Wound Healing Activity of *Ipomoea staphylina* Leaves Extract in Wistar Rats

Manjun Lu,[a] Hongchao Yang,[b] Na Yang,[c] Jun Peng,[d] and Xiuli Hou [d,*]

Wound repair is the practice of repairing the skin and other soft tissues after an injury. An inflammatory response is activated after an injury, and cells below the dermis (deepest skin layer) begin to produce more collagen (connective tissue). A scientific assessment was made for the wound-healing potential of *Ipomoea staphylina* leaves extract, using ethanol. The crude extract was made into a 2.5% and 5% (w/w) ointment and tested for wound healing activity in Wistar rats using excision and incision wound models. In the excision wound model, the period of epithelialization was reduced and an increase in wound contraction rate was observed in the extract treated groups (III and IV). On the tenth day, tissue from the excision wound area was taken and processed for the assessment of pro-inflammatory cytokines (TNF-α), and it was found that there was a notable decrease in the TNF-α concentration in the extract-treated animals. In an incision wound model, the breaking strength was significantly increased in animals treated with 2.5% and 5% (w/w) ointment of *I. staphylina* leaves extract. The extract significantly promoted fibroblast migration in an *in vitro* experiment (scratch assay), which may have been caused by the presence of flavonoids.

DOI: 10.15376/biores.19.4.9220-9233

Keywords: Wound healing; Ipomoea staphylina; Ointment; Epithelialization; Excision wound model; Pro-inflammatory cytokines (TNF-α); Incision wound model; Scratch assay

Contact information: a: Plastic and Cosmetic Surgery, Qilu Hospital of Shandong University Dezhou Hospital (Dezhou People's Hospital), Dezhou, 253000, China; b: Department of Pediatric Surgery, Qilu Hospital Of Shandong University Dezhou Hospital (Dezhou People's Hospital), Dezhou, 253000, China; c: Department of Traditional Chinese Medicine, Qilu Hospital of Shandong University Dezhou Hospital (Dezhou People's Hospital), Dezhou, 253000, China; d: Department of Dermatology, Qilu Hospital of Shandong University Dezhou Hospital (Dezhou People's Hospital), Dezhou, 253000, China;
** Corresponding author: dermhou@163.com*

INTRODUCTION

The progression of healing a wound that has been caused to the skin or other soft tissues is known as wound mending. After a lesion, an inflammatory reaction takes place, and the cells below the dermis (the deepest layer of skin) start to produce more collagen (connective tissue). The epithelial tissue, or the outer skin, regenerates later. The three phases of wound healing include proliferation, remodeling, and inflammation (Demidova-Rice *et al.* 2012a). The process also involves a number of other substances, including parenchymal blood cells, extracellular matrix, and soluble mediators (Sen *et al.* 2009). All of these stages must happen in the correct order and within the allotted time for any wound to heal, which is a natural process (Wilkins and Unverdorben 2013). The process of healing wounds will be hindered by any intervention. Both external and local variables, as well as those intrinsic to the wound, might cause interference. Conditions that may affect such

events include the following: body type, underlying chronic conditions, age, desiccation, infection or aberrant bacterial presence, maceration, necrosis, pressure, trauma, and vascular abnormalities (Edet *et al.* 2023).

Conventional medicines, such as synthetic pharmaceuticals and antibiotics, were extensively employed in wound healing in the previous few decades until plant-based medicines were developed. However, these conventional medicines have significant drawbacks when compared to plant-based remedies. Because synthetic substances can be irritating to the skin and body, especially when used consistently over time, the risk of allergic reactions and adverse effects is a big concern (Nayak and Acharjya 2008). It becomes more difficult to treat infections when antibiotic resistance develops due to the long-term use of antibiotics for wound care (Antimicrobial Resistance Collaborators 2022). Full recovery may take longer with conventional treatments, since they prioritize symptom management over holistic healing. However, there is less chance of adverse effects when using plant-based medicines, which typically contain a complex mixture of natural chemicals that aid healing through anti-inflammatory, antioxidant, and antibacterial qualities (Sen and Samanta 2015). The manufacturing and disposal of synthetic pharmaceuticals have a significant negative influence on the environment, while medicines derived from plants are often more sustainable and economical. There needs to be more thorough clinical validation of plant-based medications, though, because their effectiveness might differ.

It has long been recognized that plants have excellent medicinal characteristics including against wound healing, and many natural product studies have produced physiologically active compounds and lead structures for the creation of modified derivatives with increased activity (Saklani and Kutty 2008). Therefore, it is not unexpected that more than 75% of people worldwide rely mostly on plants and plant extracts for their healthcare (Najmi *et al.* 2022; Nwozo *et al.* 2023).

Ipomoea staphylina is a member of the Convolvulaceae family. In addition to various locations in India and China, it is present in Sri Lanka's tropical regions. The leaf extract of this plant contains anti-inflammatory activities (Firdous and Koneri 2012). The plant substances can alleviate ulcers (Banerjee and Firdous 2015) and protect against liver (Al-Haidari *et al.* 2022) and kidney damage (Feng *et al.* 2021). Studies also have shown their pharmacological effectives against ovarian cancer (Xing *et al.* 2022). Sitosteryl-3-O—D-glucoside and chiro deoxy inositol were found to be the main substances present in this plant (Reddy *et al.* 2013). However, there has been no wound healing report of the leaf extract of *I. staphylina*. Hence, in this study an attempt was made to evaluate the wound healing activity of ethanolic extract of *I. staphylina* leaves.

