JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/34089
SHORT COMMUNICATION

Evaluation of Wound Healing Activity of *Vernonia amygdalina* Extract in Diabetic Rats

Avijit Mazumder*, Bhavani Pentela, Sanjana and Vikas Gupta

Noida Institute of Engineering and Technology (Pharmacy Institute), 19 Knowledge Park II, Institutional Area, Greater Noida – 201306, Uttar Pradesh, India; avijitmazum@yahoo.com

Abstract

Excision and incision wound models were used to evaluate the wound healing activity on Wistar albino rats by applying the topical application of a prepared ointment. The wound healing potential was assessed by measuring the rate of the epithelization period, wound contraction, wound breaking strength, and histopathological parameters reference with povidone iodine ointment as standard. Higher rate of wound contraction (****P<0.0001), decrease in the period of epithelization (**P<0.01) higher wound breaking strength (****P<0.0001), and favorable histopathological changes were observed with the ointment containing 5% and 10% hydro-ethanolic extract of leaves. The data of this study indicated that hydro-ethanolic *Vernonia amygdalina* extract of leaves exhibited potent wound healing effects thus the study can be extended in future for the study of phytoconstituents which are giving the wound healing effect.

Keywords: Diabetic, Excision, Incision, Luteolin, *Vernonia amygdalina*

1. Introduction

The wound is defined as the loss or disruption of the cellular, anatomical, or functional continuity of the live tissue. It is the apparent result of individual cell death or damage, which may or may not lead to a loss of skin integrity and compromise the physiological function of the tissue. Injuries happen when the skin's normal structure and function are harmed[1]. The process of healing a wound that has been caused to the skin or other soft tissues[2].

The delay in the inflammatory process can also result in the production of reactive oxygen species, which causes harmful effects on cells and tissue. The development of free radicals and decreased antioxidant activity could exacerbate the illness and explain why healing is taking longer[3]. Now a day, a wide variety of antibiotics was used to treat wound infections, however, it has been established that these have adverse effects on the human body. The plants have drawn attention as a result, as they have demonstrated their efficacy in herbal remedies and produce extracts that contain biologically active components. In India, traditional healers have used some herbs and potential plants which having properties of wound healing, antioxidant, and anti-microbial activity. However, only a small number of them have been studied to establish their healing potential, while others were still awaiting this[4]. Therefore, it is necessary to provide scientific support for the therapeutic value of these herbs utilized in traditional medicine. *Vernonia amygdalina* has been chosen to examine and demonstrate its purported utility in traditional medicine. Previously, the extract from the leaves of this plant was successfully administered to wounds in the form of jelly, juice, or medicated ghee. The current study was designed with specific prominences to explore the wound healing potential of *Vernonia amygdalina* leaves extract by administering topically, keeping in mind the claimed benefits of *Vernonia amygdalina*. The extract prepared proved that it was of utmost active to heal wounds which have been found by monitoring wound contraction. To determine its safety profile and to choose a dose, an acute dermal toxicity investigation was conducted by taking *Vernonia amygdalina* leaf extract.

*Author for correspondence

Article Received on: 16.06.2023 Revised on: 20.07.2023 Accepted on: 25.07.2023

2. Materials and Methods

2.1 Plant Collection and Authentication

Leaves were collected from the Pragati Garden Yamuna Bank Delhi. The plant was authenticated by Dr. Priyanka Ingle, Scientist C Botanical Garden Indian Republic, sector 37, Noida, specimen no. (BSI/BGIR/1/TECH./2021/10) and a specimen was deposited (herbarium section of botanical garden, Noida).

2.2 Preparation of Extracts and Phytochemical Analysis

The *Vernonia amygdalina* leaves were congregated, cleaned, and allowed to dry in the shade. The leaves were grind with a mortar and pestle into a fine powder. 70% ethanol served as the solvent used to create the extract. The extraction was carried out with 100 g of powdered medicines using the Soxhlet method over an 18-hr period at 40˚C. The extract was thickened in a rotary evaporator at 50°C after being filtered with a No. 42 Whatman filter. The condensed extract was further air-dried to make it more solid before being placed in a desiccator for later usage. All the reagents and chemicals used were of analytical grade and taken from the chemical store of NIET (Pharmacy Institute) India.

