Kenesa *et al*., *Ethiop. J. Vet. Sci. Anim. Prod.* (2020),
Vol.4 (1): 38-45
ISSN: 2616-4647

Ethiopian Journal of Veterinary Sciences and Animal Production

Original Research Article **Open Access**

Evaluation of Crude Extract of *Argemone mexicana* and *Buddleja polystachya* on Wound Healing

KenesaTefera[1], Guesh Negash*[1.], Yohannes Hagos[1], Tilaye Demissie[2] and Haben Fisseha[3]

Abstract

This experimental study was conducted from November 2018 to May 2019 with the objective of evaluating the *in vivo* wound healing activity of *Argemone mexicana* seed and *Buddleja polystachya* seed extracts. A total of 16 white albino mice were grouped into four groups (G-I= received no any treatment; G-II= treated with simple ointment; G-III=treated with 10% *B. polystachya* seed formulated extract; and G-IV= treated with 10% *A. mexicana* formulated seed extract) each containing four mice. An excisional wound was created on the dorsal area/back of the mice. Plant extracts were prepared and evaluated for wound healing activity. The wound healing activity of each plant extract was evaluated based on wound contraction/gap filled and histopathological changes. All the data were analyzed using STATA version-13.0 statistical software. The mean ± SD of wound contraction was evaluated to determine the wound healing activity in each treatment. By day 12, treatment with 10% ointment of *B. polystachya* seed extract and *A. mexicana* enabled the wound to heal at faster rate with mean±SD of 0.20±0.00 and 0.00±0.00, respectively. Wound healing was significantly higher in groups treated with *B. polystachya* (p=0.001) and *A. mexicana* (p=0.00) compared to the control group (G-I).

Histological examination of the wounds treated with *A. mexicana* and *B. polystachya* extract ointment revealed reduced period of inflammation and enhanced fibroblast proliferation, angiogenesis and epithelialization compared to the control group. Both 10% ointment ethanolic extract of the plant seeds showed significant responses. Thus, ethanolic extract of *A. mexicana* and *B. polystachya* proved to have a potential in wound healing activity.

Key words: Albino mice, *A. mexicana*, *B. polystachya*, Histopathology, Wound Healing

Affiliations and manuscript citation:
[1]Mekelle University, College of Veterinary Medicine, Mekelle, Ethiopia
[2]Addis Ababa University, College of Veterinary Medicine and Agriculture, Addis Ababa, Ethiopia
[3]Wolaita Sodo University**,** School of Veterinary Medicine, Wolaita, Ethiopia
*corresponding author: negashgu@yahoo.com
Citation: Kenesa, T., Guesh, N., Yohannes, H., Tilaye, D. and Haben F., (2020). Evaluation of Crude Extract of *Argemone mexicana* and *Buddleja polystachya* on Wound Healing. *Ethiop. J. Vet. Sci. Anim. Prod*. (2020), Vol. 4 (1): 38-45

Date of publication: September 2020

1. INTRODUCTION

A wound is a disruption of the normal anatomical structure and the epithelial integrity of skin and cellular continuity of tissue caused by chemical, physical, thermal, microbial, or immunological injury to the tissue. Wound is very common in veterinary practice with great differences in types including incisions, abrasions, burns, bite wounds, avulsions, punctures, contusions, lacerations and shot wounds (Rupesh *et al*., 2011; Sreevani, 2012). Wound represents a serious health problem worldwide, frequently associated with high-costs and inefficient treatments (Agyare *et al*., 2016).

Wound healing is a process of regaining the integrity of cell structure and layers of the skin. Healing of wound starts from moment of injury. Normal healing process of wounds are categorized in four phases which includes hemostatic, inflammatory, fibroblastic and maturation (Alankar *et al*., 2018). According to (Boateng *et al*., 2008), wound healing consists of integrated cellular and biochemical cascades leading to reestablishment of structural and functional integrity of the damaged tissue. Many of the wounds heal by themselves, but some wounds like large wounds or wounds with a lot of necrotic tissue and infection need treatment intervention (Hengel, 2008). The main objective of wound management is healing of the injured tissue or organ within the shortest duration

along with the minimal pain and discomfort (Pather *et al*., 2011).

