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Research Article

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Comparison of the Wound Healing Activity of the Leaf and Leaf Ash Extracts of *Vernonia amygdalina* in Rats

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Abstract

Background: Species in the genus *Vernonia* (Asteraceae) are traditionally used in the treatment of different ailments, for example, leaves in both fresh and dry forms have been used in dressing wounds. Anecdotal reports show that the ash of *Vernonia amygdalina* (*V. amygdalina*) has been used in the treatment of wounds, but there are no precise reports to support this claim; therefore, the contemporary study focused on comparing the wound healing activity of its leaf extracts and leaf ash.

Methods: The study investigating the wound healing activity of *V. amygdalina* leaf was carried out in Wistar Albino rats in an excision wound model using its aqueous extract (AE) and leaf ash (LA) at concentrations of 1%, 2% and 3% (w/v). Mupirocin ointment and distilled water were used as positive and negative controls, respectively. The wound diameter was measured every 3 days from day 1 to day 22 and the results were used to calculate the percentage of wound reduction in all groups.

Results: Significant wound healing activity was observed in all groups except 3% AE (w / v), against distilled water (negative control group) ($p<0.05$). Group 2 (1% AE), group 5 (1% LA), and group 6 (2% LA) showed faster wound healing than the positive control ($p<0.05$). Leaf ash was shown to have the best wound healing activity, and its lower concentrations worked better than the category of the same concentrations in aqueous extracts.

Conclusions: Our findings have for the first time confirmed the traditional claim of using *V. amygdalina* ash in the treatment of topical wounds.

Keywords: Wound models, traditional medicine, Uganda, *Vernonia amygdalina*.

Introduction

Wounds are contemporary economic burdens on healthcare systems around the world that could increase in the next decades if no effective and safer means to treat them are found. Chronic non-healing wounds also lead to reduced quality of life, amputations, and premature deaths [1]. Wounds are defined as physical injuries that result in a break in the anatomical and physiological continuity of the skin that can arise from physical, chemical, biological, thermal, or microbiological infections that cause dysfunction [2, 3].

The treatment of wounds is expensive and is a neglected health problem in most rural settings of developing countries [4]. The use of natural products (prepared as poultices, ointments, teas, tinctures, syrups, decoctions, oils, and infusions) is common in the African indigenous traditional medicine system, and the use of plants for the treatment of wounds dates back as far as 500 years. It is documented that in Rome, Africa (Egypt), Asia and the Americas (South America and North America) herbal remedies have been sought as first-line therapy for chronic burns and wounds [5]. In this regard, several herbal formulations have been patented [6]. One of the most used plants in wound management is *Vernonia amygdalina*, especially in Africa.

Vernonia amygdalina Delile (Asteraceae) is an African medicinal herb with distinguished ethnomedicinal uses [7]. *Vernonia amygdalina* (*V. amygdalina*) is known as *Gymnanthemum amygdalimum* (Delile), named after an English botanist William Vernon [8]. It is a native tropical plant commonly known as bitter leaf plant, due to the presence of bitter antinutritional phytochemicals in its leaves [9]. It is locally known as *omubirizi* and *omululuza* in western and central Uganda [10], *ndole* in Cameroon, *tuntwano* in Tanzania, and *omugbu* in Nigeria [11]. The plant is native to tropical Africa, widely found on the riverside, lake areas, forests, grasslands, and cultivated plantations at an altitude of about 2800 m with an average annual rainfall of 750-2000 mm. It is widely grown in Yemen, Ethiopia, Brazil, Nigeria, Kenya, Tanzania, South, and Central Uganda [9, 12]. In India, it is grown in central and eastern Europe in sporadic cultivation for medicinal purposes [13]. It is also found in some African reserves, Kona National Reserve in the Tana River district in Kenya, at a height of 420 m, on the eastern side of the Mbololo forest in Taita at 1400 m, and in Narok at 2100 m [9].

