Evaluation of *in vivo* wound healing and anti-inflammatory activity of 80% methanolic extract of the leaves of *B. antidysentrica* J. F. Mill (Simaroubaceae) in mice

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**ABSTRACT**

**Objective:** The purpose of this study was to evaluate the wound healing and anti-inflammatory activities of the crude extract of the leaves of *B. antidysentrica* J. F. Mill.

**Results:** Both 2% and 4% crude extract revealed significant wound healing activity compared with negative control as evidenced by an increase in the percent of wound contraction (p < 0.01) and a decrease in epithelization period (p<0.05). In addition, both 2% and 4% extract ointments resulted in a significant increase in tensile strength (p < 0.01) compared with negative control. Hundred, two hundred and four hundred mg/kg extracts of the leaves exhibited a higher anti-inflammatory effect on the 3rd (P<0.05) and 4th hour (P<0.001) as compared to the negative control.

**Keywords:** *B. antidysentrica* J. F. Mill, wound healing, anti-inflammatory, excision, incision.

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**INTRODUCTION**

Wound is mainly defined as a loss or breaking of cellular and anatomical or functional continuity of living tissues¹ [1-3]. The loss of skin tissue integrity can cause lesions or illnesses that have a significant impact on public health and expenditure of health care resources, and can lead to disability or even death of individuals [4-6].

Wound healing is a complex process which involves various cells, the extracellular matrix (ECM), cytokines, and growth factors. As soon as injury occurred, healing is initiated by vasoconstriction and formation of a clot [7]. This homeostasis is followed by the proliferative phase [1, 8, 9]. The proliferative phase mainly involves the migration of fibroblasts that produce large quantities of collagen which in turn imparts the tensile strength and maturation of the scar [10-13].

The practice of using medicinal plants is common in developing countries like Ethiopia to meet their primary health care needs [14]. In Ethiopia, the plant, *Bracea antidysenterica* J. F. Mill (Locally known as 'Abalo') has a traditional claim of treating scabies, external parasites, dysentery, wound, gonorrhea, eczema, cancer and trypanosomosis [14, 15-24].

In addition, the crude extract of the *B. antidysentrica* (*B. antidysenterica*) had been shown to have antibacterial activity *in vitro* against the growth *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli*, *Shigella sonnei*, and *Pseudomonas aeruginosa* [18, 25, 26]. Moreover, the fruits of the plant had revealed the wound healing and anti-inflammatory activities [25]. However, to date, the *in vivo* wound healing and anti-inflammatory activities of the leaves of *B. antidysentrica* is not reported in literatures though it has been claimed, traditionally, to have wound healing activity. Therefore, the aim of this study was to scientifically validate the folklore use of the leaves of the plant for wound healing and anti-inflammatory activities in mice models and in addition, since the pharmacology of the plant extract is associated with the phytochemical patter, identifying the common phytochemical components of the extract was significantly considered.
MATERIALS AND METHODS

Collection and authentication of plant materials

The leaves of *B. antidysentrantica* were collected from areas of Debre Markos town, Ethiopia, in January, 2017 where the geographical coordinates and the altitude of the collection site was shown in Figure 1.

Then, the plant was identified by a taxonomist named Dr Getachew Addis at the National Herbarium of Ethiopian Public Health Institute, Ethiopia and deposited with a voucher specimen (number ZT-001).

Preparation of the crude extracts

The crude extract of the leaves of *B. antidysentrantica* was prepared by maceration technique according to the procedure described by previous study [25].

Ointment formulation

Ointments of the crude extract with concentrations of 2% w/w and 4% w/w was formulated following standard procedure [27] following the reduced formula as described previously [25, 28].

Experimental animals

Both sex of mice with age of 6-8 weeks and weight range of 29-40 g and rats of both sex with the weight range of 150-200g and aged 3–4 months were brought from Ethiopian Public Health Institute. The Mice and Rats were allowed to live in cages under the favorable conditions of temperature and relative humidity (22 ± 3 °C, 40-70 % relative humidity). Before using them for the experiment, they were acclimatized to the laboratory condition for a week.
Grouping and dosing of experimental animals

For excision wound model, four groups of mice (each with six mice) were used. Each group was treated following the procedure described by Tessema et al [25].