Wound management often requires topical antimicrobial agents (antiseptics or antibiotics) incorporated into wound dressings agents to enhance wound sterilization, debridement and repair process (Lipsky and Hoey, 2009; Eyarefe *et al*., 2014). Various topical antiseptics are available commercially such as chlorhexidine, hexachlorophene, iodine compounds (iodine tincture, povidone iodine, cadexomer iodine), alcohol, sodium hypochlorite, quaternary ammonium compounds, acetic acid, hydrogen peroxide and silver nitrate have been used to enhance wound healing with varying results. Topical preparations of cephazolin, bacitracin-polymyxin and B-neomycin, silver-sulphadiazinine, gentamicin, neomycin sulphate, and nitrofurazone antibiotics have been used for wound management (Liptak, 1997; Atiyeh *et al*., 2007; Lipsky and Hoey, 2009).

Alternative to such commercial products, medicinal plants have been used in medicine since ancient times and are well known for their abilities to promote wound healing (Agyare *et al*., 2016; Hosein *et al*., 2014). Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine but some of the studied medicinal plants having wound healing activity are *Alchemilla mollis* and *Alchemilla persica* (Öz *et al*., 2016), *Aloe vera* (Oryan *et al*, 2010), *Aloe littoralis* (Hajhashemi *et al*., 2012), *Caesalpinia ferrea* (Pereira *et al*., 2016), *Calendula officinalis* (Shafeie *et al*., 2015), *Citrus tamurana* (Madhyastha *et al*., 2013), *Cynodon dactylon* (Biswas *et al*., 2017), *Delonixe lata* (Sundya *et al*., 2014; Krishnappa *et al*., 2016), *Drypetesklain et* (Brusotti *et al*., 2015) and *Urtica dioica* (Babaei *et al*., 2017).

Medicinal plant application for wound healing is actually supported by many scientific studies and has many advantages; is cheap, affordable, effective, easy to manage, also safe as side effects are rare and minor, mainly less local hypersensitivity (Amini *et al*., 2010; Upadhyay *et al*., 2009). Plant based compounds are getting better attention in the pharmaceutical industry (Budovsky *et al*., 2015). According to Biswas and Mukherjee (2003), 70% of the wound healing Ayurvedic drugs are of plant origin. Besides, agents originating from natural products have been used as antiseptics, antimicrobial and wound healing agents, have advantage over synthetic ones because of their contribution in multiple drug resistance of many bacteria and fungi against antibiotics (Maver *et al*., 2015).

Plant extracts and their bioactive substances are big resource for management and treatment of wounds. *Rutin, ferulicacid, quercetin, caffeic acid* and their ester derivatives are used as protective for cutaneous neuro-vasculature, anti-inflammatory agents, accelerators of cutaneous (skin) wound healing by accelerating epithelialization process, antioxidant agents and stimulators of collagen synthesis in certain fibroblast cells (Song *et al*., 2008; Ghaisas *et al*., 2014). The presence of *rutin* in a dermatological formulation has stimulated skin wound healing by slowing down the lipid peroxidation, increasing catalase activity and decreasing protein carbonyl content (Almeidal *et al*., 2012).

There are different antiseptic and anti-inflammatory drugs that are commercially available in the market, however; commercially prepared wound dressing agents causes unwanted side effects like hypersensitivity reaction, are expensive and not easily accessible. Thus, pharmaceutical industries are searching for drugs which are less expensive, easily available and with high efficacy in wound healing activity. Recently, medicinal plants and their extracts are better solution for the above aforementioned problems. In search of different literature, efficacy of *A. mexicana* seed and *B. polystachya* seed in wound healing is not well studied. Therefore, the objectives of this study were to evaluate the effectiveness of *A. mexicana* seed and *B. polystachya* seed extract for their wound healing activity and characterize wound healing activity of *A. mexicana* and *B. polystachya* through histopathological study of skin tissue.

2. METHODOLOGY

2.1. Study Area

The study was conducted in the college of Veterinary Medicine, Mekelle University; Anatomy and pathology laboratories.

2.2. Collection and Preparation of Plant Extract

Collection of plant and Cold Maceration: The seeds of *A. mexicana* and *B. polystachya* plants were collected from natural habitat of Mekelle city. Plant materials were washed thoroughly, air-dried (dried indoor without exposure to sunlight), and coarsely powdered using electric blender. The powdered specimen was then weighed by sensitive digital balance, extracted in 96% ethanol by maceration method, and concentrated according to the procedures given by Debella (2002). A 100g of the powdered plant materials were soaked in 1000 ml of 96% ethanol in Erlenmeyer flask of two-liter capacity. The

flask containing dissolved plant materials and 96% ethanol mixture was plugged with cotton wool and agitated manually every four hours for 24 hours. After 24 hours, the supernatant was filtered with Whatman (No.1) filter paper. Then trace solvent was evaporated on water bath at +40 °C and dried in hot air oven. Finally, yield of extracts were stored at +4°C in airtight container throughout the study period. The crude ethanolic extracts of the two plants were diluted with distilled water to prepare 20% and 40% concentrations using the methodology described by Ismail *et al*., (2002) and distilled water was used for control study.