MATERIALS AND METHODS

Plant Procurement and Verification

Ipomoea staphylina stems containing leaves and flowers were taken from district Tumukur of the Karnataka state (Coordinates: 13°20'29.2"N 77°04'35.8"E) in India. The plant was taxonomically identified and confirmed by botanist; a voucher material (code number IS-2022/01) has been preserved at Herbarium of the Institute. It was observed that the leaves are green, broadly ovate, alternate about 12 to 15 cm long, base cordate, apex acute, membranous, and nerves oblique. Flowers are borne in thick clusters, more than a 100 flowers (white with a purple throat) in each bunch, in axillary or subterminal panicles.

Extraction

The *I. staphylina* leaves were powdered into smaller pieces after being air dried at room temperature for two weeks. To obtain extract, the powder was placed into a Soxhlet extractor and extracted using ethanol (99%). Prior to extraction, the powder was subjected to petroleum ether (bp 60 to 80 °C) to separate plant lipids. The yields of the ethanolic extract, which was dried using a Rotary evaporator and kept in a refrigerator, were 6.12% w/w. Analytical grade chemicals used for extraction process.

Ointment preparation

Simple ointment base 300 g was prepared by placing 15 g of hard paraffin in a beaker and melted on electric heater. Then, 15 g of cetostearyl alcohol, 255 g of white petrolatum, and 15 g of wool fat were added. All the ingredients were melted over water bath with constant stirring until they became homogeneous. Finally, 2.5 w/w (7.5 g of extract) and 5% w/w (15 g of extract) concentration ointment of the extract was created using the fusion technique. The necessary amount of ointment base was weighed and melted in a water bath at a temperature of roughly 70 °C (Umeh *et al.* 2014).

Experimental animals

For the investigation, healthy Wistar albino rats of both sexes weighing 200 to 220 g were employed. They lived separately and had unlimited access to a regular pellet meal as well as water. Prior to and during the trial, the animals were routinely weighed. Prior to and throughout the application of the experimental wounds, the rats underwent anesthesia. Every experiment was approved by the institutional animal ethics committee of Qilu Hospital of Shandong University Dezhou Hospital (Dezhou People's Hospital), Dezhou, China (Ref. No. XHX2019-04) in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication 86-23).

Excision wound model

Excision wounds were applied to the rats in accordance with Morton and Malone's descriptions (Morton and Malone 1972). After shaving the animals' dorsal fur with only an electrical clipper, the expected location of the wound was marked with methylene blue using a stainless-steel circle as a stencil. Using a surgical blade, forceps, and scissors, a complete thickness skin excision wound with a circular area of 500 mm^2 and 0.2 cm depth was made following the markings. The wound was left completely open to better understand the wound's response to microbial exposure and how the applied ointment can help prevent infections. This approach also allows the treatments to be directly applied to the wound surface (Pérez-Contreras *et al.* 2022).

The animals were divided into four groups (n=6). The basic ointment base was applied topically to animals in group 1 as a placebo treatment. The 5% w/w providone iodine ointment was applied topically to the animals in group 2 to treat them. The 2.5% and 5% ethanolic extract of *I. staphylina* made in a basic ointment base was applied topically to the animals in group 3 and 4, respectively, until complete epithelization. Using transparency sheets and a permanent marker, the wound was traced on days 0, 2, 4, 8, 12, 16, and 20 after the injury to determine the wound closure rate. Graph paper was used to measure the wound areas that were recorded. The number of days necessary for the eschar to slough off without leaving a visible raw wound determined the epithelialization period (Pérez-Contreras *et al.* 2022).

Cytokine estimation

On the tenth day, tissue from the excision incision region was removed and processed using ELISA kits for the evaluation or tumour necrosis factor (TNF-α). The pH was maintained at 7.40 and a buffer containing 50 mM tris phosphate was made. After that, a 10% w/v skin tissue homogenate was created using this buffer. The homogenate was centrifuged at 3000 rpm for 10 minutes at 4 °C. Following the manufacturer's instructions, the supernatant was gathered and used to quantify TNF-α using a commercially available kit.

Incision wound model

The animals' dorsal fur was clipped with an electrical trimmer. According to Ehrlich and Hunt, a 6-cm-long longitudinal paravertebral incision was performed through the skin and cutaneous muscle of the back. Following the incision, parted skin was stitched by nonabsorbable surgical thread and a curved needle at the intervals of 1-cm. The injuries were not covered. Animals in group 1 received a topical application of the basic ointment base as a control treatment. The animals in group 2 were treated topically with the 5% providone iodine ointment. Animals in groups 3 and 4 received topically applied 2.5% and 5% ethanolic extract of *I. staphylina* prepared in a basic ointment basis, respectively. On the eighth post-wound day, the sutures were removed, and the treatment proceeded. On the tenth day, skin-breaking strength was measured as per the previously reported procedure (Ehrlich and Hunt 1968; Espinosa-Espinosa *et al*. 2022). A line was drawn 3 mm out from the wound on either side of the sedated animal and attached to the table. Forceps were used to grasp this line, one at each end placed in opposition to the other. One of the forceps was firmly supported, while the other was attached to a light-weight metal plate that was hanging freely. Weight was gradually introduced, pulling the margins of the wound apart as it did so. The weights added were measured as a measure of breaking strength in grams and the weight addition was stopped because the wound had just begun to bleed. For a specific incision wound, three readings were taken.

