©NIJBAS
Publications 2023

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES (NIJBAS)
Volume 3 Issue 2 2023

A Comparative Study of the Topical Wound Healing Activity of Root Bark Ash and Root Bark Aqueous Extracts of *Vernonia amygdalina* on Male Mice

Umutetsi Jacklean

Faculty of Pharmacology and Toxicology Kampala International University Western Campus

ABSTRACT

Herbal medicine's acceptance into the healthcare system has been hampered by the lack of scientific proof supporting its pharmacological action, including the efficacy of *Vernonia amygdalina* to treat wounds. Despite traditional proof of its effectiveness in wound healing, the usage of root bark ash, which has been used to treat wounds since ancient times, is also a disappearing practice in modern society. By contrasting the wound-healing properties of root bark aqueous extract and root bark ash extract of *V. amygdalina*, this study aimed to fill this gap. Elastoplast ointment served as the positive control and distilled water served as the negative control as we examined the wound healing activities of these extracts on male mice in an excision wound model utilizing both root bark aqueous and root bark ash extracts at 1% w/v, 2% w/v, and 3% w/v concentrations. From day 1 to day 21, wound diameter was measured every four days, and the data was utilized to determine the diameter of the reduced wound in each group. In comparison to the negative control group, all root bark ash extracts and root bark aqueous extracts from *V. amygdalina* demonstrated a considerable amount of wound healing activity. The wounds in groups 4 (3% w/v aqueous) and 7 (3% w/v Root bark ash) healed more quickly than those in the negative control. With its greater concentrations functioning better than the same concentrations in the root bark aqueous category, root bark ash was found to have the best wound healing activity. The study's conclusion revealed the following ranking; Group 7 (3% w/v Root bark ash) > Group 4 (3% w/v aqueous) > Group 6 (2% w/v Root bark ash) > Group 3 (2% w/v aqueous), followed by Group 5 and Group 2 (1% w/v root bark ash and 1% w/v root bark aqueous), Group 4 (3% w/v Aqueous) > Group 8 (Negative control). According to our findings, both root bark ash and Root bark aqueous extracts of V. amygdalina Root barks proved effective in treating wounds, providing evidence for its traditional use in treating wounds. With 3% w/v root bark ash extract standing out among the rest, root bark ash extracts in their individual concentrations performed better than the same concentrations in the root bark aqueous extract groups.

Keywords: Herbal medicine, Wounds, Pharmacological action, *V. amygdalina*, Root barks.

INTRODUCTION

Wounds are a current burden in global healthcare systems that may worsen in the coming decades if no viable and safer treatments are identified [1, 2]. Wounds are physical injuries that cause a break in the anatomical and physiological integrity of the skin and can occur as a result of physical, chemical, biological, thermal, or microbiological diseases producing skin dysfunction [3-5]. With wounds, the protective role of the skin is disrupted, with or without loss of underlying tissue (muscle, nerves, bones), exposing the body to new infections [6, 7]. Globally, wounds are a significant social and economic burden around the world, with over 8.2 million individuals

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

suffering from infected or non-infected wounds [8]. Due to inadequate access to hospitals and financial constraints, wounds are the least reported cause of hospital visits (30–42%) and deaths (91 per year) in underdeveloped nations in Africa [9]. Wounds are frequently classed locally based on the cause, such as assault wounds, road accidents, work-related injuries (building sites, farms, and home accidents), burns, animal bites, surgery, and infections [10]. Wounds are clinically characterized by etiology, which is the reason for their presentation, such as diabetic foot ulcers caused by diabetes [11], or by the length of time, it takes for a wound to heal, such as acute or chronic wounds [12]. More literature divides wounds into open and closed wounds based on their exposure to the environment. Open wounds, for example, include deep cuts, incisions, tears or lacerations, and abrasions, whereas closed wounds can include hematomas or crush injuries [12]. Another approach to further describe wounds is by their level of cleanliness and state of contamination, such as clean, clean-contaminated, contaminated, and unclean wounds [13]. Wounds can affect the skin's important functions, making it less effective at protecting the body. In terms of wound care expenditures, protracted hospital admissions, and loss of body parts leading to physical handicaps and even death, they put physical and mental health burdens on not only the wound victims but also their caretakers and medical experts [14]. As a result, there is a need for a variety of ways to do faster-wound healing that can be used as a supplement or as an alternative. Wound healing is a normal, healthy, and self-healing response to injury [14]. Any disruption of the wound healing process poses a risk to the victim, which is why wound treatment is approached in a variety of ways, both traditional and conventional. Traditional medicine is an ancient practice that is globally accepted as herbs have been proven useful in the management of several diseases including wound healing [15-18]. Many therapeutic options and modalities are available in traditional wound management procedures, including assessment and exclusion of disease processes, wound washing, prompt dressing changes, optimal dressing selection, and antibiotic prescription. These practices include the application of wound healing products such as silver-based products such as silver nitrate and silver sulfadiazine, which are broad-spectrum antimicrobials, the use of advanced dressings that provide wound protection against contamination, antimicrobials, autolytic debridement, chemical debridement, and absorbents of wound exudates such as impregnated gauze, iodine and silver based ointments, films, and hydrocolloids [19]. Antibiotics are also used for skin and wound infections in various formulations, such as topical erythromycin, topical Elastoplast ointment, bacitracin, ciprofloxacin, ceftazidime, topical imidazole, and topical antifungals [20]. Some plants possess an antimicrobial potential and hence could be of help too [21]. The chemicals inherent in plants are responsible for these therapeutic potentials [22-24]. Other recent advances in the treatment of wounds include the use of nanotechnologies, such as metal nanoparticles like silver, gold, and zinc [25], polymeric nanoparticles like silicone and polystyrene, Nanoscafolds like hydrogels and chitosan, and peptide nanoparticles [26], but their use is associated with numerous side effects and contraindications. Many medicinal plant parts, such as leaves, flowers, and root barks, have been used in the management of wounds, and these plants include species from the following families Asteraceae, Aloaceae, Olacaeae, and Solanaceae [14]. Root bark extracts of *V. amgydalina* have been used in several parts of Africa and have proven to be useful in the treatment of both acute and chronic wounds by using its juice squeezed and applied to the lesions [27]. As with herbs, the use of root bark in wound therapy in Africa has been studied in many parts of the continent and has shown promising outcomes in wound healing [28]. The use of animal models in clinical research is a well-documented practice especially rats and mice [29-32]. There is an increasing alarm about the ineffectiveness of conventional medicines due to antimicrobial resistance, as well as high treatment expenditure on wounds in terms of care, treatment, and hospitalization, which has increased the inability to access allopathic medicines for wound management in developing countries. Thus the need to get effective wound management products that are safe, accessible, and affordable to the general public. In light of this, this study aimed at comparing the potency and efficacy of aqueous root bark extracts and root bark ash extracts of *V. amgydalina* on topical wound healing using mice model.