The different organs of *V. amygdalina* have been cited in Africa for the treatment of acute and chronic wounds [14]. Leaf ash in the treatment of wounds in Africa has shown positive results in the promotion of wound healing [15]. Some communities in Uganda use leaf ash from plants as a topical treatment for wounds [16]. For example, wounds after traditional circumcision practises [17]. Other *Vernonia* species (*V. auriculifera*, *V. colorata*, *V. scorpioides* and *V. kotschyana*) have been cited for their use in wound healing and skin eruptions [18-21] but only *V. auriculifera* and *V. scorpioides* have been investigated for their wound healing potential [19, 21]. The wound healing activity of *V. amygdalina* leaf extracts has been previously reported in male Wistar rats [22], incision wounds [23], and in combination with other plant extracts and honey 25]. In this contribution, we investigate the efficacy of aqueous extracts and ash from *V. amgydalina* to validate the use of leaf ash in African traditional medicine in topical wound healing.

Materials and methods

Collection, authentication, and preparation

Fresh leaves of *V. amygdalina* were collected from natural plants growing in Kashanyarazi (along river Rwizi; $0^0$37'1"S 30^0 39 '12'E and 1410 m elevation) near Mbarara University of Science and Technology (MUST), Kamukuzi division, Mbarara district, Uganda, on 26th July 2022. The plant material was identified and authenticated by Dr. Olet Eunice, a botanist at the Faculty of Sciences, MUST (Voucher no. WINFRED NAKABIRI 001).

The leaves were cleaned by removing any dust and extraneous matter and then shade dried for 1 week. The dried leaves were then reduced to a coarse powder using a blender and then sieved to obtain a fine powder of 601.84 g, which was separated into two portions and stored in airtight containers to prevent contamination and moisture penetration.

For the leaf extract, warm maceration was used in which 1.5 litres of distilled water was added to 150 g of the powder and placed in a shaking water bath under pre-set conditions of 500 ° C and 135 rpm for 3 hours [26]. The mixture was then filtered using a muslin cloth, followed by Whatmann No.1 filter paper, and concentrated by fun drying, producing 38.91 g of the crude leaf extract (LE). Then 1%, 2%, and 3% (w/v) concentrations were prepared using distilled water and kept in airtight plastic containers at 40 C.

For the leaf ash extracts, the powder (451.84 g) in an airtight container was placed in crucibles and transferred to a marble furnace where it was heated at 600 ° C for 24 hours. This yielded 113.562 g of leaf ash (LA). Subsequently, 100 g of LA was treated in the same way as the aqueous extract, producing 42.61 g of the ash from which 1%, 2% and 3% (w/v) concentrations were prepared and kept in airtight vials in a refrigerator at 4^0C until further use.

Experimental animals

Forty (40) white Wistar albino female rats weighing 150-250 g (8-12 weeks old) were obtained from the MUST animal research facility. They were divided into 8 groups of 5 rats each group by simple randomisation [23]. Rats were acclimatised for four weeks prior to the experiment under controlled conditions of 12 hours of light and dark cycles, a temperature of 25 ± 1°C, and a humidity of 55 ± 1% humidity [27]. The animals had free access to food and water. All procedures in this study were carried out following the National Institutes of Guidelines for the Care and Use of Laboratory Animals.

Wound healing activity

Rats were anaesthetised before wounding with 0.4 mL of injection of ketamine hydrochloride (50 mg/mL) into the dorsal thoracic region. The dorsal fur of the rats was then shaved with a pair of scissors before applying 0.1 ml of lidocaine (2% w/v) as a local analgesic to the shaved skin of the rat. Using a permanent marker, a circular outline was made in the shaved area using a centennial Ugandan shilling coin and excision wounds were applied in the range of 2.7-3.8 cm and 1.0 mm depth. The leaf aqueous extracts and the leaf ash extracts in their respective concentrations, distilled water, and mupirocin ointment were applied by soaking the extracts twice a day in their designated groups. Evaluation of the wound healing process was performed in the eight groups of rats using a diagonal wound diameter using a Vernier calliper every 3 days. These were used to calculate the areas from which the percentage of wound contraction was calculated for each rat from day 0 to day 21 [14].

$$\% \text{ Wound size reduction} = \frac{\text{initial wound surface area} - \text{wound surface area on different days}}{\text{Initial wound surface area}} \times 100$$

At this point, we considered the fall of the scab or Escher leaving no raw wound behind as the end point of complete epithelialization. Consequently, the days required for this were taken as a period of epithelialization [28].