Cytotoxicity assay of I. staphylina leaves extract

The MTT test was used to assess the cytotoxicity of *I. staphylina* leaf extract on L929 cells (Srivastava *et al*. 2017). In summary, L929 cells were cultivated for 12 h after being seeded in a 96-well plate at an initial density of 2×10^4 cells per well per 200µL of DMEM. After applying various amounts of *I. staphylina* leaf extract (as indicated by the findings), the cells were cultured for 48 h at 37 °C with 5% CO_2 supply. Following incubation, 20 µL of 5 mg/mL MTT reagent was applied to the cells, and they were incubated for 2 h in the CO_2 incubator after the spent media was removed. After solubilizing the formazan crystals in 100 µL of DMSO, a microplate reader was used to measure the absorbance at 570 nm. The cells that were only given Dulbecco's Modified Eagle Medium (DMEM) were regarded as 100% viable negative controls. Using the following formula, the percentage of cell viability was determined:

$$\%\ viability = \frac{OD\ of\ test}{OD\ of\ negitive\ control} X100 \quad (1)$$

Plotting the percentage of cell viability *versus* test sample concentrations was done. The findings were provided as mean ± SD (n = 3) after three sets of tests were performed in triplicate.

Scratch assay

In vitro cell migration experiments using L929 cells were used to test the *I. staphylina* leaf extract's capacity for wound healing (Liang *et al.* 2007). In brief, 6-well plates were seeded with 2 x 10^5cells per mL and cultivated for a whole night. After that, the cells were cleaned using Delbucco's Phosphate Buffered Saline (DPBS) and a sterile 200µL tip was used to make a scratch. The cells were washed with DPBS to remove the detached cells and other cellular debris. After applying 125 µg/mL of *I. staphylina* leaf extract and 5 µg/mL of povidone iodine as a positive control, the cells were cultured for a full day. One common medication used in wound healing is povidone iodine (James and Friday 2010). The negative controls were untreated cells. There were three replicates of the tests conducted. Using Image J software, the breadth of the scratch and wound closure at various time intervals (0, 12, 24, and 48 h) was examined.

Data analysis

Six animals from each group were used to calculate the results, which were presented as the mean (average) ± standard error of mean (SEM). The groups were separated using a one-way analysis of variance (ANOVA), which was followed by a Tukey's Multiple Comparison test in Graph Pad Prism version 8. 1 software. The P value was considered statistically significant if it was less than 0.05. In this test, the differences between each pair of means are compared while accounting for additional or repeated testing.

RESULTS

Excision Wound Model

Figure 1 represents wound area measurement at different days in all four groups. When compared to the rats given with placebo controls, the animals treated with *I. staphylina* extract ointments 2.5% and 5% showed a substantial improvement in wound-healing activity. Animals treated with the extract displayed a considerable decrease in the wound area and epithelialization period in the excision wound model. The complete epithelialization in group IV animals was achieved at day 18. Animals of group II and III achieved complete epithelialization at day 20. Hence, the 5% ointment of *I. staphylina* extract (group IV) showed rapid epithelialization of the wound when compared with 5% providone iodine ointment (group II) animals.

Table 1 shows the percentage epithelialization of the wound in different groups. From the data of wound area (in mm^2), the percentage epithelialization of the wound in different groups was calculated. The authors found that *I. staphylina* extract (2.5% and 5% ointment) showed improvement in the epithelialization in a dosage-dependent manner. The 5% ointment was found to be more potent when compared with 2.5% ointment of the extract as 5% ointment showed 100% epithelialization on day 18. Further, the 2.5% ointment of the extract was found equipotent to 5% providone iodine ointment in regards to percentage epithelialization and 5% ointment of the extract showed much more prominent effect.