METHODOLOGY

Study design

This was an experimental study to see the activity of both aqueous and ash root bark extract of *V. amygdalina* on wound healing in male Mice.

Extract Preparation

The fresh root barks of *V. amygdalina* were collected from Rukararwe Botanical Garden in Bushenyi district in December 2022. The plant material was collected and identified by a botanist at Mbarara University of Science and Technology, identified and the voucher number given as UJ 001. The collected fresh root barks of *V.* amygdalina were cleaned by washing to remove soil. This was followed by air and shade drying until constant weight was obtained. The dry root bark was then pulverized to a coarse powder using a mortar and pestle and then sieved to attain a fine powder which was stored in air-tight containers. The aqueous extract was produced by cold maceration

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

where one liter of distilled water was added to 100g of the powder for root bark separately and maintained in a water bath at about 50°C, shaken using an auto shaker for 3hrs before filtration Adiukwu *et al.* [33]. The mixture was then filtered and concentrated using a hot air oven. The powder in the air-tight container was transferred to a hot air oven and converted to ash at 400°C. The ash was treated in the same way as the aqueous extract to attain the same percentage yields.

Excision wound model

Mice were anesthetized with inhalable Halothane before any wounding was done on the male Mice. Circular excision wounds of about 14 mm length by 09 mm width were applied after shaving a 15 mm diameter area using Veet hair remover cream and the evaluation of the wound healing process in all the 08 groups of the Mice was done for the next 3 weeks [27].

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

Experimental Animals

In this experiment, the study animals were Bulb-C mice, and adult males, weighing 13–23 g.
They were purchased from the Animal House of Kampala International University-Western Campus animal facility and acclimatized (04 mice per cage) with a 12-hour light-dark cycle and a temperature of 25 ± 2°C. Animal handling and all related operations followed the Institutional Guidelines and Guide for the Care and Use of Laboratory Animals at KIU – Western Campus Uganda (8th Edition). De-ionized water was used (because ions can affect enzymes & proteins) and the water was freely available as well as food (standard mice pellets were purchased). Before any procedure, the Mice were given two weeks to adjust to the new habitat (acclimatize). In total, 32 adult male mice were used for the experiment with four (4) mice allocated per experimental group [34]. There were 08 experimental groups. The Mice were utilized to test several parameters to ascertain the impact of *V.* amygdalina root bark extract on wound healing in male mice. The test groups received aqueous and ash root bark extracts at doses of 1% w/v, 2%w/v, and 3%w/v which were applied on the excised wounds. All the doses were topically applied. The mice were randomly divided as follows: The control group received distilled water and the standard group received Elastoplast ointment 50g.

Table 1: Experimental grouping

Group	Description	Dosage	Mice no.
GROUP 1	Vehicle Control	Normal saline	04
GROUP 2	Standard control (Elastoplast ointment 50g)	50g	04
GROUP 3	Aqueous Root bark extract	1% W/V	04
GROUP 4	Aqueous Root bark extract	2% W/V	04
GROUP 5	Aqueous Root bark extract	3% W/V	04
GROUP 6	root bark ash extract	1% W/V	04
GROUP 7	root bark ash extract	2% W/V	04
GROUP 8	root bark ash extract	3 W/V	04

Selection criteria

Healthy male mice of an average weight of 13-23g were used while unhealthy and those not in appropriate weight ranges were not used.

Equipment, drugs, and materials to be used in the study

The following equipment was used during the study; an electric blender, hot air oven, auto shaker, electric weighing balance, pestle and mortar, syringes, dissection kit, and digital Vernier caliper, Materials included a sieve (500μm), beakers, filter papers, cotton, markers, gloves, and funnel. Drugs; Elastoplast ointment 50g, Veet hair remover, and inhalable Halothane were used.

Data management and analysis

The experimental results were expressed as a mean and standard variation of the mean and the significance of our results was carried out by one-way ANOVA followed by the student t-test for analysis between the groups.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

Quality control and Ethical considerations

All animals were marked using permanent markers to minimize errors. A control group(s) was added for reference purposes. All the equipment used was calibrated before use. The reagents used were of the analytical grid. Validated methods were used in the study. The study was permitted by the School of Pharmacy Kampala International University Western campus. Anesthetics used were to minimize pain during the application of the wounds and the medicine. The use of these Mice was determined by considering the 3Rs that is: Replacement, Reduction, and Refinement [35]. The choice of Mice as a research model was based on their similarity to humans for the specific character investigated, which was wound healing [36].

RESULTS

Observations

Eschars had formed on the wounds in all groups by the 4th day, this fell off faster in groups containing root bark ash by the 8th day, followed by the groups containing 3% aqueous root bark 12th day. The positive control had the fastest fall on day 6. The negative control had its eschars falling off last by the 17th day.

Wound healing result analysis

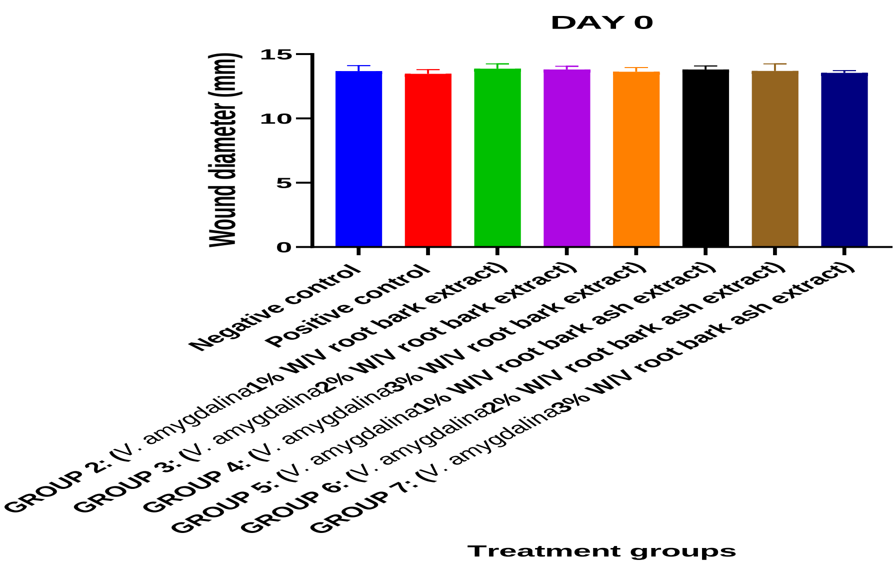


Figure 1: Showing the mean wound diameter (mm) on day 0. Results are expressed in Mean ± SEM. n = 4.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

