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Original Article

Wound-Healing Activity of Herbal Ointment Containing the Root Extracts of *Astragalus propinquus* in Sprague Dawley Rats

Varatharajan Rajavel[1,*], Fazlina Mustaffa[1], Foo Yau Rou[2], Lim Jia Qi[2], Catherine Khor Khai Lin[2], Mari Jothi[3], Karthikeyan Jayabalan[4]

[1]Department of Pharmacology, Toxicology and Basic Health Sciences Unit, Faculty of Pharmacy, AIMST University, Semeling, Bedong, Kedah Darul Aman, MALAYSIA.
[2]Undergraduate Students, Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, MALAYSIA.
[3]Research Scholar, Government Siddha Medical College and Hospital, Palayamkottai, Tirunelveli, Tamil Nadu, INDIA.
[4]School of Physiotherapy, Faculty of Allied Health Professions, AIMST University, Semeling, Bedong, Kedah Darul Aman, MALAYSIA.

ABSTRACT

Background: *Astragalus propinquus* is an herb belongs to the family of Fabaceae. *Astragalus propinquus* is commonly known as Mongolian milkvetch in English and in Chinese it is called huang qi. *Astragalus propinquus* is a traditional Chinese herbal medicine with ancient origins, derived from the various roots of *Astragalus* species native to China Mongolia, North America and Korea. *A. propinquus* possesses cardiotonic, immunostimulant, hepatoprotective and anti-aging activities. The aim of our study was to assess the wound-healing properties of herbal ointment containing the root extracts of A. propinquus in Sprague Dawley (SD) rats. **Materials and Methods:** *A. propinquus* roots were collected dried, powdered and extracted with ethanol and methanol for 7 days by cold maceration method. *A.propinquus* ointment was prepared using wool fat, hard paraffin, cetostearyl alcohol and white soft paraffin. Ointment formulation was applied once daily on the excision wound on SD rats for 20 days. During the study, the animals were weighed on a regular basis every 5 days. Every fourth day after wound formation, wound contraction was measured as a percentage contraction. In the excision wound model, the wound area was measured by tracing the wound margins onto a transparent sheet and assessing it using millimeter-based graph paper. **Results:** On day 20, the non-treatment group showed a wound contraction was 90.04±1.06 %. The ethanolic extract group and methanolic extract group showed a significant increase in wound contraction which was 98.44±0.99% and 96.63±0.17 % as compared with non-treatment group, moreover the standard drug treatment group also showed the significant contraction with the percentage of 99.88±0.12%. **Conclusion:** Plant based ointment exhibited shows significant wound healing activity in rat excision wound model.

Keywords: *Astragalus propinquus*, Plant based ointment, Sprague Dawley rats, Wound healing activity.

Correspondence:
Dr. Varatharajan Rajavel
Associate Professor, Department of Pharmacology, Toxicology and Basic Health Sciences Unit, Faculty of Pharmacy, AIMST University, Semeling 08100 Bedong, Kedah Darul Aman, MALAYSIA.
Email: varadharajeen@gmail.com

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INTRODUCTION

Astragalus propinquus is referred to as Huang Qi in China "yellow leader." The name highlights both the root's distinctive inner coloration and its esteemed status among practitioners of Chinese medicine. The dried root of Astragalus membranaceus, first recorded in the *Shennong Bencao Jing* (*Shennong's Classic of Materia Medica,* 200-300 AD), has been one of the most widely used health-promoting herbal remedies in China for over 2,000 years. The contemporary Pharmacopoeia of the People's Republic of China lists *astragalus* as an official drug and as one of the fifty essential herbs still used in Traditional Chinese herbal Medicine (TCM). Although it is frequently prescribed daily, *astragalus* is typically used as a Qi tonic. It is frequently given to tonify the lungs and as also support for recurrent colds in addition to being consumed as a tea during illness to hasten recovery.[1] *Astragalus*, which is tonifying and energising, is frequently referred to as the "young person's ginseng." *Astragalus* has recently garnered significant attention as an adaptogen a plant known for its balancing effects, particularly on the immune, neurological and hormonal systems despite being a relatively new area of focus in eclectic American herbalism.[2] *Astragalus* was principally valued for its immunomodulating, antibacterial, diuretic and antiviral activities prior to this classification.[3,4] To further help fight infection, *Astragalus* on antiviral, anti-inflammatory

