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WOUND HEALING POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *SISYMBRIUM IRIO*

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ABSTRACT -A wound is the loss of the normal integrity, structure, and functions of the skin due to a physical, chemical, or mechanical agent. Wound repair consists of an orderly and complex process divided into four phases: coagulation, inflammation, proliferation, and remodeling. The potential of *sisymbrium irio* in the treatment of wounds has been reported in numerous studies, emphasizing those with antioxidant, anti-inflammatory, and antimicrobial properties, e.g., alkaloids, saponins, terpenes, essential oils, and these compounds can interact in the various stages of the wound healing process. This research work addresses the most current in vitro and in vivo studies on the wound healing potential of *sisymbrium irio*, as well as the main mechanisms involved in this activity. We observed sufficient evidence of the activity of these compounds in the treatment of wounds; however, we also found that there is no consensus on the effective concentrations in which the *sisymbrium irio* exert this activity. For this reason, it is important to work on establishing optimal treatment doses, as well as an appropriate route of administration.

KEYWORDS: *Sisymbrium irio*, Wound Healing, Proliferation, antioxidant, Proliferation, Remodeling Inflammation

INTRODUCTION

Various treatments are employed to address the process of wound healing. Various treatments have been applied both locally and systemically to promote wound healing. Antibiotics and antiseptics, desloughing agents (chemical debridement, such as hydrogen peroxide, eusol, and collagenase ointment), wound healing promoters, certain materials like tissue extracts, vitamins, and minerals, and a variety of plant products are among the various agents used to promote wound healing. Medicinal plants speed up the healing process of wounds by encouraging blood clotting, thwarting infection, and improving wound healing. It is true that plants and plant-derived chemicals enhance care and control the healing of wounds. Medicinal plants have the ability to heal wounds through a variety of mechanisms, including modulation of wound healing, reduction of bacterial count, and enhancement of collagen (Gupta and Jain, 2010).

Some important phytochemical wound healing activity

- Asiatocide
- Chlorogenic Acid

- Quercetin
- Gallic acid

PLANT PROFILE

Sisymbrium irio :Plants have shown a vital role in curing the human diseases all throughout the world. One of the cruciferae members named sisymbrium isused in treatment of rheumatoid, voice disorder inflammation etc. Few members also showed anti-microbial, antioxidant, analgesic and antipyretic activities. Researches done throughout the world revealed that this particular genus is characterized by presence of various metabolites such as, steroid, oil, anthraquinone, alkaloid and flavonoid (Al-Jaber, 2011). *Sisymbrium irio* is one of the 90 species of the genus, which is found in various part of the world Sisymbrium genus has four well known invasive species they are *S. irio, S. officinale, S. orientale* and *S. altissimum*. *S. irio* has been listed for official medicinal plant use in both Ayurveda and Unani therapies. Seeds of *S. irio* are utilized as Febrifuge, expectorant and against voice disorders. It has been reported that *S. irio* is used in the treatment of rheumatism, inflammation, antimicrobial, antipyretic, analgesic and antioxidant activity. *S. irio* is also used in some places for dietary purposes. In a study *Sisymbrium irio* seeds were subjected to instrumental neutron activation for their elemental analysis, as a result the seeds were found to contain high levels of Iron, Manganese and sodium**.**

Figure 1: Seeds of *S. irio*

World-wide distribution

Sisymbrium irio is indigenous species of North Africa, temperate Asia and Europe but it has been transferred by migrants to South Africa, North America and Australia, *Sisymbrium irio* L is a polytypic plant and it grows during winters in Punjab plains. It is found in different ploidy levels such as 2n, 3n, 4n, 6n, 8n. All these races are highly effected and modified in response of changing amount of sunlight and moisture content of the soil (Akhtar *et al.*, 2020).

Pharmacological activities of *Sisymbrium irio* L

Antibacterial and antifungal activities

Crude ethanolic extracts of *S. irio* seeds showed antibacterial activities against both gram negative (*Salmonella typhi* and *Salmonella paratyphi* A) and gram positive (*Staphylococcus aureus*) bacterial strains (Akhtar *et al.*, 2020). The antimicrobial activities of the five compounds isolated from the methanolic extracts of the roots of *S.irio* was determined and found to be active antimicrobials against 3 Gram positive bacteria (*Staphylococcus*

aureus, Bacillus subtilis and *B.pumilus*), Gram-negative bacteria (*Pseudomonas aeruginosa, Proteus vulgaris* and *E.coli*) and against a yeast.