Preparation of Formulation: The topical formulations in the form of ointment were prepared from plant extracts. These formulations were prepared by using simple ointment (cetostearyl alcohol, wool fat, hard paraffin and yellow soft paraffin) and plant extract (Table 1).

Method of preparation: All the constituents (cetostearyl alcohol, wool fat, hard paraffin and yellow soft paraffin) were mixed together and molten at 70°C. The plant extract was added slowly, mixed thoroughly and gently and cooled down according to (British Pharmacopoeia 2004, p 2453). The prepared ointments were packed in small wide mouth container and stored at +4°C until use. In this study three types of ointments formulated were simple ointment, 10% (W/W) of *A. mexicana* seed extract and 10% (W/W) of *B. polystachya* seed extract.

2.3. Experimental Animals

White albino mice between 35-40gm of body weight were used in this study. These mice were procured from Mekelle University, College Veterinary Medicine and School of Pharmacy. They were housed in plastic cages under standard environment at room temperature and natural day/night cycle with free access to pelleted food and clean water.

2.4. Experimental Design

The mice were anesthetized by ether. Dorsal fur below the ear of animals was disinfected with 70% ethanol and shaved with surgical blade. In this experiment, the excisional wound model was used for wound creation. About 1 cm diameter of wound was created and the mice were observed regularly (Al-Henhena *et al*., 2011).

2.5 Administration of Treatment

The mice were divided into four groups in separate plastic cages each group containing four mice. All treatments were administered topically/ointment once a day at the rate of 0.1 gm on every wound (Al-Henhena *et al*., 2011). These mice were grouped based on the treatment types as follows:

Group-I: Four mice with no treatment
Group-II: Four mice treated with simple ointment only
Group-III: Four mice treated with 10% ointment formulated from *B. Polystachya* seed
Group-IV: Four mice with 10% ointment formulated from *A. mexicana* seed.
At the end of the experiment the mice were sacrificed with Pentobarbitol sodium.

2.6. Parameters for Wound Evaluation

Wound contraction: The reduction in wound area was measured by caliper daily starting from the day of wound creation. To evaluate healing process, reduction in wound size (measure of wound contraction) and outcomes were recorded at day 0, day 3, day 7, day 9 and day 12 (Al-Henhena *et al*., 2011).

Histopathological assessment: One mouse from each group was scarified with Pentobarbitol sodium for histopathology at day 0, 4, 9, and 12 for histological analysis. The skin biopsy was excised from the center of the wound area. All samples taken for histopathological analysis were labeled according to the group to which the mice belonged to and fixed in 10% neutral buffer formalin. After fixation, skin tissues were dehydrated through graded alcohol series, cleared in xylene, tissue infiltration and embedded in paraffin wax. The tissues were sectioned at 5µm thick perpendicular to the wound sample. These were dewaxed in xylene and followed by staining with standard hematoxylin and eosin (H & E) staining. The degree of cellular infiltration, collagen production, neovascularization and the thickness of epithelium were assessed. The cells were observed by 40X magnification power (Maregesi *et al*., 2016).

2.7. Statistical Analysis

The data collected from laboratory were recorded in the format developed for this purpose and later entered into Microsoft Excel 2016 and analysis was carried out using a standard statistical software program (STATA version 13). The data summarized using descriptive statistics and all the values were expressed as means ± standard deviation of the mean. The data obtained in the study were subjected to one way of analysis of variance (ANOVA) for determining the significant difference and p-value <0.05 was considered to be significant.

3. RESULTS

3.1. Wound Closure (Contraction)

The results of this study revealed that higher rate wound closure progress have been registered in G-III and G-IV. Beginning from day 3, up to day 12, the mean± SD of the wound diameter was decreasing in all of the groups but the fastest complete healing was achieved in G-IV on day 12. Mean of reduction in the wound diameter or mean± SD of wound contraction responding to treatment used is shown in (Table 2).