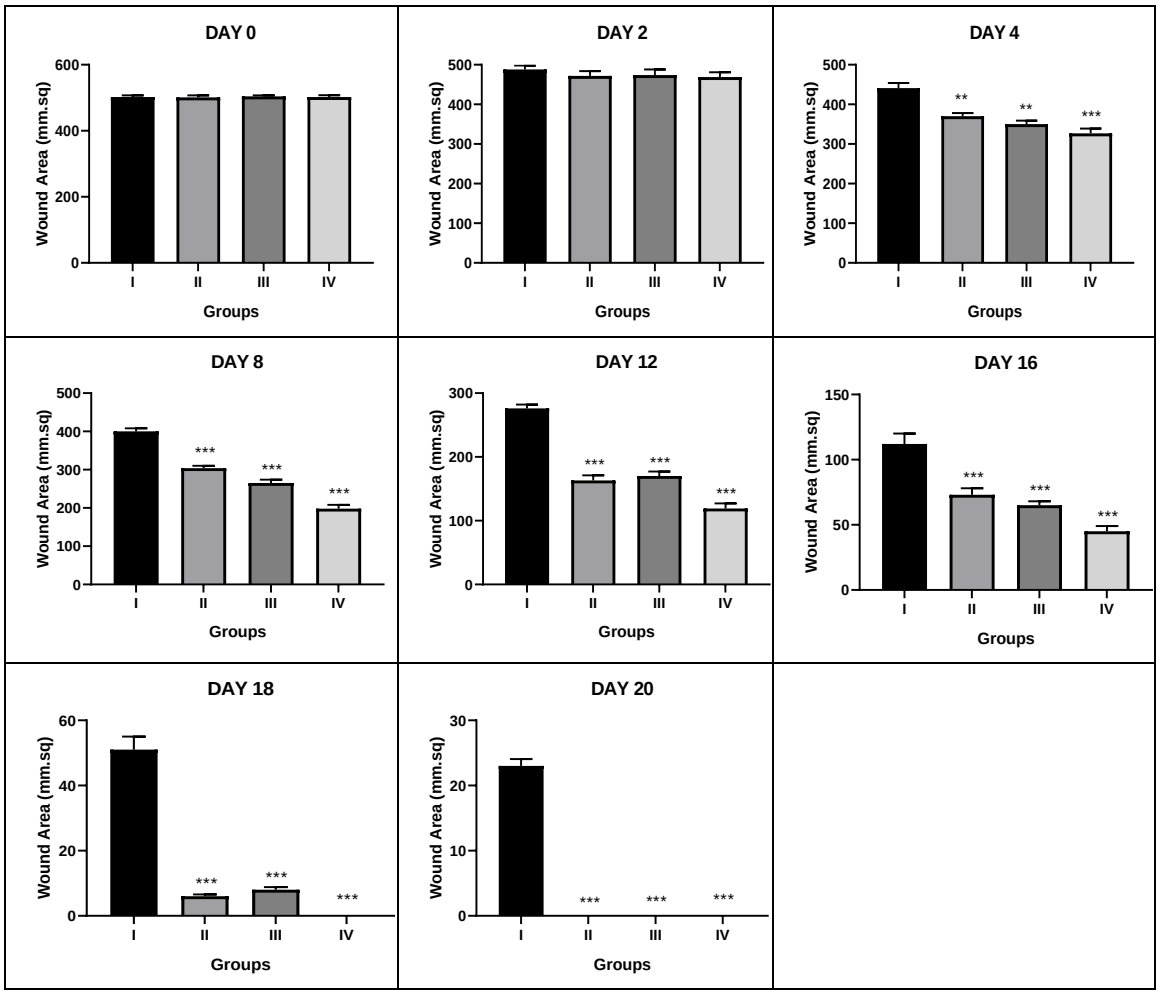

Fig. 1. Wound healing effect of ethanolic extract of *I. staphylina* leaves (wound area measurement at different days) in excision wound model
Values are represented as mean ± SEM; it was found that **P>0.01 and ***P>0.001 when group II, III, and IV were compared with group I.

Table 1. Effect of Ethanolic Extract of *I. staphylina* Leaves of Percentage Epithelialization

Groups	Percentage Epithelialization							
	Day 0	Day 2	Day 4	Day 8	Day 12	Day 16	Day 18	Day 20
I	0	2.97	10.51	22.61	46.42	76.19	89.88	95.04
II	0	6.37	30.27	42.62	68.12	85.65	98.80	100
III	0	6.36	36.38	46.32	67.19	85.88	98.40	100
IV	0	6.95	40.35	60.43	82.70	92.04	100	100

It is known that TNF-α, which is released by macrophages during the proliferative stage of wound healing, promotes the development of the extracellular matrix in injured tissues by stimulating fibroblasts to produce proteoglycan and fibronectin.

Based on this concept, on the tenth day, tissue from the excision incision region was removed and processed using ELISA kits for the evaluation of pro-inflammatory cytokine (Fig. 2). It was found that in group I, the level of TNF-α was high compared with other groups (II, III, IV). Both the 2.5% and 5% ointment of the extract showed a notable decrease in the TNF-α concentration when compared with group I. Moreover, in 5%

providone iodine ointment-treated animals (group II), a notable decrease in TNF-α concentration was also found when compared with group I. However, 5% ointment of the extract showed a considerable increase in the TNF-α level.

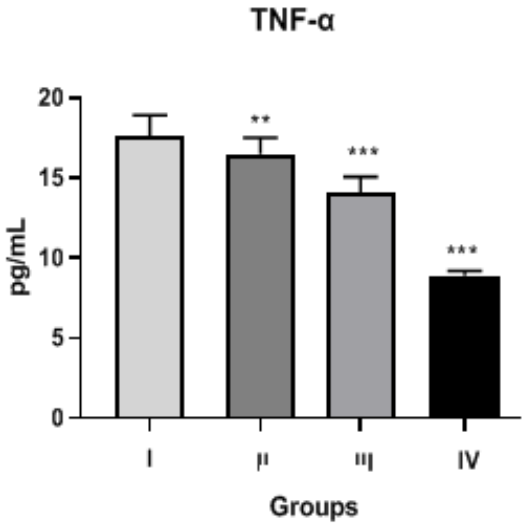

Fig. 2. Effect of ethanolic extract of *I. staphylina* leaves on proinflammatory cytokine TNF-α in excision wound model
Values are represented as mean ± SEM; it was found that ***P>0.001 when group II, III, and IV were compared with group I.

Incision Wound Model

In comparison to the control (group I), the 5% crude extract treatment group's tensile strength was significantly higher. Tensile strength was increased by 40.0% and 42.5% in the 2.5% and 5% ointment of the extract treated groups, respectively, in comparison to group I.