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and immunostimulating characteristics.[5] *Astragalus* contains polysaccharides that increase white blood cell activity, promote pituitary adrenal-cortical function and replenish bone marrow's production of red blood cells that have been depleted.[6,7] High antioxidant concentrations found in *astragalus* boost the cardiovascular system and help to shield cells from oxidative stress damage. *Astragalus* may lower cholesterol levels, enhance cardiovascular health and minimise the signs and symptoms of serious heart disease, according to recent Chinese studies.[8] *Astragalus* has also been demonstrated to protect the kidneys and liver against harm brought on by drugs and viruses.[9] *Astragalus* may benefit us by regulating how our bodies use dietary glucose, which makes it advantageous for people with diabetes.[10] *A. propinquus* reveals the presence of *astragalus* polysaccharides, coppers, amino acids, sucroses, flavonoids, saponins, seleniums, zincs, triterpenoids, several minor isoflavonoids, vitamin B_9 and other biogenic amines.[11] Cuts on the skin are repaired by a variety of cell strains and their metabolites as part of a crucial physiological process.[12] In the very early stages of the inflammatory stage, attempts are made to repair the damage brought on by a local aggression. Ultimately, these processes result in regeneration and repair, involving cell proliferation and subsequent differentiation through pre-existing tissue cells and/or stem cells. Repair involves the replacement of specialised structures caused by the deposition of collagen.[13] Accordingly, depending on the cell strains impacted by the injury, regeneration and repair may take place following a skin lesion in the same tissue. A few of the therapy alternatives for wound management include analgesics, antibiotics and nonsteroidal anti-inflammatory medicines; nevertheless, the majority of these medications have harmful side effects.[14,15] Several studies on herbal drugs have been conducted in recent years to explain their potential in wound management and these natural remedies have proven their efficacy as an alternative to available synthetic drugs for wound treatment.[16] Many natural herbs have been shown to have strong wound-healing properties in pharmacological studies.[17] This research work aims to evaluate the wound healing activity of plant-based ointment in Sprague Dawley rats using *A. propinquus* extracts.

MATERIALS AND METHODS

Plant Collection

The dried root sample, *A. propinquus* was collected at Kedai Ubat and Farmasi Yit Min Sdn Bhd, Kangar, Perlis after verifying the plant which is used for the research. The plant root sample was identified and authenticated by Dr. Mari Jothi (M.D Research Scholar), Government Siddha Medical College and Hospital, Tirunelveli, Tamil Nadu, India. The dried roots were gradually milled to get coarse powder. The coarse powder was kept in a clean closed container.

Preparation of Extract (Cold Maceration Technique)

Ethanol and methanol extract are prepared from the coarse powder *of A. propinquus* root. During the cold maceration, powdered sample was weighed and separated into a conical flask with the addition of 95% ethanol and methanol as a medium for the extraction process. The conical flask used was slightly larger than the total volume of solvent extract which was 500 mL conical flask to allow uniform shaking when placed on the mechanical shaker. Then they were allowed to stand with continuous agitation of 135 rpm at room temperature for 7 days until the soluble matter has dissolved. After 7 days, the mixtures were strained and marc was compressed to retrieve any excess solvent extract. The filtrate was then collected separately in a round bottom flask and evaporated using a rotary evaporator. The temperature and rpm for ethanolic and methanolic set at 60°C and 110 rpm in the rotary evaporator. To get a concentrated crude product, the extract was pour into China dish and heated in a water bath at 60°C. The successfully obtained finishing product is then sealed with aluminium sheet, labelled and placed in the desiccator.