The wound healing activity of each treatment group was evaluated against G-I. On day three, there was no significant difference observed in wound contraction in simple ointment treated group (p= 0.282). Whereas wound healing activity in mice treated with *B. polystachya* (p= 0.009) and *A. mexicana* (p=0.004) was significantly higher compared to G-I. On day seven in G-II; there was wound contraction but not significant (p=0.500). whereas, in G-III (p= 0.001) and IV (p= 0.00); a better result was observed with highly significant wound healing activity in group IV. On day nine, the wound healing (contraction) was significant in all groups (II, III and IV). Nevertheless, in case of group III and IV; the wound healing was significantly higher than the other two groups (Table 3; Fig 1).

3.2. Histopathological Examinations

The degree of cellular infiltration, collagen production, neovascularization and the thickness of epithelium over the wound has been assessed. Observation was made through 400 X magnification power. The histology of skin biopsies taken at day zero was normal so it was used as a comparison for the activity of each treatment in re-constitution of the skin to its normal architecture throughout consecutive days of histopathological examination.

The histopathology of the group which received no treatment (G-I) and simple ointment only (G-II) showed fewer collagen fibers and fibroblast, and higher inflammatory cells on day three (Fig. 2: I-B and II-B). In G-III (treated with 10% *B. polystachya* ethanol extract ointment), there were fibroblast infiltration into the dermal layer, capillaries looked increased in number and incomplete re-epithelization was also observed (Fig. 2: III-B). G-IV which were treated with *A. mexicana* seed ethanol extract ointment, there were an increase in blood vessels, collagen fiber and fibroblast and in epithelium thickness (Fig. 2: IV-B).

Histological examination of skin biopsy from mice in G-I and G-II at day nine revealed deposition of collagen fiber and fibroblasts dominated the dermal layer and blood vessels were also increased (Fig. 2: I-C and II-C). In G-III, there were high number of blood vessels, dense collagen fiber, and fibroblast and incomplete re-epithelization (Fig. 2: III-C). In G-IV; well organized collagen fiber, high number of blood vessels and increased rate of re-epithelization were observed (Fig. 2-D).

On day 12 post wounding, histology sample from skin of mice taken from G-I and G-II showed increased number of collagen fiber, fibroblasts, blood vessels and incomplete re-epithelization (Fig. 2: I-D and II-D). In G-III and G-IV; the histology of skin possessed well organized structure with re-epithelization and collagen fiber (Fig. 2: III-D and IV-D).

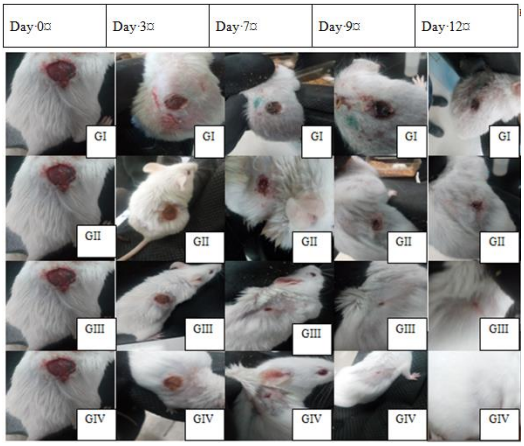

Figure 1: Morphology and size of wound per days of observation

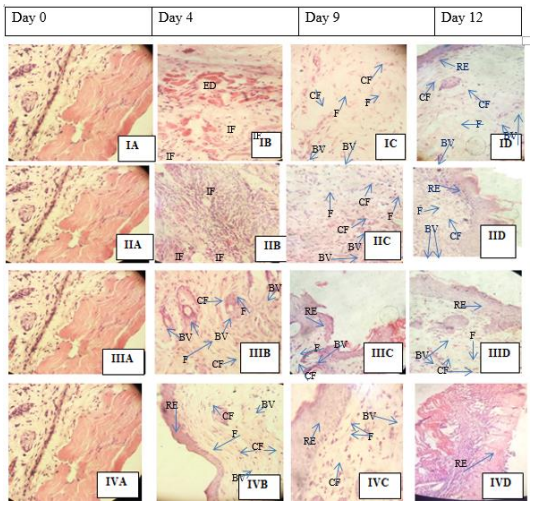

Figure 2: Histopathology of wounds in different treatment groups

Description: ED= edema, IF= Inflammatory cells, CF= Collagen fiber, F= Fibroblast, BV= Blood vessel, RE= Re-epithelization.