2.3 Phytochemical Screening

The test tubes containing the leaf extracts were then individually examined for saponins, tannins, triterpenoids, anthraquinones, reducing sugar (Fehling's test), steroids, flavonoids, cardiac glycosides, alkaloids, phytosterols, polyphenols. All of these phytoconstituents were identified by color changes[5,6].

2.4 Experimental Rats

Male or female albino Wistar rats, 8–10 weeks old and weighing 150–180g were used in this investigation. It was decided to follow the protocol while employing animals in research at the NIET IAEC (Pharmacy Institute). Polypropylene cages were used to acquire and house animals for 90 days in Animal House. The NIET (Pharmacy Institute) was used under typical circumstances, such as 27°C, 40–70 % relative humidity, and a 12–12-hour cycle for darkness and light. They consumed their regular pellet feeds and drank water as needed throughout the operation. Surgery was performed in a clean environment under watchful observation. The animals were given inhalational anesthesia with diethyl ether. The garbage in the cages was changed every three days to guarantee the cleanliness and comfort of the animals.

2.5 Acute Dermal Toxicity

In compliance with OECD guidelines No. 434, acute dermal toxicity research was created and administered to adult female albino rats utilizing the constant dosage approach. After each animal was weighed, the outer layer of its body was estimated. 10% of the body's surface area was appropriately shaved, and extracts were then administered topically (2000 mg/kg B.W.) to the shaved area after being blended with a foundation of hard paraffin, wool fat, soft paraffin, and ceto-stearyl alcohol. Using gauze or a non-irritating tape, the ointment was kept to the shaved region. Animals were periodically kept under observation for a whole day to look for any signs of poisoning[7].

2.6 Ointment Preparation

The ointment base was once made from hard paraffin, which was precisely weighed and melted in a water bath. Hard paraffin is still present today. The components melted better when the addition was made while stirring slowly. After thoroughly blending, let the ointment base cool. The *Vernonia amygdalina* leaves extract was accurately weighed and added to make a smooth paste. The base was then progressively added until the ointment was homogeneous. It was then placed into the proper disinfectant container[8].

2.7 Induction of Diabetes

Rats from Groups 2, 3, 4, and 5 were weighed and had their fasting blood glucose levels checked after going without food all night. The rats were then intraperitoneally administered one dose of STZ, freshly made in 0.1 M citrate buffer (pH 4.5) to induce diabetes. Animals in group 2 received an introduction of 0.1 M citrate buffer. Additionally, fasting blood sugar levels were rechecked after 5 days (>270mg/dL) using an ACCU-CHEK Instant S blood glucose meter. To evaluate blood sugar levels, blood was taken from a rat tail vein[9].

2.8 Wound Healing-Activity

2.8.1 Excision Wound Healing

The animals have been divided into groups, with six animals in each group and dosed per additional information:

Group 1: Control
Group 2: Diabetic control group
Group 3: Standard (Povidone Iodine)
Group 4: Test 5% leaf extract
Group 5: Test 10% leaf extract

Diethyl ether was administered to the animal to cause anesthesia. The dorsal thoracic region of albino Wistar rats was depilated, as well as an area on the posterior back of the rats was chosen, which was 1 cm from the spinal column. The 500mm^2 of skin's full thickness was then removed from the chosen region. The animals were divided into 5 groups of six each. On translucent paper, the wound area was easily measured, and the result was calculated on 1mm graph paper. To quantify wound repair, the method was repeated every third day till the wound had healed completely. Calculations were done to figure out where the wound was contracting. The location of the wound on the day of the injury was treated as day 0, as well as the day of the injury was treated as 100%[10–12]. The wound contraction rate is calculated by the formula given below:

Formula for calculation of wound closure:

$$\% \text{ wound closure} = \frac{\text{Initial wound area - Final wound area}}{\text{Initial wound area}} \times 100$$

2.8.2 Incision Wound Model

The animals have been divided into groups, with six animals in each group, and dosed under additional information:

