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Research Article

Wound healing effect of aqueous extracts of *Brucea antidysenterica* and *Croton marcostachyus* from Northwest Ethiopia in albino mice

Zewdie Mekonnen [a, b,*], Asmare Amuamuta [a,b], and Yohannes Abere [a,b]

[a] *Department of Biochemistry, College of Medicine and Health Sciences, Bahir Dar University, Ethiopia*
[b] *Department of Biomedical Research, Biotechnology Research Institute, Bahir Dar University, Ethiopia*

* **Corresponding author**: Department of Biochemistry, College of Medicine and Health Science, Bahir Dar University, P.O. Box: 1151, Bahir Dar, Ethiopia; **Tel:** +251-91-1073157; **Email**: zewdmek2007@gmail.com

Back ground: The practice of traditional medicine to treat wound and bleeding disorders in Ethiopia is based on use of numerous herbs. In Ethiopia, the traditional healers use the leaves of *Brucea antidysenterica* and *Croton marcostachyus* for wound healing purposes. But no scientific study is reported on the in vivo evaluation of the pharmacological effect of the two selected plants collected locally from Zegie and around Lake Tana monasteries, Northwest Ethiopia.

Objective: The purpose of this study was to evaluate the wound healing effects of Brucea *antidysenterica* and *Croton marcostachyus* on a mouse model.

Methods: 50mg/ml and 100mg/ml of *Brucea antidysenterica* and 50mg/ml and 100mg/ml of *Croton marcostachyus leaf* aqueous extract solutions were applied on the 1.5cm inflicted wound on the trunk part of the mice of different groups. The degree of wound healing as a percentage was calculated from the wound diameter for each experimental animal.

Result: The aqueous extract 50mg/ml and 100mg/ml of *Brucea antidysenterica* and *Croton marcostachyus achieved a* relatively higher degree of wound healing percent compared to the control group. Moreover, the 100mg/ml aqueous leaf extract of *Croton marcostachyus and Brucea antidysenterica* have shown higher degree of wound healing percent compared to the 50mg/ml concentrations of the two plants.

Conclusion: The current study concluded that the aqueous extracts of *Brucea antidysenterica* and *Croton marcostachyus* possess excellent wound healing potential. Hence, the results of the current study support the traditional use of leaf extracts of *Croton marcostachyus and Brucea antidysenterica* as remedies for wound healing. Further studies are required to isolate the active compounds and determine safety margin of the plant extracts.

Key words: Wound healing, *Brucea antidysenterica*, *Croton marcostachyus*

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1. Introduction

Across the world, a large segment of the population has accepted traditional medical practice that includes use of phytomedicines obtained from different medicinal plants (Joy et al, 2001, Saxena et al, 2011). Similarly, Ethiopia has long history of using medicinal plants for the treatment of various diseases (Dawit et al, 2003;

Giday et al, 2007). In contrast to synthetic compounds, herbal products are deemed to be safer and hence they are preferred for treatment of various diseases (Joy et al, 2001). Discovering agents that accelerate wound healing either when it is progressing normally, or when it is suppressed by various agents like corticosteroids, anti-neoplastics, or non-steroidal anti-inflammatory agents is very important (Wicke et al*,* 2000; Sasidharan et al, 2012).

The treatment of wounds involves the usage of synthetic and herbal plants as alternative and complementary medicine (Nagori and Solank, 2011). Wound healing herbal plants promote blood clotting, fight infection and accelerate the healing of wounds. Many medicinal plants have been reported to have very important roles in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way compared to the synthetic ones (Shenoy et al*,* 2009).

Croton macrostachyus ('bessana') is native to some Eastern African countries such as Eritrea, Ethiopia, Kenya, Tanzania, Uganda and Nigeria. It is one of the eight *Croton* species found in Ethiopia (Kapingu et al, 2000; Bantie et al*,* 2014). It is a deciduous tree 3-25 m high, although more commonly 6-12 m; crown rounded and open with large spreading branches. In areas where it is native, the plant (or its parts) is used for treatment of several human health problems that include symptoms of diabetes (Ocvirk et al, 2013), malaria (Giday et al 2007; Mesfin et al, 2009; Gelaw et al, 2012), dysentery, stomach ache , ascariasis and taeniasis (Giday et al, 2009), abdominal pain (Yirga et al, 2011), gonorrhoea (Geyid, 2002), wounds, ringworm infestation, hemorrhoids (Wubetu et al*,* 2017), venereal diseases (Bantie et al, 2014), rheumatism (Giday et al, 2009) and as a purgative in cases of ascariasis (Giday et al, 2009; Gidey and Samuel, 2011; Bantie et al, 2014). It is also used to stop bleeding in child birth (Giday et al, 2009). The seeds are eaten to induce abortion while the seed oil is used as a purgative (Bantie et al, 2014). In addition, the leaves of *Croton marcostachyus* has been reported to have antimicrobial effect (Taye et al, 2011).