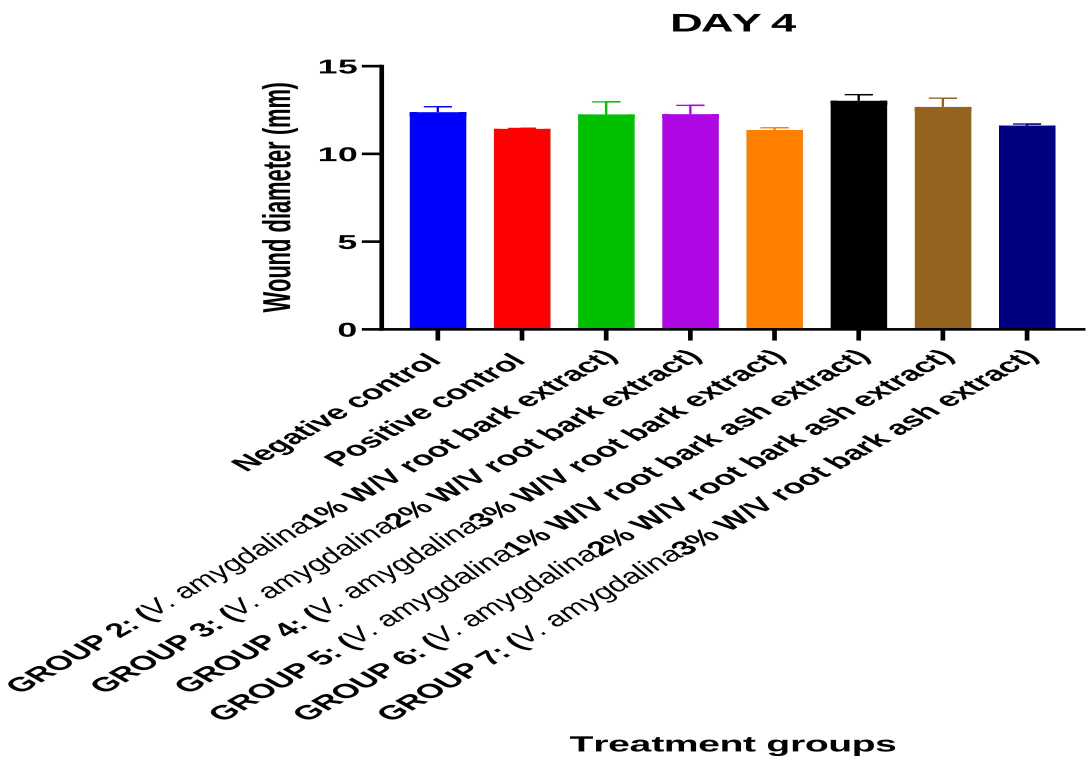


Figure 2: Showing the mean wound diameter (mm) on day 4. Results are expressed in Mean ± SEM. n = 4.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

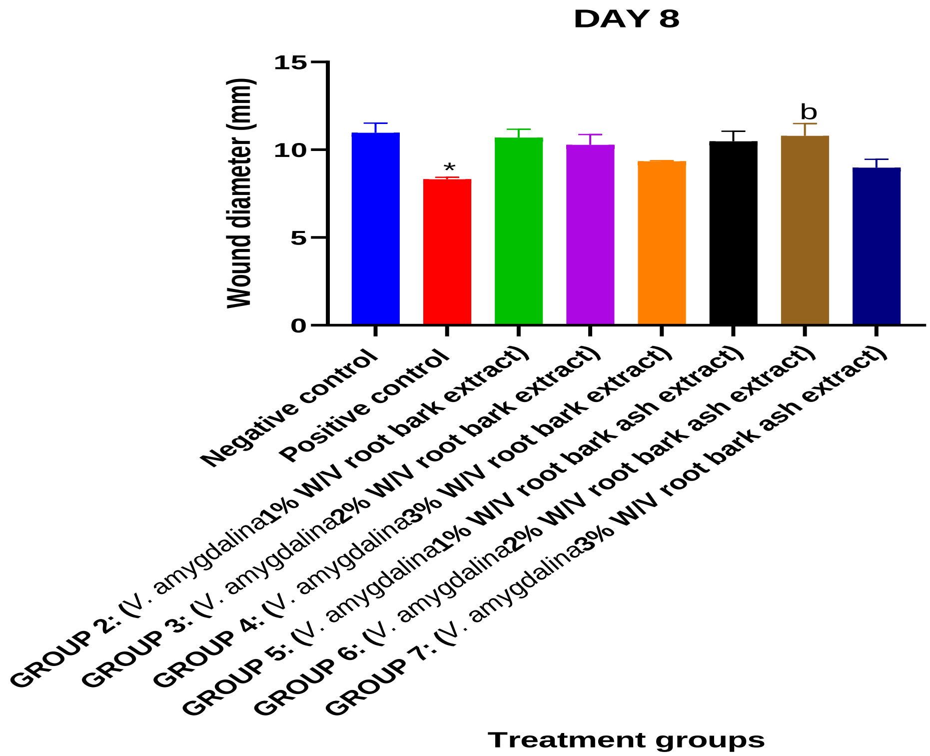


Figure 3: Showing the mean wound diameter (mm) on day 8. Results are expressed in Mean ± SEM. n = 4. * = p ≤ 0.05 vs negative control; b = p ≤ 0.05 vs positive control.

On days 4 and 8, no significant difference in wound healing in the different groups was observed in relation to the negative control except the positive control group. Also, group 6 was statistically significant ($p<0.05$) in relation to the positive on day 8.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