Group 1: Control
Group 2: Diabetic control group
Group 3: Standard (Povidone Iodine)
Group 4: Test 5% leaf extract
Group 5: Test 10% leaf extract

Diethyl ether was used to administer anesthesia both before and during the trial. Dorsal fur has been removed. On the other side of the spinal column, 2 cm-long incisions were done over the depth of the skin in the paravertebral region. By the use of surgical thread as well as a curved needle, the split skin was kept together and sewn at intervals of 0.5 cm. For 10 days, the ointment as described earlier was applied topically to the wounds of animals in different groups. To promote better wound healing, the continual fibers along the margins of both wounds were tightened. Day 0 was the day the injury occurred. Once the incisions had entirely healed, the sutures were removed. 10 days following the injury, a consistent, constant water flow technique was used to calculate the wound-breaking strength[10–12].

2.8.3 Histopathological Activity

Animals from the control group, standard group, and treated groups of excision wound and incision wound models are anesthetized by di ethyl ether and then samples of their healed skin tissue are removed for histological analysis and kept in 10% formalin. Re-epithelization, fibroblast cells, collagen fibers, and new blood vesicle parameters were observed in histological analysis[13].

2.8.4 Statistical Evaluation

Every single parameter's mean S.E.M (Standard Error Mean) was determined for each observation. ANOVA and Dunnett's test were used to statistically analyze the values.

3. Results

3.1 Phytochemical Screening

In the phytochemical screening of this plant Saponins, Tannins, Triterpenoids, Alkaloids, Flavonoids, Glycosides, Reducing sugar were present (Table 1).

Table 1. Phytochemical screening of *Vernonia amygdalina* leaves

S. No.	Chemicals phytoconstituent test	Tests performed	Test procedure	Observation	Hydro-Ethanol Extract
1.	Saponins	Foaming test	0.5g of extract + 5ml of H_2O, shake for 15min	Frothing	+
		Lead acetate test	1ml extract + 1% lead acetate	White precipitate	

Table 1. to be Continued...

2.	Tannins	Ferric chloride test	0.5g of extract+ 10 ml of H_2O,+ few drops of 0.1% $FeCl_3$	Blue-black colour	+
3.	Triterpenoids	Libermann-buchard test,	0.5g of extract + few drops of acetic anhydride +strong sulphuric acid	Deep red colour	+
		Salkowski test	0.5g of extract+ 1ml chloroform+ strong H_2SO_4	Reddish violet colour	
4.	Alkaloids	Dragendroff test	leaves extract + chloroform, Vaporize the $CHCl_3$ + few drops of Dragendroff reagent	Dark brown	+
		Mayers test	3 mL of extract+ Few drops of Mayer's	Precipitation	
5.	Flavonoids	Shinodas test,	Few pieces of magnesium ribbon + concentrated HCL drop to a 5 ml extract solution	Reddish colour	
		Alkaline reagent test	Add sodium hydroxide to 5 ml of the extract	Yellow colour	+
6.	Glycosides	Keller-killiani	5ml water + 0.5g of the extract+ few drops of $FeCl_3$+ 2 ml of glacial acetic acid + 2 ml of concentrated sulphuric acid.	Reddish brown colour layer seen at the intersection	+
7.	Reducing sugar	Fehlings test	Add 0.5g of the extract to 10 ml of Deionized H_2O and filter it. To filtrate add 5 ml of chloroform and 1 ml of diluted ammonia.	Red colour	+

3.2 Acute Dermal Toxicity Test

It was found to be safe to use leaf extracts up to 2000mg/kg B.W. because there was no sign of harm even after 24 hrs of cutaneous application, the extracts were hence considered to be safe at the dose tested. To conduct the analysis, 0.1 mg/kg, or one-tenth of the maximum tested dose, was used.

3.3 Incision Wound Model

On the tenth Post-Wounding Day (PWD), better WBS and diabetic wound healing patterns were seen for each test group in comparison to the control, and statistically significant outcomes were found. On the tenth PWD, the control and diabetic control animals had Wound Breaking Strength (WBS) of 285.8 g ± 1.4 g, and 279.6 ± 1.2 g, respectively, but the treated animals had a standard WBS of 401.6±1.3 g (****P<0.01). 10% Hydroethanolic Leaf Extract *Vernonia amygdalina* (HELEVA) exhibited 395±2.11 (****P<0.01), whereas 5% HELEVA showed 377.6±1.8 g (***P<0.01). The outcome demonstrates that 10% HELEVA ointment has a significant impact on diabetic wound repair (Table 2, Figure 1).

