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Effects of Aqueous and Alcoholic Extracts of Russian Knapweed on Cutaneous Wound Healing Process in Rabbits

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ABSTRACT

Several drugs and natural products are used to treat wounds. However, ranchers in Afghanistan do not have enough access to Veterinary services (medicine). On the other hand, medicine is expensive. In this case, the best option is homemade drugs made from medicinal plants. Therefore, this study aims to evaluate the effects of the Aqueous and Alcoholic extracts of Russian Knapweed on the cutaneous wound-healing process in rabbits. Twenty-five local rabbits were used in this experiment and were randomly divided into five groups: control, 5% aqueous extract, 10% Aqueous extract, 5% alcoholic extract, and 10% Alcoholic extract. At first, all rabbits were clinically examined. The wound area was surgically prepared, and after induction of local anesthesia, a 15mm x 15mm incision was made on the back part of all rabbits. The Russian Knapweed extracts were made using the maceration method. After the wounds were created, the ointments were applied twice a day. Measurement of the wound area was done using a ruler on days 1st, 4th, seventh, 10th, 13th, and 16th of wound creation. 5% Aqueous and Alcoholic Russian Knapweed had a significantly ($p<0.05$) greater effect on wound healing on days 4, 7, and 16 compared to the control group. Meanwhile, the 10% Aqueous group results were also significantly ($p<0.05$) higher than the control group on days 7 and 16. The results of this experiment suggest that Aqueous and alcoholic extracts of Russian Knapweed have healing effects on the cutaneous wound-healing process in rabbits.

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INTRODUCTION

The integumentary system serves as the body's outer covering and first line of defense (Allen & Harper, 2011). Because of its external position, it is highly exposed to injury. Every day, numerous humans and animals experience damage to their body tissues from various sources, which disrupts tissue nutrition and integrity. Such disturbances—caused by physical, chemical, thermal, microbial, or immune agents—disrupt standard tissue structure and function and are collectively referred to as wounds (Masson-meyers et al., 2020).

Following an injury, the body immediately begins a restorative process to rebuild the tissue's original form and function. This process, known as healing, involves a sequence of overlapping biological stages: hemostasis (coagulation), inflammation, proliferation (granulation), and maturation or contraction (Ackermann, 2012; Mohan, 2015; Vegad, 2008).

Since ancient times, the treatment of wounds has been one of humanity's most significant medical concerns. Although a wide range of synthetic drugs is available to accelerate wound recovery, many have undesirable effects, limitations, or high costs. Consequently, attention has increasingly shifted toward medicinal plants, which are considered safer, less expensive, and more effective alternatives. Traditional medicine has long utilized a variety of herbs and natural products to promote wound healing due to their rich content of therapeutic bioactive compounds (Babaei-ghaghelestany et al., 2022). In many developing countries, people trust the healing and antimicrobial properties of plants—an idea supported by scientific laboratory research. Recent studies have shown that plant extracts may possess promising activity against cancer, microbial, and viral diseases (Akhgari et al., 2022). The global issue of antimicrobial resistance has further spurred scientists to explore herbal medicines as alternatives to chemical drugs. Research suggests that using plant-based products can significantly reduce drug resistance, as plants naturally produce antimicrobial compounds to protect themselves against pathogens (Babaei-ghaghelestany et al., 2022).

In Afghanistan, livestock owners living in remote rural areas often lack access to Veterinary medicines and services. In addition, high drug prices make treatment difficult for most animal keepers. Therefore, there is a growing need to develop a treatment that is affordable, accessible, effective, and free of significant side effects. In light of these challenges, the current research aims to evaluate the effects of Aqueous and Alcoholic extracts of Russian Knapweed (Acroptilon repens) on open cutaneous wound healing in rabbits (Olfat et al., 2025).

Acroptilon repens is a perennial herbaceous species in the Asteraceae family, native to Turkey, Central Asia, and China (Callaway & Schaffner, 2011). The plant features upright stems reaching 30–80 cm in height (Koloren et al., 2008) and produces pink to purple flowers (Akhgari et al., 2022). It grows best in moist environments and can generate up to 1,200 mature seeds per shoot (Mangold et al., 2007). Seeds are mainly spread through agricultural equipment or contaminated hay and less frequently by wind (Gaskin & Littlefield, 2017). Moreover, essential oils extracted from its aerial parts have demonstrated antimicrobial effects against Gram-positive bacteria (Quintana et al, 2008; Nadaf et al., 2013).