Preparation of Ethanolic and Methanolic *A. propinquus* Ointment

Simple ointment B.P. was formulated using hard paraffin, cetostearyl alcohol, white soft paraffin and wool fat. The master formula for its preparation (Table 1) was sourced from the British Pharmacopoeia.[18] A 10 g simple ointment base was prepared by melting 0.5 g of hard paraffin in a china dish over a water bath maintained at 80°C. Subsequently, cetostearyl alcohol (0.5 g), white soft paraffin (8.5 g) and wool fat (0.5 g) were added sequentially in descending order of their melting points. All components were melted together with continuous stirring until a homogeneous mixture was achieved. The mixture was then removed from the water bath and stirred until it cooled completely. For the preparation of methanol and ethanol extract ointments, 1 g of each extract was individually incorporated into a portion of the simple ointment base to achieve a 10% (w/w) formulation via levigation. The remaining simple ointment base was gradually mixed in to ensure thorough blending. Finally, the prepared extract ointments were transferred into clean containers (Figure 1) for topical application during the experiment.[19]

Experimental Animals

Healthy male Sprague Dawley rats weighing between 200 g to 215 g were used in this study. The animal house of AIMST University provided the animals. A total of 24 male Sprague Dawley rats are divided equally among 4 animal cages, each housing 6 rats. Animals were housed in tidy, dry cages. During the research, the animals were acclimatised to standard laboratory conditions at 25±2°C and 12:12 hr light and dark cycle, were fed a standard rats pellets and water *ad libitum*. The experiment protocol was approved by AIMST University Human and Animal Ethics

Figure 1: The smooth texture of ethanolic and methanolic *A. propinquus* ointment.

Committee (AUAEC/FOP/2022/04). Every five days during the course of the study, the animals were weighed.

Excision Wound Creation

On wounding day, the animals were anesthetized by using diethyl ether. The animal's dorsal fur was removed after a brief period and the anticipated area (about 300 mm^2) of the wound was marked on their backs. Then, the wound was formed by cutting away full thickness of skin along the markings area (300 mm^2) and the wound was left exposed to the open environment and each rat was housed in an individual cage following wound creation.[20]

Wound Healing Activity

Following wound creation, the animals were divided into four groups, each consisting of six rats. For the excision wound model, the groups were designated as follows:

- Group 1: No drug treatment (Negative Control).
- Group 2: Received ethanolic extract ointment.
- Group 3: Received methanolic extract ointment.
- Group 4: Received Povidone-iodine ointment (0.5% w/w) (Positive control-standard drug).

The ointment was applied topically to the animals once daily for 20 days, beginning on the second day post-wound creation. The wound healing activity of *A. propinquus* was evaluated using the excision wound model. The wounds were monitored on a regular basis until they healed completely. Every 4th day after wound formation, wound contraction was measured as a percentage contraction.

Table 1: Formula of Simple ointment British Pharmacopoeia (BP).[18]

Ingredients	Master Formula (g)	Working Formula (g)
Wool fat	50.0	0.5
Hard paraffin	50.0	0.5
Cetostearyl alcohol	50.0	0.5
White soft paraffin	850	8.5
Total	1000	10

Measure of Wound Contraction

On days 0, 4, 8, 12, 16 and 20 for all groups in the excision wound model, the wound area was determined by tracing the excision wound margin with a transparent sheet using millimeter-based graph paper.[21] Every 4th day until complete wound healing, wound contraction was measured and expressed as a percentage of the healed wound area. The changes in wound area were expressed in mm^2. Using the following formula, wound contraction was quantified as the percentage reduction in the original wound size.

Statistical Analysis

The data were presented as mean±Standard Error of the Mean (SEM) for 6 animals per group. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test, with a p-value<0.05 considered statistically significant.