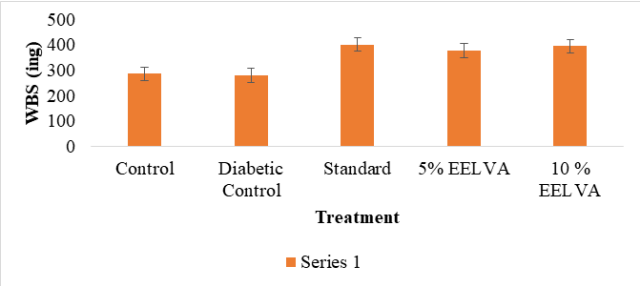

Figure 1. Graphical representation of WBS on incision wound model.

Table 2. Effect of ointment prepared from hydroethanolic leaf extract *Vernonia amygdalina* (HELEVA) on WBS of incision wound model

Treatment	WBS (in g) (Mean ±SEM)
Control	285.83 ±1.4
Diabetic control	279.66 ±1.2**
Standard	401.6 ±1.3****
5% HELEVA	377.66 ±1.8***
10% HELEVA	395 ±2.11****

(****P<0.0001) and (***P<0.001) evaluation by one-way ANOVA following Dunnett's assessment, (n = 6)

3.4 Excision Wound Model

Applying HELEVA ointments topically demonstrated strong wound healing potentials, as seen by the shortened epithelization time and expanded wound contraction area compared to the control group. The findings clearly show that *Vernonia amygdalina* could effectively treat wounds when compared to the control, supporting its usage in traditional medicine.

The wounds on the control and diabetic control animals shrank from 404±1.4 mm^2 and 406.5±1.4 at (0 PWD) to 27±1.5 mm^2 and 23.5±1.2 at 21 PWD, respectively, while full epithelization was noted at 28 PWD. Control and diabetic controls had mean epithelization times of 27.6± 0. 7 and 28.3±0. 6 respectively. The animals in the standard group had wounds that shrank from 416.8±2.5 mm^2 at baseline to 0 ≠ 0 mm^2 at post-mortem day 21 (PWD), and the epithelization duration was recorded as 21±0.7 days (**P<0.01). Rats treated with 10% HELEVA epithelization period was 20.83 ±0.6 days (**P<0.01) and wound closure was 99.5 % as shown in the table. Rats treated with 5% HELEVA wound contraction 407.3±1.2 mm^2 (0 PWD) to 0.7±0.2 (21 PWD) and epithelization period was reported as 22±0.5 (Figures 2 and 3, Table 3).

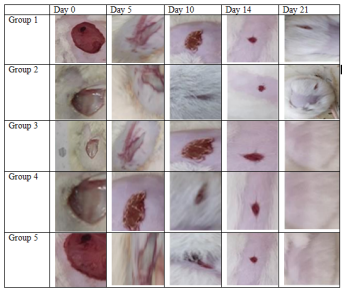

Figure 2. Wound area of different groups treated with formulation.

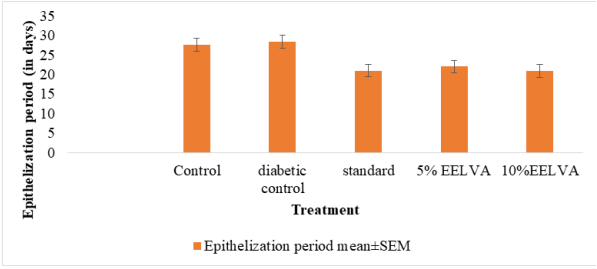

Figure 3. Graphical representation epithelisation period in a wound model.