Studies have also demonstrated the larvicidal potential of Russian knapweed extract against Anopheles stephensi, Culex pipiens, and Culex quinquefasciatus (Toolabi et al., 2018). In traditional medicine, Russian knapweed has been utilized worldwide for its emetic, antiepileptic, and antimalarial properties. Recent investigations indicate that this plant possesses multiple pharmacological effects, including antimicrobial, antipyretic, lipid-lowering, antioxidant, and antidiabetic activities. Furthermore, extracts of Russian knapweed have shown strong cytotoxicity against the P-388 and HL-60 tumor cell lines and contain abundant polyphenolic compounds (Dashti et al., 2022; Babaei-ghaghelestany et al., 2022).

Owing to its antioxidant, anti-inflammatory, and antimicrobial characteristics, Russian knapweed may serve as a promising natural agent for promoting wound healing. The study will answer to following research question;

- Does the extract of Russian Knapweed accelerate the wound healing process?

METHODS AND MATERIALS

This experimental and mixed research was conducted on 25 Dutch rabbits in the Faculty of Veterinary Sciences of Kabul University. The materials used in this experience are: Russian Knapweed plant, Distilled water, Alcohol, Whatman filter paper, Water bath, Vaseline, and Surgical tools.

Study Area

Russian Knapweed plant was collected at the time of flowering from the north of Afghanistan, Faryab province, Dawlat Abad district, and transferred to Kabul University, Faculty of Veterinary Sciences. In this experimental research, 25 Dutch rabbits were used. After purchasing the rabbits from the market, they were transferred to the Veterinary Sciences Faculty of Kabul University and kept at room temperature under natural light until the end of the research. During this time, they had enough water and food.

Preparation of Russian Knapweed Extract

Russian Knapweed was washed three times to remove the dust. After washing, it was left in the dry air for 10 days. Its leaves were separated from the stem and ground into powder using a mortar and pestle. Aqueous extract was prepared by the maceration method: 100 g of Russian Knapweed powder was added to 1000 ml of distilled water and left for a week to dissolve completely. The solution was filtered by Whatman filter paper. The solvent from the obtained extract was removed in a water bath to yield the active ingredients. Five grams of active ingredients were mixed with 95 grams of 100% pure Vaseline, and 10 grams of effective ingredients were mixed with 90 grams of 100% pure Vaseline, yielding 5% and 10% ointments of Russian Knapweed, respectively, at room temperature over 4 hours in the laboratory of the veterinary faculty. An alcoholic extract was prepared by the maceration method, similar to the aqueous extract, with the difference that 80% ethyl alcohol was used instead of distilled water. Ointments at 5% and 10% were prepared as described above (Golam Rasul, 2018; Abu Bakar & Haque, 2020).

Preparation for Wounding

Prior to initiating the experiment, the health status and body weight of the rabbits (average 730 g) were assessed through physical examinations. The animals were then allowed to acclimate to the new environment for three days. Subsequently, the fur on the dorsal area of each rabbit was carefully shaved to prepare the site for surgery. The animal was restrained in a fixed position, and a 15 mm × 15 mm square was marked on the left dorsal region, slightly below the spine, using a ruler and a fine-tipped pen. To induce local anesthesia, 0.5 ml, 2% lidocaine was injected subcutaneously at each of the four corners of the square. Following anesthesia, a full-thickness skin wound was created in the designated area (Zaki *et al.*, 2005). After completion of the surgical procedure, Vaseline and other mentioned ointments were applied to the wound surface twice a day (morning and evening) at certain times.

Grouping

The animals were randomly divided into the following five groups:

- (Control): The wounds of this group were treated with pure Vaseline.
- (5% Aqueous) In this group, 5% Aqueous extract ointment was used.
- (10% Aqueous): The wound of this group was treated with 10% Aqueous extract ointment.
- (5% Alcoholic): The wounds of this group were treated with 5% Alcoholic extract ointment.
- (10% Alcoholic): 10% Alcoholic extract ointment was applied to the wound surface of this group.