Table 2. Wound Healing Effect of Ethanolic Extract of *I. staphylina* Leaves in Incision Wound Model

Parameter	Groups			
	I	II	III	IV
Skin-breaking or Tensile strength (g)	263.28±4.12	446.74±7.86***	438.46±5.91***	458.19±4.24***
% increase of Tensile strength	-	41.1	40.0	42.5

Values are represented as mean ± SEM; it was found that ***P>0.001 when group II, III, and IV were compared with group I

Cytotoxicity Assay of *I. staphylina* Leaves Extract

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to evaluate the cytotoxic impact of *I. staphylina* leaf extract on L929 cells. The test compound's various concentrations were applied to the cells for 48 h, during which time the cytotoxic potential of the extract was assessed. At 31.25 and 62.50 µg/mL treatment concentrations of *I. staphylina* leaf extract, the percentage viability of L929 cells was reported to be 99.357±0.23 and 98.51±0.29. Table 3 lists the *I. staphylina* leaf extract

concentrations that were employed in the cell migration assay at doses of 31.25 and 62.50 µg/mL.

Table 3. Outcome of *I. staphylina* Leaves Extract on Viability of L929 Cells.

Extract (µg/mL)	% Viable Cells
31.25	99.357±0.23
62.5	98.51±0.29
125	88.29±0.31
250	63.98±0.48
500	48.12±0.35
1000	26.40±0.25

Data are shown in a triplicate manner (mean±SEM)

Scratch Assay

The main processes in wound healing, which involve a variety of cell types as well as other microenvironmental variables, are fibroblast activation, proliferation, and migration. An extensively used *in vitro* method for determining a compound's capacity to heal wounds is the scratch test. In the present investigation, 48 h were spent treating L929 cells with doses of *I. staphylina* extract of 31.25 and 62.50 µg/mL.

Table 4. Percentage of Cells Migrated Towards the Wound

Treatment	Concentrations	% Cell Migration			
		0 h	12 h	24 h	48 h
Vehicle control	-	0	11.35 ± 0.93	25.04 ± 1.35	42.73 ± 1.81
Providone iodine	5 µg/mL	0	40.26 ± 1.12	73.39 ± 1.74	94.51 ± 1.97
I. staphylina extract	31.25 µg/mL	0	29.38 ± 1.08	51.65 ± 1.03	75.54 ± 1.72
I. staphylina extract	62.50 µg/mL	0	37.62 ± 1.51	66.31 ± 1.23	82.92 ± 1.86

Data are shown in a triplicate manner (mean±SEM).

Image J software was used to determine the wound closure distance after cell migration was recorded at 0, 12, 24, and 48 h. The findings showed that after 48 h, *I. staphylina* leaf extract at 31.25 µg/mL filled the hole left by the scratch 75.54±1.72%. Conversely, *I. staphylina* leaf extract, at 62.50 µg/mL, filled up the scratch's gap by 82.92±1.86%in 48 h. Table 4 shows the percentage of wound closure in untreated, extract-treated, and control drug-treated cells at various time periods. *I. staphylina* leaf extract caused L929 cells to migrate, which closed the lesion. In the cells treated with conventional medication (5 µg/mL), 94.51±1.97%of the gap had healed after 48 h (Table 4).

DISCUSSION

Two models, including excision and incision wound models, were used to assess the plant extract's ability to cure wounds. Excision wound models measured factors, such as initial wound contractions, epithelialization time, and TNF-α concentration, while incision wound models measured parameters such as tensile strength. The ability of the treatments to speed up wound healing was shown by improvements in wound contraction

rate, quick epithelialization, a positive rise in the inflammatory cytokine (TNF-α), and an increase in tensile strength.

A reduction in the percentage of the initial wound size represents wound contraction, which aids in wound closure. Contraction is an important factor in healing because it reduces the size of the wound and speeds up recovery. Additionally, by shortening the distance that migratory keratinocytes must travel, contraction lessens the amount of extracellular matrix required to heal the defect and aids in re-epithelization (Mukherjee *et al.* 2000; Collier 2004).

Both 2.5% and 5% ointments of the extract resulted in a dose-dependent increase in wound contraction rate and a reduction in epithelialization periods in the excision wound model. This might be connected to the bioactive component concentration in the extract ointment. The study's results demonstrated unequivocally that the 2.5% and 5% ointments of the extract substantially promoted wound healing in the excision wound model as contrasted to the group that received simple ointment base treatment (group I). On day 12, the 5% extract ointment had a much better impact on wound healing than the 5% providone iodine ointment-treated animals (group II). On day 16, the 5% extract ointment-treated group showed substantial healing with a percentage wound closure of 92.02%; on day 18, it was 100%.

The presence of flavonoids in the extract may explain why animals treated with it experienced a faster rate of wound contraction and a shorter duration of epithelialization. By preventing both cyclooxygenase and lipoxygenase activities, flavonoids lessen the production of inflammatory metabolites. Additionally, flavonoids stop neutrophil degranulation, a direct method of reducing the release of arachidonic acid by neutrophils and other immune cells, which contributes to the extract's ability to heal wounds (Nijveldt *et al.* 2001; Mulisa *et al.* 2015). This increased rate of wound contraction and shorter time needed for full epithelialization suggest that the extract of *I. staphylina* leaves is more effective at promoting wound healing. These might be connected to the individual or combined actions of the bioactive compounds found in the plant extract. It might be possible that the wound healing effect of the extract of *I. staphylina* leaves may be due to presence of flavonoids.