3.5 Histopathology Evaluation

The two models, in contrast to control rats, animals treated with normal medication plus an ethanolic extract of *Vernonia amygdalina* demonstrated considerable wound healing as evidenced by fibroblast cells, collagen fibers, and new blood vesicles. When compared to the control group, the standard and extract-treated groups are significantly more likely to have collagen fibers, and new blood vessels (Figures 4 and 5).

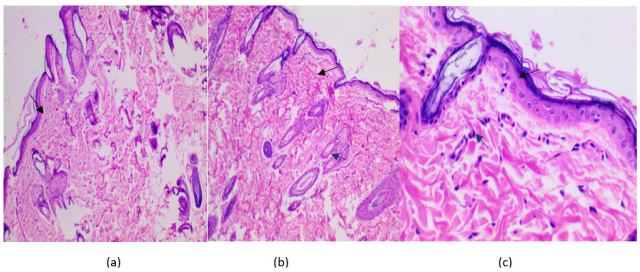

Figure 4. Skin histopathology of control group (a) standard (b) ethanolic extract treated group 10% dose (c); in excision wound models black colour arrow shows re-epithelization indicates newly formed epidermis covering the wounds in (b) and (c); as well as the blue colour arrow shows immature granulation tissue in wound (b) and (c); newly forming blood vessels have been shown better in (b) and (c) as compared to (a).

Table 3. Effect on the epithelization period in days of excision wound model

Treatment	Epithelialization period (in days)	Wound contraction (mm^2/rat) (Mean ± SEM)					Wound closure
		PWD 0	PWD 5	PWD 10	PWD 14	PWD 21	
Control	27.66 ±0.7	404±1.4	364±1.9	288.8±1.2	147±1.5	27±1.5	93.3%
Diabetic control	28.33 ±0.6	406.5±1.4	365.5±1.6	289±1.9	149.8±2.2	23.5±1.2	94%
Standard	21 ± 0.7**	416.8±2.5**	343±1.5 **	250.8±1.4**	89.3±2.8**	0≠0	100%
5% HELEVA	22 ± 0.5**	407.3±1.2	354.5±2.2**	263.3±1.7**	108.1±1.1**	0.7±0.2**	99.8%
10% HELEVA	20.83 ± 0.6**	411.1±1.6*	345.5±2.4**	257.3±1.1**	100.1±1.4**	0.2±0.1**	99.9%

(***P<0.001)(*P<0.05) and **P<0.01. Evaluation by one way ANOVA following Dunnett's assessment, (n = 6)

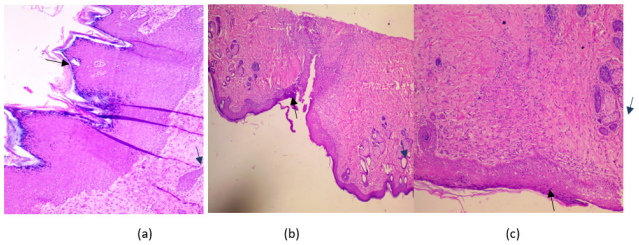

Figure 5. Skin histopathology of control group (a), standard (b), ethanolic extract treated group 10% dose (c), in incision wound models black colour arrow shows re-epithelization indicates newly formed epidermis covering the wounds in (b) and (c) as well as blue colour arrow shows immature granulation tissue in wound (b) and (c) newly forming blood vessels have been shown better in (b) and (c) as compare to (a).

4. Discussion

The healing process is impacted by an under or overactive inflammatory response. Chemotaxis and phagocytosis lead to a reduced ability of wounds to heal and a higher risk of infection in people with Diabetes Mellitus (DM), there is not only an increase in glycemic index level, but also a decrease in permeability and vascular flow, and this affects both the synthesis of collagen fibres and the remodelling of wounded tissue. Increased blood sugar levels have been linked to endothelial damage[14]. Wound healing processes require coordination of overlapping distinct cellular activities, involving phagocytosis, chemotaxis, mitogenesis, angiogenesis, synthesis of collagen and other matrix constituents, and interaction of extracellular matrix molecules, soluble mediators, and various cells that cooperate in the repair procedure[15]. When a wound heals, a series of procedures occur, named epithelialization, wound contraction, collagenization, coagulation, and inflammation[16,17]. Wound contraction and epithelialization are independent of one another and happen concurrently, but coagulation and collagenization are closely connected stages[18,19].