Wound healing studies

a. Excision wound model

The excision wound model was done following the procedure described by previous studies [25, 29] and the wound healing effect of the extracts was calculated as follows [30, 31].

\[ \text{% Wound contraction} = \left( \frac{\text{Wound area on day } n}{\text{Wound area on day } 0} \right) \times 100 \]

Where n = the days where measurement was taken

b. Incision wound model

The incision wound model was conducted following the procedure of previous studies [25, 32]. The percent tensile strength was calculated using the following formula [27].

\[ \text{Tensile strength (TS) of extract (\%) = } \left( \frac{\text{TS extract} - \text{TS simple ointment}}{\text{TS simple ointment}} \right) \times 100 \]

\[ \text{Tensile strength (TS) of reference (\%) = } \left( \frac{\text{TS reference} - \text{TS simple ointment}}{\text{TS simple ointment}} \right) \times 100 \]

\[ \text{Tensile strength (TS) of vehicle (\%) = } \left( \frac{\text{TS simple ointment} - \text{TS left untreated}}{\text{TS left untreated}} \right) \times 100 \]

Anti-inflammatory activities

The anti-inflammatory activity of the plant was performed following the procedure stated by previous studies [25, 33]. Edema percent inhibition was calculated as follows:

\[ \text{Percentage of inhibition (\%) = } \left( \frac{1 - x/y}{} \right) \times 100 \]

Where x = mean increase in paw circumference of treated mice and y = mean increase in paw circumference of control mice.

Phytochemical screening

The presence of secondary metabolites has been conducted qualitatively in the crude extract of the plant. The screening test for the presence of selected secondary metabolites of the crude extract of B. antidysentrica, was done using standard tests described previously [34-40]. The presence of alkaloids, tannins, flavonoids, saponins and phenols were tested by using Wagner's test, Lead acetate and ferric chloride test, NaOH test, Frothing test, and Ferric chloride test, respectively.

Acute toxicity studies

i. Acute oral toxicity study

The oral acute toxicity study was conducted on female mice by administering solutions of the extract following OECD 425:2008 guideline [41].

ii. Skin irritation test

Skin irritation test was done according to standard dermal irritation test [25, 42, 43].

Statistical analysis

The raw data obtained were expressed as mean ± SEM and analyzed using ANOVA followed by Post Hoc Tukey tests using SPSS version 20 software. Statistically significant was considered at \( p < 0.05 \).

RESULTS

Wound Healing Effect of the Extracts

a. Excision wound model

Two and four percent of the plant extracts which were preparing using ointment bases, and the standard drug (Nitrofurazone) were found to be comparably effective (\( P<0.05 \)) compared to the simple ointment on days six & eight, and the effect was maintained till the 14th day with increased degree of significance (Table 1). The 4% plant

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wound area (mm2)±SEM (% contraction)</th>
<th>EP (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>217.13±13.18 (30.90)</td>
<td>28.26±8.92 (91.00)</td>
</tr>
<tr>
<td>4</td>
<td>175.72±16.24 (44.00)</td>
<td>17.60±1.08</td>
</tr>
<tr>
<td>6</td>
<td>146.05±21.96 (53.50)</td>
<td>14.00±0.63</td>
</tr>
<tr>
<td>8</td>
<td>100.36±18.96 (68.00)</td>
<td>14.00±0.63</td>
</tr>
<tr>
<td>10</td>
<td>60.99±13.05 (80.60)</td>
<td>14.00±0.63</td>
</tr>
<tr>
<td>12</td>
<td>39.25±9.83 (87.50)</td>
<td>14.00±0.63</td>
</tr>
<tr>
<td>14</td>
<td>28.26±8.92 (91.00)</td>
<td>14.00±0.63</td>
</tr>
</tbody>
</table>

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 base; LE=methanol leaves extract; NFO=nitrofurazone ointment, *: compared against the control. \( \text{r} \text{p}<0.05; \text{p}<0.01; \text{p}<0.001. \)
extract showed significant (P<0.001) and maximum percentage (99.9 %) of wound contraction was found on the last day of treatment as compared to the negative control (simple ointment; ointment without the extract) (Table 1). As compared to the control, a significantly shorter healing time was achieved by both of the extracts (2%; P<0.05 & 4%, P<0.01). The difference of healing time between extract formulations and standard (nitrofurazone) drug was no significant.

b. Incision wound model

The 80% methanolic extracts, 2% and 4%, significantly increased the percent tensile strength (P<0.01) by 84.04%, and 89.62%, respectively, compared to negative control. Similarly, both 2% and 4% leaves extracts, and the standard (P<0.001) increased the tensile strength (Table 2).