Table 1: Ingredients of the formulations

Simple ointments		**10% *A. mexicana***		**10% *B. polystachya***	
Ingredients	**Amount (gm)**	**Ingredients**	**Amounts (gm)**	**Ingredients**	**Amount (gm)**
Hard Paraffin	2	Hard Paraffin	2	Hard Paraffin	2
Cetostearyl Alcohol	2	Cetostearyl Alcohol	2	Cetostearyl Alcohol	2
Wool Fat	2	Wool Fat	2	Wool Fat	2
Yellow/White Soft Paraffin	34	Yellow/White Soft Paraffin	34	Yellow/White Soft Paraffin	34
		A. mexicana seed	4	*B. polystachya*	4

Table 2: Rate of wound contraction in the experimental mice

Experimental groups	**Wound contraction (cm)**			
	Group- I	Group-II	Group-III	Group-IV
Day-0	1.00±0.00	1.0±0.00	1.00±0.00	1.00±0.00
Day-3	0.97±0.06	0.90±0.10	0.77±0.06	0.73±0.06
Day-7	0.87±0.06	0.83±0.06	0.63±0.06	0.53±0.06
Day-9	0.80±0.00	0.65±0.07	0.40±0.0	0.30±0.00
Day-12	0.70±0.00	0.50±0.00	0.20±0.00	0.00±0.00

Table 3: Comparison of wound healing activity of the formulated ointment versus G-I (without treatment)

Group	Days of observation and with their respective p-value							
s	3	p-	7	p-	9	p-	12	p-
Group	6%	0.28	13	0.50	15	0.01	20	-
Group	20	0.00	23	0.00	40	0.00	50	-
Group	23	0.00	33	0.00	50	0.00	70	-

4. DISCUSSION

Wound healing occurs in three stages: inflammatory, proliferative, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast (Swaim *et al.,* 2001). It occurs as the myofibroblasts contract (Lawrence and Diegelmann, 1994).

In the present study, the evaluation of the progress of the wound contraction induced by each plant extract is shown in Table 2. The improved progress of wound contraction was seen in G-III and G-IV as compared to other groups. This might be due to they would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area (Patil *et al*., 2001). The wound contraction was totally completed on day 12 in G-IV (mean± SD=0.00±0.00) and in G-III it was very narrow but not totally closed (mean± SD= 0.20±0.00) whereas in G-I (mean± SD= 0.700±0.00) and G-II (mean± SD= 0.500±0.00) the wound contraction was delayed. Treatment with 10% of *A. mexicana* and *B. polystachya* seed extract helped for faster wound healing (contraction). The earlier wound contraction rate of the ethanolic extract of both plants may be due to stimulation of interleukin-8, an inflammatory a-chemokine that affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes (Moyer *et al*., 2002).

The efficacy of each treatment has been evaluated against control group (G-I). Only, 10% *A. mexicana* showed significant wound healing on day three (p=. 0.004). This might be due to the bioactive substances found in this plant that induced fastest wound healing. Similarly, significantly promoted wound healing activity was seen in G-III ($p=0.001$) and G-IV on day seven. On day nine, all groups of mice in G-II, G-III and G-IV have shown significant wound contraction which is accounted to 15%, 40% and 50%, respectively. The treatment with simple ointment only showed significant wound healing activity from day nine. This might be due to effect of the simple ointment added on the age of the wound on wound healing. On day 12, the recorded wound contraction exceeded the control group by 20% in G-II, 50% in G-III and 70% in G-IV. This study result is in agreement with the Patil *et al*., (2001) who reported significant wound healing activity of petroleum ether and butanol fractions of ethanol extract of *A. mexicana*, containing some sterols, alkaloids, proteins and carbohydrates in albino mice model.

Wound healing is a physiological response to injury and an intricate process following damage to the skin and other soft tissues of the body, required for reconstruction of damaged tissue (Ji *et al*., 2016). It involves the dynamic process of multiple biochemical consequences towards restoration of the damaged

cellular structure to its regular and original state. A classical cascade of wound healing involves three sequential and overlapping phases: inflammation, proliferation, and remodeling (Hemant *et al*., 2016). It depends on precise coordination of connective tissue repair, re-epithelialization, and angiogenesis (Reinke and Sorg, 2012). The aim of these processes is to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin (Geethalakshmi *et al*., 2013). Several plants extracts containing proanthocyanidins, polyphenolic flavonoids and polyphenols are expected to provide enabling support to the healing process initially by the moderation of superoxide anions and later by enhancing the expression of vascular endothelial growth factor (VEGF), thereby enhancing angiogenesis and flow of blood as the repair process advances (Prasanta and Anjali, 2013).