Brucea antidysenterica is similarly used in traditional medicine for multiple purposes. Different parts of the plant are widely used against malaria, helminthic infections, fever, dysentery and other disorders (Dawit et al, 2003; Grace and Fowler, 2008; Kefe et al, 2016). In addition, the root of *Brucea antidysenterica* has been reported to have antibacterial effect studied on culture of different wound infection causing bacteria (Taye et al, 2011).

In Ethiopia, the traditional healers use the leaf of *Brucea antidysenterica* ('Aballo') and *Croton marcostachyus* ('Bissanna') for wound healing purposes. However, no scientific study has been reported on the in vivo evaluation of the pharmacological effect of the two selected plants collected locally from zegie and around Lake Tana monasteries, Northwest Ethiopia.

Therefore, the purpose of this study was to evaluate the wound healing effects of *Brucea antidysenterica* ('Aballo') and *Croton marcostachyus* ('Bissanna') plants material collected form Zegie and Lake Tana monasteries.

2. Methods

2.1 Collection and preparation of plant material

Fresh leaves of *Brucea antidysenterica* and *Croton marcostachyus* were collected from Zegie and other Lake Tana monasteries and the botanical identity of the plants were done. After the botanical authentication, the leaves were dried under shade in the laboratory for 7 days and were ground to powder using an electric blender. About 400 gram of powdered material of *Croton marcostachyus* was taken in a large, clean beaker and soaked in 2500ml of distilled water. In addition, about 200gram of powdered material of *Brucea antidysenterica* was taken in a large, clean beaker and soaked in 1400ml of distilled water. The beakers were sealed and kept for a period of 24hours with occasional shaking and stirring. The solutions of the two beakers were then filtered through filter paper (Whatman No.1) and the filtrates were lyophilised. The extracts were kept in separate dissectors until used. The crude aqueous extract of Croton *marcostachyus and Brucea antidysenterica* were studied for their wound healing effects with different concentration.

2.2 Preparation of test solutions

The 50mg/ml of *Brucea antidysenterica* and *Croton marcostachyus* leaf extract aqueous solutions were prepared by measuring 5 gram of each extract and dissolving the two extract in 100 ml solution separately. Similarly, the 100mg/ml solutions of the two plant extract were prepared by weighing 10 g of the two extracts in 100ml of solution.

2.3 Wound healing effects using the excision wound model

15 male Albino mice (27-36g) were taken and divided into five groups of 3 mice each. Each group was housed in a separate cage. All animal procedures were conducted with strict adherence to the National Institute of Health (NIH) Guide for the care and use of Laboratory Animals (NIH Publication #85-23 Rev. 1985).

Animals were anaesthetized and an area with a diameter 1.5cm (15mm) was marked using a frame marker pen. The required area of the dorsal fur of the animals was shaved with an electric clipper. Sterilization of the area was achieved by spraying with 70% alcohol in water. A full thick skin was excised from the predetermined area by removing the epidermis and dermis layers up to the level of the subcutaneous fat. All treatments in the five groups were given every 2 days until the wound was completely healed.

Petroleum jelly (Vaseline) was applied topically on the wounds of Group 1 mice; 50mg/ml and 100mg/ml of *Brucea antidysenterica* leaf extract was applied on the wounds of group 2 and 3 mice respectively, and 50mg/ml and 100mg/ml of *Croton marcostachyus* leaf extract was applied on the wounds of group 4 and 5 mice respectively. The treatments were repeated every two days on each group and the diameter of the wounds was measured on the vertical and horizontal planes to assess the healing response. The degree of wound healing (decrease in wound area) was determined and expressed as a percentage of wound area on Day zero.

Wounds were considered closed (completely healed) if moist granulation tissue was no longer apparent and the wound was covered with new epithelium (Tsala et al*,* 2016).

2.4 Statistical Analysis

All statistical calculations were performed on the Statistical Package for Social Sciences (SPSS) version 20.0 software (IBM Corp, USA) with analysis of variance followed by Dunett post-hoc test. All data were expressed as the mean±SD. Independent sample T-test was also used. In all the statistical tests a confidence level of 95% and $p< 0.05$ was considered significant.