Table 2: Effect of topical application of 80 % methanol extracts of B. antidysentrica leaves on breaking strength of an incision wound on day 10 of wounding

<table>
<thead>
<tr>
<th>Dose</th>
<th>Breaking strength (g)</th>
<th>% tensile strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>LU</td>
<td>141.0±10.6</td>
<td>-</td>
</tr>
<tr>
<td>SO</td>
<td>160.4±13.9</td>
<td>13.75</td>
</tr>
<tr>
<td>2% LE</td>
<td>295.1±29.1</td>
<td>84.04</td>
</tr>
<tr>
<td>4% LE</td>
<td>304.1±27</td>
<td>89.62</td>
</tr>
<tr>
<td>0.2% NFO</td>
<td>315.5±7</td>
<td>96.75</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6 mice in each group), SO=simple ointment base; LU=left untreated control; LE= leaves extract NFO=nitrofurazone ointment, *: compared against the control, +: compared against left untreated1p<0.05; 2p<0.01; 3 p<0.001.

*Here LU represents the group receiving either the simple ointment or the medicated ointment

Anti-inflammatory effect of the extracts

On the 2nd (P<0.05), 3rd (P<0.01) and 4th (P<0.001) hour of administration, the 400mg/kg of the extract reduced the edema volume significantly compared to the negative control. Similarly, on the 3rd and 4th h, the remaining (100,200 mg/kg) doses of the extract reduced the edema volume significantly (P<0.05) compared to the negative control (Table 3).

Table 3: Anti-inflammatory effect of 80 % methanol extract of leaves of B. antidysentrica on carrageenan-induced paw edema following oral administration in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean change in the hind paw volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV 1h 2h 3h 4h</td>
</tr>
<tr>
<td>Control (2% Tween 80)</td>
<td>0.6±0.01 1.15±0.07 1.2±0.07 1.20±0.07 1.17±0.07</td>
</tr>
<tr>
<td>LE (100mg/kg)</td>
<td>0.65±0.06 (9.55%) 0.87±0.04 (7.41%) 0.84±0.08 (31.10%) 0.76±0.05 (34.87%)</td>
</tr>
<tr>
<td>LE (200mg/kg)</td>
<td>0.64±0.08 (11.81%) 0.86±0.03 (27.74%) 0.85±0.06 (30.44%) 0.68±0.04 (42.22%)</td>
</tr>
<tr>
<td>LE (400mg/kg)</td>
<td>0.63±0.03 (18.40%) 0.77±0.03 (36.05%) 0.73±0.02 (39.93%) 0.59±0.03 (49.23%)</td>
</tr>
<tr>
<td>Indomethacin (10mg/kg)</td>
<td>0.77±0.02 (32.81%) 0.76±0.02 (37.21%) 0.68±0.03 (44.19%) 0.57±0.03 (51.28%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6 animals in each group) and analyzed by one-way ANOVA followed by Tuckey post hoc test; BV=basal volume; LE=methanol leaves extract, *: compared against the control, 1p<0.05; 2p<0.01; 3p<0.001.

Phytochemical screening

From the Phytochemical screening, the extract of the leaves of B. antidysentrica was found to be positive for the presence various secondary metabolites including alkaloids, triterpenoids, saponins, flavonoids, phenols, tannins, steroids and glycosides.

Acute toxicity studies

a. Acute oral toxicity study

The death of none of the mice and absence of any sign of toxicity till the 14th day confirmed that the plant extract was appeared to be safe at 2000mg/kg dose and hence the LD₅₀ of both leaves extract is greater than 2000 mg/kg.

b. Skin irritation study

The score of skin irritation, after 24h of treatment with both (2% and 4%) of the leaves extract, in terms of edema and Erythema, was found to be zero and ranged from zero to two, respectively signifying that none of the animals developed edema or Erythema after application of any of the ointments.

The range of scoring of Primary Irritation (SPI) for the medicated ointments was from 0.067 to 0.33 confirming negligible irritant nature of the extracts.
DISCUSSION

The traditional use of medicinal plants as wound healing agents needs scientific validation of their pharmacological parameters [44]. According to excision wound model, the topical application of ointments prepared from 80% methanol extracts of leaves of B. antidysentrica showed dose dependent wound contraction rate as compared to mice received simple ointments. This effect of the extract might be related to reduction of the amount of extracellular matrix needed to repair the defect and hence assisted re-epithelization by shortening the distant migrating keratinocytes travel plays paramount role as it decreases the dimension of the wound and hence shortens the healing time. [2]. Moreover, the wound contraction effect of the extracts could be associated with inhibition of microbial growth particularly in the inflammatory phase and its mitogenic activity which enhance fibroblast motility and its cellular proliferation as well as subsequent transformation to myofibroblasts during wound healing [45]. Stimulation of fibroblasts is believed to be one of the mechanisms of plant extracts to facilitate wound healing as result of their migration from the wound edge to the wound site, proliferation and consequently production of collagen which is considered to be the main constituent of extracellular matrix [46].