Antioxidant activity

This plant exhibits significant antioxidant properties. A comparative antioxidant activities of different part of *S.irio* extracts dissolved in different solvent was determined. It was found out that the aqueous, butanol and ethylacetate extracts of flowers, stems and leaves showed high levels of antioxidant activities in comparison of others extracts used in the study.

Antipyretic Activity

The seeds of the *S. irio* had proven to have marked Antipyretic effects. In a study the antipyretic activity of the crude ethanolic extracts of seeds of *S.irio* was investigated by using the technique that is yeast induced pyrexia in rats. The significant antipyretic effects were shown (onset: less than 1 hour, peak: at 3 hours, duration: less than 5 hours (Al-Jaber, 2011).

Analgesic effects

Analgesic is an agent to reduce pain. It has been reported that the *S.irio* has profound analgesic effects. In a study, the analgesic effects of seeds of *S.irio* were investigated.The technique of writhing in mice induced by acetic acid was used to test the analgesic activity. Ethanolic and sodium salicylate extracts made from the seeds of *S.irio* showed marked analgesic effects in comparison to other extracts used in the study (Akhtar *et al.*, 2020).

Anti-inflammatory effects

Plants and their chemical constituents are used as anti-inflammatory agents in traditional medicine. The anti-inflammatory effect of crude ethanolic extract of *Sisymbrium irio* seeds were tested on albino rats (Wistar strain) using cotton pellet granuloma assay. The dose of 100mg/kg and 200mg/kg, p.o for 7days of crude extract was taken in a study, while Diclofenac and double distilled water were used as standard and control respectively. As a result, *Sisymbrium irio* seeds showed marked antiinflammatory effects at 100-200mg/kg (Al- Mazroa *et al.,* 2015)

MATERIALS AND METHODS

1. Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Seeds of *Sisymbrium irio* were collected from local market

2. Drying

Drying of fresh plant parts was carried out in sun but under the shade.

3. Extraction by soxhlation extraction process

Procedure was adopted for the preparation of extracts from the shade dried and powdered herbs.

4. Phytochemical Screening

Detection of alkaloids,carbohydrates, glycosides, saponins, phenols, tannins,flavonoids etc.

5. Quantitative study of bioactive compound

The estimation of total alkaloid content is an essential step in evaluating the bioactive potential of plant extracts, as alkaloids are known for their pharmacological properties, including analgesic, antipyretic, and anti-inflammatory effects.

6. In vitro antioxidant activity using nitric oxide method

The in vitro antioxidant activity assay using nitric oxide (NO) scavenging is widely used to evaluate the free radical scavenging capacity of plant extracts and other natural compounds.

7. Wound healing activity of ethanolic extract of *Sisymbrium irio*

7.1 Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%).

7.2 Experimental model

Excision wounds were created after shaving the left dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear

7.3 Data Analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one- way ANOVA followed by Dunnet's test.

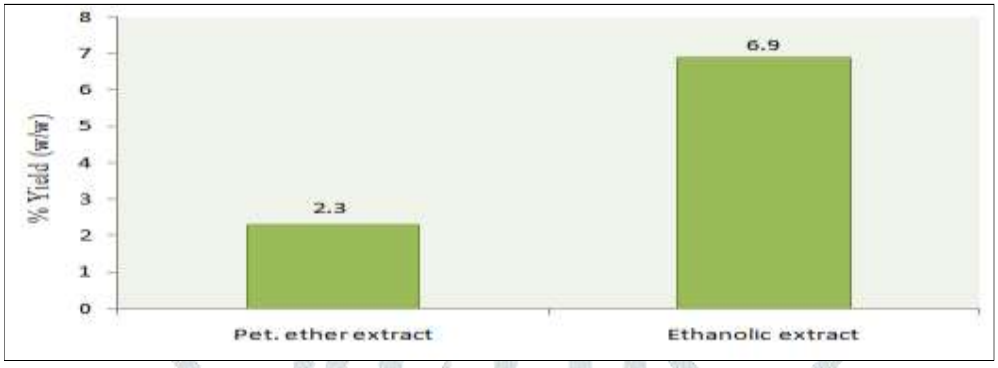
RESULTS AND DISCUSSION

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used.