Statistical analysis

The different group diameter of the wounds of the rats on different days was recorded in Microsoft Excel (Microsoft Corporation, USA) and used to calculate the area of the wound and the percentage of contraction. Then the data was exported to GraphPad Prism for Windows (version9.2.0 of Graph Pad software in San Diego, U.S.A.), where the average value is expressed as Standard Error. The statistical significance of the percentage of total wound contraction of each rat per group was assessed by ANOVA, followed by Dunnett's multiple comparison test for group analysis against negative control, and Tukey's multiple comparison test between groups. The statistical assessment was performed at $P<0.05$.

Results

Formation of eschars

Based on daily observations, eschars had formed in the wounds in all groups on the 4th day; this fell faster in the groups containing leaf ash extract on day 7, followed by the groups containing leaf aqueous extract on day 9. The controls had their eschars falling off last, with the positive control by the 11th day and the negative control by the 13th day.

Wound contraction

On the 4th and 7th day, no significant differences in healing were observed in the different groups and compared to the negative control (**Table 1**). On the 10th day, groups (2 & 6) and group 5 showed moderate and high significant wound healing compared to the negative control (group 8). On the 13th day, groups 2 and 5 showed moderate and highly significant wound healing compared to the negative control ($P<0.05$). On day 16, groups 2, 5, and 6 showed significant low, high, and moderate wound healing, respectively, against the negative control. On day 19, groups (1,2 & 6), groups (3 & 5) showed low and moderate significant wound healing, respectively, compared to the negative control. On the 22nd day, groups (1, 2, 3 & 5), group 6 and group 7 showed moderate, high, and low significant wound healing compared to the negative control. It was also noted that on the 7th day, groups 2, 3, and 5 all showed low significant wound healing activity compared to the positive control (group 1). On day 13, group 5 showed moderate and low significant wound healing activity compared to group 1 and group 7 respectively.

Table 1. Mean percentage contraction of the wound diameter

Day	Negative control	G_2 (1% w/v)	G_3 (2 % w/v)	G_4 (3% w/v)	G_5 (1 % w/v)	G_6 (2% w/v)	G_7 (3% w/v)	Positive control
4	29.55 ± 2.077	35.36 ± 4.033	29.05 ± 2.343	34.11 ± 2.174	36.62 ± 2.924	31.49 ± 2.709	31.26 ± 2.765	23.84 ± 6.721
7	44.42 ± 2.898	54.60 ± 3.152[a]	53.01 ± 2.379[a]	47.85 ± 1.747	55.06 ± 1.976[a]	50.56 ± 1.976	48.93 ± 2.498	33.59 ± 8.669
10	65.33 ± 2.63	77.84 ± 2.331**	72.04 ± 2.380	69.83 ± 2.174	80.22 ± 2.345***	78.08 ± 1.865**	71.51 ± 1.851	70.99 ± 3.933
13	83.2 ± 1.382	89.58 ± 0.6836**	87.65 ± 2.381	86.49 ± 2.244	92 ± 0.642***[aab]	86.4 ± 1.119	85.62 ± 0.7366	84.9 ± 0.722
16	88.78 ± 1.497	93.06 ± 0.4935*	92.67 ± 2.382	92.42 ± 1.875	95.78 ± 0.806***[a]	94.18 ± 0.771**	92.52 ± 0.3708	90.71 ± 1.236
19	93.00. ± 0.7199	96.37 ± 0.7322*	96.88 ± 2.383**	95.67 ± 1.419	97.38 ± 0.524**	96.05 ± 0.9061*	95.59 ± 0.4104	96.2 ± 0.461*
22	95.06 ± 0.7885	98.11 ± 0.5722**	98.33 ±02.384**	97.15 ± 1.018	98.9 ± 0.243***	98.24 ± 0.763**	97.86 ± 0.562*	98.17 ± 0.288**

