doi.org/10.36721/PJPS.2024.37.5.REG.1053-1061.1

Evaluation of wound healing activity of hydromethanolic extract of *Jacobaea maritima* leaves in rabbits

Rimsha Shahzad, Saba Kousar*, Imtiaz Gull, Malaika Bukhari,
Mishal Fatima and Rameen Malik
Fatima-Tul-Zahra Department of Life Sciences, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan

Abstract: The study investigated the wound healing efficacy of *Jacobaea maritima* leaf extract in rabbits through various wound models. A methanol-based extract was prepared from air-dried *J.maritima* leaves and used to formulate ointments at concentrations of 5%, 10% and 20%. Acute dermal toxicity tests confirmed no adverse reactions at the highest dose (2000mg/kg). The extract's wound healing activity was assessed using excision, incision, and burn wound models. In the excision wound model, all extract concentrations significantly enhanced wound contraction compared to the control, with the 20% extract showing the highest efficacy (100% contraction by day 22). The extract also reduced epithelialization time, with the 20% formulation performing similarly to nitrofurazone. In the burn wound model, the extract ointments led to substantial wound contraction and shorter epithelialization times compared to controls, with the 20% concentration achieving 100% contraction by day 24. Histopathological examinations revealed improved healing in extract-treated wounds, with enhanced neovascularization and collagen deposition. These results suggest that *J.maritima* leaf extract possesses potent wound healing properties, effectively reducing healing times and enhancing tissue repair across different wound models. The study supports the potential of *J.maritima* as a valuable ingredient in wound care formulations.

Keywords: *Jacobaea maritima*, wound healing, hydromethanolic extract, rabbit models.

Submitted on 27-06-2024 – Revised on 10-9-2024 – Accepted on 10-9-2024

INTRODUCTION

The largest organ in the body, the skin serves as an essential barrier that keeps the outer world out of the inside environment (Wang *et al*. 2023). Wound is described as a rupture or opening in the skin barrier that compromises the structural and functional integrity of live tissue (Saroya 2013). Wound created by external mechanical force-induced wound, surgical procedures, burn, and chemical damage. When the wound is severe then harm the epidermis of skin. The skin can sustain different degrees of damage from the many processes that the body goes through, including ulcers brought on by several chronic conditions (Liu *et al*. 2021). Acute or chronic wounds are also classified according to how long it takes for them to heal. The chronic wounds usually have underlying medical issues that inhibit and obstruct healing, acute wounds heal normally (Abeje *et al*. 2022). Hemostasis, inflammation, proliferation and remodeling represent the four fundamental phases within the intricate and dynamic process of tissue regeneration, recognized as wound healing (Liu *et al*. 2021).

Jacobaea maritima, or sea ragwort, is native to the Mediterranean region, including countries like Spain, Portugal, Italy, France and Greece. It naturally grows in coastal areas with sandy or rocky soils and can tolerate saline conditions. The plant is well-adapted to its native environment, thriving in well-drained soils and often found in coastal and rocky habitats. It is also widely cultivated elsewhere for ornamental purposes due to its attractive silvery foliage (Zhang and Gong 2023).

Jacobaea maritima is an effective gargle for burns, wounds and eye infections. It can also be used to treat mouth ulcers and sore throats (Fetzer 2018). Traditionally, this plant is used to make a homoeopathic remedy that is used to cure wounds, various illnesses such intestinal worms, internal bleeding, menstrual disorders, and spasms. This plant is thought to have a cooling and astringent effect and this plant is thought to have a cooling and astringent effect. As a laxative, to initiate menstruation and for cleansing and purification, it is also utilized (Delgadillo Valdez 2018). The most beneficial nutrient in *J. maritima* is alkaloids. Plants have alkaloids that protect them from predators and regulate growth. Alkaloids are particularly well-known for their therapeutic uses as anti- inflammatory, cardiovascular, and cosmetic agents (Kurek 2019). The antimicrobial and antioxidant characteristics of *J. maritima's* leaves, which have been used for wound healing traditionally and have been scientifically shown, are two elements that can contribute to the wound-healing actions of numerous medicinal plants (Badalamenti *et al*. 2023). There is currently no scientific evidence to confirm the safety or wound healing properties of *J. maritima.* Therefore, the goal of this investigation was to examine the potential toxicity of the crude extract of *J. maritima* in animal models as well as its capacity to heal wounds.

**Corresponding authors:* e-mails: Sabakousar888@gmail.com

MATERIALS AND METHODS

Materials

Drugs and chemicals

Wool fat, formalin 10% buffered solution, cetostearyl alcohol, bees wax, 6M HCL, normal saline, eosin, paraffin, methanol, sodium hydroxide, white soft paraffin, distilled water, per chloric acid, injection of diazepam and ketamine, hematoxylin. Everything that was used in the preparations, chemicals and reagents met the necessary standards for analytical quality.

Plant material

Fresh foliage from the *J. maritima* plant was collected and identified from Faiz-e-Amm nursery Multan on 14th December 2023. The leaves underwent a cleaning process using tap water and were subsequently air-dried in a shaded location. The resulting dried material was crushed into a fine powder, measured, and stored in a sealed container until the extraction process.