STZ administration was discovered to be sufficient to cause non-insulin-dependent diabetes mellitus (Type 2) in rats. It was also found to be capable of causing peripheral insulin resistance or inhibiting insulin production from pancreatic cells. Additionally, the pancreatic beta-cells of the Islets of Langerhans are harmed by STZ due to unchecked Reactive Oxygen Species (ROS) production, which reduces insulin synthesis and release[20].

To determine the healing capability of consecutive ethanolic extracts of *Vernonia amygdalina* in distinct phases, two distinct wound models were used in the current investigation. The findings of the present investigation show that, when measured by assessing wound contraction using an excision wound model, all subsequent extracts showed wound healing efficacy. The ethanolic extract was chosen for further investigation because it has demonstrably superior wound-healing capability. Increased epithelialization and wound contraction rates higher healed area in the subsequent ethanolic extract results in comparison to the control results support the idea that this can speed up healing[19].

Additionally, it was found that incised wounds had stronger skin than untreated controls following treatment with ethanolic extract. This results in new collagen concentration and fiber stability[21]. Reactive oxygen species and bacteria surrounding a wound had synergistic effects that slow healing. Such infected wounds pose a serious health risk. The results show that the ethanolic extract of leaves repeatedly has strong antibacterial and antioxidant activities.

The results above are further supported by histopathological studies, which show greater epithelialization and collagenization in the extract-treated wounds compared to controls.

According to the preliminary phytochemical examination, the consecutive ethanolic extract contained flavonoids, triterpenoids, steroids, alkaloids, saponins, tannins, and phenolic compounds. These agents speed up the healing process by influencing one or more phases of it. In addition to reducing or delaying the start of cell necrosis, flavonoids have been shown to minimize lipid peroxidation through increasing vascularity. Therefore, it is thought that any medication that prevents lipid peroxidation will boost the survival of collagen fibers by enhancing circulation, reducing cell damage, and fostering DNA synthesis[22]. Because of their antibacterial and free radical scavenging properties, may be flavonoids are also known to speed up wound healing. These properties are thought to be responsible for wound closure and an accelerated rate of epithelialization[23].

5. Conclusion

The hydro-ethanolic extract of leaves has flavonoid as the component, the research work confirms the wound-repairing activity in diabetic rats showed may be due to

the presence of flavonoid found in the plant *Vernonia amygdalina*, as demonstrated by a notable increase in the rate of wound contraction and by enhancing epithelization in STZ-induced diabetic rats. The potential advantage in enhancing wound healing in diabetic rats, maybe owed to the free-radical scavenging activity of the plant. As an alternative to synthetic medicine with side effects, our results showed hydro ethanolic extract treated groups having inhibitory effects on inflammatory cells, appear to be promising wound healing agents. Hence, it is concluded that the *Vernonia amygdalina* has wound-healing activity so could be helpful in patients suffering from diabetes.

6. Acknowledgement

The authors would like to express deepest gratitude to the Noida Institute of Engineering and Technology (Pharmacy Institute) for providing the necessary facilities.