Statistical significance for the negative control against the other groups whose significance is indicated by * and Tukey's multiple comparison test (between the groups and the level of significance was indicated by (a) for the significant groups against the positive control and (b) for the group against 3% leaf ash).
NB: G_2- Group 2, G_3-Group 3, G_4-Group 4, G_5-Group 5, G_6-Group 6, G_7- Group 7

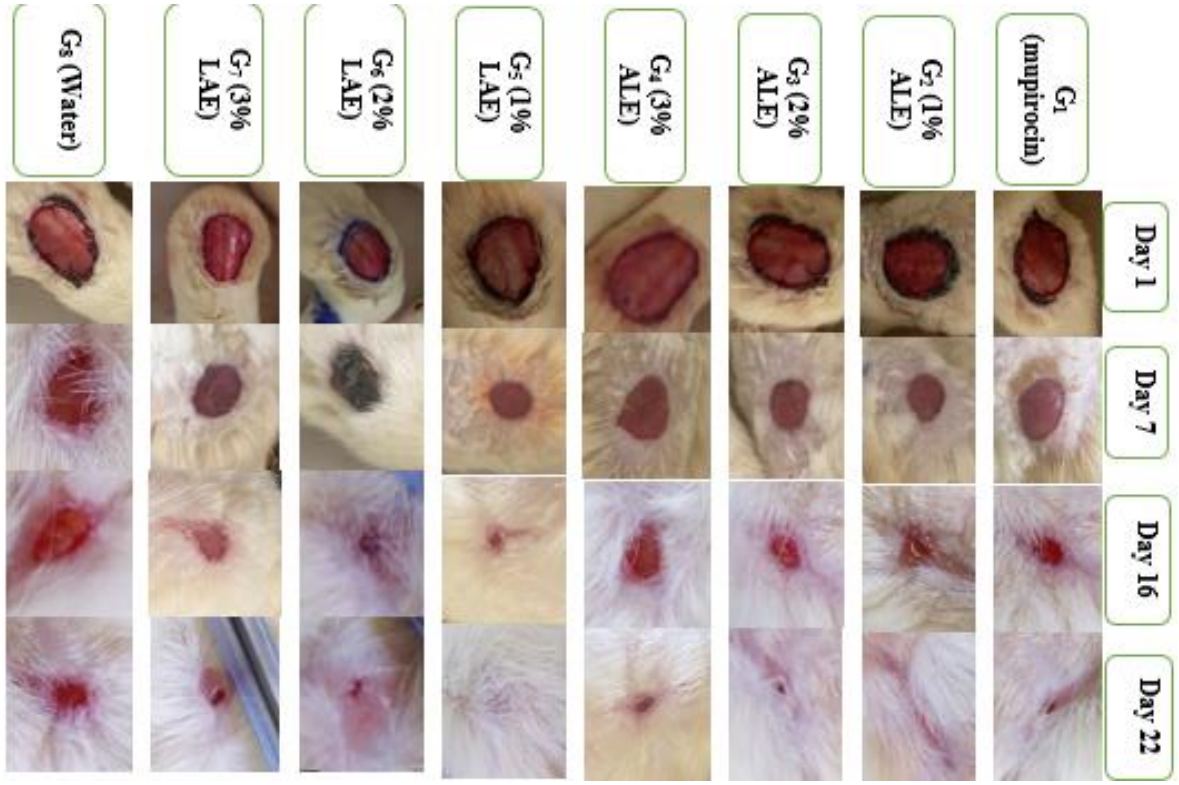

Figure 1. Progression of wound healing in the different groups under study. ALE = aqueous leaf extract and LAE = leaf ash extract.