RESULTS

Effect of Body Weight of Sprague Dawley Rats on Wound Healing Activity

The body weights of Sprague Dawley rats were measured every 5 days after the wound creation. It is used to determine whether the

Table 2: Excision wound area (mm²).

Group	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Negative control	292.67±24.73	245.33±34.67	194.67±26.93	130.67±21.63	92.12±17.08	27.33±12.81
Ethanolic Extract	302.41±18.74	232.23±19.99	167.64±13.24 *	81.62±12.04 *	32.82±17.31 *	04.21±2.53 **
Methanolic Extract	298.67±81.37	224.67±66.42	171.33±28.54 *	110.21±12.93 *	54.67±14.43 *	12.33±9.69 **
Standard Drug	296.06±56.27	212.33±33.89	125.33±20.18*	42.67±13.45*	14.06±2.48*	0.67±0.3**

All the values are given as mean±SEM. *p<0.05 and **p<0.01 compared with negative control group.

Table 3: Wound contraction percentage (%).

Group	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Negative control	0	16.42±8.48	32.29±9.83	55.86±7.32	68.86±2.74	90.04±1.06
Ethanolic Extract	0	23.63±4.71	46.68±3.42	73.24±5.10 *	89.49±3.14 *	98.44±0.99 **
Methanolic Extract	0	24.72±6.78	44.27±3.15	65.79±1.84 *	82.71±0.46 *	96.63±0.17 **
Standard Drug	0	28.87±3.02	57.32±4.33	84.31±4.75*	95.82±0.61*	99.88±0.12**

All the values are given as mean±SEM. *p<0.05 and **p<0.01 compared with negative control group.

weight of animals will affect wound healing activity. There is no significant change between the 4 groups.

Measure of Wound Contraction

Wound excision from each group was taken every 4th day after wound creation and data's are compared in Table 3. The excision wound rats applied with ethanolic extract ointment, methanolic extract ointment and standard drug showed a significant wound healing activity against negative control group on day 8th onwards. The significant results were continued for the following days 12th, 16th and 20th days as compared with negative control group (Figure 2).

Area of Excision Wound

The measurements of wounds treated with ointment were presented in Table 2. The area of excision wound of ethanolic extract ointment, methanolic extract ointment and standard drug treatment groups shows significant effect when compare with the negative control group.

Percentage of Wound Contraction

The measurements of wound contraction percentage were presented in Table 3. The wound contraction percentage of ethanolic extract ointment, methanolic extract ointment and standard drug treatment groups shows significant effect when compare with the negative control group.

DISCUSSION

Wound healing is the progression of restoring damaged tissue to its pre-injury state. The process of repairing connective tissue goes through four stages: coagulation to stop blood loss, inflammation and debridement of foreign material from the skin, epithelium repair and finally tissue remodelling and collagen deposition. Agents that have the ability to speed up these stages are referred to as wound-healing promoters.[22,23] Wound healing is a complex and dynamic biological process focused on restoring cellular structures and re-establishing tissue integrity of injured tissues as closely as possible to their original condition. This process is influenced by factors such as anti-inflammatory effects, fibroblast proliferation, keratinocyte activity, fibroblast protein expression, collagen matrix formation, antibacterial properties and antioxidant functions.[24]

The principal active antioxidants are *astralagus* flavonoids, which have high activity against superoxide anion.[25] According to Jiangwei *et al.*, 2011[26] reported that the consumption of *Astragalus membranaceus* improves lipid profiles, suppresses lipid peroxidation and enhances antioxidant enzyme activity, potentially mitigating the risk of coronary heart disease linked to hyperlipidemia and oxidative stress. Chen *et al.*, 2011[27] found that the ethanol extract of *Astragalus membranaceus* exhibited greater free radical-scavenging activity compared to its crude extracts.