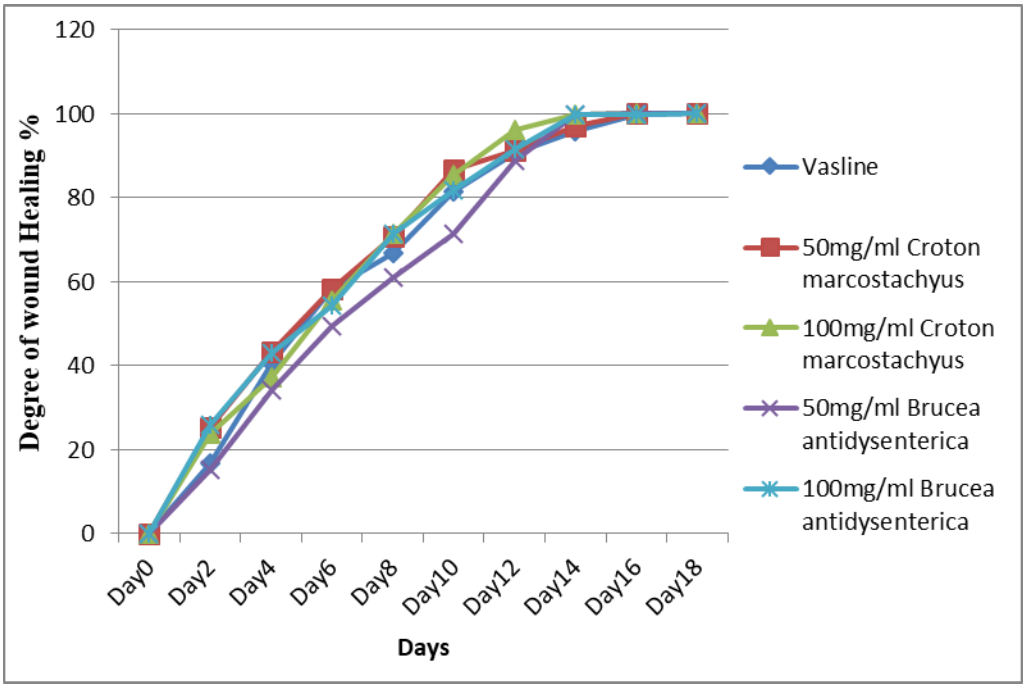
3. Results

The aqueous crude extracts of the plant materials were weighed and the yields calculated as a percent of the dried plant material. The yields were 12.8% and 9.8% for *Brucea antidysenterica* and *Croton marcostachyus*, respectively.

The wound healing activities of the two plants extracts, and progressive degree of wound contraction on the Vaseline treated group are shown in **Figure 1** and **Table 1**.

The 50mg/ml *Croton marcostachyus* showed significantly higher degree of wound healing percent compared with the Vaseline treated group except at day 6 and 12 in which the difference in the mean of degree of wound healing percent were not significant (p-value at day 6=0.80, at day 12, p=0.262). Similarly, the 100mg/ml *Croton marcostachyus* showed significantly higher degree of wound healing percent as compared to the vaseline treated group at day 2 and it became non-significant up to day 6, and finally it showed significant difference up to complete healing of the wound (p-value at day 4=1.000, at day 6, p=1.000). Moreover, the difference in mean of the degree of wound healing percent between 50mg/ml and 100mg/ml *Croton marcostachyus* was significant except at day 10 and 16 in which the difference was not significant (p-value at day 10=0.186,at day 16=0.047). The mean degree of wound healing percents was relatively higher for the 100mg/ml *Croton marcostachyus* treated group.

The 50mg/ml *Brucea antidysenterica* showed significantly higher degree of wound healing percent compared to the Vaseline treated groups up to day 10 and became non-significant at day 12 (p=0.059), finally the mean in the degree of wound healing percent become significant up to complete wound healing. Similarly, the 100mg/ml *Brucea antidysenterica* showed significantly higher degree of wound healing percent compared to the Vaseline treated groups except at day 10 and 12 in which the mean degree of wound healing percent difference was non-significant (p-value at day 10=0.677, at day 12=0.115). Moreover, the mean degree of wound healing percent difference was significant up to day 12 between 50mg/ml and 100mg/ml *Brucea antidysenterica*. In addition, non-significant degree of wound healing percent was observed from day 14 up to complete wound healing(p-value >0.05).



Each value represents mean ± SD of the mean, n =3

Figure 1: Effect of the aqueous extract of the leaves of *Croton marcostachyus* and *Brucea antidysenterica* on the degree of wound healing percent of excised wounds on mice model.

Table 1: Effect of aqueous extract of the leaves of *Croton marcostachyus and Brucea antidysenterica* on the degree of wound healing percent of excision wound on mice model.