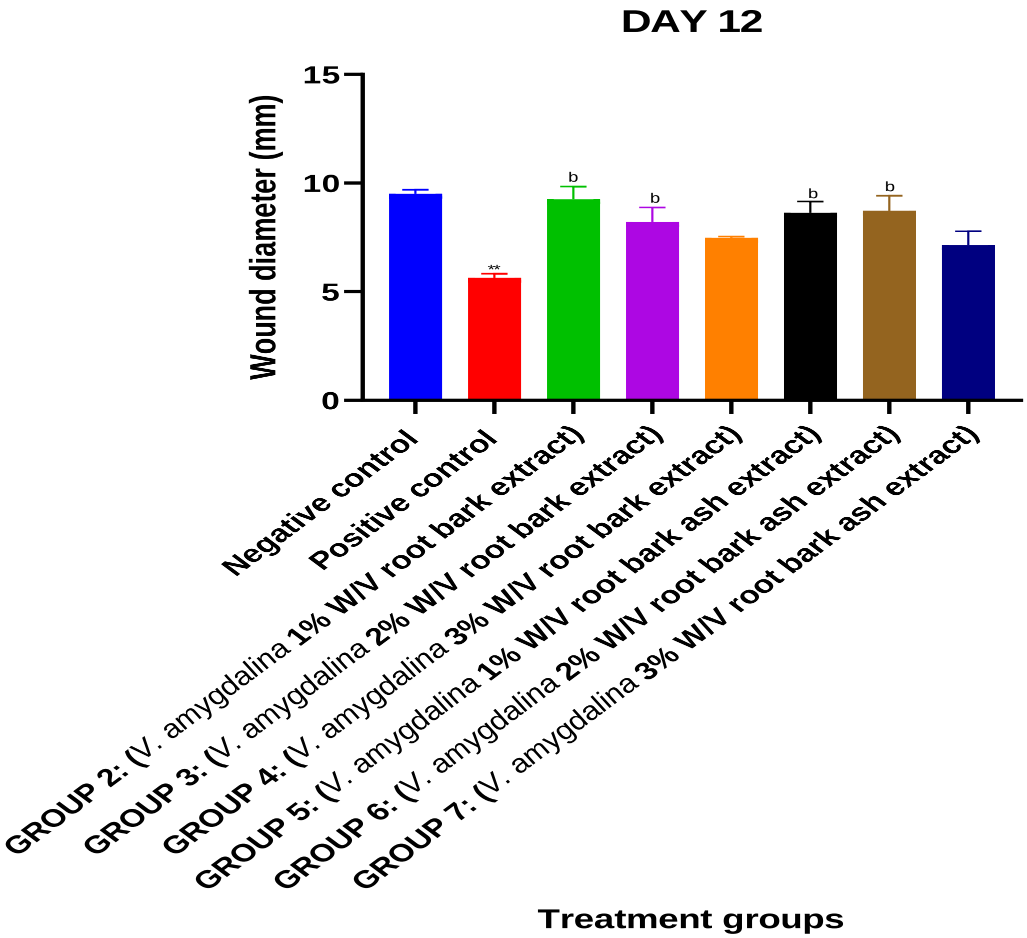


Figure 4: Showing the mean wound diameter (mm) on day 12. Results are expressed in Mean ± SEM. n = 4. ** = p <0.01 versus negative control; b = p < 0.05 vs positive control.

On day 12, the positive control group showed moderately significant effect ($p<0.01$) in relation to the negative control. Group 2, group 3, group 5, and group 6 showed significant ($p<0.05$) wound healing in relation to the positive control.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

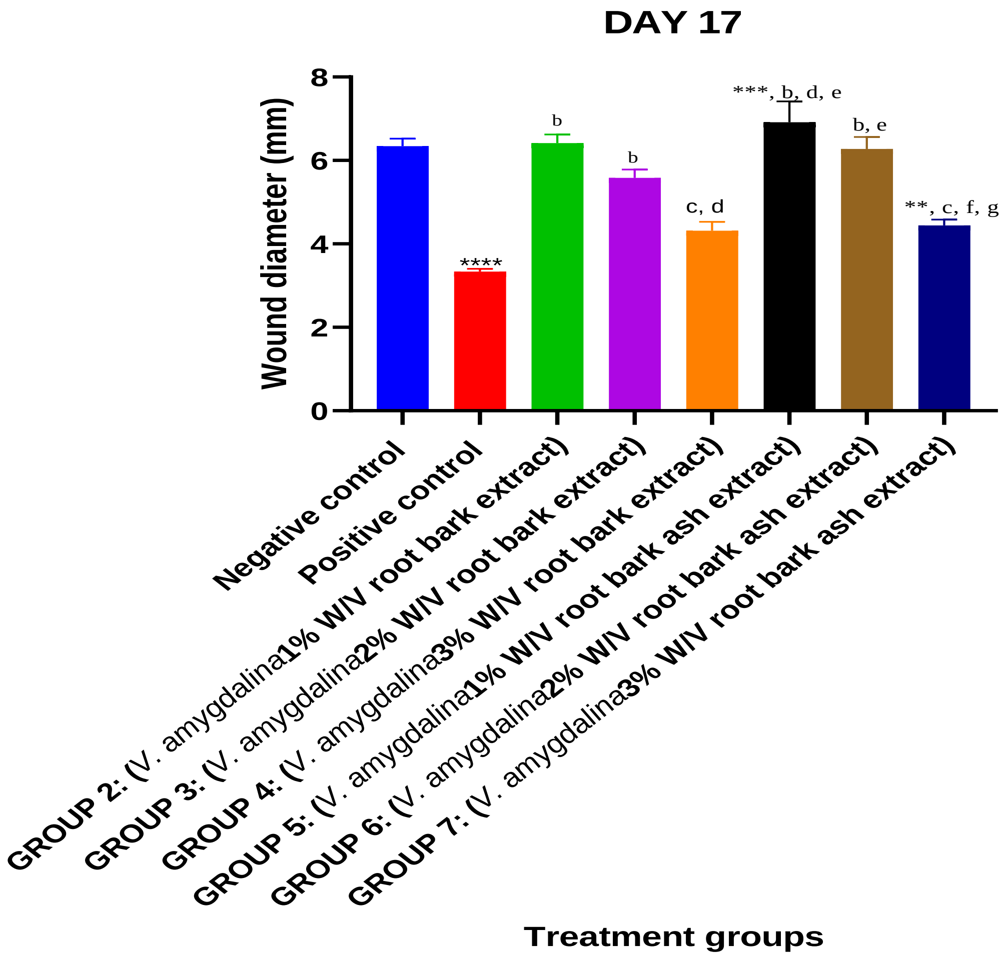


Figure 5: Showing the mean wound diameter (mm) on day 17. Results are expressed in Mean ± SEM. n = 4. ** = p ≤ 0.01; *** = p ≤ 0.001; **** = p < 0.0001 vs negative control; b = p ≤ 0.05 vs positive control; c = p ≤ 0.05 vs group 2; d = p ≤ 0.05 vs group 3; e = p ≤ 0.05 vs group 4; f = p ≤ 0.05 vs group 5; g = p ≤ 0.05 vs group 6.