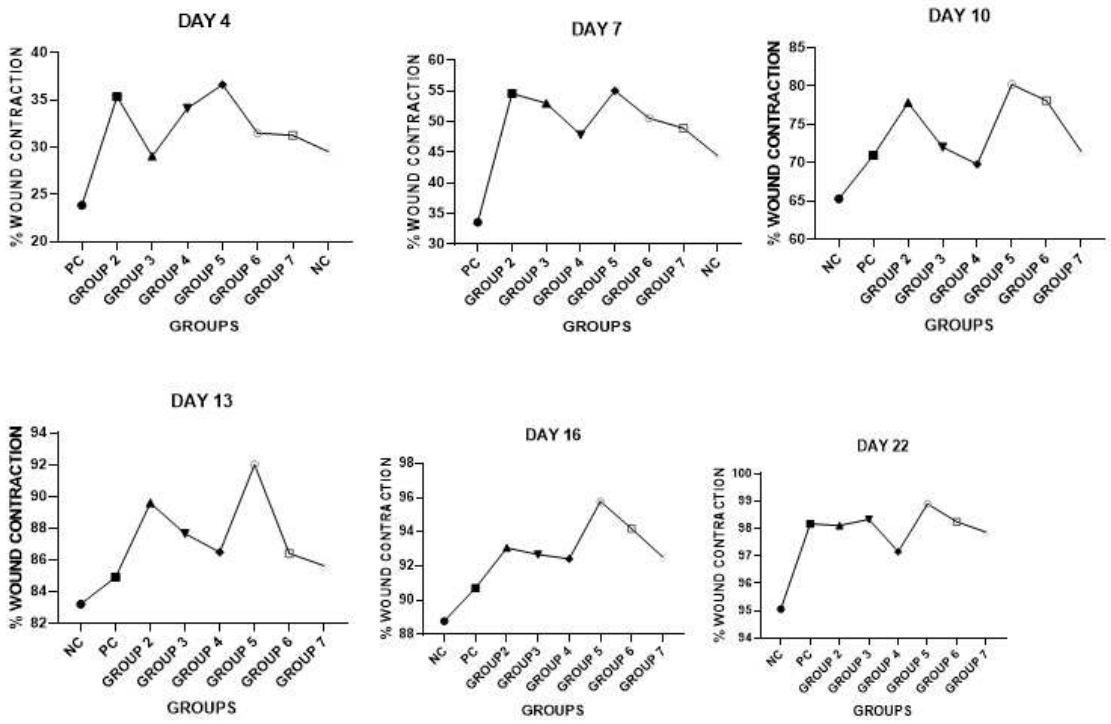

Figure 2. Wound contraction in different groups of rats treated with different concentrations of extracts.

Discussion

The use of medicinal plants in wound healing has been traditionally practised in indigenous communities [29]. However, the validation of the wound healing potential of most medicinal plants has not been performed. It is known that such bioactivities may be influenced by the method of preparation of the sample. In this perspective, we investigated the wound healing activity of aqueous and leaf ash extracts of *V. amygdalina* in rats. In all study groups, the area of wound contraction was found to be time-dependent and improved with the duration of exposure to the extracts. The aqueous extracts and leaf ash of *V. amygdalina* in their respective concentrations (1% w/v, 2% w / v and 3% w/v) showed better wound healing activity compared to the negative control, as shown in Table 1.

