WOUND HEALING EFFICACY OF METHANOLIC EXTRACT OF ABHAL (*JUNIPERUS COMMUNIS*)- A TOPICAL EXPERIMENTAL STUDY.

ABSTRACT:

Background:-A cross-sectional study (2022–2023) screening 10,003 individuals found an overall chronic wound prevalence of 1.89 per 1,000, higher in rural (2.64) than urban (1.57) areas, are chiefly caused by diabetic foot ulcers and neglected trauma, often arising from inadequately managed acute injuries or comorbidities, and impose a substantial burden on quality of life. Excessive use of topical antibiotics has contributed to resistant organisms, underscoring the need for herbal alternatives.. Abhal (*Juniperuscommunis L.*), anUnani drug with reported wound-healing potential yet limited scientific validation, was therefore investigated in this study.

Methods: This study evaluated the wound-healing efficacy of a methanolic extract of Abhal (*Juniperuscommunis*L.) using 36 Wistar rats divided into three groups: negative control, standard antibiotic, and Abhal test group. A 20 mm circular excision wound was created on each rat, and treatments were applied topically once daily. Healing progress was tracked through photographs and graph paper measurements on days 4, 8, 12, 16, and 20 to determine wound contraction percentage and epithelialization time (analysed via one-way ANOVA and Tukey-Kramer tests).

Results: The negative control group showed the lowest healing rate across all time points. Conversely, both the standard and test groups demonstrated maximum wound healing on days 8 and 12. In the Abhal group, wound contraction on days 12 and 16 was highly significant ($P < 0.001$) compared to day 4, closely mirroring the standard antibiotic group's performance. Furthermore, the test group and standard group achieved complete epithelialization in significantly less time than the negative control.

Key words:- Abhal (*Juniperuscommunis L.*), methanolic extract, Tukey-Kramer tests, wound contraction percentage, epithelialization time.

1 | Introduction

Skin diseases constitute an increasingly significant global health concern, with the burden of skin and subcutaneous conditions rising sharply over the past decade. Data from the Global Burden of Disease (GBD) study indicate that the global prevalence of these

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conditions rose by 22.7% between 2005 and 2015, underscoring their rapid escalation across 195 countries and territories[1]. Many disorders affect the skin's ability to serve as a protective barrier, often leading to wounds that may be acute or chronic. Wound-related skin disorders, such as diabetic foot ulcers, venous leg ulcers, and pressure sores, pose major clinical challenges due to prolonged healing times, susceptibility to infection, and frequent recurrence. These chronic wounds are often associated with systemic diseases including diabetes, vascular disorders, or immobility, making them not only a dermatological issue but also a multidisciplinary healthcare concern. Chronic wounds, defined as those that fail to progress in an orderly and timely manner to restore the anatomic and functional integrity of the injured site, represent a significant burden to patients, clinicians, and healthcare systems. Often referred to as hard-to-heal wounds, they typically require more than three months to reach chronicity[3]. Globally, chronic wounds affect an estimated 1.51 to 2.21 per 1000 population, a figure expected to rise with aging populations worldwide. In developing countries such as India, prevalence rates are even higher, with chronic wounds affecting approximately 4.5 per 1000 and overall wounds 15.03 per 1000 of the general population. This growing burden highlights the urgent need for improved prevention, management, and innovative therapeutic strategies to address wound-related skin diseases[4].

Since ancient times, humanity has sought effective wound healing agents, and modern science now emphasizes faster recovery with fewer complications. Traditional medicine, still central to healthcare in Asia and Africa, relies on natural compounds from plants, animals, and minerals, often applied topically for localized benefits such as reduced toxicity, enhanced bioavailability, prolonged drug action, and improved therapeutic efficacy. Unlike oral therapies with fluctuating plasma levels, topical delivery ensures sustained pharmacological activity and better outcomes. Yet, conventional treatments and advanced therapies remain limited, costly, and often ineffective, leaving many wounds chronic and leading to amputations—highlighting the urgent need for natural wound care approaches to improve healing and reduce disability[5-7].