Table 1: % Yield of *Sisymbrium irio*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	2.3%
2.	Ethanolic	6.9%

Figure 2: Comparative graph of % yield of *Sisymbrium irio*



The percentage yield of *Sisymbrium irio* extracts highlights the efficiency of different solvents in extracting phytochemicals from the plant material. As shown in Table 7.1, the petroleum ether extract produced a yield of 2.3% (w/w), while the ethanolic extract resulted in a significantly higher yield of 6.9% (w/w). This disparity suggests that ethanol, being a polar solvent, is more effective in extracting a broader range of compounds, including polar and semi-polar phytochemicals, from *Sisymbrium irio*. In contrast, petroleum ether, a nonpolar solvent, primarily extracts nonpolar components such as lipids and certain hydrocarbons, which are typically present in lower concentrations. The higher yield with ethanol indicates its suitability for comprehensive phytochemical studies aimed at capturing a wider variety of bioactive compounds.

Phytochemical screening of extract

Small portion of the dried extracts was subjected to the phytochemical tests using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed in the table

Table 2:Phytochemical screening of extracts of *Sisymbrium irio*

S. No	Constituents	Ethanolic Extract	Observation
1	**Alkaloids** Dragendroff's test Hager's test	 +ve +ve	 Red precipitate Yellow precipitate
2	**Glycosides** Legal's test	 -ve	 Brown coloured
3	**Flavonoids** Lead acetate Alkaline test	 -ve -ve	 Brown coloured Brown coloured
4	**Phenol** Ferric chloride test	 -ve	 Brown coloured

5	**Proteins** Xanthoproteic test	+ve	Yellow colored
6	**Carbohydrates** Fehling's test	-ve	Brown coloured
7	**Saponins** Foam test	-ve	No foam
8	**Diterpenes** Copper acetate test	+ve	Green colored
9	**Tannins** Gelatin Testssss	-ve	Brown coloured

The phytochemical screening of *Sisymbrium irio* ethanolic extract, as presented in Table 2, reveals the presence and absence of various bioactive constituents based on specific chemical tests. Alkaloids were positively identified in the ethanolic extract through Dragendorff's and Hager's tests, indicated by red and yellow precipitates, respectively, suggesting the presence of nitrogenous compounds with potential pharmacological activities. Proteins were also detected using the xanthoproteic test, shown by a yellow coloration, which may imply a nutritional or functional role in medicinal applications.

Diterpenes were positively identified through the copper acetate test, as indicated by a green coloration, pointing to the presence of these bioactive compounds, often noted for anti- inflammatory and antimicrobial properties. However, other major phytochemical classes such as glycosides, flavonoids, phenols, carbohydrates, saponins, and tannins tested negative, indicated by either brown coloration or lack of reaction, which suggests that these compounds are either absent or present in trace amounts within the ethanolic extract. This profile provides insight into the composition of the *Sisymbrium irio* ethanolic extract, emphasizing its potential for further pharmacological investigation, particularly for its alkaloid and diterpene content.

Results of total alkaloid content of *Sisymbrium irio* extract

Estimation of total alkaloid content (TAC)

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: **$y = 0.007x - 0.003$, $R^2 = 0.999$**, where X is the Atropine equivalent (AE) and Y is the absorbance.

Calibration curve of Atropine

Table 3: Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	40	0.285±0.003
2	60	0.447±0.004
3	80	0.613±0.005
4	100	0.754±0.002
5	120	0.892±0.001

***Average of three determination, Mean ± SD**

The calibration curve for atropine was established by measuring the absorbance of different concentrations of atropine solutions, as shown in Table 7.3. The concentrations ranged from 40 to 120 µg/ml, with corresponding mean absorbance

values increasing proportionally, indicating a direct relationship between atropine concentration and absorbance. At 40

µg/ml, the mean absorbance was recorded as 0.285 ± 0.003, gradually rising to 0.892 ±

0.001 at 120 µg/ml. This linear trend suggests that absorbance measurements for atropine can be reliably used to estimate unknown concentrations within this range by referencing the calibration curve. The consistent standard deviations (SD) across triplicate measurements confirm the reproducibility of the assay, ensuring accurate determination of atropine concentration in similar experimental set ups.