Animal model and housing conditions

Male rabbits weighing 1.5kg were procured from a nearby market in Multan. They were granted unlimited access to commercially produced, standardized food, and water. These rabbits were housed at the Department of Biochemistry, *Muhammad Institute of Medical and Allied Sciences in Multan, Pakistan,* maintaining a constant temperature of 25 degrees Celsius. The experiments adhered to guidance from the National Research Council and received approval from the Ethical Committee of Muhammad Institute of Medical and Allied Sciences Multan, Pakistan (approval No. MIMAS/03/Biochem/ 315/20).

Methods

To create an extract, 200ml of methanol 70% was mixed with 50g of edible *J. maritima* leaf powder until it completely dissolved. The resulting solution was then continuously stirred with an electric shaker at a temperature of 20 to 25 degrees Celsius. 72 hours late, Whatman filter paper was used to filter the mixture and the filtered solution was then added to the solvent in the rotating evaporator. Up to the time of usage, the produced extract was stored at a temperature of 4 degree Celsius (Kooti *et al*. 2014).

Preliminary phytochemicals screening

The crude extract of the plant *J. maritima* underwent qualitative phytochemicals screening to determine the presence of numerous secondary metabolites, including flavonoids, alkaloids, carbohydrates, triterpenoids, saponins, tannins and proteins (Naveed *et al*. 2022).

Preparation of topical formulation

After studying the acute dermal toxicity, three types of ointment for the crude extract were developed; one with a strength of 5%, 10% and another with a strength of 20%.

Simple ointment formulation

The first step was to make a simple ointment for each model using white soft paraffin, hard paraffin, wool fat, and cetostearyl alcohol. Utilize electronic balance to weigh each ingredient. Prior to melting in a water bath, solid paraffin was first placed into a dish, evaporated, and then melted. The following ingredients were then added until they were all melted. For uniformity, the mixture was continually stirred. The unfavorable side effects of this simple cream matched those of the abrasive control cream (Namunana *et al*. 2018).

Acute dermal toxicity

Total six normal skinned rabbits were divided in to two groups of three rabbits each. Before the test, the animals were spend five days in individual cages being acclimated to the laboratory environment. Then, 24 hours before the test procedure, around 10% of the body surface area of the test animals' fur was removed from the dorsal region of their trunks. Each test group first received 2000 mg/kg of an ointment containing 10% crude extract, while the control group initially received 2000 mg/kg of simple ointment base. A non-occlusive bandage with gauze were applied as soon as it was finished, and it was remained on for 24 hours. Each animal test site was thoroughly cleaned with distilled water after each item and any lingering test chemical had been present for 24 hours. The appearance of any unflavored skin reactions was observed in animals for 24 hours.

Two rabbits from each group were collected on the same day, given the same dose, covered, and cleaned, and when the same 24-hour period of time was up, they were similarly inspected to check for any skin reactions. After being washed, all animals were watched carefully for the emergence of any negative skin responses, including erythema or edoema, for 24 hours, and then every 14 days thereafter (Mielke *et al*. 2017).

Grouping and dosing of experimental animals

For the purpose of assessing the potential of crude extract to treat the burn, excision and incision wound models, animals were randomly separated into four groups. Each group was consisting of three rabbits. Four groups of three rabbits each was used in each wound model to test the crude extract the ability to speed up the healing of incision, excision and burn wounds. Group I: (negative control) ointment, Group II: 5% crude extract ointment, Group III: 10% crude extract ointment, Group IV: 20% crude extract ointment, Group V: Positive control ointment containing 0.2% nitrofurazone (Abeje *et al*. 2022; Ravishankar *et al*. 2019).

Wound healing activity

Excision wound mode

Intraperitoneal injections of diazepam (5mg/kg) and ketamine (80mg/kg) were used to anaesthetize rabbits. After that shaved the skin on the dorsal limb with a razor and disinfected with 70% alcohol. Mark a predictable 400mm^2 circular wound site with a fine permanent marker and follow the mark using forceps full thickness of marked skin was then cut carefully. To stop the bleeding, the wound was wiped with a cotton swab dipped in saline before being left fully exposed. The test group's wound was topically administered once a day until it healed completely, with the day of the injury serving as day zero (Abeje *et al*. 2022; Ravishankar *et al*. 2019). Wound closure rate, duration of epithelization, histopathological studies, and tensile strength were used to evaluate the progress of wound healing. To evaluate the wound closure rate, measurements were made on days 0, 2, 4, 6, 8, 10, 12 and 16 following the injury. The extract's ability to cure wounds, expressed as a percentage of wound contraction, was calculated using the formula below.

$$\%\ \text{wound contraction} = \frac{\text{wound area on day0 - wound area on day n}}{\text{wound area on day0}} \times 100$$

Where, n is the number of post-injury wounds (Abeje *et al*. 2022; Ravishankar *et al*. 2019). Period of epithelization. The duration required for a scar to completely shed without any lingering raw wound debris was assessed (Abeje *et al*. 2022).

Histopathological examination

At the conclusion of the study, each group of rabbits received intraperitoneal injections of 320mg/kg of ketamine and 20mg/kg of diazepam, which is four times the anaesthetic dose. Following that, full-thickness skin samples were cut into sections. These samples were cut into 5 µm slices and then fixed in 10% buffered formalin, paraffin-blocked, then hematoxylin and eosin stained. A pathologist who wishes to remain anonymous assessed the phases of wound healing, and the results were compared to those of the control group (Abeje *et al*. 2022).