In this study, inflammatory cell was increased in G-I and G-II on day four with few number of collagen fiber and fibroblast compared to group treated with 10% *A. mexicana* and *B. polystachya* seed extract ointment (Fig. 2: IB and IIB). In group III and IV the collagen fiber and fibroblasts were dominant and inflammatory cells were fewer in number; but collagen fibers were more organized in group IV (Fig. 2: IIIB and IVB). This result showed that both plant extracts have anti-inflammatory activity. This finding is similar to Houghton (1999) who reported that aqueous extract of other Buddleja *spp*. promoted growth of dermal fibroblasts and reduce inflammation (two aspects of the cascade of events associated with the wound healing process) and Asthana *et al*. (2001) who reported that aqueous extract of leaves of *A. mexicana* possess anti-inflammatory properties.

In present study, histopathological evaluation showed that improved wound healing activity with *B. polystachya* and *A. mexicana* seed by reducing inflammatory cells, re-epitalization period, and inducing proliferation. The wound healing properties of these medicinal plants might be due to their phyto-constituents such as tannins, saponins, terpenes and triterpenes that assist the wound healing process in many ways (Muhammad *et al*., 2015). Flavonoids and triterpenoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for the increased wound contraction and epithelialization (Tsuchiya *et al*., 1996).

5. CONCLUSION AND RECOMMENDATIONS

In this study, the seed extracts of *A. mexicana* and *B. polystachya* have shown significant wound healing activity. These herbs promoted wound healing by inducing wound contraction, reducing inflammatory and re-epitalization period. These herbs also promoted angiogenesis and deposition of collagen fiber and fibroblast proliferation. Even though both plants have wound healing activity, 10% *A. mexicana* seed extract ointment has shown better wound healing activity in relation to 10% *B. polystachya* seed extract ointment. Therefore, based up on the above findings *A. mexicana* and *B. polystachya* could be recommended as a new candidate as wound healing agent; However, further study on phytochemical analysis of each plant should be done and pharmacological and toxic effect of these plants should be studied.

6. REFERENCES

Agyare, C., Boakye, D., Bekoe, O., Hensel, A., Dapaah, O., and Appiah, T. (2016). Review: African medicinal plants with wound healing properties. *Journal of Ethnopharmacolology*, **177**: 85-100.

Alankar, S., Arun, M., Syed, A., Aftab, A., Mohammed, F., Abuzinadach, M., and Najam, K. (2018). *In vivo* models for assessment of wound healing potential: A systematic review. *Wound medicine*, **20**: 43-53.

Al-Henhena, N., Mahmood, A., Al-magrami, A., Nor-Syuhada, A., Zahra, A., Summaya, M., Suzi M., and Salmah, I. (2011). Histological study of wound healing potential by ethanol leaf extract of Strobilanthe scrispus in rats. *Journal of Medicinal Plants Research*, **5**(16): 3660-3666.

Almeida, S., Benvegnu, M., Boufleur, N., Reckziegel, P., Barelos, C., Coradini, K., Carvalho, M., Burger, E., and Beck, C. (2012). Hydrogels containing rutin intended for cutaneous administration: efficacy in wound healing in rats. *Drug Development and Industrial Pharmacy*, **38** (7): 792-799.

Amini, M., Kherad, M., Mehrabani, D., Azarpira, N., Panjehshahin, R., and Tanideh, N. (2010). Effect of Plantago major on burn wound healing in rat. *Journal of Applied Animal Research*, **37** (1): 53-56.

Asthana, A., Mall, H., Dixit, K., and Gupta, S. (2001). Fungitoxic properties of latex of plants with special reference to that of Croton bonplandianus Baill. *International Journal of Crude Drug Research*, **27** (1): 25-58.

Atiyeh, S., Costagliola, M., Hayek, N., and Dibo, A. (2007). Effect of silver on burn wound infection control and healing: Review of the literature. *Burns*, **33** (2):139–148.

Babaei, E., Mohammad, A., Mehdikhani, F., Milad, M., and Emad, G. (2017). The healing effects of herbal preparations from *Sambucusebulus* and *Urticadioica* in full-thickness wound models. *Asian Pacific Journal of Tropical Biomedicine*, **7**: 421-27.

Biswas, K. and Mukherjee, B. (2003). Plant medicines of Indian origin for wound healing activity: a review. *The International Journal of Lower Extremity Wounds*, **2** (1): 25–39.

Biswas, K., Pandit, S., Chakrabarti, S., Banerjee, S., Poyra, N., and Seal, T. (2017). Evaluation of Cynodon dactylon for wound healing activity. *Journal of Ethnopharmacology*., **197**: 128-37.

Boateng, J., Matthews, H., Stevens, E., and Eccleston, M. (2008). Wound healing dressings and drug delivery systems: a review. *Journal of Pharmaceutical Sciences*, **97** (8): 2892–2923.

