of 80 % hydroalcoholic extracts of the of leaves of *Dodonaea angustifolia*, *Rhamnus prinoides* and root barks of *Brucea antidysentrica* on breaking strength of an incision wound model on day 10 of wound creation.

Dose	Breaking strength (g)	% Tensile strength
LU	131.04 ± 2.25	-
SO (control)	161.0 ± 9.2	12.86
5% w/w DA	262.5 ± 34.4***2** $^{+}$ **3**	**63.04**
10% w/w DA	288.5 ± 19.8***2** $^{+}$ **3**	**79.19**
5% w/w RP	241.9 ± 10.9^{+}**3**	50.25
10% w/w RP	252.1 ± 18.6***1** $^{+}$ **3**	**56.58**
5% w/w BA	234.3 ± 9.9^{+}**3**	45.52
10% w/w BA	248.1 ± 18.6*1 $^{+}$ **3**	54.10
0.2%NF	310.6 ± 4.924***3** $^{+}$ **3**	**92.92**

Values are expressed as mean ± SEM (n = 6 animals in each group) and analyzed by one-way ANOVA followed by Tuckey post hoc test; tensile strength was measured on the 10th post-wounding day using continuous water flow technique; SO = simple ointment base; LU = left untreated control; DA = *Dodonaea angustifolia*; RP = *Rhamnus prinoides*; BA = *Brucea antidysentrica*, NF = nitrofurazone ointment, *: compared against the control, +: compared against left untreated 1p < 0.05; 2p < 0.01; 3p < 0.001.

residues of 52.3 and 41.7 and 36.4 g were gained making the yields 17.43%, 13.9%, and 12.13% respectively.

3.2. Wound-healing effect of the extracts

3.2.1. Excision wound model

As depicted in Table 2, 10% medicated formulations of *Rhamnus prinoides* showed significant wound contraction against the control (P < 0.05) on days 2 and 4. On day 6, all doses of medicated extracts except 5% w/w extracts of *Brucea antidysentrica* and *Rhamnus prinoides* contracted the wound significantly (P < 0.05). Both doses (5% &10% w/w) of *Dodonaea angustifolia* and 10% of *Rhamnus prinoides* as well as *Brucea antidysentrica* increases percentage of wound contraction with an increasing significant level on days 8 &10 (P < 0.01) and 12 &14 (P < 0.001). All doses of the extracts were comparably effective (P < 0.05) against the simple ointment on days 12 and 14. On the other hand, 5% w/

Table 4. Results of phytochemical screening of the hydroalcoholic extracts of leaves **of** *Dodonaea angustifolia*, *Rhamnus prinoides* and root barks of *Brucea antidysentrica*

Phytochemicals	Test used	Phytochemical screening test results from the hydroalcoholic extracts					
		5% DA	10% DA	5%RP	10%RP	5%BA	10%BA
Alkaloids	Mayer's and Wagner's test	+	+	+	+	+	+
Tannins	Braymer's Test	+	+	+	+	+	+
Terpenoids	–	+	+	+	+	+	+
Flavonoids	Alkaline reagent test	+	+	+	+	+	+
Saponins	foam test	+	+	+	+	–	–
Phenols	Ferric chloride test	+	+	+	+	+	+

Note: (+) indicates the presence and (–) indicates the absence of particular metabolites.
DA = *Dodonaea angustifolia*; RP = *Rhamnus prinoides*; BA = *Brucea antidysentrica*
Unit of Concentration = w/w.

w *Rhamnus prinoides* showed a significant wound contraction effect on 12^{th} and 14^{th} days ($P < 0.01$ and $P < 0.001$, respectively). On the same day, similar dose of Brucea *antidysentrica* contracted the wound with different significant level ($P < 0.05$ and $P < 0.001$, respectively). On the last day of treatment, 10% medicated formulation of *Dodonaea angustifolia* showed greatest percent (99.9%; $P < 0.001$) of wound contraction followed by 5% w/w *Dodonaea angustifolia* (99.15%; $P < 0.001$) and 10% w/w *Rhamnus prinoides* (99.00%; $P < 0.001$). The data from (Table 2) confirmed that considerably shorter healing time was recorded by 5% &10% w/w of *Dodonaea angustifolia* ($P < 0.01$) and 10% w/w *Rhamnus prinoides & Brucea antidysentrica* ($P < 0.05$) against the control. The healing time due to 5% w/w *Rhamnus prinoides & Brucea antidysentrica* was not significant (Figure 2).

3.2.2. Incision wound model

As shown in Table 3, the hydroalcoholic extracts were effective in increasing the breaking strength of the healing wound. Ten percent of *Rhamnus prinoides & Brucea antidysentrica* ($P < 0.05$) and 5%&10% (w/w/) of *Dodonaea angustifolia* ($P < 0.01$) increased the tensile strength significantly by 54.10%, 56.58%, 63.04%, and 79.19%, respectively. Comparing with the animals remain untreated, all medicated preparations had a greater increasing effect on the tensile strength ($P < 0.001$).

3.3. Phytochemical screening

Phytochemical screening of leaves of *Dodonaea angustifolia, Rhamnus prinoides*, and root barks of *Brucea antidysentrica* was done to qualitatively identify the presence or absence of secondary metabolites and the results showed the presence of various secondary metabolites as publicized below (Table 4).

3.4. Acute oral toxicity study

The results of the oral acute toxicity study revealed that all tested extracts of the medicinal plants were appeared safe to the dose of 2000 mg/kg as none of the mice was died and even did not show any sign of toxicity to the final date of the experiment. Therefore, the Lethal Dose 50% (LD50%) of both leaves of *Dodonaea angustifolia* and *Rhamnus prinoides* and root barks of *Brucea antidysentrica* are greater than 2000 mg/kg.