Wounds are anatomical and functional disturbances of tissues, and the healing process is mediated through complicated mechanisms involving tissue haemostasis, inflammation, regeneration (proliferation), and responsive remodelling [30, 31]. The wound healing activity of the leaf aqueous extracts of *V. amygdalina* could be due to the availability of different phytochemicals such as flavonoids (luteolin, luteolin-7-*O*-b-glucuronoside and luteolin 7-*O*-b-glucoside)[32], sesquiterpene lactones (vernolide, vernolepin, and vernomenin) [33], tannins, saponins (vernoamyosides A-D)[34], terpenes, glycosides, and coumarins. Furthermore, the phytochemical groups mentioned above have shown other relevant pharmacological potentials for wound healing, for example anti-inflammatory activity [35], antioxidant activity (mainly due to flavonoids) through the elimination of free radicals [36], and antimicrobial activities [37, 38].

The wound healing potential of leaf ash extracts could be attributed to the possession of trace elements and minerals such as zinc, iron, copper, chromium, lead, calcium, manganese and cadmium [39, 40], which act as enzyme co-factors, and thus enhance structural components in tissue repair and wound healing [15].

Leaf ash extracts at the concentrations tested had better wound healing activity compared to their leaf aqueous counterparts against negative control, as observed graphs embedded in Figure 2. No research provided results we could compare with, hence there was no justifiable explanation for this, thus more research has to be done to clarify this phenomenon.

Furthermore, lower concentrations had better wound healing activity than higher concentrations for leaf ash and aqueous extracts, as seen in Figure 2 where groups 2 (1% aqueous extract) and 5 (1%

w/v leaf ash). There was no study explaining this, but it could be due to the increase in the levels of certain phytochemicals or trace elements that have antagonistic activity against the wound healing process or the activity of those phytochemicals that accelerate the process. More studies are needed on this to confirm the reasons.

At the end of the experiment, it was found that the 1% leaf ash extract had the best observable wound healing activity, with the highest percentage area of wound healing contraction than all other groups after the period of exposure to different extract concentrations.

4. Conclusions

Our findings support the use of leaf ash from *V. amygdalina* and aqueous extracts that stimulated wound healing activity. Leaf ash extracts at their specific concentrations worked better than the same concentrations of leaf aqueous extract groups, with 1% leaf ash extract standing out among all the rest. Other parameters such as day of epithelization, tensile strength, and histomorphology should be performed. Studies for different formulations (creams, ointments, and sprays) for leaf aqueous and leaf ash extracts should be carried out to provide more scientific evidence of these extracts when incorporated into modern formulations. Further studies on the wound-healing activity of *V. amygdalina should be performed* using other solvents such as ethanol and methanol during the extraction process.

Data availability

Data sets for this study may be provided by the corresponding author on request.

Conflicts of interest

The authors declare that there is no conflict of interest with respect to the publication of this article.

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References

1. Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, Car J. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. Syst Rev. 2017; 6: 15.
2. Markiewicz-Gospodarek A, Kozioł M, Tobiasz M, Baj J, Radzikowska-Büchner E, Przekora A. Burn Wound Healing: Clinical Complications, Medical Care, Treatment, and Dressing Types: The Current State of Knowledge for Clinical Practice. Int J Environ Res Public Health. 2022;19 :1338.
3. Chhabra S, Chhabra N, Kaur A, Gupta N. Wound Healing Concepts in Clinical Practice of OMFS. J Maxillofac Oral Surg. 2017; 16:403-423.
4. Toppino S, Koffi DY, Kone BV, N'Krumah RTAS, Coulibaly ID, Tobian F, Pluschke G, Stojkovic M, Bonfoh B, Junghanss T. Community-based wound management in a rural setting of Côte d'Ivoire. PLoS Negl Trop Dis. 2022; 16 :e0010730.
5. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound Healing and the Use of Medicinal Plants. Evid Based Complement Alternat Med. 2019; 2019: 2684108.
6. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Futur J Pharm Sci. 2021; 7: 53.
7. Ugbogu EA, Okezie E, Dike ED, Agi GO, Ugbogu OC, Ibe C, Iweala EJ. The Phytochemistry, Ethnobotanical, and Pharmacological Potentials of the Medicinal Plant-Vernonia amygdalina L. (bitter Leaf). Clin Complement Med Pharmacol. 2021; 1: 100006.
8. Agbogidi O, Akpomorine M. Health and nutritional benefits of bitter leaf (Vernonia amygdalina Del.). Int JA. 2013; 2: 164-70.
9. Kaur D, Kaur N, Chopra A. A comprehensive review on phytochemistry and pharmacological activities of Vernonia amygdalina. J Pharmacogn Phytochem. 2019; 8: 2629-36.
10. Njan AA. Herbal medicine in the treatment of malaria: Vernonia amygdalina: an overview of evidence and pharmacology. In: Toxicology and Drug Testing. IntechOpen, pp. 167-186, 2012.
11. Audu SA, Taiwo AE, Ojuolape AR, Sani AS, Bukola AR, Mohammed I. A study review of documented phytochemistry of Vernonia amygdalina (Family Asteraceae) as the basis for pharmacologic activity of plant extract. J Nat Sci Res. 2012; 2: 1-9.
12. Alem S, Woldemariam S. A comparative assessment on regeneration status of indigenous woody plants in Eucalyptus grandis plantation and adjacent natural forest. J Forest Res. 2009; 20: 31-36.