British Pharmacopoeia, (2004). The Stationery Office (18thed.). Pp. 2453. London, UK.

Brusotti, G., Andreola, F., Sferrazza, G., Grisoli, P., Merelli, A., Cuna, D., Calleri, E., Nicotera, G., Pierimarchi, P., and Serafino, A. (2015). *In vitro* evaluation of the wound healing activity of Drypetesklainei stem bark extracts. *Journal of Ethnopharmacology*., **175**: 412-21.

Budovsky, A., Yarmolinsky, L., and Ben-Shabat, S. (2015). Effect of medicinal plants on wound healing. *Wound Repair and Regeneration*, **23** (2): 171-183.

Debella, A. (2002). Manual for phytochemical screening of medicinal plants. Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia, Pp. 35-47.

Eyarefe, D., and Dei, D. (2014). Retrospective study of prevalence and pattern of surgical conditions presented at the Ashanti Regional Veterinary Clinic, Kumasi, Ghana. *Global Veterinaria*, **13** (3): 408-413.

Geethalakshmi, R., Sakravarthi, C., Kritika, T., Arul M., and Sarada, L. (2013): Evaluation of antioxidant and wound healing potentials of *Sphaeranthusa maranthoides* Burm. *BioMed Research International*, 2013.

Ghaisas, M., Kshirsagar, B., and Sahane, S. (2014). Evaluation of wound healing activity of ferulic acid in diabetic rats. *International Wound Journal*, **11** (5): 523-532.

Hajhashemi, V., Ghannadi, A., and Heidari, H. (2012). Anti-inflammatory and wound healing activities of Aloe littoralis in rats. *Research in Pharmaceutical Sciences*., **7**: 73-78.

Hemant, N., Amit, S., Rajnish, S., Madan, K., Harinarayan, C., and Mahendra, R. (2016). Pharmacological Investigation of the Wound Healing Activity of *Cestrum nocturnum (L.)* Ointment in Wistar Albino Rats. *Journal of Pharmaceutics*, **2016**: 8.

Hengel, A. (2008). Wound management: A new protocol for companion animals. Utrecht University.

Hosein, F., Abbasabadi, Z., Reza, M., Shams-Ardekani, M., Abdollahi, R., and Rahimi, M. (2014). A comprehensive review of plants and their active constituents with wound healing activity in traditional Iranian medicine. *Wounds*, **26** (7): 197- 206.

Houghton, J. (1999). Wound-healing properties of *Buddleja globosa* (Buddlejaceae). Paper presented at Congress: 2000 Years of Natural Products Research: past, present and future, Amsterdam.

Ismail, H., Chitapa, K., and Gebre, S. (2002). Toxic effect of Ethiopian neem oil on larvae of cattle tick, Rhipicephalus pulchellus Gerstaeker. *Kasetsart Journal of Natural Science*, **36:** 18-22.

Ji, Y., Zhang, A., Chen, X., Che, X., Zhou, K., and Wang, Z. (2016): Sodium humate accelerates cutaneous wound healing by activating TGF-β/Smads signaling pathway in rats. *Acta Pharmaceutica Sinica B*, **6** (2): 132-140.

Krishnappa, P., Venkatarangaiah, K., Rajanna, S., and Balan K. (2016). Wound healing activity of Delonixelata stem bark extract and its isolated constituent quercetin-3-rhamnopyranosyl-(1-6) glucopyranoside in rats. *Journal of Pharmaceutical Analysis*, **6**: 389-95.

Lawrence, T. and Diegelmann, F. (1994). Growth factors in wound healing. *Clinics in Dermatology*, **12**: 157–69.

Lipsky, A. and Hoey, C. (2009). Topical antimicrobial therapy for treating chronic wounds. *Clinical Infectious Diseases*, **49** (10): 1541-1549.

Liptak, M. (1997). An overview of the topical management of wounds. *Australian Veterinary Journal*, **75** (6): 408-413.

Madhyastha, M., Masatoshi, Y., Hiroshi, S., Tsuyomu, I., and Masugi, M. (2013): Revealing the mechanism of in vitro wound healing properties of Citrus tamurana extract. *BioMed Research International*, **2013**: 1-8.

Maver, T., Maver, U., Stana, K., Smrke, D., and Kreft, S. (2015). A review of herbal medicines in wound healing. *International Journal of Dermatology*, **54** (7): 740-751.