4. Discussion

Peoples of the world utilized numerous plants and/or their derivatives as wound-healing agents despite proven evidence of their safety and effectiveness were under question [11]. All the wound-healing phases cannot be halted using conventional wound healing agents, indicating the necessity of developing newly proven effective and safe wound-healing drugs. Fast wound contraction, shorter epithelization period, and satisfactory improvement of breaking strength characterized rapid wound –healing process. Biochemical markers including tissue DNA, RNA, total protein, and hydroxyproline are of good quality of drugs for enhanced healing [52, 53].

In the excision wound model, the topical application of the hydroalcoholic extracts resulted in an enhanced wound reduction rate and this may be attributed to better wound healing progression and noticeable wound margin hydration due to tissue regeneration. On the last day of treatment, the percentage closure of all extracts was fallen between 97.7%-99.9% while the group treated with simple ointment was 89.9%. Wound contraction that contributes to wound closure is articulated as a reduction in the percentage of original wound size [21].

Wound contraction role is crucial as it decreases wound dimension and increases amount of extracellular matrix required to repair the defect cell and assists re-epithelization [3]. The wound reduction effect of the extracts here may be due to inhibition of microbial growth particularly in the inflammatory phase, their mitogenic activity which enhances motility of the fibroblast and its cellular proliferation as well as subsequential conversion to myofibroblasts through the healing process mainly being dermal [21]. Fibroblasts stimulation is one of the mechanisms of plant extracts to assist wound healing owing to their migration from the wound edge to its site, proliferation, and consequently formation of collagen; the main constituent of extracellular matrix [54].

In this study, animals treated with all medicated crude extracts except those treated with 5% *Rhamnus prinoides and Brucea antidysentrica* significantly shortened an essential component of wound healing; the period of epithelization [53]. As this essential element proceeds, different biochemical processes are conducted and this attributed to the momentous effect of the extracts (88). This significant effect of extracts on the period of epithelization proved their potential healing activity.

Furthermore; the considerable effect of the extracts on the tensile strength that revealed their wound-healing activity may be due to collagen synthesis, its maturation, angiogenesis, and stabilization of fibers where all these cumulative effect improves circulation, thus providing oxygen and nutrients, essential for the healing process of the wound site [55, 56].

Phytochemical screening test of plant material extracts revealed the presence of various secondary metabolites including alkaloids, saponins, tannins, flavonoids, phenols, and terpenoids with the exception that root bark of *Brucea antidysentrica* lacks saponins.

These biologically active compounds are directly accountable for antioxidant, antimicrobial, antifungal, and anticancer activities [57]. Tannins for example encourage wound healing process by chelating of free radical or reactive oxygen species, shrinking of proteins, promoting wound contraction, increasing capillary vessels and fibroblasts formation [58, 59]. Besides; phenolic compounds act as antimicrobials, antioxidants, and anti-inflammatory [60]. Restrain the reactive species produced by phagocytic cells is also important in wound healing process [61].

Another metabolites termed as flavonoids reduce lipid peroxidation by preventing or slowing the onset of cell necrosis and improving vascularity which in turn increases the viability of collagen fibrils by increasing the circulation, preventing cell damage, and promoting DNA synthesis. These compounds and Triterpenoids are also promote the wound-healing process mainly due to their antimicrobial and antioxidant ability, which seems to be responsible for wound contraction and increased rate of epithelialization period [62]. As reported by another study; certain flavonoids hold back enzymes such as phospholipaseA2 and have astringent effects playing an important role in wound contraction and rate of epithelialization [63, 64, 65]. The relationship between antibacterial activity to steroids, anti-inflammatory activity to saponins, and anti-bacterial and anti-analgesic activities to alkaloids is also reported by Kumar Bargah where all these metabolites attributed to fast wound contraction and shorter epithelialization period [66].

According to the results from the acute oral toxicity test, the median lethal doses (LD50) were greater than >2000 mg/kg for both leaves and the root bark extracts. Generally, the [67] guideline recommends the test chemical to be categorized under experimentally safe substance for use if the LD50 value of the test chemical is more than 3 times the minimum effective dose. Since the hydroalcoholic extract of the leaves and the root bark had an LD50 value of more than the recommended dose (100 mg/kg), it was taken as a good candidate for further studies. Based on the LD50 value, (LD50 > 2000 mg/kg) both extracts can be designated as "unlikely to be hazardous" [68].

5. Conclusion and recommendations

The present study indicated that the hydroalcoholic extracts of the leaves of *Dodonaea angustifolia*, *Rhamnus prinoides*, and *root bark of Brucea antidysentrica* possess wound healing activity established by a significant rate of wound contraction and shorter epithelization period that may be due to the presence of biologically active secondary metabolites acting as either individually or collectively to bring about the overall effect. These findings provide scientific support for the folkloric repute of the leaves of *Dodonaea angustifolia, Rhamnus prinoides*, and root bark of *Brucea antidysentrica* as a wound-healing agent. Therefore, based on the results of this study, the authors would like to forward the following recommendations.

- Performing wound healing activity tests with various solvent fractions.
- Carrying out in vitro tests for the wound healing activity of the crude extract and its fractions.
- Performing out a quantitative phytochemical study to quantify the active components against wounds from the plant.
- Carrying out chronic toxicity studies of the extract in animal models.

Declarations

Author contribution statement

Z. Tessema: Conceived and designed the experiments; Performed the experiments, Wrote the paper.

Y. Molla: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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