Incision wound model

As with the excision wound model, anesthesia was administered to the animals used in the experiment. After shaving the dorsal limbs of every rabbit, 70% alcohol was used to disinfect them. After that incision of 3 cm long through the full thickness of the skin; surgical sutures and curved needle was used to close wound with interrupted sutures spaced 1 cm apart and wound was left undressed after being stitched (Demilew, Adinew, and Asrade 2018; Ravishankar *et al*. 2019). The day of wounding was count as day 0 and the treatment was start on next day of wounding. According to the above grouping dosage section, the animals in groups I through IV were given the appropriate topically administered medications. From the day after the wound was first created until the ninth day, treatment was given and the sutures were taken out at the day eighth. The test group's tensile strengths were contrasted with those of the negative and positive controls. The following formulas were used to determine the group's percent tensile strengths (%TS) (Abeje *et al*. 2022).

$$\%\ \text{TS of extract} = \frac{\text{TS of extract - TS of vehicle}}{\text{TS of vehicle}} \times 100$$

$$\%\ \text{TS of reference} = \frac{\text{TS of reference - TS of vehicle}}{\text{TS of vehicle}} \times 100$$

$$\%\ \text{TS of vehicle} = \frac{\text{TS of vehicle - TS of group left untreat}}{\text{TS of group left untreat}} \times 100$$

Burn wound model

The rabbits were given intraperitoneal injections of ketamine (80mg/kg) and diazepam (5 mg/kg). After that shaved the skin on the dorsal limb with a razor and disinfected with 70% alcohol. Rabbits were partial burn from the hot melted wax. For this purpose, the hot wax was heated to 80 degrees Celsius and then poured into a cylinder with an opening that was 300mm^2, after that put it on the shaved skin and leave it there until it hardens. Normally the wax solidify within 10-12 minutes, afterward the cylinder was taken out and each animal was get a circular burn wound of partial thickness. Animals were housed in individual cages and from the day of the damage until the test group's scabs fell off, therapy was administered at the wound site in the appropriate groups as specified in grouping and dosage (Ravishankar *et al*. 2019). Every two days, the wound contraction, which begins to occur after two days after the wound, was measured to track the healing process. After healing, histopathology analysis and epithelialization time were performed (Fahimi *et al*. 2015).

Histopathology analysis

Pathological examination was conducted utilizing the techniques outlined in the previously mentioned excision wound model's pathology analysis (Abeje *et al*. 2022).

STATISTICAL ANALYSIS

The standard error of the mean (SEM), which was computed for every group using the raw data supplied by SPSS version 23 software, was used to express the results. Turkey's post hoc test and one-way analysis of variance (ANOVA) were used in the statistical study to look for meaningful differences in group averages. A statistically significant criterion of $p<0.05$ was employed (Abeje *et al*. 2022).

RESULTS

Percentage yield of crude extract

The crude extract obtained from 1kg of coarse powder derived from *J. maritima* leaves amounted to 162g. Consequently, the percentage yield of the crude extract was 16.2%.

Phytochemicals screening test

The qualitative screening test for phytochemicals in the plant's crude extract indicated the existence of alkaloids, flavonoids, tannins, triterpenoids, carbohydrates, aldehydes, vitamins, saponins and proteins.

Table 1: Ingredients for preparing a simple ointment

Ingredient	Master formula (g)	Reduced formula (g)
Hard paraffin	50	5
White soft paraffin	850	85
Wool fat	50	5
Cetostearyl alcohol	50	5
Total	1000	100

Table 2: Impact of ointment derived from crude extract on contraction of excision wounds.

Days	Group I (SO)		Group II (5% CEO)		Group III (10% CEO)		Group IV (20% CEO)		Group V (NFO 0.2%)	
	WoundArea (mm^2)	% Wound contraction	WoundArea (mm^2)	% Wound contraction	WoundArea (mm^2)	% Wound contraction	WoundArea (mm^2)	% Wound contraction	WoundArea (mm^2)	% Wound contraction
2	388.31 ±0.67	2.92	382.63 ±1.64	4.34	373.57 ±1.04	6.60	363.5 ±1.05	9.12	368.04 ±0.79	7.99
4	351 ±1.0	12.25	345± 1.0	13.75	334.02 ±14	16.49	325.39 ±0.64	18.65	328.74 ±0.58	17.81
6	298.52 ±0.71	25.37	281± 1.0	29.75	258.97 ±1.0	35.25	269.53 ±0.73	32.61	267.14 ±0.57	33.21
8	251.07 ±0.22	37.23	198.94± 0.72	50.26	181.81 ±0.52	54.54	172.83 ±0.54	56.79	176.80 ±0.69	55.8
10	206.78 ±0.63	48.30	168± 1.0	58	153.33 ±0.45	61.66	146.56 ±1.22	63.36	148± 0.82	63
12	183.30 ±1.05	54.17	139.83 ±0.36	65.04	121.53 ±0.52	69.61	114.36 ±0.71	71.41	116.72 ±1.08	70.82
14	155.74 ±0.53	61.06	122.22 ±0.66	69.44	104.63 ±0.70	73.84	98.05 ±0.58	75.48	101.77 ±0.64	74.55
16	135 ±1.0	66.25	110.86 ±0.26	72.28	95.81 ±0.0.69	76.047	66.39 ±1.18	83.40	78.04 ±0.82	80.49
18	108.72 ±0.48	72.82	103.93 ±0.51	74.01	74.96 ±0.76	81.26	30.55 ±0.69	92.36	44.87 ±1.05	88.78
20	83.38 ±0.67	79.15	78.52 ±0.32	80.37	33.48 ±1.02	91.63	8.46 ±0.79	97.88	21.16 ±0.33	94.71
22	55±1.0	86.25	48.57±0.	87.85	6.24±0.73	98.44	0.00±0.0	100	4.68±0.5	98.83