Herbal extracts may speed up the healing process of wounds by promoting fibroblast proliferation and anti-inflammatory activities during the healing process. Based on this context the concentration of TNF-α was measured on day 10 in excision wound model. The production of collagen by the fibroblasts that are multiplying and being stimulated by TGF-β further improves this process. Thus, proteases secreted by macrophages and fibroblasts play a key role in the conversion of pro-transforming growth factor-beta (TGF-β), which fibroblasts synthesize, into bioactive TGF-β (Ritsu *et al.* 2017). In the current study, it was found that 2.5% and 5% ointment of the extract showed a notable decrease in the TNF-α concentration when compared with group I. Hence, the wound healing activity of the extract ointment of *I. staphylina* leaves may be due to acceleration of the inflammatory phase of wound healing.

Tensile strength measures how well the tissue under repair resists tension and can reveal the tissue's quality. A wound's tensile strength is primarily influenced by an increase in collagen concentration and fiber stability. In comparison to ointment base-treated group (group 1), the tensile strength of both the 2.5% and 5% extract ointment treated groups (III and IV) was considerably higher. It has been reported that in addition to their ability to reduce proteins due to their astringent properties, tannins and other phytochemicals like them also encourage wound contraction, the growth of capillary vessels and fibroblasts,

and the healing of open wounds. Additionally, flavonoids reduce lipid peroxidation by stopping or delaying cell necrosis and enhancing vascularity, which raises the viability of collagen fibrils by boosting circulation, minimizing cell damage, and encouraging DNA synthesis (Demidova-Rice *et al.* 2012b; Odukoya *et al.* 2012; Dutt *et al.* 2023). Preliminary phytochemical study showed that *I. staphylina* leaves contain flavonoids and polyphenolic compounds. Thus, it can be said that an increase in the tensile strength may be due to the presence of tannins or flavonoids in *I. staphylina* leaves extract.

The MTT assay was also used to evaluate the extract's cytotoxicity. The cytotoxicity test is predicated based on the notion that assessing a biological material's biological and therapeutic significance can be aided by early toxicity screening. Evaluating the plant extract's cytotoxic effects on cells or an *in vivo* model is essential because certain plant metabolites may be harmful to cells due to intermolecular interactions inside the cell. A measurement of the half maximum inhibitory concentration (IC_{50}) value provides evidence for this effect. A low IC_{50} value indicates the extract's capacity to be cytotoxic at lower dosages, whereas a high IC_{50} value indicates that a large concentration of the extract is necessary to have a negative impact on the cell. The test compound's various concentrations were applied to the cells for 48 h, during which time the extract's cytotoxic potential was assessed. At 31.25 and 62.50 µg/mL treatment concentrations of *I. staphylina* leaf extract, the percentage viability of L929 cells was reported to be 99.357% ± 0.23 and 98.51% ± 0.29.

Table 3 lists the *I. staphylina* leaf extract concentrations that were employed for therapy along with the associated percentage of cell viability. Based on these findings, the extract's cytotoxic effects were evaluated at doses of 31.25 and 62.50 µg/mL, which were also utilized to evaluate the extract's ability to promote wound healing. Additionally, the IC_{50} level of 30 µg/mL concentration has been determined by the American National Cancer Institute (NCI) for the extract to be deemed toxic to the cells (Rosidah and Hasibuan 2014). In the current investigation, L929 cells were subjected to extract at various doses far closer to these suggested limits, and at 31.25 and 62.50 µg/mL concentrations, the percentage viability of the cells was largely unaffected. This supports our assertion that *I. staphylina* extracts may be utilized to treat wounds without raising concerns about toxicity. However, the range of these assertions is restricted to the greatest extract concentration and the kind of cell line employed in the investigation.

In vitro studies show that wound healing is correlated with cell migration, which also accounts for the wound closure rate. One simple way to quantify cell migration *in vitro* is to use the *in vitro* scratch assay (Pekmez and Milat 2020). Consequently, a wound region was created and tested extract treatments *in vitro* using the *in vitro* scratch assay. Cell migration and proliferation are essential for wound healing, particularly during re-epithelization, as the quickly proliferating fibroblasts will produce enough cells to fill the wound site and travel quickly (Qing 2017). The results indicate that both extract concentrations exhibited a substantial cell proliferative activity over the spectrum of tested doses.

Reports indicate that flavonoids induce fibroblast migration to facilitate wound healing. For instance, flavonoid C-glycosides produced from oil palm leaves promote the migration and proliferation of fibroblast cells (Zain *et al.* 2020). This finding provides a more profound understanding of the correlation between the wound closure activity of the extract used in the present investigation and this phenomenon. Thus, the study of wound closure potentially serves as a valuable tool for acquiring knowledge about natural remedies that accelerate the process of wound healing.

CONCLUSION

1. The excision and incision wound models both improved with the use of the *I. staphylina* leaf extract. In the excision wound model, the rate of epithelialization increased, while the healing time decreased.
2. An *in vitro* experiment (scratch assay) revealed that the extract induced a significant migration of fibroblasts, may be attributed to the presence of flavonoids. Preliminary phytochemical study established that *I. staphylina* leaves contain flavonoids and polyphenolic compounds.
3. Therefore, it may be concluded that the extract from *I. staphylina* leaves may include tannins or flavonoids, which could be responsible for wound healing activity.

Author's Contribution

B.Y. - Supervision; S.M.F. - Experiment and Supervision, A.A.A.E.S. - Statistic; W.S. - Review and Writing Manuscript; R.A.A.H. - Data Curation; A.A.- Experiment and Statistic; M.A.H.M. - Writing and Results Analysis

REFERENCES CITED

Al-Haidari, R. A., Sayeed Mohammed, F., Alhaddad, A., Samman, W. A., Aa El-Sayed, A., Ah Abdellatif, A., and Ah Mostafa, M. (2022). "Hepatoprotective activity of Ipomoea staphylina against d-glan/lps-induced acute hepatic failure in experimental rats," *Pakistan Journal of Pharmaceutical Sciences* 35(6), 1663-1668.