On day 17, group 7 and group 5 showed moderate and high significant ($p<0.01$ and $p< 0.001$) wound healing respectively against negative control. Also, positive control showed the most significant ($p<0.0001$) in relation to the negative control group. Group 2, Group 3, Group 5, and Group 6 showed significant effects ($p<0.05$) in relation to the positive control group. Group 4 and Group 7 were significant ($p<0.05$) in relation to Group 2. Group 4 and Group 5 showed a significant effect in comparison to Group 3. Then group 5 and group 6 have significant effects in relation to group 4. Group 7 was significant against Group 5 and Group 6.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

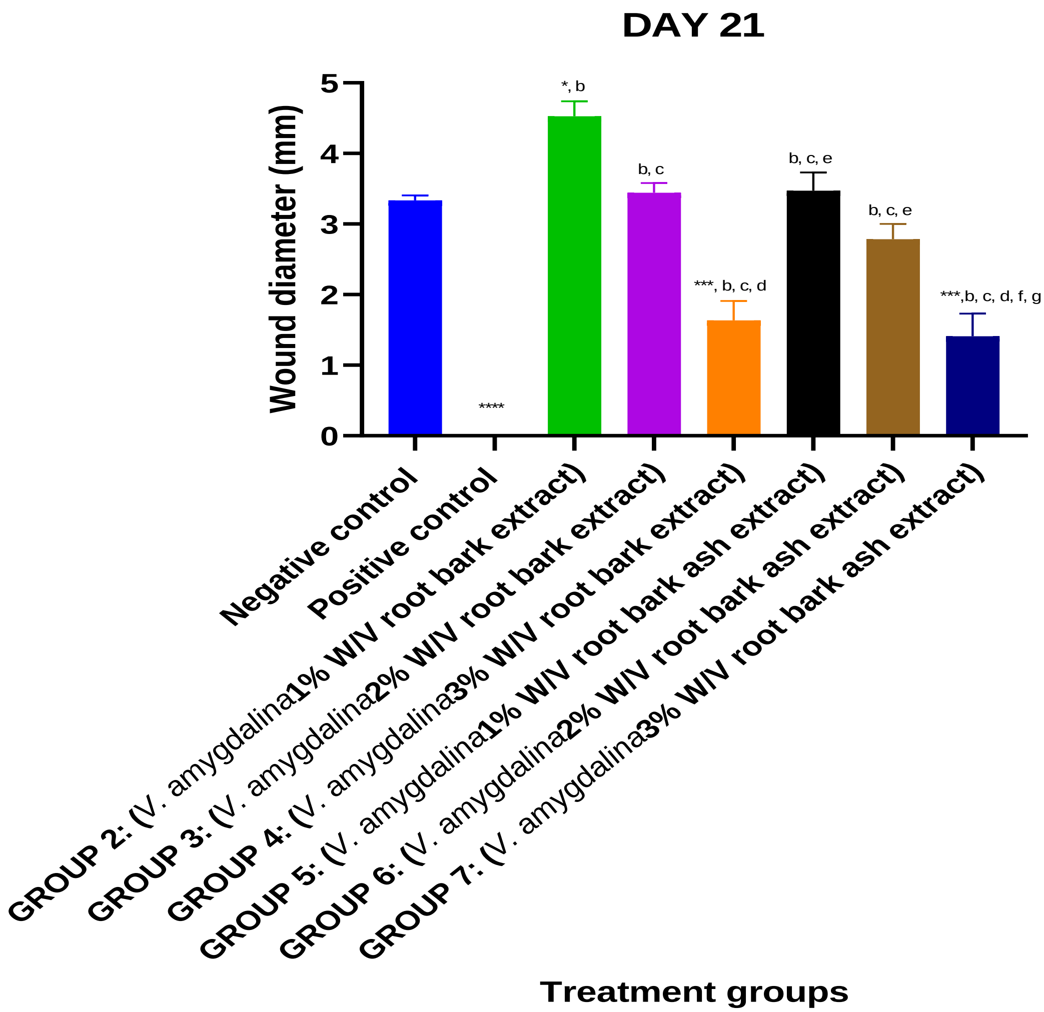


Figure 6: Showing the mean diameter of the wound (mm) on day 21. Results are expressed in Mean ± SEM. n = 4. ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p < 0.0001$ vs negative control; b = $p \leq 0.05$ vs positive control; c = $p \leq 0.05$ vs group 2; d = $p \leq 0.05$ vs group 3; e = $p \leq 0.05$ vs group 4; f = $p \leq 0.05$ vs group 5; g = $p \leq 0.05$ vs group 6. On day 21, group 2 showed a low significant effect ($p<0.05$) while group 4 and group 7 showed highly significant ($p<0.001$) wound healing effect against the negative control and also positive control group showed a most significant effect ($p<0.0001$) in relation to the negative control group. Groups 2 to group 7 all showed a significant effect ($p<0.05$) in relation to the positive control group. From group 3 to group 7 all showed significant effect ($p<0.05$) in relation to group 2. Group 4 and Group 7 were significant ($p<0.05$) against Group 3. Groups 5 and 6 showed significant effects against group 4. Lastly, Group 7 showed a significant effect ($p<0.05$) against Group 5 and Group 6.

ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

Figure 7: Showing pictures representing the progression of wound healing in the different groups on different day

Day 8 **Day 12** **Day 17** **Day 21**

Negative Control

Positive Control

GROUP 2 (1 % W/V Root Bark aqueous Extract)