Acute dermal toxicity test

The topical administration of a maximum dose of 2000mg/kg of the 10% extract ointment formulation revealed no indications of skin reactions during meticulous monitoring for 24 hours following the application's washing and daily observations for a duration of 14 days.

Wound healing activity of the crude extract excision wound model

Wound contraction

When ointments with crude extract were applied topically instead of just a simple ointment, there was a noticeable reduction in wound size. Specifically, at a 5% concentration, significant wound contraction was observed on day-7 ($p<0.01$) and at a 10% concentration, the contraction was even more substantial ($p<0.001$). Moreover, at a 20% concentration, the contraction was highly significant ($p<0.0001$). From day-14 to day-22, ointments with 5%, 10% and 20% crude extract consistently demonstrated progressive and significant ($p< 0.0001$) wound contractions compared to the negative control. Furthermore, compared to the 5% and 10% crude extract ointments, the 20% crude extract ointment and the conventional treatment caused noticeably more wound contractions from day 6 to day 14. With each post-wounding day, the percentage of wound contractions improved in all groups. Day 22 saw 86.25% percent wound contractions for the normal ointment, 87.8% for 5% crude extract ointment, 100% for 20% crude extract ointment, 98.4% for 10% crude extract ointment, and 98.8% for the negative control (NFO 0.2%)(table 2).

Period of Epithelization

Groups treated with 5%, 10% and 20% crude extract ointments as well as nitrofurazone 0.2% ointment showed significantly shorter times for the excision site to fully epithelialize. The 20% crude extract ointment and nitrofurazone both significantly ($p<0.0001$) reduced the epithelialization duration when compared to the 5% and 10% crude extract groups as well as the negative control. In addition, the 10% extract significantly ($p<0.05$) decreased the time required for epithelialization in comparison to the negative control.

Table 3: Influence of ointment formulated from crude extract on contraction of burn wounds.

Days	Group I(SO)		Group II (5% CEO)		Group III (10% CEO)		Group IV (20% CEO)		Group V (NFO 0.2%)	
	Wound Area (mm^2)	% Wound contraction	Wound Area (mm^2)	% wound contraction	Wound Area (mm^2)	% wound contraction	Wound Area (mm^2)	% wound contraction	Wound Area (mm^2)	%Wound contraction
2	294.27 ±1.18	1.91	293.36 ±0.91	2.21	293 ±0.81	2.3	292.41 ±0.77	2.53	291.38 ±0.80	2.87
4	285.97 ±1.07	4.67	285.35 ±0.87	4.88	285.21 ±0.89	4.93	284.37 ±0.57	5.21	284.19 ±0.92	5.27
6	277.54 ±1.10	7.48	276.56 ±0.97	7.81	275± 0.81	8.33	274.24 ±0.64	8.58	273.69 ±0.82	8.77
8	267.39 ±0.79	10.87	266.20 ±0.77	11.26	263.95 ±0.90	12.01	262.66 ±0.92	12.44	261.43 ±0.61	12.86
10	247.42 ±0.81	17.52	246.49 ±0.55	17.83	234.27 ±0.76	21.91	234.36 ±1.13	21.88	231.37 ±0.81	22.87
12	237.93 ±1.23	20.69	225.38 ±0.85	24.87	170.48 ±1.03	43.17	169.58 ±0.73	43.47	160.31 ±0.98	43.56
14	206.99 ±0.78	31.00	175.8 ±0.84	41.40	109.62 ±0.72	63.46	108.56 ±0.91	63.81	107.93 ±0.87	64.02
16	176.04 ±0.81	41.32	137.50 ±0.75	54.16	67.24 ±0.77	77.58	66.25 ±0.81	77.91	65.91 ±0.58	78.03
18	160.27 ±0.89	46.57	111.42 ±0.65	62.86	33.57 ±0.64	88.81	32.55 ±0.95	89.15	31.87 ±0.81	89.37
20	137.20 ±0.91	54.26	77.62 ±0.98	74.12	3.74 ±0.78	98.75	2.12 ±0.35	99.29	2.51± 0.79	99.16
22	106.69 ±1.04	64.43	38.58 ±0.74	87.14	1.62± 0.63	99.46	0.28± 0.15	99.90	0.58± 0.28	99.80
24	72.24 ±0.86	75.92	19.62 ±0.82	93.46	0.00± 0.00	100	0.00± 0.00	100	00.00± 00.00	100

Table 4: Impact of ointments derived from crude extracts on tensile strength in the incision wound model.