Wound Measurement

The wound surface was measured by a ruler on the 1st, 4th, 7th, 10th, 13th, and 16th days after surgery.

Statistical Analysis

The collected data were presented as mean ± standard deviation and analyzed using SPSS version 23. In addition, wound healing progress was calculated as a percentage using the following formula:

$$\text{Wound healing (\%)} = \frac{\text{Initial wound area} - \text{Current wound area}}{\text{Initial wound area}} \times 100$$

To compare outcomes across different experimental groups, the Kruskal-Wallis non-parametric test was employed. Differences were considered statistically significant when the P-value < 0.05.

FINDINGS

In the results section of this study, two main parameters were evaluated: (i) changes in clinical signs and body weight of the animals, and (ii) the process and degree of wound healing. Each parameter is presented and discussed separately in the subsequent sections.

Vital Signs and Body Weights

The vital signs "temperature, heart rate, respiratory rate", and body weights of all rabbits were evaluated on the 1st, 4th, 7th, 10th, 13th, and 16th days of the experiment (Table.1). According to the non-parametric Kruskal-Wallis test, there was no significant difference in all vital signs and body weights of rabbits throughout the research between the groups but the 10% Alcoholic group had lost weight on 16th day compared to the 13th day (Table.1).

Table 1. *Vital Signs and Weights of Rabbits*

Parameters	Day	Control	5% Aqueous	10% Aqueous	5% Alcoholic	10% Alcoholic
Temperature (c)	1	38.72±0.44	39.32±0.21	38.62±0.25	38.98±0.47	38.62±0.59
	4	39.16±1.08	39.24±0.54	38.74±0.41	39.36±0.85	38.78±1.09
	7	39.1±1.08	39.34±0.37	38.74±0.41	39.36±0.83	38.4±0.8
	10	38.8±0.4	37.7±4.08	39.4±0.4	39.2±0.1	39.4±0.4
	13	38.07±0.09	38.6±0.3	38.2±0.1	38.9±0.7	38.3±0.09
	16	38.3±0.3	38.6±0.3	38.2±0.1	38.1±0.08	38.1±0.1
Heart Rate (bpm)	1	202±20.1	205.20±25.7	215±22.9	214±21.9	218±23.8
	4	203±15.6	210±20	218±20.4	202±14.8	208±17.8
	7	203±15.6	210±20	218±20.4	202±14.8	210±20
	10	204±5.4	216±20.7	210±10	200±14.1	205±10
	13	193.7±7.5	198±4.4	205±11.1	202±4.4	197.5±5
	16	195±5.7	202±8.3	204±8.9	200±7.07	197.5±5
Respiratory Rate (brpm)	1	56.2±4.1	51.4±4.1	47.6±5.9	49±4.1	49±2.2
	4	51±4.1	51±4.1	48±5.7	47±2.7	45.6±0.89
	7	51±4.1	50±3.5	48±5.7	46±2.2	45.2±0.5
	10	51±2.2	50±3.5	47±2.7	47.6±2.5	47.5±5
	13	45.5±1.2	44±2.2	45.2±0.4	48.6±2.1	47±2.4
	16	46.2±1.7	46.6±2.3	45.8±1.3	47.4±2.5	47±2.4
Body Weight (gr)	1	680±148.3	660±151.6	790±216.2	640±167.3	820±164.3
	4	700±143	680±168	790±216.2	640±167.3	820±164.3
	7	714±131.07	700±183.7	814±221.8	694±192.8	885±118.1
	10	728±136.8	740±181.6	900±226.3	750±158.1	962.5±110.8
	13	717.5±102.7	760±185	900±226.3	760±151.3	962.5±110.8
	16	750±108.01	800±209.1	950±226.3	830±130.3	950±91.2

The data were presented as Means ± SD.

Normal range of Temperature 37.8°-39.4c°; Heart Rate 130-325 (bpm); and Respiratory Rate 32-60 (brpm); (Vennen & Mitchell, 2009).