Dose	Degree of Wound healing%									
	Day 0	**Day 2**	**Day 4**	**Day 6**	**Day 8**	**Day 10**	**Day 12**	**Day 14**	**Day 16**	**Day 18**
Vaseline	0.00±00.00	16.64±0.46	40.49±0.64	58.37±0.73	66.79±0.78	81.57±0.18	90.62±0.56	95.9±0.414	99.98±0.006	0.00±00.00
50mg/ml *Croton marcostachyus*	0.00±00.00	25.27±0.58*	43.23±1.0*	58.37±0.73	70.84±1.0*	86.81±0.74*a	91.31±0.52	97.04±1.07*	100.0±0.006*[a]	0.00±00.00*
100mg/ml *Croton marcostachyus*	0.00±00.00	23.82±0.23*	37.25±0.91	55.56±1.0	71.32±0.42*	85.45±0.28*b	96.26±0.28 *	99.97±0.023*	100.0±0.006*[b]	0.00±00.00*
50mg/ml *Brucea antidysenterica*	0.00±00.00	15.34±0.48*	34.2±0.65*	49.33±0.2*	61.19±0.74*	71.56±1.0*	88.89±1.0	99.89±0.045*a	100.0±0.006*[a]	0.00±00.00*
100mg/ml *Brucea antidysenterica*	0.00±00.00	26.04±0.15*	42.9±0.56*	54.35±0.55*	71.32±0.42*	81.89±0.39	91.56±0.58	99.96±0.04*b	99.98±0.006*[b]	0.00±00.00*

*Each value represents mean ± SD of the mean, n =3, *P < 0.05: Difference significant when compared to Vaseline treated group. Values with different superscript letters in the same column indicate non-significant degree of wound healing percent difference between the two doses of the same plant.*

4.0 Discussion

A Wound is defined as the disruption of the cellular and anatomic continuity of a tissue (Guyton and Hall, 2006; Sabale et al, 2012). It may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue or typically associated with loss of function (Sabale et al, 2012). The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue involving different overlapping phase (Kumar et al, 2007, Sabale et al, 2012, Patel, 2014). Several factors delay or reduce the wound healing process including bacterial infection, necrotic tissue & interference with blood supply, lymphatic blockage & diabetes mellitus (Nandanwar et al, 2010; Hashemi et al, 2015). Clinically, one often encounters non-healing, under-healing or over healing. Therefore, the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences (Wicke et al, 2000; Sasidharan et al, 2012).

Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair (Raina et al, 2008). The topical agents used include antibiotics and antiseptics, desloughing agents (chemical debridement, e.g. hydrogen peroxide,eusol and collagenase ointment wound healing promoters (e.g.Tretinoin, *aloe vera* extract, honey, comfrey, benzoyl peroxide, *chamomilia* extract, dexpanthenol, tetrachlordecaxide solution, clostebol acetate and the experimental cytokines(Raina et al, 2008). Many substances like vitamins, minerals and a number of plant products have been reported by various workers, to possess pro-healing effects (Dahanukar et al, 2000; Kumar et al 2007; Varoglu et al, 2010).

The current study was designed to evaluate the wound healing effect of the aqueous extract of two selected locally used plants using excisional wound models. In the current study *Croton marcostachyus and Brucea antidysenterica* have shown comparably higher degree of wound healing percent as compared to the vaseline treated groups. From the degree of wound healing percent the two plants leaf extracts have shown good progress of tensile strength and collagen synthesis. From the measurement of wound healing percent, higher degree of wound healing percents were observed in the 100mg/ml *Croton marcostachyus* and *Brucea antidysenterica* compared to the 50mg/ml *Croton marcostachyus* and *Brucea antidysenterica* respectively. Studies reported that fourteen different secondary metabolite including phenolics and triterpenes of the lupane and hopane groups have been isolated from *Croton macrostachyus* (Tala et al, 2013). In addition, phytochemical screening of the two plant leaf extract also revealed the existence of flavinoids and other metabolite (Amuamuta et al, 2015). The observed higher degree of wound healing percent in higher concentration compared to lower concentration might be because of the concentration difference of these bioactive molecules present in the crude extracts of the two plants. Moreover, the observed wound healing effects of the plant leaf extracts might be because of the synergistic effect of the bioactive molecules present in the extract such as alkaloids, flavonoids, glycosides, proteins sugars and vitamin C (Gelaw et al, 2012; Bantie et al, 2014; Amuamuta et al, 2015; Kefe et al, 2016). The reduced wound healing effect observed in the lower concentration of the two plant extracts might be because of the lower concentrations of bioactive molecules present in the extract.

5.0 Conclusion

The study indicated the wound healing effect of the aqueous leaf extracts of the two plants that supported their traditional use. The healing effect is found to be effective in the functional recovery of wound healing in dose dependent manner. The observed result might be either due to their individual or cumulative effect bioactive molecules in the extract that enhanced wound healing. Further studies on the isolation and characterization of bioactive molecules are required.

Conflict of Interest declaration

The authors declare no conflict of interest.

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