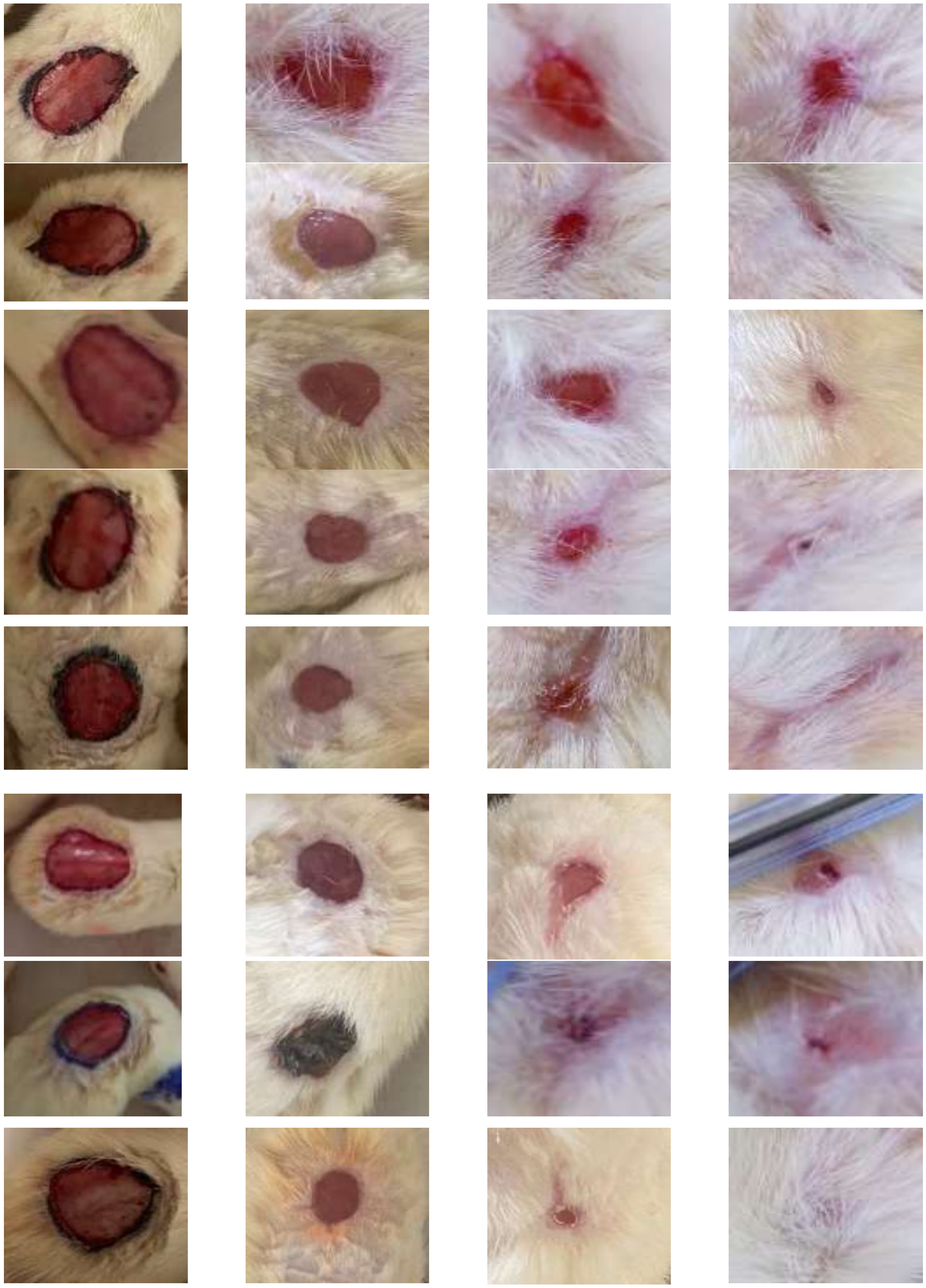
GROUP 3 (2 % W/V Root Bark aqueous EXTRACT)

GROUP 4 (3% W/V Root Bark aqueous extract)

GROUP 5 (1 % W/V Root Bark ash Extract)

GROUP 6 (2 % W/V Root Bark ash Extract)

Group 7 (3% w/v Root bark ash extract)



OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

DISCUSSION

Area of wound contraction was observed to be time-dependent in all groups and to get better with longer exposure to the extracts. In contrast to the negative control, the results showed that root bark aqueous and root bark ash extracts of *V. amygdalina* in their respective concentrations (1% w/v, 2% w/v, and 3% w/v) demonstrated greater wound healing activity. The findings of this study were related to the wound-healing properties of methanolic extracts of *Ageratum conyzoides* L. (Asteraceae) and *Anthocleista djalonensis* according to Nicolaus *et al.* [37]. The presence of phytochemicals in the plant, such as flavonoids (such as luteolin, luteolin7-0b-glucuronoside (most abundant component), and luteolin 7-o-b-glucoside), maybe the cause of the root bark aqueous extracts of V. amygdalina's wound healing properties [38]. Other phytochemicals in *V. amygdalina* include Vernolide, vernolepin, and vernomenin, among other sesquiterpene lactones; tannins; vernoamyosides; saponins; terpenes; glycosides; and coumarins [39]. These are seen to provide wound healing activity through their anti-inflammatory [40], antioxidant activity (mainly due to flavonoids) through scavenging of free radicals [41], and antimicrobial activities [42] and antifungal [43]. The presence of trace elements and minerals in root bark that function as enzyme co-factors and boost the structural components in tissue repair could be the cause of the wound-healing activity of root bark ash and root bark aqueous extract [23, 44, 45]. These elements include Zinc, Iron, Copper, chromium, calcium, and manganese. Ash also possesses anti-septic and anti-infective activity and thus the improved wound healing noticed in its treatment groups. Figures 5 and 6 demonstrate that as compared to their root bark aqueous counterparts, root bark ash extracts exhibited greater wound healing efficiency. On observation high concentrations had better wound healing activity than the lower concentrations for both root bark ash and the root bark aqueous extracts this is seen in the graphs of day 17 and day 21 where groups 4 (3 % w/v root bark aqueous extract) and 7 (3 % w/v root bark ash) have the higher peaks when compared to lower concentrations. These findings were in agreement with those from a study that was carried out by Nicolaus *et al.* [37] using *Calendula officinalis* extracts where the wound healing activity increased with the increase in concentration of the extracts and this was explained by the antioxidant properties that significantly suppress the proliferation of excision wounds hence preventing pathogenic microorganism invasion. By the end of the experiment, 3% w/v root bark ash extract exhibited the highest percentage area of wound healing contraction compared to all other groups, demonstrating the strongest observable wound healing activity.

CONCLUSION

This research revealed that both the root bark aqueous extracts and root bark ash of *V. amygdalina* had wound-healing activities. With 3% w/v root bark ash extract standing out among the rest, root bark ash extracts in their individual concentrations performed better than the same concentrations in the root bark aqueous extract groups.

RECOMMENDATIONS

More research should be done on root bark aqueous and root bark ash extracts in various formulations (creams, ointments, and sprays), in order to show the effectiveness of these extracts in contemporary formulations. It is important to conduct additional research on *V. amygdalina's* capacity to heal wounds when extracted using different solvents like ethanol and methanol. To measure wound healing activity, the same study should be conducted with other parameters, such as the day of epithelization, tensile strength, and histo-morphologic analyses. Following more thorough research showing clear success, the creation of herbal wound treatments should be promoted and approved as an alternative to conventional therapy in areas where access to and affordability of traditional medicine are issues.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