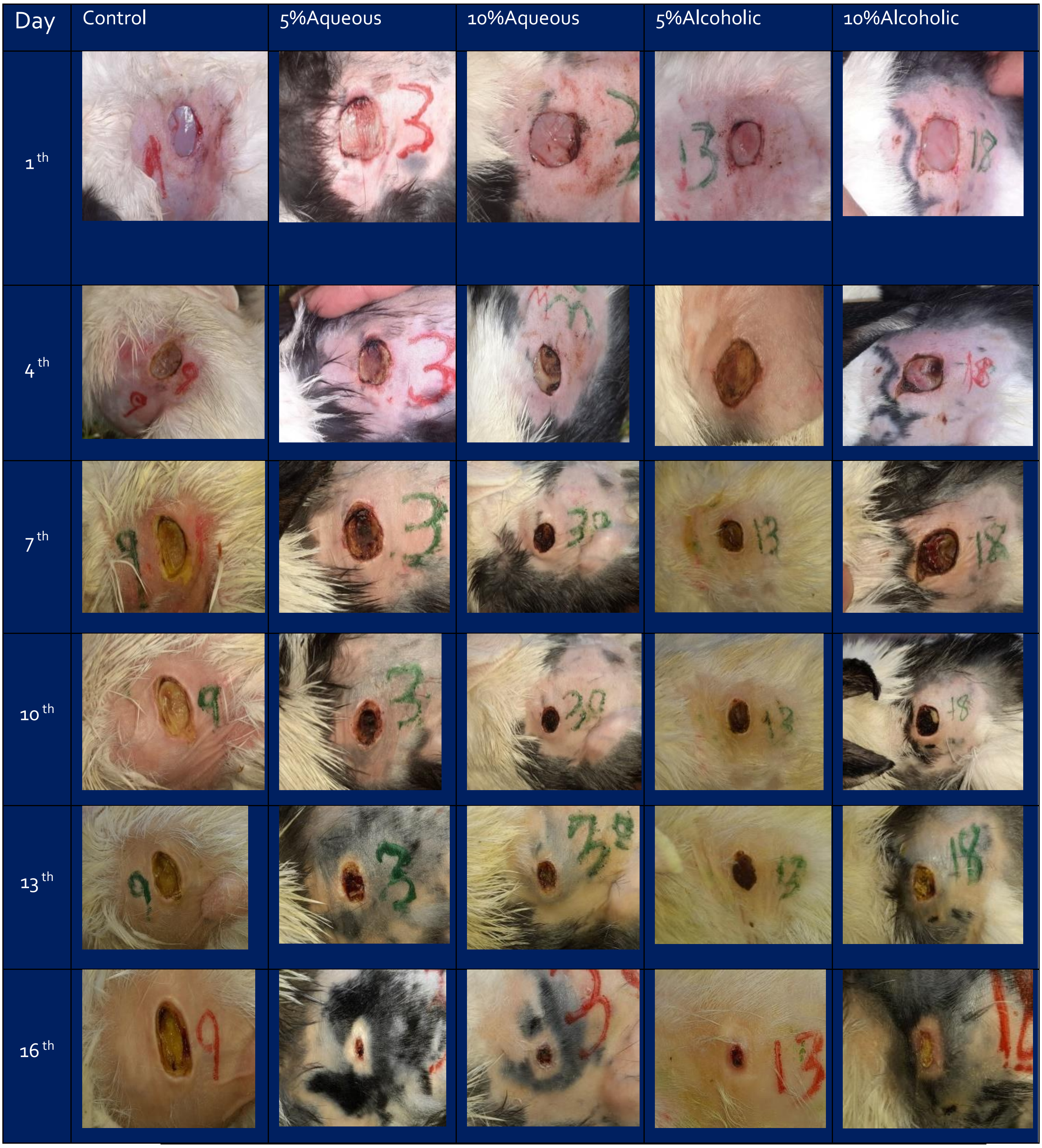
Wound Healing

On the first day, a cutaneous wound was created in all rabbits. The size of the wound was 15mm×15mm (Table.2; Figure.1.1). In addition to the mean and standard deviation, the wound healing rate has been shown as a percentage (%), therefore, the percentage of healing on the first day was considered (0), as the size of the wound decreases, the percentage of healing rate increases.