Group (treatment)	Tensile strength (g)	% Increase in tensile strength
Group I (untreated)	266.27±0.71	—
Group II (SO)	280.23±0.70	6.59
Group III (5% CEO)	352.57±0.61	17.52
Group IV (10% CEO)	421.33±0.71	40.44
Group V (20% CEO)	424.80±0.74	41.60
Group VI (NFO 0.2%)	428.22±0.56	42.74

Histopathology analysis

On day sixteen, histology specimens of the removed wound from groups treated with crude extract were collected. In every group, one animal was used to gather the sample specimens. Based on histological investigation, wounds treated with 20% CEO showed signs of better healing, including neovascularization, decreased inflammatory cell count and a modest concentration of fibroblasts and collagen deposits. The animal's nitrofurazone-treated material had low levels of polymorphonuclear and mononuclear cells, significant collagen deposition and moderate fibroblast proliferation. Additionally, the physical improvements in the rabbits' wound textures that were given different treatments are depicted in fig. 1.

Burn wound model

Wound contraction

The crude extract ointments significantly influenced wound contraction compared to the negative control at concentrations of 5%, 10% and 20% on days 14, 12 and 10, respectively ($p<0.0001$). The regular treatment showed greater wound contraction on day 8 ($p<0.05$) and continually induced more substantial ($p<0.0001$) contractions from day 10 onward when compared to negative control between days 10 and 24, the 20% crude extract ointment plus the conventional treatment caused more substantial ($p<0.0001$) wound contractions than the 5% and 10% crude extract ointments. In the days following the wound, the proportion of wound contractions rose and by day 24, it had reached 100% for both the 10% and 20% crude extract ointments and the nitrofurazone therapy. On day 24, the basic ointment achieved 75.92% contraction, whereas the 5% crude extract ointment produced 93.46% contraction (table 3).

Period of epithelization

In the treatment groups, complete epithelialization took notably less time, which is in line with the outcomes observed in the excision wound model. Consequently, the

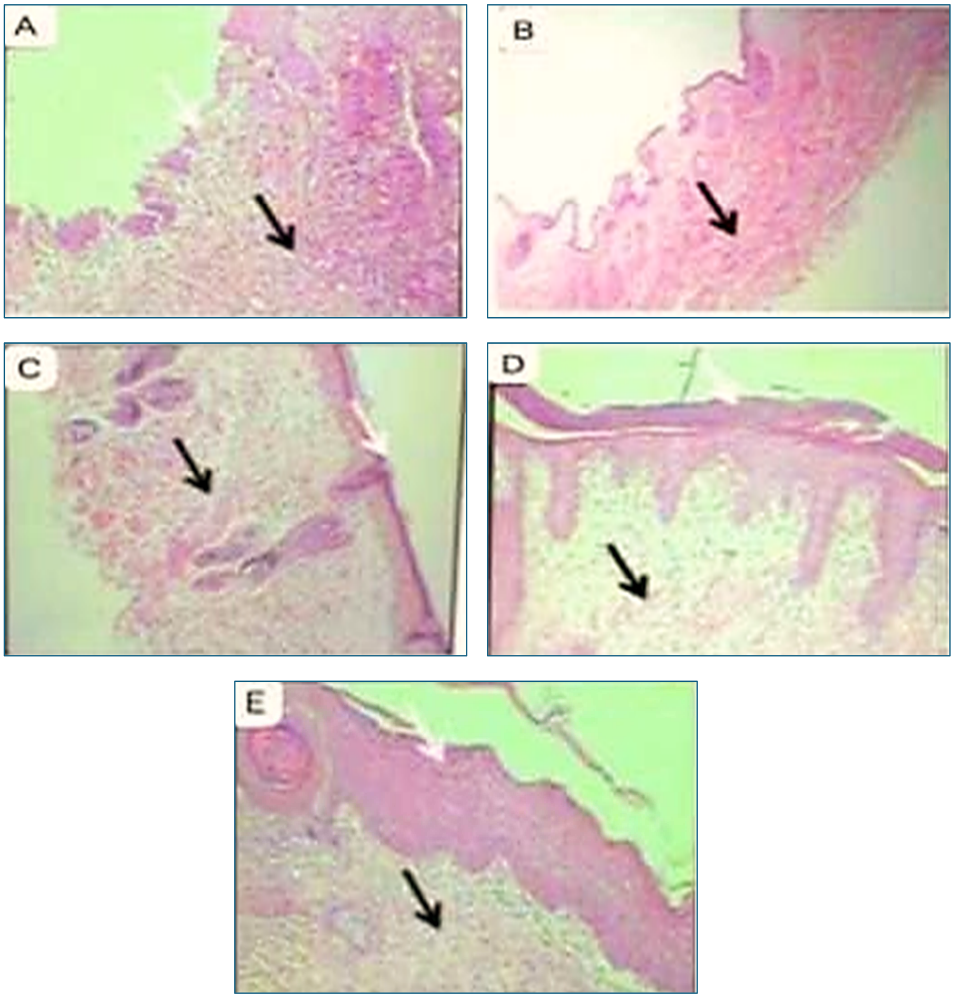

Fig. 1: The histological sections of excision wound tissue. (A) negative control (B) treated with 5% CEO (C) treated with 10% CEO (D) treated with 20% CEO (E) treated with 0.2% nitrofurazone.

groups treated with 5% (p0.01), 10% CEO (p0.001), 20% CEO (p0.0001) and NFO 0.2% (p0.0001) saw a much shorter epithelialization duration than the negative control. The 20% CEO and NFO 0.2% treatments outperformed the 5% and 10% CEO treatments in terms of significantly reducing the epithelialization period. Remarkably, there was no discernible difference between the effects of the 20% CEO and nitrofurazone treatments on the epithelialization time.