Figure 2: Graph of calibration curve of Atropine

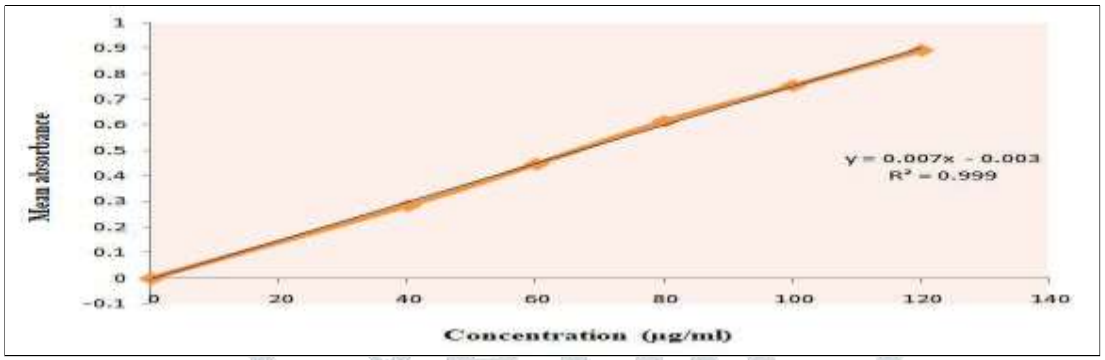


Table 4: Estimation of total alkaloid content of *Sisymbrium irio*

S. No.	Extract	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.52

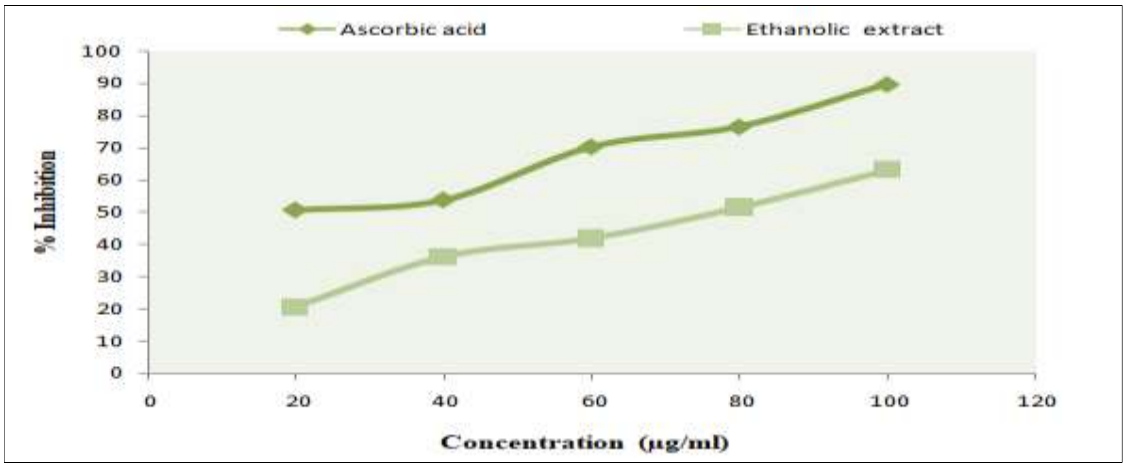
The total alkaloid content of *Sisymbrium irio* ethanolic extract, as shown in Table 7.4, was determined to be 0.52 mg per 100 mg of dried extract. This concentration indicates that alkaloids are present in moderate amounts within the ethanolic extract, contributing a measurable fraction of the extract's overall phytochemical composition. Given the known pharmacological potential of alkaloids, including anti-inflammatory, antioxidant, and wound healing effects, this quantification supports the possible therapeutic application of *Sisymbrium irio* for further pharmacological studies.