Antimicrobial Resistance Collaborators (2022). "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis," *Lancet* (*London, England*) 399(10325), 629-655. DOI:10.1016/S0140-6736(21)02724-0

Banerjee, A., and Firdous, S. M. (2015). "Antiulcer activity of hydroalcoholic extract of *Ipomoea staphylina* plant in rats," *Bangladesh Journal of Pharmacology* 10(3), 652-653. DOI: 10.3329/bjp.v10i3.24034

Collier, M. (2004). "Recognition and management of wound infections," (http://www.worldwidewounds.com/2004/january/Collier/Management-of-Wound-Infections.html).

Demidova-Rice, T. N., Durham, J. T., and Herman, I. M. (2012a). "Wound healing angiogenesis: Innovations and challenges in acute and chronic wound healing," *Advances in Wound Care* 1(1), 17-22. DOI:10.1089/wound.2011.0308

Demidova-Rice, T. N., Hamblin, M. R., and Herman, I. M. (2012b). "Acute and impaired wound healing: Pathophysiology and current methods for drug delivery, Part 1: Normal and chronic wounds: Biology, causes, and approaches to care," in: *Advances in Skin and Wound Care* 25(7), 304-314. DOI: 10.1097/01.ASW.0000416006.55218.d0

Dutt, Y., Pandey, R. P., Dutt, M., Gupta, A., Vibhuti, A., Raj, V. S., Chang, C. M., and Priyadarshini, A. (2023). "Silver nanoparticles phytofabricated through *Azadirachta indica*: Anticancer, apoptotic, and wound-healing properties," *Antibiotics* 12(1), 121. DOI:10.3390/antibiotics12010121

Edet, U. O., Mbim, E. N., Ezeani, E., Henshaw, O. U., Ibor, O. R., Bassey, I. U., Asanga, E. E., Antai, E. E., Nwaokorie, F. O., Edet, B. O., Bebia, G. P., Tega, C., Mboto, C. I., Nkang, A., and Nneoyi-Egbe, A. F. (2023). "Antimicrobial analysis of honey

against *Staphylococcus aureus* isolates from wound, ADMET properties of its bioactive compounds and in-silico evaluation against dihydropteroate synthase," *BMC Complementary Medicine and Therapies* 23(1), article 39. DOI: 10.1186/s12906-023-03841-z

Ehrlich, H. P., and Hunt, T. K. (1968). "Effects of cortisone and vitamin A on wound healing," *Annals of Surgery* 167(3), 324-328. DOI:10.1097/00000658-196803000-00004

Espinosa-Espinosa, L., Garduño-Siciliano, L., Rodriguez-Canales, M., Hernandez-Portilla, L. B., Canales-Martinez, M. M., and Rodriguez-Monroy, M. A. (2022). "The wound-healing effect of mango peel extract on incision wounds in a murine model," *Molecules* 27(1), article 259. DOI: 10.3390/molecules27010259

Feng, H., Feng, Q., Xiao, T., Liu, T., Guan, B., Firdous, S. M., and Huang, J. (2021). "*Ipomoea staphylina* attenuates potassium dichromate-induced nephrotoxicity in Wistar rats via antioxidant and antiapoptotic effects," *Doklady. Biochemistry and Biophysics* 499(1), 289-295. DOI: 10.1134/S1607672921040074

Firdous, S. M., and Koneri, R. (2012). "*In vivo* and *in vitro* antiinflammatory activity of leaves of *Ipomoea staphylina*," *International Journal of Pharmacy and Pharmaceutical Sciences* 4(SUPPL. 5), 339-343.

James, O., and Friday, E. T. (2010). "Phytochemical composition, bioactivity and wound healing potential of *Euphorbia heterophylla* (Euphorbiaceae) leaf extract," *International Journal on Pharmaceutical and Biomedical Research* 1(1).

Liang, C. C., Park, A. Y., and Guan, J. L. (2007). "*In vitro* scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro," *Nature Protocols* 2(2), 329-333. DOI: 10.1038/nprot.2007.30

Mukherjee, P. K., Verpoorte, R., and Suresh, B. (2000). "Evaluation of in-vivo wound healing activity of *Hypericum patulum* (Family: Hypericaceae) leaf extract on different wound model in rats," *Journal of Ethnopharmacology* 70(3), 315-321. DOI: 10.1016/S0378-8741(99)00172-5

Mulisa, E., Asres, K., and Engidawork, E. (2015). "Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* J. (Polygonaceae) in mice," *BMC Complementary and Alternative Medicine* 15(1), article 341. DOI: 10.1186/s12906-015-0878-y

Najmi, A., Javed, S. A., Al Bratty, M., and Alhazmi, H. A. (2022). "Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents," *Molecules* 27(2), article 349. DOI: 10.3390/molecules27020349

Nayak, S., and Acharjya, B. (2008). "Adverse cutaneous drug reaction," *Indian Journal of Dermatology* 53(1), 2-8. DOI: 10.4103/0019-5154.39732

Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E. C., Boelens, P. G., Van Norren, K., and Van Leeuwen, P. A. M. (2001). "Flavonoids: A review of probable mechanisms of action and potential applications," *American Journal of Clinical Nutrition* 74(4), 418-425. DOI:10.1093/ajcn/74.4.418

Nwozo, O. S., Effiong, E. M., Aja, P. M., and Awuchi, C. G. (2023). "Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review," *International Journal of Food Properties* 26(1), 359-388. DOI: 10.1080/10942912.2022.2157425

Odukoya, O. A., Sofidiya, M. O., Samuel, A. T., Ajose, I., Onalo, M., and Shuaib, B. (2012). "Documentation of wound healing plants in Lagos-Nigeria: Inhibition of lipid

peroxidation as *in-vivo* prognostic biomarkers of activity," *Annals of Biological Research* 3(4), 1683-1689.