Histopathology Analysis

On day 22, samples of wound tissues were collected for histological analysis. Animal tissues treated with 20% CEO and the conventional medication showed a low number of inflammatory cells, polymorphonuclear cells, and mononuclear cells, combined with a moderate quantity of fibroblasts and collagen deposits. The animals' specimens treated with 10% CEO likewise showed modest fibroblast fibrillation, but considerable neovascularization and collagen deposition. In contrast, necrotic tissue, a moderate concentration of inflammatory cells and neovascularization were seen in rabbit tissue treated with basic ointment (negative control), but neither fibroblast growth nor collagen deposition were present (table 3, fig. 2).

Incision wound model

On day ten following the incision, the tensile strength (wound breaking strength) of the wound was assessed. On this specific day, groups treated with 20% crude extract and nitrofurazone exhibited significantly higher tensile strength ($p<0.0001$) compared to those receiving simple ointment and the untreated groups. Furthermore, both the 20% crude extract and nitrofurazone treatments significantly ($p<0.001$) elevated tensile strength compared to the 5% and 10% crude extract treatments. Tensile strength was also considerably greater in the groups treated with 5% and 10% crude extract compared to the untreated ($p<0.01$) and negative control ($p<0.05$) groups. Percentages of tensile strength increased in the 5%, 10%, and 20% crude extract and nitrofurazone 0.2% treated groups compared to the untreated group were 17.52%, 40.44%, 41.60% and 42.74% (table 4).

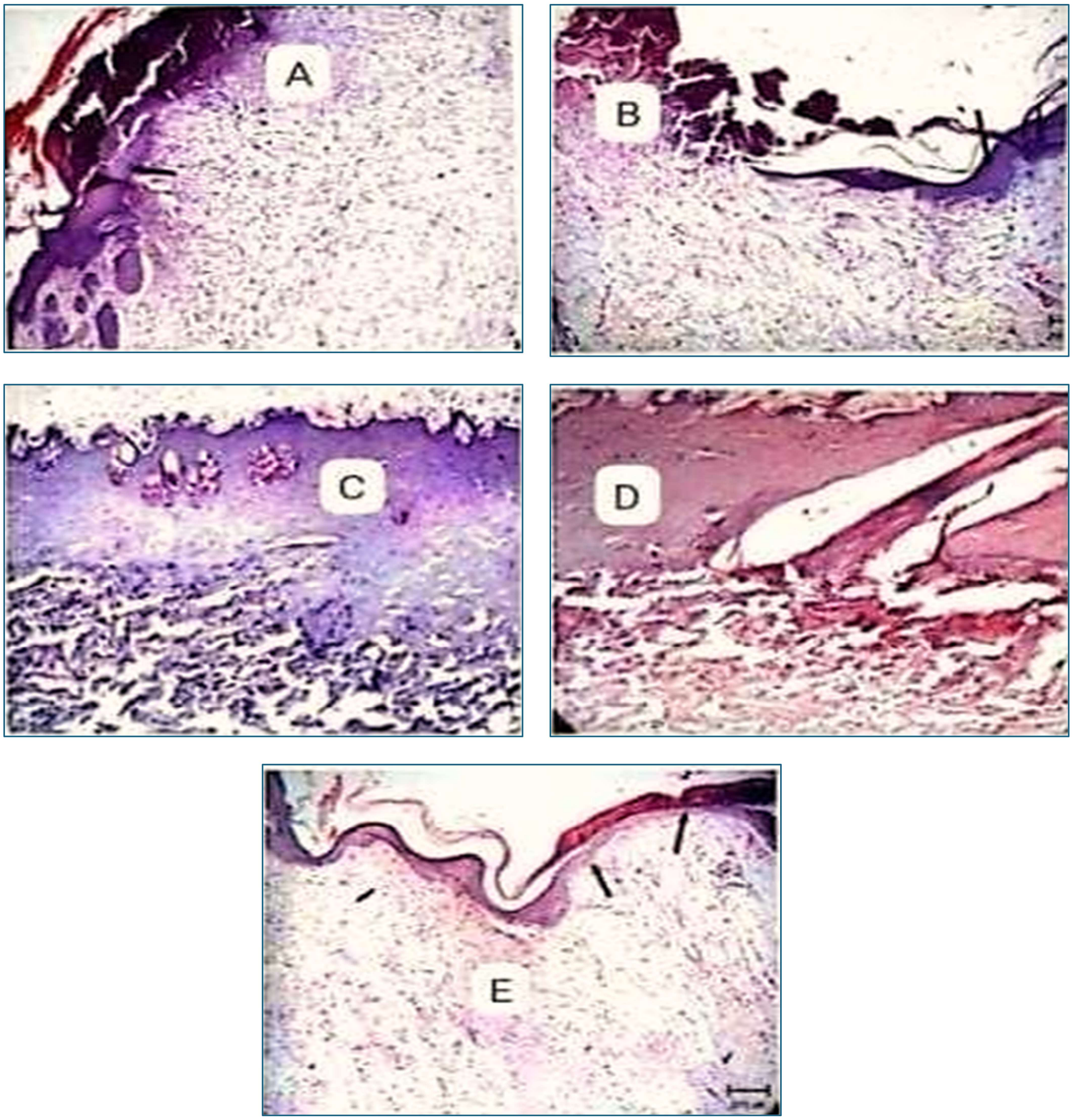

Fig. 2: Histopathology of skin at day 22, stained with H&E (100x). (A) treated with 20% CEO (B) treated with 10% CEO (C) treated with 5% CEO (D) treated with SO (E) treated with 0.2% NFO.