Results of antioxidant activity using NO model

Table 5: % Inhibition of ascorbic acid and extract of *Sisymbrium irio*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	50.87	20.74
2	40	53.96	36.29
3	60	70.42	42.02
4	80	76.64	51.67
5	100	89.79	63.25
IC 50 value		**23.54**	**74.36**

Figure 3: % Inhibition of ascorbic acid and extract of *Sisymbrium irio*



The nitric oxide (NO) scavenging activity of *Sisymbrium irio* ethanolic extract and ascorbic acid (used as a standard antioxidant) was evaluated at varying concentrations, as summarized in Table 7.5. The percentage inhibition of nitric oxide radicals increased with concentration for both ascorbic acid and the ethanolic extract, indicating a dose-dependent antioxidant effect.

Ascorbic acid showed a significantly higher NO scavenging activity, with % inhibition values ranging from 50.87% at 20 µg/ml to 89.79% at 100 µg/ml, achieving an IC_{50} (half- maximal inhibitory concentration) value of 23.54 µg/ml. In comparison, the *Sisymbrium irio* ethanolic extract exhibited lower % inhibition values, from 20.74% at 20 µg/ml to 63.25% at 100 µg/ml, with an IC_{50} value of 74.36 µg/ml.

The higher IC_{50} value for the ethanolic extract indicates that it has a lower NO scavenging capacity compared to ascorbic acid, suggesting that while the extract does possess antioxidant activity, it is less potent than the standard antioxidant. These findings support the potential antioxidant properties of *Sisymbrium irio*, though further concentration adjustments or synergistic combinations with other antioxidants may be necessary for enhanced

Results of *in vivo* wound healing activity of ethanolic extract of *Sisymbrium irio*

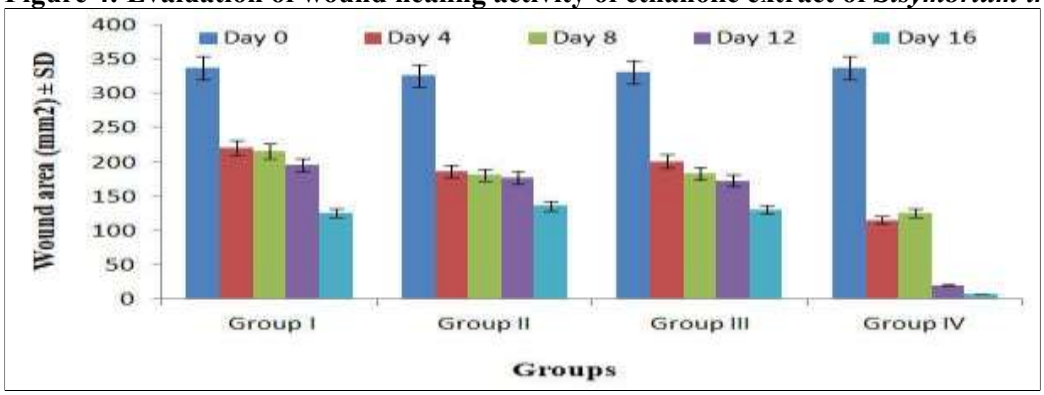
In this study, significantly improved wound-healing activity has been observed with the ethanolic extract of *Sisymbrium irio*, compared to that of the reference standard and control group of animals.

Table 6: Evaluation of wound healing activity of ethanolic extract of *Sisymbrium irio* on rats

Group	Wound area (mm^2) ± SD				
	Day 0	Day 4	Day 8	Day 12	Day 16
I	336.5±6.5	220.3±3.5	215.6±3.6	195.5±3.6	125.5±3.8
II	325.4±8.2	185.6±5.2	180.8±5.8	176.6±5.5	135.8±5.6
III	330.5±5.6	200.5±6.3	183.3±9.2	172.2±6.3	130.3±6.9
IV	336.6±7.4	115.4±5.6	125.56±7.4	20.3±7.4	7.6±7.5

All values are expressed as mean ± SD

Figure 4: Evaluation of wound healing activity of ethanolic extract of *Sisymbrium irio* on rats



The results of this study demonstrate that the ethanolic extract of *Sisymbrium irio* has significant wound-healing activity in rats when compared to both the positive control group (untreated) and the standard treatment group (vitamin C). The data obtained from the wound area measurements clearly show that the extract facilitated wound healing over the 16-day observation period.