Our findings indicate that from the 8th day onwards, wound contraction in the groups treated with ethanol and methanol extract-based ointments demonstrated significant improvement compared to the negative control group. The observed

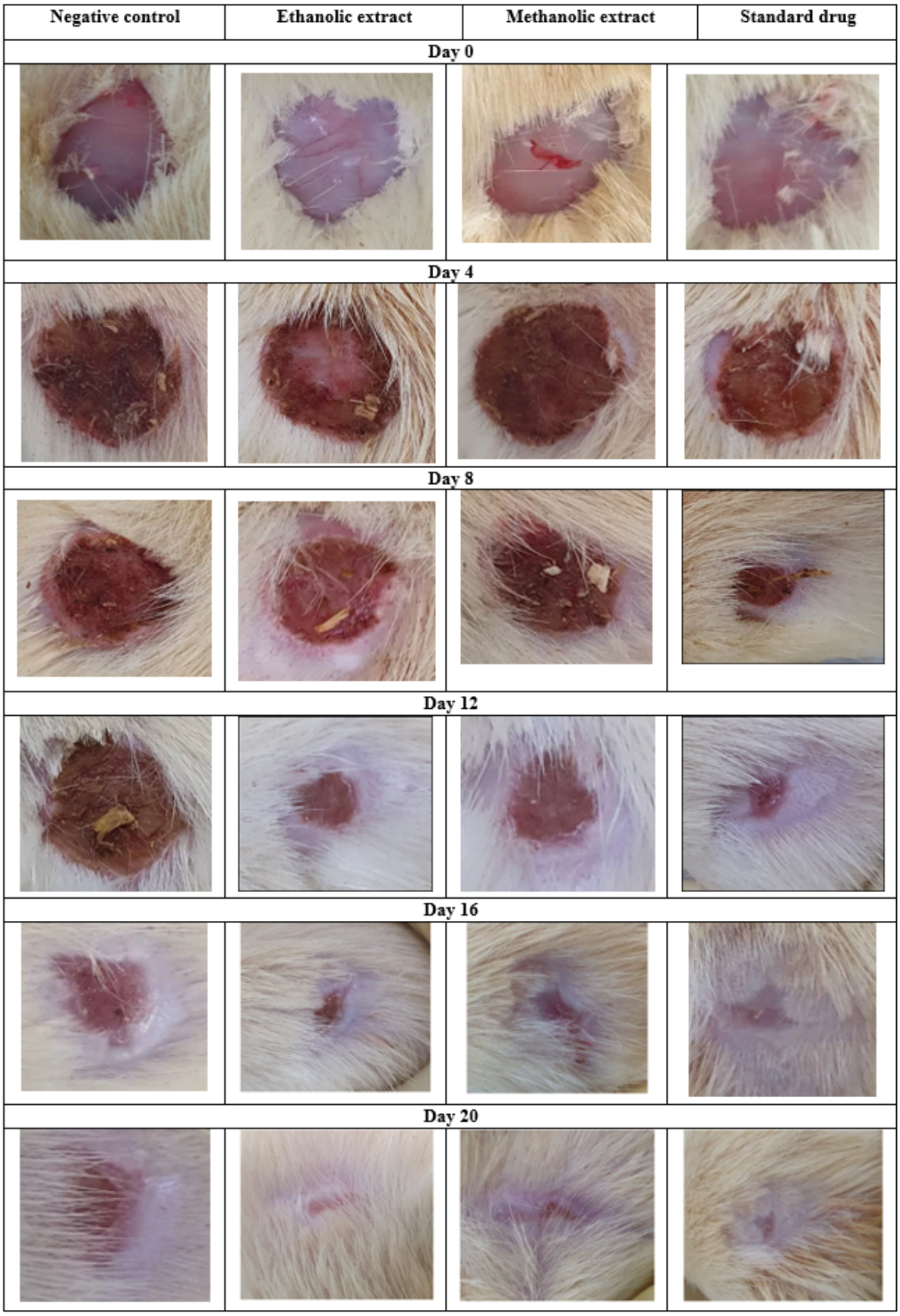

Figure 2: Areas of excision wound every 4th day after wound creation.