In this study, the animal groups received medicated ointments significantly shortened the period of epithelization which is used as a defining parameter of successful wound closure ability [56]. As epithelialization proceeds, contractile property of myofibroblasts is enhanced, epithelial cells are proliferated and crawl across the wound bed to cover it where all these phenomena might attributed to the significant effect of the extracts on the epithelialization period [47].

From the incision wound model, the extracts able to increase the tensile strength that might be due to collagen synthesis, its maturation, angiogenesis and stabilization of fibers showing their wound healing effect. The cumulative effect of all these phenomena improves circulation, thus providing oxygen and nutrients, essential for the healing process of the wound site [49, 50].

Moreover, the wound healing effect of the study plant might be related to its anti-inflammatory effects on wounds as uncontrolled inflammation could compromise the wound healing process [50]. Carrageenan-induced inflammation is supposed to be biphasic. An initial phase lasting up to 2 h is attributed to the release of serotonin, histamine, bradykinin and Substance-P. In contrast, the late phase, lasting from 3–5h, is mainly due to the neutrophils infiltration, the expression of COX-2 and the production of large amounts of pro-inflammatory mediators such as PGE, and various cytokines (IL-1β, IL-6 and TNF-α) [51-53]. In this study, 100 and 200mg/kg of 80% methanol extract resulted in a significant anti-inflammatory effect on carrageenan induced mouse paw edema on the 3rd & 4th h while the 400mg/kg showed such effect at the 2nd h of administration. Therefore, the study revealed that the extract affect the second phase of inflammation i.e., the extract might inhibit the release of inflammatory mediators like prostaglandins through inhibition of cyclooxygenase pathway [54]. The other possible mechanism for the anti-inflammatory action of the plant could be attributed to the up regulation of anti-inflammatory cytokines (IL-10, IL-4, and IL-13) which have negative effect on the production of prostaglandins [54]. All these cumulative effects of the extracts might be attributed due to the presence of the metabolites including alkaloids, tannins, flavonoids, phenols, steroids, glycosides, and triterpenoids that were confirmed during the study.

From acute oral toxicity test, the median lethal dose (LD₅₀) of this extract was found to be >2000 mg/kg suggesting that the extract could be designated as ‘unlikely to be hazardous’ [55]. In addition, the SPI value and absence of hypersensitivity reaction in the form of Erythema and edema also indicated that the extracts are non-irritants which might have contributed to the quick healing process and safety of the formulation [43, 56].

CONCLUSIONS

The present study indicated that 80% methanol extract of the leaves of B. antidysentrica possess wound healing activity and anti-inflammatory effect. These findings provide a scientific evidence for folkloric importance of the leaves of B. antidysentrica as wound healing and anti-inflammatory agent. Thus, further studies aimed at isolation and characterization of the active principle(s) of the crude extract responsible for the wound healing and anti-inflammatory activities has to be conducted.

LIMITATIONS

The study did not perform further isolation and characterization of the 80% methanol extract due to the shortage of budget.

DECLARATIONS

Acknowledgments

The authors would like to acknowledge Addis Ababa University and Debre Markos University for the financial support and EPHI for their kind welcome and consent to use premises and facilities at Pharmacology laboratory.

Funding

This is already described in acknowledgement.

Availability of data and materials

The data is available in public library of Addis Ababa University in a form of graduate student thesis.
Authors’ contributions
ZT designed and conducted all laboratory experiments; analyzed and interpreted experimental results. EM, YM, DY and AD participated in the proposal development and final paper write up. YM prepared the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not Applicable.

Ethical statement and consent to participate
The study protocol was approved by the Scientific and Ethics Committee of the Department of Pharmacology in Addis Ababa University but no consent was needed. Based on the international laboratory animal use and care guideline [33], the mice were pre-anesthesized for wounding purpose by providing ketamine and diazepam intraperitoneally. In addition, cervical dislocation method was used to euthanize the animals after finishing the experiment.

REFERENCES
17. Getahun A. Some common medicinal and poisonous plants used in Ethiopian folk medicine. 1976.
22. Gebeeyehu, G, Asfaw Z, Enyew A, Raja N. Ethno botanical study of traditional medicinal plants and their conservation...