DISCUSSION

The current research employed excision, incision, and burn wound models. In the excision and burn wound models, parameters such as wound contractions, epithelialization periods, and histopathological changes were assessed, tensile strength was assessed using the incision wound model. Accelerated wound contractions, quick epithelialization, favorable histopathological alterations and increased tensile strength were all indicators of each treatment's efficacy. The plant material crude extracts (20% w/w), (10% w/w) and (5% w/w) showed dose-dependent increases in wound contraction rate and reductions in epithelialization durations in the excision wound model. The concentration of bioactive ingredients in the extract ointment may be responsible for this result. The study revealed significant ($p<0.01$), ($p<0.001$) and ($p<0.0001$) wound contractions with the 5%, 10% and 20% crude extract on the 16th day, aligning with findings from other studies on various plant sources (Murthy *et al*. 2013). The accelerated wound contraction and reduced epithelialization time imply an enhanced effectiveness of the plant extract in promoting wound healing, potentially due to the individual or combined impacts of bioactive compounds. The extract might stimulate fibroblast proliferation and exhibit anti-inflammatory properties, contributing to the overall wound repair process (Thakur *et al*. 2011). Plant extracts have the potential to be therapeutic due to their phytochemical contents, which contain bioactive chemicals with potential antibacterial and antioxidant activities. A prior examination focusing on *J. maritima's* leaves, specifically a 70% methanolic crude extract rich in polyphenolic content, showcased notable antioxidant effects. The anti-oxidant properties of *J. maritima* crude extract are responsible for the healing effects that have been reported. These effects include reducing the risk of oxidants causing damage to extracellular proteins, lipids, and DNA, which can extend the inflammatory phase of wound healing.

As compared to the negative control, the study results showed a significant reduction in the mean days of epithelialization together with a notable influence on wound contraction. The extract may have the ability to promote collagen synthesis, fibroblast proliferation, or have anti-inflammatory and antioxidant characteristics, which would explain the enhanced epithelialization and wound contraction. In comparison to the group that received simple ointment treatment, there were histopathological improvements such as moderate fibroblast growth and collagen deposition. The wound healing efficacy of the 5%, 10% and 20% extract ointment closely mirrored that of the standard drug during treatment, indicating the healing impact of the crude extract on burn wounds. These results are consistent with a previous investigation on nettle extract, which found substantial differences in collagen deposition and angiogenesis over an 18-day treatment period when compared to a negative control (Mascharak *et al*. 2021). The efficacy of the crude extract in expediting wound healing was further substantiated in the incision wound model by assessing its impact on tensile (breaking) strength. The groups treated with crude extract ointments exhibited significantly better tensile strength compared to the simple oinment-treated and untreated groups. The presence of many phytochemicals found during screening is primarily responsible for the crude extract's apparent wound-healing abilities. Notably, certain phytochemicals such as protein, alkaloids, carbohydrates, triterpenoids, saponins, flavonoids and tannins are frequently identified. Among these, alkaloids, considered the most beneficial nutrient in *J. maritima*, play a crucial role.

The therapeutic applications of alkaloids, including their use as analgesics, cardio protectants and anti-inflammatory agents, have been extensively documented (Kurek 2019). Tannins are important for wound healing and reducing complications from infections because of their astringent, antimicrobial and angiogenic qualities. Alkaloids help to maintain the development of fibroblasts; flavonoids increase vascularity and decrease lipid peroxidation; terpenoids have antibacterial and astringent properties; and glycosides have actions that are immunomodulatory, antioxidant, analgesic and antimicrobial. Collectively, these activities contribute to the effective wound healing properties of *J. maritima* leaves, which are rich in minerals such as phosphorus, iron, aluminum, calcium, magnesium and potassium. Additionally, it harbors compounds like caryophyllene oxide, hydrocarbons and specific pyrrolizidine alkaloids (Voynikov *et al*. 2023). Significantly, in traditional medicine, this plant is acknowledged for its various health advantages, encompassing antispasmodic qualities, the treatment of cataracts, alleviation of anxiety, and addressing severe eye conditions such as opacity, conjunctivitis and corneal clouding (Althubiti *et al*. 2023). Experimental evidence supporting the anti-inflammatory, immunomodulatory, and antioxidant activities within the *Jacobaea* genus substantiates the plant's efficacy in the process of wound healing.

CONCLUSION

The study concludes that the methanol extract of *J. maritima* leaves demonstrates significant wound healing properties across various wound models in rabbits. The extract, particularly at a 20% concentration, was effective in promoting wound contraction, reducing epithelialization time and enhancing tissue repair compared to control treatments. The improved histopathological outcomes, including increased neovascularization and collagen deposition, further support the extract's efficacy in accelerating wound healing. These findings suggest that *J. maritima* leaf extract has potential as a natural and effective wound healing agent, warranting further investigation for its application in wound care products.