7. References

1. Zulkefli N, Zahari CNMC, Sayuti NH, Kamarudin AA, Saad N, Hamezah HS, Bunawan H, Baharum SN, Mediani A, Ahmed QU, Ismail AF. Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. International Journal of Molecular Sciences. 2023; 24(5):4607. https://doi.org/10.3390/ijms24054607
2. Fulzele SV, Sattuwar PM, Joshi SB, Dorle AK. Wound healing activity of Hingvadya Ghrita in rats. Indian Drugs. 2002; 39(11):606-9.
3. Adly AA. Oxidative stress and disease: an updated review. Res J Immunol. 2010; 3(2):129-45. https://doi.org/10.3923/rji.2010.129.145
4. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. Journal of Ethnopharmacology. 2000; 70(3):343-9. https://doi.org/10.1016/S0378-8741(99)00187-7
5. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 2008; 7(3):1019-24. https://doi.org/10.4314/tjpr.v7i3.14686
6. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum books Limited. Ibadan, Nigeria; 1993. p. 150-6.
7. Rajasree PH, Vishwanad V, Cherian M, Eldhose J, Singh R. Formulation and evaluation of antiseptic polyherbal ointment. International Journal of Pharmacy and Life Sciences. 2012; 3(10).
8. Bostani M, Rahmati M, Mard SA. The effect of endurance training on levels of LINC complex proteins in skeletal muscle fibers of STZ-induced diabetic rats. Scientific Reports. 2020; 10(1):8738. https://doi.org/10.1038/s41598-020-65793-5
9. Yaduvanshi B, Mathur R, Mathur SR, Velpandian T. Evaluation of wound healing potential of topical formulation of leaf juice of Tridax procumbens L. in mice. Indian Journal of Pharmaceutical Sciences. 2011; 73(3):303-6.
10. Neuman RE, Logan MA. The determination of collagen and elastin in tissues. Journal of Biological Chemistry. 1950; 186(2):549-56. https://doi.org/10.1016/S0021-9258(18)56248-7
11. Lee KH. Studies on the mechanism of action of salicylates III. Effect of vitamin A on the wound healing retardation action of aspirin. Journal of Pharmaceutical Sciences. 1968; 57(7):1238-40. https://doi.org/10.1002/jps.2600570736
12. Nayak BS, Pereira LMP. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complementary and Alternative medicine. 2006; 6(1):1-6. https://doi.org/10.1186/1472-6882-6-41
13. Murti K, Kumar U. Enhancement of wound healing with roots of *Ficus racemosa* L. in albino rats. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(4):276-80. https://doi.org/10.1016/S2221-1691(12)60022-7
14. Corrêa AC, Oliveira AK, Dourado DM, Facco GG, Bento LM, Rivero-Wendt CL, Matias R. Pouteria ramiflora leaf extract on emulgel in wound healing activity in diabetic rats. Brazilian Journal of Biology. 2021; 82. https://doi.org/10.1590/1519-6984.239378
15. Güzel S, Özay Y, Kumaş M, Uzun C, Özkorkmaz EG, Yıldırım Z, Ülger M, Güler G, Çelik A, Çamlıca Y, Kahraman A. Wound healing properties, antimicrobial and antioxidant activities of *Salvia kronenburgii Rech. f.* and *Salvia euphratica Montbret, Aucher & Rech. f.* var. euphratica on excision and incision wound models in diabetic rats. Biomedicine and Pharmacotherapy. 2019; 111:1260-76. https://doi.org/10.1016/j.biopha.2019.01.038
16. Arun M, Satish S, Anima P. Herbal boon for wounds. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(2):1-2.
17. Bairy KL, Rao CM. Wound healing profiles of Ginkgo biloba. Journal of Natural Remedies. 2001:25-7.
18. Shirwaikar A, Shenoy R, Udupa AL, Udupa SL, Shetty S. Wound healing property of ethanolic extract of leaves of Hyptissuaveolens with supportive role of antioxidant enzymes. *Indian J Exp Biol*. 2003; 41(3):238-241.
19. Gurung S, Škalko-Basnet N. Wound healing properties of Carica papaya latex: In vivo evaluation in mice burn model. Journal of Ethnopharmacology. 2009; 121(2):338-41. https://doi.org/10.1016/j.jep.2008.10.030

20. Gobinath R, Parasuraman S, Sreeramanan S, Enugutti B, Chinni SV. Antidiabetic and antihyperlipidemic effects of methanolic extract of leaves of Spondias mombin in Streptozotocin-induced diabetic rats. Frontiers in Physiology. 2022; 13:870399. https://doi.org/10.3389/fphys.2022.870399
21. Pravin S, Kale MK, Mahendra G, Nikhil A, Pathak AK. Evaluation of wound healing activity of *Arisaema leschenaultii Blume*. in rats. Der Pharmacia Lettre. 2011; 3(4):200-6.
22. Getie M, Gebre-Mariam T, Rietz R, Neubert RH. Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Sapindaceae). Die Pharmazie. 2002; 57(5):320-2.
23. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. Journal of Ethnopharmacology. 1996; 50(1):27-34. https://doi.org/10.1016/0378-8741(96)85514-0