From the data in Table 7.6, it is evident that the animals in Group IV (treated with vitamin

C) exhibited the most significant reduction in wound area. The wound size in this group decreased from 336.6 ± 7.4 mm² on Day 0 to 7.6 ± 7.5 mm² by Day 16, demonstrating the well-known wound healing properties of vitamin C, which is essential for collagen synthesis and tissue repair. Vitamin C's effectiveness in wound healing is consistent with previous studies, where it has been shown to promote tissue regeneration and reduce inflammation. In comparison, the *Sisymbrium irio* extract-treated groups (Groups II and III) showed notable wound area reductions as well, albeit at a slower rate. Group II (treated with 100 mg/kg of the extract) showed a decrease in wound size from 325.4 ± 8.2 mm² on Day 0 to 135.8 ± 5.6 mm² on Day 16. Group III (treated with 200 mg/kg of the extract) also demonstrated effective wound healing, with a decrease from 330.5 ± 5.6 mm² on Day 0 to

130.3 ± 6.9 mm² on Day 16. While these groups did not heal as rapidly as the vitamin C group, the wound healing progress observed in these extract-treated groups was still substantial and indicates that *Sisymbrium irio* possesses potential therapeutic properties for wound healing (Chambial *et al.*, 2013).

The positive control group (Group I), which received no treatment, showed the least improvement in wound healing. Wound areas in this group decreased only from 336.5 ± 6.5 mm² to 125.5 ± 3.8 mm² by Day 16, indicating a slower, less effective healing process compared to the other treated groups.

The differences in healing rates between the extract-treated groups and the vitamin C group could be attributed to the varying bioactive components of *Sisymbrium irio* and their different mechanisms of action. While vitamin C is a well-established antioxidant and collagen synthesis enhancer, *Sisymbrium irio* might promote healing through other mechanisms, such as its anti-inflammatory, antimicrobial, or antioxidant properties. It is possible that the higher dose (200 mg/kg) of the extract (Group III) might have shown even better healing if given more time or at a higher concentration.

The observed wound-healing effect of *Sisymbrium irio* could be due to the presence of phytochemicals such as flavonoids, alkaloids, and glycosides, which are known to have anti-inflammatory, antibacterial, and antioxidative effects. These compounds might reduce oxidative stress, enhance tissue repair, and prevent infection, all of which are critical for optimal wound healing

SUMMARY AND CONCLUSION

This study aimed to evaluate the wound-healing properties of the ethanolic extract of *Sisymbrium irio* in rats, as well as its chemical composition and potential biological activities. The study provided valuable insights into the efficacy

of the extract in wound healing, its phytochemical profile, and its inhibitory effects on oxidative stress.

The ethanolic extract of *Sisymbrium irio* had a higher yield (6.9%) compared to the petroleum ether extract (2.3%), indicating a better extraction of bioactive compounds in ethanol.

The ethanolic extract showed dose-dependent inhibition of oxidative stress, with an IC50 value of 74.36 µg/ml, higher than the ascorbic acid standard (IC50 = 23.54 µg/ml). This suggests that while the extract is an effective antioxidant, it is less potent than ascorbic acid, which is known for its strong antioxidative properties.

The ethanolic extract of *Sisymbrium irio* significantly reduced wound size over the 16-day period compared to the untreated control group. Group IV (treated with vitamin C) exhibited the most significant reduction in wound area, but the extract groups (II and III) also showed substantial improvement. Specifically, Group II (100 mg/kg) and Group III (200 mg/kg) demonstrated effective healing, with wound areas decreasing from 325.4 ± 8.2 mm^2 to 135.8 ± 5.6 mm^2 and from 330.5 ± 5.6 mm^2 to 130.3 ± 6.9 mm^2, respectivelAlthough the extract was less effective than vitamin C, it still significantly improved wound healing in the test animals.