13. Bhattacharjee B, Lakshminarasimhan P, Bhattacharjee A, Agrawala DK, Pathak MK. Vernonia amygdalina Delile (Asteraceae)–An African medicinal plant introduced in India. Zoo's Print. 2013; 28: 18-20.
14. Nafiu A, Akinwale O, Owoleye B. Histomorphological Evaluation of Wound Healing-Comparison between Use of Honey and Vernonia Amygdalina Leaf Juice. Nigeria Trop J Health Sci. 2016; 23: 10-11.
15. Shaikh DM, Shaikh HZ. Ash as a unique natural medicine for wound healing. Isra Med J. 2009; 1: 72-78.
16. Ssegawa P, Kasenene JM. Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. J Ethnopharmacol. 2007; 113: 521-540.
17. De Wolf JJ. Circumcision and initiation in western Kenya and eastern Uganda: Historical reconstructions and ethnographic evidence. Anthropos. 1983; 369-410.
18. Cioffi G, Sanogo R, Diallo D, Romussi G, De Tommasi N. New compounds from an extract of Vernonia colorata leaves with anti-inflammatory activity. J Nat Prod. 2004; 67: 389-94.
19. Molon A, Biavatti M, Kreuger M. Effects of the topical application of the extract of Vernonia scorpioides on excisional wounds in mice. Revista Brasileira De Farmacognosia. 2005; 15: 82-87.
20. Nergard CS, Diallo D, Michaelsen TE, Malterud KE, Kiyohara H, Matsumoto T, Yamada H, Paulsen BS. Isolation, partial characterisation and immunomodulating activities of polysaccharides from Vernonia kotschyana Sch. Bip. ex Walp. J Ethnopharmacol. 2004; 91(1):141-52.
21. Ashenafi E, Abula T, Abay SM, Arayaselassie M, Sori M. Evaluation of the Antioxidant and Wound Healing Properties of 80% Methanol Extract and Solvent Fractions of the Leaves of Vernonia auriculifera Hiern. (Asteraceae). Clin Cosmet Investig Dermatol. 2023; 16:279-299.
22. Ruslim AK, Anitasari S, Ismail S, Oli EM, Yani S. Effect of African leaves extract (Vernonia amygdalina Del.) on wound healing velocity after tooth extraction in Rattus norvegicus. Jurnal sains dan kesehatan. 2017; 1: 408-414.
23. Eyo JE, Uzoibiam BO, Ogbanya KC, Nnaji TO. Comparative Evaluation of Wound Healing Effects of Ocimum gratissimum, Vernonia amygdaline and Zingiberofficinalis extracts on Incision Wound Model in Rats. PhOL. 2014; 3: 44-50.
24. Mboto C, Eja ME, Adegoke AA, Iwatt GD, Asikong BE, Takon I, Udo SM, Akeh M. Phytochemical properties and antimicrobial activities of combined effect of extracts of

the leaves of Garcinia Kola, Vernonia amygdalina and honey on some medically important microorganisms. Afr J Microbiol Res. 2009; 3: 557-559.