In Unani medicine, wound **(***jarāḥat*)is defined as the separation (*tafarruq-e-ittiṣāl*) of muscle (*laḥam*) without pus. It refers to severe tissue disruption, especially in delicate structures. ulcer (*Qurūḥ*)**,** on the other hand, is the same separation but accompanied by pus formation. Ulcers are classified into three types: simple ulcer (*Qurūḥ-e-Baseet*):Free from complicating factors that delay healing. compound ulcer (*Qurūḥ-e-Murakkab*):

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Associated with pain, pus formation, and darkening of surrounding tissue. Non-healing ulcer (*Qurūḥ-e-Aseer-ul-Indimal*): Resistant to closure and prolonged in duration[8].

The Unani system of medicine encompasses a diverse range of effective therapeutic approaches for wound management. It is characterized by affordability, accessibility, cultural acceptance, physiological compatibility, and a comparatively lower incidence of adverse effects. Within this framework, wounds are treated using pharmacological agents endowed with specific properties, including *mujaffif-i-qurūḥ* (desiccant), *jalī* (detergent), *mundammil-i-qurūḥ* (cicatrizant), *muḥallil* (resolvent), *man-i-alam* (analgesic), and *daf-i-taffun* (antiseptic)[7]. Approximately 163 plant species have been traditionally used for wound healing in Indian systems of medicine such as Ayurveda, Siddha, Unani, and folk medicine. Among the most commonly used plants by tribal communities in India are Aloe *(Aloe vera),* Neem *(Azadirachtaindica),* Papita *(Carica papaya),* Dalchini*(Cinnamomumzeylanicum),* Haldi *(Curcuma longa),* Asgarghas *(Cynodondactylon), Thuhar(Euphorbia nerifolia),* Bargad *(Ficusbengalensis),* Goolar *(Ficusracemosa),* Asl-us-soos *(Glycyrrhizaglabra),* Qaranfulhindi *(Nelumbonucifera),* Tulsi *(Ocimum sanctum),* Amla*(Phyllanthusemblica),* Sheet raj *(Plumbagozeylanica),* Sandal surkh *(Pterocarpussantalinus),* Mjeeth *(Rubiacordifolia),* Lodhpathani *(Symplocosracemosa),* Arjun tree *(Terminaliaarjuna), and* Haleela *(Terminaliachebula)*[9]

Abhal (fruit of *Juniperuscommunis Linn.*) is one such drug described in classical Unaniliterature including *Al-Qanoon Fit-Tibb*, *Muheet-e-Azam*, *Khazain-ul-Advia*, and *Makhzan-ul-Mufradat*.This plant has been extensively documented for its multifaceted pharmacological activities, which include Analgesic activity[10], Anti-inflammatory activity[11], Antimicrobial activity[12], Antioxidant activity[13, 14]. Such properties underscore its therapeutic potential in wound healing and broader clinical applications.

The berries, aerial part, fruit and bark of the Abhal possess different pharmacological activities and hence being used as carminative, antiseptic, diuretic, emmenagogue, digestive and anti- inflammatory. So Abhal is used in acute and chronic cystitis, renal suppression, albuminuria, leucorrhoea, amenorrhoea, infantile tuberculosis, piles, asthma, gonorrhoea, cough, and skin infections[15].

According to classical Unani literature, the drug *Abhal* (*Juniperuscommunis*) is recognized for its effectiveness in treating wounds[17-19]. This study was undertaken to

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evaluate its therapeutic potential, with the primary objective of identifying a treatment that is expedient, affordable, and efficient.

2 | MATERIALS AND METHODS

Collection of plant samples and Authentication

The dried fruit of *Abhal* (*Juniperuscommunis*) was procured from the local market in Patna, Bihar, and subjected to authentication procedures to ensure its botanical identity and quality. The test drug was scientifically validated by Cognosmed Laboratories Private Limited, AIC; Bihar Patna – 800010, Bihar, India (CIN: U24230BR2018PTCO40001). Authentication was performed using High-Performance Thin Layer Chromatography (HPTLC), and the sample was certified under Authentication No. CML/AYUSH/24-25/010 dated 11 October 2024. The issuance of this certificate confirmed the authenticity and reliability of the drug material, thereby establishing its suitability for subsequent pharmacological and analytical investigations.