The ethanolic extract of *Sisymbrium irio* exhibited promising wound-healing activity, likely due to its alkaloid and diterpene content, which may exert anti-inflammatory and antioxidant effects. Although it was less effective than the standard vitamin C treatment, the extract still showed a significant improvement in wound healing compared to the control group. The study supports the potential use of *Sisymbrium irio* as a natural therapeutic agent for wound healing, with future research recommended to isolate and identify the specific active compounds responsible for its effects.

BIBLIOGRAPHY

1 Gupta N, Jain UK. Prominent wound healing properties of indigenous medicines. J Nat Pharmaceuticals 2010; 1: 2–13.

2 Nair RV (1995) Indian medicinal plants- a compendium of 500 species. (Mentha arvensis Linn, Warrier PK, Nambiar VPK, Ramankutty C editors). Hyd: Ori Long Pvt Ltd 4:15–17

3 Gopinath D, Rafiuddin A, Gomathi M, Chitra K, Sehgal PK, Jayakumar R (2004) Dermal wound healing processes with curcumin incorporated collagen films. Biomaterials 25(10):1911–1917

4 Ahmad O, Aboutorab TN, Naeini T, Behrooz NE (2010) Effect of aqueous extract of aloe vera on experimental cutaneous wound healing in rat. Vet Arhiv 80(4):509–522

5 G. Amish Burn Study, N. M. Kolacz, M. T. Jaroch et al., "The effect of Burns & Wounds (B&W)/burdock leaf therapy on burn-injured Amish patients: a pilot study measuring pain levels, infection rates, and healing times," Journal of Holistic Nursing, vol. 32, no. 4, pp. 327–340, 2014.

6 R. Wang, M. Lechtenberg, J. Sendker, F. Petereit, A. Deters, and A. Hensel, "Wound- healing plants from TCM: in vitro investigations on selected TCM plants and their influence on human dermal fibroblasts and keratinocytes," Fitoterapia, vol. 84, pp. 308– 317, 2013.

7 Zakaria Z, Reezal I, Mat Jais A, et al. The anti-inflammatory, anti-pyretic and wound healing activities of *Cocos nucifera* L.(MATAG types) fresh juice and kernel extract in experimental animals. *J Pharmacol Toxicol*. 2006;1:516–526

8 An H, Peng K, Wang Q, et al. Effect of pomegranate peel polyphenol gel on cutaneous wound healing in alloxan-induced diabetic rats. Chin Med J. 2012; 126:1700–1706.

9 Mukherjee PK, Mukherjee K, Pal M, Saha BP. Wound healing potential of Nelumba nucifera (Nymphaceae) rhizome extract. Phytomedicine. 2000;7:66-73

10 Farahpour MR, Hesaraki S, Faraji D, Zeinalpour R, Aghaei M. Hydroethanolic Allium sativum extract accelerates excision wound healing: Evidence for roles of mast-cell infiltration and intracytoplasmic carbohydrate ratio. Brazilian Journal of Pharmaceutical Sciences. 2017.

11 Preethi KC, Kuttan R. Wound healing activity of flower extract of *Calendula officinalis*.

12 Journal of Basic and Clinical Physiology and Pharmacology. 2009; 20(1):73-80

13 Hemmati AA, Aghel N, Rashidi I, Gholampur-Aghdami A. Topical grape (Vitis vinifera) seed extract promotes repair of full thickness wound in rabbit. International Wound Journal. 2011;8(5):514-520

14 Criollo-Mendoza MS, Contreras-Angulo LA, Leyva-Lopez N, Gutierrez-Grijalva EP, Jiménez-Ortega LA, Heredia JB. Wound healing properties of natural products: Mechanisms of action. Molecules. 2023 Jan 6;28(2):598.

15 El-Sherbeni SA, Negm WA. The wound healing effect of botanicals and pure natural substances used in in vivo models. Inflammopharmacology. 2023 Apr;31(2):755-72.

16 Mancia FH, Kim JS, Cabahug RA, Hwang YJ. Cytomolecular analysis of repetitive DNA provides insight into the chromosome structure of *Sisymbrium irio*. Horticulture, Environment, and Biotechnology. 2022 Apr;63(2):249-61.