25. Amarachukwu EL, Ogheneochuko P. Screening of wound healing effect of Elaeis guineensis Oil, extract of Vernonia amygdalina mixed with dried egg albumin on burn wound inflicted guinea pig. World J Biol Pharm Health Sci. 2021; 8: 013-028.
26. Adiukwu PC, Amon A, Nambatya G, Adzu B, Imanirampa L, Twinomujuni S, Twikirize O, Amanya M, Ezeonwumelu JO, Oloro J. Acute toxicity, antipyretic and antinociceptive study of the crude saponin from an edible vegetable: Vernonia amygdalina leaf. Int J Biol Chem Sci. 2012; 6: 1019-1028.
27. Council NR. Guide for the care and use of laboratory animals. 2010.
28. Lambebo MK, Kifle ZD, Gurji TB, Yesuf JS. Evaluation of Wound Healing Activity of Methanolic Crude Extract and Solvent Fractions of the Leaves of Vernonia auriculifera Hiern (Asteraceae) in Mice. J Exp Pharmacol. 2021; 13:677-692.
29. Albahri G, Badran A, Hijazi A, Daou A, Baydoun E, Nasser M, Merah O. The Therapeutic Wound Healing Bioactivities of Various Medicinal Plants. Life (Basel). 2023;13 :317.
30. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010; 89: 219-29.
31. Michels M, Córneo E, Rocha LBG, Dias R, Voytena APL, Rossetto M, Ramlov F, Dal-Pizzol F, Jesus GFA. Paraprobiotics strains accelerate wound repair by stimulating re-epithelialization of NIH-3T3 cells, decreasing inflammatory response and oxidative stress. Arch Microbiol. 2023; 205 :134.
32. Yeap SK, Ho WY, Beh BK, San LW, Ky H, Yousr AHN, Alitheen NB. Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities. J Med Plants Res. 2010; 4: 2787-2812.
33. Luo X, Jiang Y, Fronczek FR, Lin C, Izevbigie EB, Lee KS. Isolation and structure determination of a sesquiterpene lactone (vernodalinol) from Vernonia amygdalina extracts. Pharm Biol. 2011; 49: 464-70.
34. Quasie O, Zhang Y, Zhang H, Luo J, Kong L. Four new steroid saponins with highly oxidized side chains from the leaves of Vernonia amygdalina. Phytochem Lett. 2016; 15: 16-20.
35. Georgewill O, Georgewill U. Evaluation of the anti-inflammatory activity of extract of Vernonia amygdalina. Asian Pac J Trop Med. 2010; 3: 150-151.
36. Habtamu A, Melaku Y. Antibacterial and Antioxidant Compounds from the Flower Extracts of Vernonia amygdalina. Adv Pharmacol Sci. 2018; 2018: 4083736.

37. Inusa A, Sanusi SB, Linatoc AC, Mainassara MM, Awawu JJ. Phytochemical analysis and antimicrobial activity of bitter leaf (Vernonia amygdalina) collected from Lapai, Niger State, Nigeria on some selected pathogenic microorganisms. Sci World J. 2018; 13: 15-18.

38. Ghildiyal S, Gautam MK, Joshi VK, Goel RK. Wound healing and antimicrobial activity of two classical formulations of Laghupanchamula in rats. J Ayurveda Integr Med. 2015; 6 :241-7

39. Ayoola P, Adeyeye A, Onawumi O. Trace elements and major minerals evaluation of Spondias mombin, Vernonia amygdalina and Momordica charantia leaves. Pakistan J Nutr. 2010; 9: 755-758.

40. Sheikh DM, Shaikh HZ. Topical application of dung cake ash as innovative therapy in skin wound healing in rabbit model. Pakistan J Physiol. 2009; 5; 68-72.