Experimental animals

Thirty-six healthy adult Wistar albino rats of either sex were randomly selected for the present study. The animals were procured from M/S Chakraborty Enterprises, Laboratory Animal Supplier, GirishVidyaratna Lane, Narkeldanga, Kolkata-700011, West Bengal, India (Registration No. 1443/PO/Bt/s/11/CPCSEA). They were maintained in the Laboratory Animal House of the Department of Veterinary Pharmacology and Toxicology, Bihar Animal Sciences University (BASU), Patna. Rats aged 12 weeks, with body weights ranging from 150–200 g, were utilized for the experiment. Each animal was housed individually in a polypropylene cage with a controlled floor area, under standardized environmental conditions (temperature 23 ± 2°C, regulated humidity, and a 12 h light/dark cycle). Prior to initiation of the study, the rats were acclimatized for one week and housed separately to avoid estrous cycle synchronization. Animals were provided a standard vegetarian diet fortified with vitamins and minerals in accordance with National Research Council (NRC) guidelines for laboratory rats, along with unrestricted access to potable water. The experimental protocol was conducted in compliance with NRC recommendations and received prior approval from the Institutional Animal Ethical Committee (IAEC) under reg. no:-1365/GO/Re/S//10/CPCSEA Dated 07.04.2022

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Experimental design

The rats were randomly and evenly distributed into three groups, each consisting of 12 animals. Group-1 rats were left untreated and considered as the negative control. Group-2 rats were administered with standard drug 1% w/w Framycetinsulphate cream (FSC) while group-3 received Test Drug (*Abhal* methanolic extract)**.**

Group	Treatment	No. of Animal	Mode of Application	Duration of Experiment
1	Untreated	12	---	21 days
2	1% w/w Framycetinsulphate cream	12	Topical	21 days
3	Methanolic extract of *Abhal*.	12	Topical	21 days

Table1:-Experimental Design and Treatment Groups

Extraction procedure

The test drug was subjected to coarse grinding using an electric grinder in the Pharmacy Laboratory of Government Tibbi College and Hospital, Patna. The material was air-dried at room temperature and subsequently pulverized into a fine powder. For extraction, 20 g of the powdered drug were macerated in 200 mL of methanol at 50 °C for 8 hours under continuous agitation (700 rpm) in the absence of light. This process was repeated twice to maximize yield. The resulting mixtures were filtered, and the combined filtrates were concentrated to dryness using a rotary evaporator maintained at 40°C[20].

Concentration of extract:

A stock solution of the *Abhal* extract was prepared by dissolving 8 gm of the dried extract in 4 ml of dimethyl sulfoxide (DMSO), resulting in a final concentration of 2 gm/ml. The solution was thoroughly mixed to ensure complete dissolution and stored at 4 °C in a dark container to protect it from light and degradation[21].

Standard drug:

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Framycetin 1% w/w (Soframycin, Aventis Pharma, Goa) was purchased from local market patna.

Preliminary phytochemical screening

The crude methanolic extract was assessed for secondary metabolites such as alkaloids, flavonoids, saponins, tannins, proteins, starch, carbohydrates, and steroids using standard methods.

Methodology for Induction of wound

The rats were kept without food overnight before being subjected to excision wounds. They were anesthetized using the open-mask method with anesthetic ether, and their backs were shaved prior to the procedure[22]**.**A circular incision, approximately 20 mm in diameter, was made on the sanitized skin surface, and the skin was carefully removed from predetermined area in the dorsal inter scapular region,using a toothed forceps, scalpel and pointed scissor.

The wound area, was then immediately measured by placing a transparent tracing paper over it and outlining its shape. This traced paper was transferred onto a 1mm² graph sheet, where, the outline was retraced. The number of squares within the traced boundary was counted, and the area was recorded accordingly[23].The procedure followed standard protocol and each rat was housed individually in disinfected polypropylene cages. All steps complied with the Institutional Animal Ethics Committee guidelines.

Treatment methods

The ointment was topically applied once a day starting from the day of the operation till complete epithelialization. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percentage reduction in wound area. The progressive changes in wound area were monitored planimetrically by measuring the diameter every 4th day. The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound[24].

Wound healing evaluation parameters

I. Measurement of wound contraction

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The dynamic change in wound area was monitored by camera on the 4th, 8th, 12th, and 16th days[25], with wound size traced on transparent paper, transferred to millimetre-scale graph paper for surface area evaluation, and measured under light diethyl ether anesthesia[23], the evaluated surface area was then used to calculate the percentage of wound contraction, taking the initial wound size of 314 mm² as 100%, according to the following formula.

$$WC\ (\%) = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

II. Period of epithelialization

Falling of scab deserting no raw wound behind was taken as end point of complete epithelialization and the days required for this was taken as period of epithelialization[24].