Pekmez, M., and Milat, N. S. (2020). "Evaluation of *in vitro* wound healing activity of thymoquinone," *European Journal of Biology* 79(2), 151-156. DOI: 10.26650/EurJBiol.2020.0044

Pérez-Contreras, C. V., Alvarado-Flores, J., Orona-Ortiz, A., Balderas-López, J. L., Salgado, R. M., Zacaula-Juárez, N., Krötzsch, E., and Navarrete, A. (2022). "Wound healing activity of the hydroalcoholic extract and the main metabolites of *Amphipterygium adstringens* (cuachalalate) in a rat excision model," *Journal of Ethnopharmacology* 293, article 115313. DOI: 10.1016/j.jep.2022.115313

Qing, C. (2017). "The molecular biology in wound healing & non-healing wound," *Chinese Journal of Traumatology - English Edition* 20(4), 189-193. DOI: 10.1016/j.cjtee.2017.06.001

Reddy, D. P., Kota, R., Renuka, S., Anarthe, S. J., and Raghavendra, N. M. (2013). "Isolation, characterization of phytoconstituents and pharmacological screening of *Ipomoea staphylina*," *Asian Journal of Pharmaceutical and Clinical Research* 6(1), 30-33.

Ritsu, M., Kawakami, K., Kanno, E., Tanno, H., Ishii, K., Imai, Y., Maruyama, R., and Tachi, M. (2017). "Critical role of tumor necrosis factor-α in the early process of wound healing in skin," *Journal of Dermatology & Dermatologic Surgery* 21(1), 14-19. DOI: 10.1016/j.jdds.2016.09.001

Rosidah, and Hasibuan, P. A. Z. (2014). "Cytotoxic effect of n-hexane, ethylacetate and ethanol extracts of *Plectranthus amboinicus*, (Lour.) Spreng.) on HeLa and vero cells lines," *International Journal of Pharm. Tech. Research* 6(6), 1806-1809.

Saklani, A., and Kutty, S. K. (2008). "Plant-derived compounds in clinical trials," *Drug Discovery Today* 13(3–4), 161-171. DOI: 10.1016/j.drudis.2007.10.010

Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K., Gottrup, F., Gurtner, G. C., and Longaker, M. T. (2009). "Human skin wounds: A major and snowballing threat to public health and the economy: Perspective article," *Wound Repair and Regeneration* 17(6), 763-771. DOI:10.1111/j.1524-475X.2009.00543.x

Sen, T., and Samanta, S.K. (2014). "Medicinal plants, human health and biodiversity: A broad review," in: *Biotechnological Applications of Biodiversity*, J. Mukherjee (ed.), *Advances in Biochemical Engineering/Biotechnology*, Vol. 147. Springer, Berlin, Heidelberg. DOI: 10.1007/10_2014_273

Srivastava, J. K., Pillai, G. G., Bhat, H. R., Verma, A., and Singh, U. P. (2017). "Design and discovery of novel monastrol-1,3,5-triazines as potent anti-breast cancer agent *via* attenuating epidermal growth factor receptor tyrosine kinase," *Scientific Reports* 7(1), article 5851. DOI: 10.1038/s41598-017-05934-5

Umeh, V., Ilodigwe, E., Ajaghaku, D., Erhirhie, E., Moke, G., and Akah, P. (2014). "Wound-healing activity of the aqueous leaf extract and fractions of *Ficus exasperate* (Moraceae) and its safety evaluation on albino rats," *Journal of Traditional and Complementary Medicine* 4(4), 246-252. DOI:10.4103/2225-4110.139105

Wilkins, R. G., and Unverdorben, M. (2013). "Wound cleaning and wound healing: A concise review," *Advances in Skin and Wound Care* 26(4), 160-163. DOI: 10.1097/01.ASW.0000428861.26671.41

Xing, L., He, L., Zhang, T., Ruan, H., Zhang, Y., Li, Y., and Mohammed, F. S. (2022). "Evaluation of the effects of *Ipomoea staphylina* on ovarian cancer cell line," *Pakistan Journal of Pharmaceutical Sciences* 35(1), 49-52.

Zain, M. S. C., Lee, S. Y., Sarian, M. N., Fakurazi, S., and Shaari, K. (2020). "*In vitro* wound healing potential of flavonoid c-glycosides from oil palm (*Elaeis guineensis* Jacq.) leaves on 3t3 fibroblast cells," *Antioxidants* 9(4), article 326. DOI: 10.3390/antiox9040326

Article submitted: June 23, 2024; Peer review completed: July 31, 2024; Revised version received and accepted: September 7, 2024; Published: October 16, 2024.
DOI: 10.15376/biores.19.4.9220-9233