Moyer, E., Saggers, C., and Ehrlich, P. (2002). Effects of interleukin-8 on granulation tissue maturation. *Journal of Cellular Physiology*, **193**: 173–179.

Oryan, A., Naeini, T., Nikahval, B., and Gorjian, E. (2010). Effect of aqueous extract of Aloe vera on experimental cutaneous wound healing in rat. *Veterinarski Arhiv*., **80**: 509-22.

Öz, E., Ilhan, M., Özbilgin, S., Akkol, K., Acıkara, B., Saltan, G., Keleş, H., and Süntar, I. (2016). Effects of *Alchemilla mollis* and *Alchemilla persica* on the wound healing process. *Bangladesh Journal of Pharmacology*, **11**: 577-84.

Pather, N., Viljoen, M., and Kramer, B. (2011). A biochemical comparison of the in vivo effects of Bulbine frutescens and Bulbine natalensis on cutaneous wound healing. *Journal of Ethnopharmacology*, **133** (2): 364–370.

Patil, B., Jalalpure, S., and Ali, A. (2001): Preliminary phytochemical investigation and wound healing activity of the leaves of Argemone mexicana Linn. (Papaveraceae). *Indian Drugs*, **38**: 288-293.

Pereira, P., Mario, M., Brizeno, C., Nogueira, C., Ferreira, M., Pereira, G., and Assreuy, M. (2016). Modulator effect of a polysaccharide-rich extract from Caesalpinia ferrea stem barks in rat cutaneous wound healing: Role of TNF-α, IL-1β, NO, TGF-β. *Journal of Ethnopharmacology*, **187**: 213-23.

Prasanta, G. and Anjali, G. (2013): Phyto-Extracts in Wound Healing. *Journal of Pharmacy and Pharmacology*, **16** (5) 760-820.

Reinke, M. and Sorg, H. (2012). Wound repair and regeneration. *European Surgical Research*, **49** (1): 35-43.

Rupesh, T., Nikita, J., Raghavendra, P., and Sandhu, S. (2011). Practices in wound healing studies of plants. *Evidence-based Complementary and Alternative Medicine*, Article Id: 438056, epublished. www.ecam.org.uk.

Serarslan, G. (2007). Pustularpsoriazis-like tinea incognito due to Tricophytonrubrum. *Mycoses*, **50** (6):523-524.

Shafeie, N., Naini, T., and Hossein, J. (2015). Comparison of different concentrations of Calendula officinalis gel on cutaneous wound healing. *Biomedical and Pharmacology Journal*, **8**: 979-92.

Song, S., Park, W., Sohn, D., Shin, K., Choi, K., and Sim, S. (2008). The effect of caffeic acid on wound healing in skin-incised mice. *Korean Journal of Physiology and Pharmacology*, **12**(6): 343-347.

Sreevani, P. (2012). Comparative evaluation of wound healing properties of cinnamomumverumj. s. presl mediated phytogenic and chemically derived nanosilver in wistar rats. Thesis Submitted to the Sri Venkateswara Veterinary University, India.

Sunday, G., Ifeanyi, E., and Ezeja, I. (2014). Wound healing potentials of leaf and bark extracts of Delonixregia. *World Journal of Pharmaceutical Sciences*, **3**: 133-42.

Swaim, F., Hinkle, H., and Bradley, M. (2001). Wound Contraction: Basic and Clinical Factors. *Compendium on Continuing Education for the Practicing Veterinarian*, **23**: 20-33.

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T., and Iinuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. *Journal of Ethnopharmacology*, **50**:27–34.

Upadhyay, K., Kumar, R., Mandotra, K., Meena, N., Siddiqui, S., Sawhney, C., and Gupta, A. (2009). Safety and wound healing efficacy of sea buckthorn (Hippophaerhamnoides L.) seed oil in experimental rats. *Food and Chemical Toxicology*, **47**(6): 1146-1153.

Acknowledgements
We would like to extend our gratitude to the College of Veterinary Sciences for all facilitation and to all contributor of this work.

Contribution of Authors
KT collected data. GN project initiator and advisor. YH analyzed the data. TD read histopathology data. HF prepared tissue for histopathology.

Authors Declaration
The authors declare that this work entitled **"**EVALUATION OF CRUDE EXTRACT OF *ARGEMONE MEXICANA* AND *BUDDLEJA POLYSTACHYA* ON WOUND HEALING**"** submitted to EJVSAP is the result of our original research work. we also declare that this research work or part thereof has not been published earlier elsewhere in any manner.

The authors have declared that they have no competing interest.

Funding: Mekelle University has covered the financial costs of this project.