wound-healing effects of the plant may be attributed to its antioxidant properties, which play a critical role in tissue repair. Wound healing is a multifaceted process comprising a series of interconnected cellular and biochemical events designed to restore the structural and functional integrity of injured tissues. Numerous medicinal plants have been increasingly utilized for their efficacy in promoting wound healing, each acting through different mechanisms. Medicinal herbs hold great potential in enhancing wound repair processes. The primary objective in wound healing is to accelerate contraction while reducing patient pain, discomfort and scarring. The antioxidant and antibacterial properties of the plant may contribute to reducing the inflammatory phase and minimizing the risk of infection, abscess formation and recurrence.[28-31]

To scientifically validate the traditional use of plant-based topical therapies as wound-healing agents, they must exhibit multiple effects on the physiological systems involved in wound healing.[32] The current study's outcomes align with earlier research that highlights the usefulness of ethanol and methanol extracts in promoting wound healing. Studies show that both extracts significantly accelerate wound contraction and epithelialization in animal models, with the ethanol extract demonstrating a comparatively stronger effect. Similar results have been observed in the treatment of excision wounds, where plant-based ointments enhanced the rate of healing by reducing inflammation and promoting tissue repair.[32] Methanol extract ointments have also shown promising results in wound contraction, though their efficacy, while significant, may be slightly less potent than ethanol extracts. Both extracts contribute to wound healing process through their antioxidant and antimicrobial properties, which aid in reducing the inflammatory phase and preventing infections. Concurrently, the antioxidant activity mitigates oxidative stress by neutralizing Reactive Oxygen Species (ROS) and excess proteases, thereby protecting protease inhibitors from oxidative damage, a crucial step in facilitating tissue repair and wound closure.[24,33-35]

Flavonoids present in *Astragalus membranaceus* root extract have demonstrated the ability to mitigate lipid peroxidation by delaying or reducing the onset of cell necrosis and promoting increased vascularization. These bioactive compounds are believed to facilitate wound contraction and enhance the rate of epithelialization observed with the application of *Astragalus membranaceus* ointment. This accelerated wound healing is attributed to the flavonoids astringent and antibacterial properties, which play a critical role in tissue regeneration and the prevention of infection.[11,35] The effect of ethanol and methanol root extract ointment on wound contraction and healing in experimental rats provides a scientific basis to justify the traditional usage of *Astragalus membranaceus*. The wound healing properties of *Astragalus membranaceus* are likely attributable to its bioactive phytoconstituents. These compounds, either individually or through synergistic interactions, contribute to the quickening of the wound healing development. Their combined effects may influence various stages of tissue repair, including inflammation, cell proliferation and tissue remodeling, ultimately enhancing the rate of wound closure and recovery.[36-39] This work shows that formulating ethanol and methanol extract of *Astragalus propinquus* ointment demonstrates efficacy in wound repair, supporting the potential utilisation of the extracts in the development of commercial dermatological formulations.

CONCLUSION

This study demonstrated that ointments containing ethanol and methanol extracts of *A. propinquus* exhibit properties that significantly accelerate wound healing compared to the negative control group. Observations of wound contraction and percentage of closure confirmed the effectiveness of *A. propinquus* root extract ointments in promoting wound closure during topical treatment and wound management. The herbal ointment demonstrated significant wound healing activity in excision wound models in rats. However, further investigation is needed to elucidate the underlying mechanisms, whether through modulation of the body's defensive processes or direct pharmacological effects on the wound.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***A. propinquus*:** *Astragalus propinquus*; **ANOVA:** Analysis of variance; **B.P:** British Pharmacopoeia. **g:** Gram**; mm:** Millimeter; **ROS:** Reactive oxygen species; **SD:** Sprague Dawley; **SEM:** Standard error of the mean; **RPM:** Revolutions per minute; **TCM:** Traditional Chinese herbal medicine; **w/w:** Weight by weight.

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