REFERENCES

Abeje BA, Bekele T, Getahun KA and Asrie AB (2022). Evaluation of wound healing activity of 80% hydromethanolic crude extract and solvent fractions of the leaves of *Urtica simensis* in mice. *J. Exp. Pharmacol.*, **14**: 221-241.

Althubiti AA, Alsudir SA, Alfahad AJ, Alshehri AA, Bakr AA, Alamer AA, Alrasheed RH and Tawfik EA (2023). Green synthesis of silver nanoparticles using *Jacobaea maritima* and the evaluation of their antibacterial and anticancer activities. *Int. J. Mol. Sci.*, **24**: 16512.

Badalamenti N, Merra R, Schicchi R, Geraci A, Sgadari F and Bruno M (2023). The chemical composition of the aerial parts essential oil of *Jacobaea delphiniifolia* (Asteraceae) growing in Sicily (Italy). *Nat. Prod. Res.*, **2023**: 3075-3079.

Delgadillo Valdez G and Jhardy G (2018). Reducción del ruido mediante barreras vegetales con las especies Jacobaea maritima y Aptenia cordifolia en condiciones controladas-Lima. **2018**: 2345.

Demilew W, Adinew GM and Asrade S (2018). Evaluation of the wound healing activity of the crude extract of leaves of *Acanthus polystachyus* Delile (Acanthaceae). *Evid. Based Complement. Altern. Med.*, **2018**: 2047896.

Fahimi S, Abdollahi M, Mortazavi SA, Hajimehdipoor H, Abdolghaffari AH and Rezvanfar MA (2015). Wound healing activity of a traditionally used poly herbal product in a burn wound model in rats. *Iran Red Crescent Med. J.*, **17**: 123-128.

Fetzer TM (2018). Appellative use of first names in Swiss German: Denominations for animals, plants, parts of the body, objects and concepts. *Onomastica Uralica*, **13**: 303-316.

Kooti W, Ghasemiboroon M, Asadi-Samani M, Ahangarpoor A, Abadi A, Afrisham R, Dashti N (2014). The effects of hydro-alcoholic extract of celery on lipid profile of rats fed a high fat diet. *Adv. Environ. Biol.*, **8**: 325-330.

Kurek J (2019). Alkaloids: their importance in nature and human life. *BoD-Books on Demand*.

Liu X, Xu H, Zhang M and Yu DG (2021). Electrospun medicated nanofibers for wound healing. *Membranes*, **11**: 770.

Mascharak S, DesJardins-Park HE, Davitt MF, Griffin M, Borrelli MR, Moore AL, Chen K, Duoto B, Chinta M and Foster DS (2021). Preventing engrailed-1 activation in fibroblasts yields wound regeneration without scarring. *Science*, **372**: eaba2374.

Mielke H, Strickland J, Jacobs MN and Mehta JM (2017). Biometrical evaluation of the performance of the revised OECD Test Guideline 402 for assessing acute dermal toxicity. *Regul. Toxicol. Pharmacol.*, **89**: 26-39.

Murthy S, Gautam MK, Goel S, Purohit V, Sharma H and Goel RK (2013). Evaluation of *in vivo* wound healing activity of *Bacopa monniera* on different wound model in rats. *Biomed Res. Int.*, **2013**: 972028.

Namunana S, Lutoti S, Nyamaizi G, Agaba G, Apun I, Ssebunnya C, Tenywa GM, Wangalwa R, Kaggwa B and Kamba PF (2018). Formulation, development and validation of a wound healing herbal ointment from extracts of *Bidens pilosa* and *Aloe barbadensis*. *J. Pharm. Pharmacol. Res.*, **2**: 32-38.

Naveed M, Batool H, Rehman SU, Javed A, Makhdoom SI, Aziz T, Mohamed AA, Sameeh MY, Alruways MW and Dablool AS (2022). Characterization and evaluation of the antioxidant, antidiabetic, anti-inflammatory and cytotoxic activities of silver nanoparticles synthesized using *Brachychiton populneus* leaf extract. *Processes*, **10**: 1521.

Ravishankar K, Kiranmayi GVN, Prasad YR and Devi L (2019). Wound healing activity in rabbits and antimicrobial activity of *Hibiscus hirtus* ethanolic extract. *Braz. J. Pharm. Sci.*, **54**: e17075.

Saroya AS (2013). Controversial herbal drugs of Ayurveda. *Sci. Publ.*

Thakur R, Jain N, Pathak R and Sandhu SS (2011). Practices in wound healing studies of plants. *Evid. Based Complement. Altern. Med.*, **2011**: 438056.

Voynikov Y, Balabanova V, Gevrenova R and Zheleva-Dimitrova D (2023). Chemophenetic approach to selected Senecioneae species. *Plants*, **12**: 390.

Wang J, Gull I, Kousar S and Shahzad R (2023). Evaluation of anti-tyrosinase activity of Pyrus communis leaves extract and cosmetic formulation. *Int. J. Pharmacol.*, **19**: 421.

Zhang K and Gong S (2023). The complete chloroplast genome and phylogenetic analysis of *Jacobaea maritima* (Asteraceae). *Mitochondrial DNA Part B*, **8**: 771-776.