On the 4th day, there was a significant difference in wound healing rate between the 5% Aqueous group compared to the control group, $P < 0.05$ (Table.2; Figure. 1.4). On the 7th day, the 5% Alcoholic group had the highest healing rate, and the difference of this group with the control group was significant $P < 0.05$. The difference between the 10% Aqueous and 5% Aqueous groups compared to the control group was also significant $P < 0.05$ (Table.2; Figure. 1.7).On the 10th day, wound healing was increasing in all groups, but the difference in healing between groups was not significant $P > 0.05$ (Table.2; Figure. 1.10).On the 13th day, 10% Aqueous, and 5% Alcoholic had the highest healing rate and the control group had the lowest, although this difference between the groups was not significant statistically $P > 0.05$ (Table.2; Figure. 1.13). On the 16th day, 10% Aqueous, 5% Alcoholic, 5% Aqueous 10% Alcoholic, and control groups had the highest healing rate, respectively. The 10% Aqueous and 5% Alcoholic groups were almost 100% healed. Based on the non-parametric Kruskal-Wallis test, this difference was significant $P < 0.05$ (Table.2; Figure. 1.16).

Table 2. *Wound Healing Process of Rabbits*

Parameter	Day	Control	5% Aqueous	10% Aqueous	5% Alcoholic	10% Alcoholic
Wound Size (mm)	1	225±0	225±0	225±0	225±0	225±0
Percentage of healing %		0	0	0	0	0
Wound Size (mm)	4	217.5±15	171.2±23.33*	219±8.21	202.2±28.66	200.2±29.06
Percentage of healing %		3.3	23.9	2.6	10.1	11
Wound Size (mm)	7	210±12.2	136.2±27.7*	158.6±10.8*	133.8±18.5*	161.7±42.6
Percentage of healing %		6.6	39.4	29.5	40.5	28.1
Wound Size (mm)	10	139.7±14.8	101.2±38.5	91±19.5	100.8±18.3	122.7±38.04
Percentage of healing %		37.9	55	59.5	55.2	45.4
Wound Size (mm)	13	79.2±46.8	63.4±28.8	32.8±13.7	43±21.3	61.2±36.5
Percentage of healing %		64.8	71.8	85.4	80.8	72.8



Wound Size (mm)	16	52±56.1	21.2±17.7*	3.8±3.2*	7.6±7.9*	36.5±24.7
Percentage of healing %		76.8	90.5	98.3	96.6	83.7

The data were represented as Means ± SD and percentages.* P<0.05

Figure 1. Wound healing process

DISCUSSION

Natural products with anti-inflammatory, antioxidant, and antimicrobial properties are widely recognized for their role in promoting wound repair (Davoodi *et al.*, 2022). Considering that *Russian Knapweed* exhibits these characteristics (Babaei-ghaghelestany *et al.*, 2022; Akhgari *et al.*, 2022). This study aimed to evaluate its effects on cutaneous wound healing in rabbits. The wound-healing efficacy of Russian Knapweed is attributed to bioactive compounds, such as flavonoids and triterpenes (Quintana *et al.*, 2008; Nadaf *et al.*, 2013). In addition, other plant constituents, including tannins, alkaloids, saponins, vitamins, minerals, and steroids, have been reported to contribute significantly to tissue repair (Nasa & Kumar, 2020; Ekradi *et al.*, 2021; Mazhar *et al.*, 2022).

During the study, weight gain in rabbits correlated with improved wound healing, with the 5% Alcoholic and 10% Aqueous extract groups demonstrating the highest healing rates and the most significant weight gain. These observations align with findings by Premarathna et al. (2021).

Among the ointments formulated from *Russian Knapweed*, the 5% concentration exhibited superior wound healing compared to the 10% Alcoholic extract. This result is consistent with studies on *Calendula officinalis* (*Asteraceae*), in which gels containing 5%, 7%, and 10% ethanolic flower extracts were applied to cutaneous wounds in Sprague-Dawley rats. The experimental gels significantly reduced wound size compared to controls, with the 7% gel achieving the highest healing rate (Firdous & Sautya, 2018).