REFERENCES

1. Sen, C. K. Human Wounds and Its Burden: An Updated Compendium of Estimates. Adv Wound Care (New Rochelle). 2019;8(2):39-48.
2. Ilesanmi, R. E. and Ogundeji, K. D. Nursing Intensity per Wound Care episode: A case of Poor Costing of Nursing Care in Nigeria. *West African Journal of Nursing*, 2020; 30(2): 36-46.
3. Nakibuule, M. K., Ntulume, I., Mwandah, D. C., Tibyangye, J., Bashir, A., Odoki, M., Okoche, D., Maniga, J. N., Emmanue, E., Kwizera, E., Richard, B., & Aliero, A. A. Anti-bacterial Activity of Crude Flavonoid Fraction from Bidens pilosa Leaves against Selected Chronic Wound Bacterial Pathogens. *Journal of Complementary and Alternative Medical Research*, 2019; *8*(1): 1–13.
4. Sunday, A. G., Ifeanyi, O. E., & Ezeja, M. I. Wound healing potentials of leaf and bark extracts of Delonix regia. *World J Pharm Pharm Sci*, 2014; 3: 133-42.
5. Sunday, A. G., Ifeanyi, O. E., & Uzor, O. F. Wound Healing Potential Of Water And Ethanol Extract Of Fresh Leaves And Bark Of gambia Albidum In Albino Rats. *IOSR J. Dental Med. Sci*, 2014; 13:16-22.
6. Oyabambi, A. O., Nafiu, A. B., Okesina, A., Babatunde, S. S., Dominic, E. K., & Oreoluwa, D. O. Corn silk methanolic extract improves oxidative stress and inflammatory responses in rats' excision wound model. *Ceylon Journal of Science*, 2021; *50*(1): 39-46.
7. Ogundeji, K. D., Akinyemi, K. F., Adeyemo, A., Oluwaleke, A. K., & Ilesanmi, R. E. Economic burden of wound care among patients in a Nigerian teaching hospital: Implications for Insurance Coverage in Nigeria. *African Journal of Nursing and Health*, 2018, 9.
8. De Francesco F., Riccio V., Biswas R., Busato A., Di Bella C., Serri E., Sbarbati A., Zavan B., Riccio M., Palumbo Piccionello A. In Vitro Characterization of Canine Microfragmented Adipose Tissue Non-Enzymatically Extracted from the Thigh and Lumbar Regions. *Animals*. 2021;11: 3231.
9. Builders, M. I. & Oseni-Momodu, E. 2017. Pattern of wounds at Bingham University Teaching Hospital, Jos. *Journal of Medicine and Medical Sciences*. 2017; 8 (5): 060-068.
10. Builders, P. F. & Builders, M.I. 2016. Wound Care: Traditional African Medicine Approach. Worldwide Wound Healing: Innovation in Natural And Conventional Methods. Intech, Rijeka, Croatia**,** 1-24.
11. Noor S, Zubair M, Ahmad J. Diabetic foot ulcer--A review on pathophysiology, classification and microbial etiology. Diabetes Metab Syndr. 2015;9(3):192-9.
12. Percival, N. J. Classification Of Wounds And Their Management. Surgery (Oxford). 2002; 20**:** 114-117.
13. Truskett, P. Bailey And Love's Short Practise Of Surgery;26th Edition. Edited By N. S.Williams, C. J. K. Bulstrode, and P. R.O'connell. Boca Raton, FL: Crc Press, 2013. 1517 pages. Isbn 978-1-4441-2128-5. Price: $199.95: Media Review. Anz Journal Of Surgery. 2014; 84**:** 193-193.
14. Nagori, B. P. and Solanki, R. Role of medicinal plants in wound healing.Research Journal Of Medicinal Plant,2011; 5**:** 392-405.
15. Ibiam, U. A., Alum, E. U., Orji, O. U., Aja, P. M., Nwamaka, E. N., Ugwu, O. P. C., & Ekpono, E. U. Anti-Inflammatory Effects of Buchholzia coriacea Ethanol Leaf-Extract and Fractions in Freund's Adjuvant-Induced Rheumatoid Arthritic Albino Rats. *Indo American Journal of Pharmaceutical Sciences*, 2018; *5*(7): 6341-6357.
16. Alum, E. U., Umoru, G. U., Uti, D. E., Aja, P. M., Ugwu, O. P., Orji, O. U., Nwali, B. U., Ezeani, N., Edwin, N. and Orinya, F. O. Hepato-protective effect of Ethanol Leaf Extract of *Datura stramonium* in Alloxan-induced Diabetic Albino Rats. *Journal of Chemical Society of Nigeria*, 2022; 47 (3): 1165 – 1176.
17. Ugwu, O. P.C., Alum, E. U., Okon, M. B., Aja, P. M., Obeagu, E. I. and Onyeneke, E. C. Ethanol root extract and fractions of *Sphenocentrum jollyanum* abrogate hyperglycemia and low body weight in Streptozotocin-induced diabetic Wistar albino Rats, *RPS Pharmacy and Pharmacology Reports*, 2023, rqad010.
18. Alum, E. U., Inya, J. E., Ugwu, O. P. C., Obeagu, I.E., Aloke, C., Aja, P. M., Okpata, M. G., John, E. C., Orji, M. O. and Onyema, O. Ethanolic leaf extract of *Datura stramonium* attenuates Methotrexate-induced Biochemical Alterations in Wistar Albino rats. *RPS Pharmacy and Pharmacology Reports*, 2023; 2(1):1–6.
19. Jaffe, L. and Wu, S. C. Dressings, Topical Therapy, and Negative Pressure Wound Therapy. Clinics In Podiatric Medicine And Surgery, 2019; 36**:** 397-411.
20. O'dell, M. L. Skin And Wound Infections: Overview. American Family Physician. 1998; 57:2424.
21. Asogwa, F. C., Okoye, C. O. B., Ugwu, O. P. C., Edwin, N., Alum, E. U. and Egwu, C. E. Phytochemistry and Antimicrobial Assay of *Jatropha curcas* Extracts on Some Clinically Isolated Bacteria - A Comparative Analysis. *European Journal of Applied Sciences*,2015*;* **7**(1): 12-16.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122
22. Ibiam, U. A., Alum, E. U., Aja, P. M., Orji, O. U., Nwamaka, E. N., & Ugwu, O. P. C. Comparative analysis of chemical composition of Buchholzia coriacea ethanol leaf-extract, aqueous and ethylacetate fractions. *Indo American Journal of Pharmaceutical Sciences*. 2018; 5(7): 6358-6369.
23. Alum, E. U., Oyika, M. T., Ugwu, O. P. C., Aja, P. M., Obeagu, E. I., Egwu, C. O. and Okon, M. B. Comparative analysis of mineral constituents of ethanol leaf and seed extracts of *Datura stramonium. IDOSR Journal of Applied Sciences*, 2023; **8**(1):143-151.
24. Alum, E. U., Mathias, C. D., Ugwu, O. P.C., Aja, P.M., Obeagu, E. I., Uti, D. E. and Okon, M. B. Phytochemical composition of *Datura stramonium* Ethanol leaf and seed extracts: A Comparative Study. *IAA Journal of Biological Sciences*, 2023; **10**(1):118-125.
25. Mihai, M. M., Dima, M. B., Dima, B. & Holban, A. M. 2019. Nanomaterials for wound healing and infection control. *Materials*. 2019; 12**:** 2176.
26. Rajendran, N. K., Kumar, S.S. D., Houreld, N. N. & Abrahamse, H. A Review On Nanoparticle-Based Treatment For Wound Healing. *Journal Of Drug Delivery Science And Technology*. 2018; 44**:** 421-430.
27. Nafiu, A., Akinwale, O. and Owoleye, B. Histomorphological evaluation of wound healing-comparative use between honey and Vernonia Amygdalina Root barkJuice. Nigeria. *Trop. J. Health Sci*, 2016; 23**:** 10-11.
28. Shaikh, D. M. & Shaikh, H. Z. Ash As A Unique Natural Medicine For Wound Healing. Isra Med J., 2009; 1**:** 72-78.
29. Uti, D. E., Igile, G. O., Omang, W. A., Umoru, G. U., Udeozor, P. A., Obeten, U. N., Ogbonna, O. N., Ibiam U. A., Alum, E. U., Ohunene, O. R., Chukwufumnanya, M. J., Oplekwu, R. I. and Obio, W. A. Anti-Diabetic Potentials of Vernonioside E Saponin; A Biochemical Study. *Natural Volatiles and Essential Oils*, 2021; **8**(4): 14234-14254.
30. Alum, E. U., Famurewa, A. C., Orji, O. U., Aja, P. M., Nwite, F., Ohuche, S. E., Ukasoanya, S. C., Nnaji, L. O., Joshua, D., Igwe, K. U. and Chima, S. F. Nephroprotective effects of *Datura stramonium* leaves against methotrexate nephrotoxicity via attenuation of oxidative stress-mediated inflammation and apoptosis in rats. *Avicenna Journal of Phytomedicine*. 2023; 13(4): 377-387.
31. Ugwu, O. P.C., Alum, E. U., Obeagu, E. I, Okon, M. B., Aja, P. M., Samson, A. O., Amusa, M. O. and Adepoju, A. O. Effect of Ethanol leaf extract of *Chromolaena odorata* on lipid profile of streptozotocin induced diabetic wistar albino rats. *IAA Journal of Biological Sciences*, 2023; **10**(1):109-117.
32. Aja, P. M., Ogwoni, H. A., Agu, P. C., Ekpono, E. U., Awoke, J. N., Ukachi, O. U., Orji, O. U., Ale, B. A., Nweke, C. P., Igwenyi, I. O., Alum, E. U., Chukwu, D. C., Offor, C. E., Asuk, A. A., Eze, E. D., Yakubu, O. E., Akobi, J. B., Ani, O. G. and Awuchi, C. G. *Cucumeropsis mannii* seed oil protects against Bisphenol A-induced testicular mitochondrial damages. *Food Science & Nutrition*, 2023; 00: 1– 11.
33. Adiukwu, P. C., Amon, A., Nambatya, G., Adzu, B., Imanirampa, L., Twinomujuni, S., Twikirize, O., Amanya, M., Ezeonwumelu, J. O. & Oloro, J. Acute Toxicity, Antipyretic And Antinociceptive Study Of The Crude Saponin From An Edible Vegetable: Vernonia Amygdalina Root bark. *International Journal Of Biological And Chemical Sciences*. 2012, 6**:** 1019-1028.
34. Charan, J., & Kantharia, N. How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics, 2013; 4(4): 303–306.
35. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 2002;43(4):244-58.
36. Kuramoto, T., Nakanishi, S., Ochiai, M., Nakagama, H., Voigt, B. and Serikawa, T. Origins of albino and hooded rats: implications from molecular genetic analysis across modern laboratory rat strains. PLoS One. 2012;7(8):e43059.
37. Nicolaus, C., Junghanns, S., Hartmann, A., Murillo, R., Ganzera, M., & Merfort, I. In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts. *Journal of Ethnopharmacology*. 2017; 196: 94-103.
38. Lubis, M. F., Hasibuan, P. A. Z., Syahputra, H., Astyka, R., & Baruna, I. Phytochemical Profile and Pharmacological Activity of *Vernonia amygdalina* Delile Stem Bark Extracts Using Different Solvent Extraction. *Open Access Macedonian Journal of Medical Sciences*. 2022; 10(A): 860-866.
39. Tijjani, M. A., Mohammed, G. T., Alkali, Y. T., Adamu, T. B., & Abdurahaman, F. I. Phytochemical analysis, analgesic and antipyretic properties of ethanolic Root barkextract of Vernonia amygdalina Del. *Journal of Herbmed Pharmacology*. 2017; 6(3): 95-99.