The findings of the present research indicate that ointments prepared from *Russian Knapweed* accelerated wound closure and prevented infections or inflammation, likely due to the plant's antimicrobial, antioxidant, and anti-inflammatory activities (Dashti *et al*., 2022; Babaei-ghaghelestany *et al.*, 2022).

This study showed that there is no significant difference between Aqueous and Alcoholic extracts of Russian Knapweed in the studied groups on different days. However, the 5% Aqueous, Alcohol, and 10% Aqueous groups had significant differences with the control group on the 4th, 7^{th,} and 16th days. These findings are inconsistent with the study, which showed that the aqueous and methanolic extracts of each weed have different antioxidant properties. In general, the methanolic extracts of weeds had higher antioxidant and antibacterial properties than their aqueous extracts. As the concentration of the Aqueous extract increased, its effectiveness also increased, but not for the alcoholic extract, whose effectiveness did not increase with concentration. These findings in the Aqueous extract are not in conflict with the findings of that study, but they conflict with the findings in the Alcoholic extract, because they found that the antioxidant properties of weed extracts increased with increasing concentration of Aqueous and methanolic extracts (Babaei-ghaghelestany et al.. Overall, the 5% Aqueous, 5% Alcoholic, 10% Alcoholic, and 10% Aqueous extract groups were more effective in promoting wound healing than controls, consistent with Gou, who noted that plants in the *Asteraceae* family generally exhibit wound repair activity (Gou *et al.*, 2023).

In terms of healing duration, control group wounds healed more slowly than those in the treated groups. Notably, in the 5% Alcoholic and 10% Aqueous groups, some rabbits achieved complete wound closure by the 16th day. This observation supports findings by Vitor et al, highlighting that medicinal plants such as *Aloe vera*, *Salvia miltiorrhiza*, *Mimosa tenuiflora*, *Alchemilla vulgaris*, *Angelica sinensis*, and *Radix astragali* can significantly enhance skin lesion healing compared to controls (Vitor *et al.*, 2018).

Also, the plant's strong healing effect may be due to its phenolic and flavonoid compounds, as the hydro-ethanol extract from Vitis labrusca leaves was found to promote wound healing, likely due to its total phenolic and flavonoid content (El Sherbeni, 2023).

Finally, the result of this research is in conflict with "The results of this study show that the Russian Knapweed root and stem extract obtained by decoction method did not have a good effect on wound healing" (Olfat et al., 2025).

CONCLUSION

The results of this research showed that ointments made from Russian Knapweed using the maceration method accelerate wound healing and prevent infection, inflammation, and other complications. Therefore, it can be a potential alternative treatment for wounds. Despite the promising results of this study, several limitations should be considered. First, the study had a small sample size, which may limit the generalizability of the findings. Second, only one extraction method (maceration) was evaluated, and the effects of different extraction methods were not assessed. Third, the study used a single animal model, and the results may not be directly applicable to other animal species or to different physiological conditions. In addition, long-term evaluations to assess potential adverse effects or the durability of the wound-healing response were not performed. Finally, the precise cellular and molecular mechanisms involved in the accelerated wound-healing process were not thoroughly investigated. Therefore, it is recommended to include larger sample sizes and different animal models to enhance the validity and generalizability of the results. Investigating various concentrations of Russian Knapweed extract and comparing their effects may help determine the optimal therapeutic dose. In addition, the use of different extraction methods and their comparative efficacy is suggested. Long-term studies are necessary to evaluate safety, potential side effects, and the sustainability of the therapeutic effects. Furthermore, future research should focus on elucidating the biological and molecular mechanisms underlying wound healing to understand the extract's mode of action. Ultimately, conducting clinical trials could facilitate the potential use of this ointment as an alternative therapeutic option in medical practice. Finally, research on combining this ointment with other pharmaceutical agents may reveal synergistic effects or reduce side effects.

AUTHORS CONTRIBUTIONS

All authors contributed significantly to this study: Conceptualization and design (Olfat), analysis and interpretation (Olfat and Tawfeeq), original draft preparation (Olfat), review and editing (Olfat, Zabuli, and Sangary), supervision (Olfat, Tawfeeq, and Zabuli), data collection and entry (Olfat and Sangary). All authors reviewed the manuscript and approved the final version.

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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