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

40. Ugbogu, E. A., Emmanuel, O., Dike, E. D., Agi, G. O., Ugbogu, O. C., Ibe, C., & Iweala, E. J. The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant-Vernonia amygdalina L.(bitter Root bark). *Clinical Complementary Medicine and Pharmacology*. 2021; 1(1): 100006.
41. Alum, E. U., Ibiam, U. A., Ugwuja, E. I., Aja, P. M., Igwenyi, I. O., Offor, C. E., Orji, O. U., Aloke, C., Ezeani, N. N., Ugwu, O. P. C. and Egwu, C. O. Antioxidant Effect of *Buchholzia coriacea* Ethanol Leaf Extract and Fractions on Freund's Adjuvant-induced Arthritis in Albino Rats: A Comparative Study. *Slovenian Veterinary Research*. 2022; 59 (1): 31–45.
42. Tula, M. Y., Azih, A. V., Iruolaje, F. O., Okojie, R. O., Elimian, K. O., & Toy, B. D. Systematic study on comparing phytochemicals and the antimicrobial activities from different parts of V. amygdalina. *African Journal of Microbiology Research*. 2012; 6(43): 7089-7093.
43. Mahmud, T. M. M. A review: medicinal values, agronomic practices and postharvest handlings of *Vernonia amygdalina*. *Food Research*. 2019; 3(5): 380-390.
44. Aja, P. M., Ugwu, O. P. C., Ekpono, E. U., Mbam, M. L., Alum, E. U. and Ibere, J. B. Proximate and mineral compositions of Phoenix dactylifera (fruit sold in Hausa Quarter Abakaliki, Ebonyi state, Nigeria. *IDOSR Journal of Scientific Research*. 2017;2(1):53-65.
45. Aja, P. M., Nwobasi, C. S., Alum, E. U., Udeh, S.M.C., Edwin, N., Orinya, O.F., Abara, P.N. and Aloke, C. Mineral and Proximate Compositions of Nauclae latifolia Root bark from Abakaliki, Ebonyi State Nigeria. *International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)*, 2017; 6 (2): 375- 382.

Umutetsi Jacklean (2023). A Comparative Study of the Topical Wound Healing Activity of Root Bark Ash and Root Bark Aqueous Extracts of *Vernonia amygdalina* on Male Mice. NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES (NIJBAS) 3(2): 164-177.