Statistical Analysis

The data on percentage wound healing was statistically analyzed using ANOVA one way followed by Tukey-Kramer Multiple comparison test. All data were reported as means ±

3 |RESULT

Phytochemical Screening:

Qualitative phytochemical screening of Abhal berries revealed the presence of alkaloids, tannins, proteins, starch, carbohydrates, flavonoids, and steroids. However, saponins were found to be absent.

Wound Healing Effect

Wound area was measured by tracing the wound margin using 1mm² graph sheet in each 4^{th} day-interval and healed area was calculated by subtracting from the original wound area.

The mean± SEM of wound healing (%) values on day 4^{th} were 24.09 ± 1.64, 47.29 ± 1.104 and 38.45 ± 1.77 for negative control, Standard and test group respectively in which maximum wound healing (%) was seen in standard group and minimum in negative control group. By day 8^{th}, the mean ± SEM values were 36.48± 1.77 for negative

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control, 66.87± 0.863 for standard group and 58.64± 1.34 for test group, here also maximum wound healing (%) was seen in standard group and test group and minimum in negative control group. On day 12th the mean ± SE values were 61.30±1.19 for negative control, 88.47± 1.235 for standard group and 83.75± 1.24 for test group, here also maximum wound healing (%) was seen in standard group and test group and minimum in negative control group. On day 16th the values were 81.78±0.83, 98.87± 0.097 and 97.50± 0.31 for negative control, standard group and test group respectively, in which maximum wound healing (%) was seen in standard and test group and minimum in negative control group. From day 4 onwards, a significant reduction in wound siz**e** was observed in the treated groups. This early onset of wound contraction indicates that the treatments enhanced fibroblast activity, collagen deposition, and angiogenesis (formation of new blood vessels), all of which are essential for tissue repair. The control group, in contrast, showed slower wound contraction and delayed healing.

It was also observed that epithelialization period of test group 19 ± 0.365 and standard group 18 ± 0.00 were less in comparison to negative control group 20.83 ± 0.542(Table 5). The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing process. The studies on excision wound healing model revealed that all the three groups showed day to day decrease in wound area. However, on 16th post wounding day, negative control animals group showed 58.33mm^2 reduction of wound area (which may be due to self-immunity of the animals) whereas Standard group-Framycetin treated animal, showed 3.1 mm^2 wound area reduction and the test group exhibited 7.9mm^2 wound areas.

Days	Negative control	Standard dug	Test drug
0th day			

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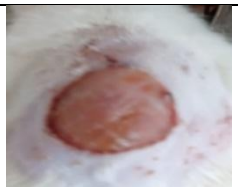
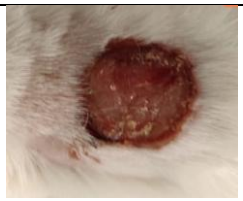
4th day			
8th day			
12th day			
16thday			

Fig 1:-Photographic representation of wound contraction on various post excision days (0-21 days)

Groups	Percentage (%) wound contraction in days			
	Day 4	Day 8	Day 12	Day 16
Negative Control	24.09 ± 1.64 (238.33)	36.48± 1.77 (199.41)	61.30±1.19 (121.5)	81.78±0.83 (58.33)
Standard group	47.29 ± 1.10** (165.5)	66.87± 0.86** (104)	88.47 ± 1.23** (36.16)	98.87± 0.09** (3.1)
Test group (Methanolic extract of Abhal)	38.45 ± 1.77 * (193.33)	58.64± 1.34** (129.83)	83.75± 1.24** (51)	97.50± 0.31** (7.9)

Table 4: Effect on Post-Wound Area in the Excision Wound Model

Values are expressed as mean ± SEM, n=12. Statistically significant as compared to control; P<0.05(*), P<0.01 (**) Values in Parenthesis are post wounding reduction in wound area in mm^2.

Group	epithelialization period (in days)
Negative Control	20.83 ± 0.54

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Standard group (1% w/w framycetinsulphate)	18 ± 0.00
Test group (MEJC)	19 ± 0.365

N= 12 wistar rats per group; values represents mean SEM.

Table 5:- Effect on period of epithelization in excision wound model

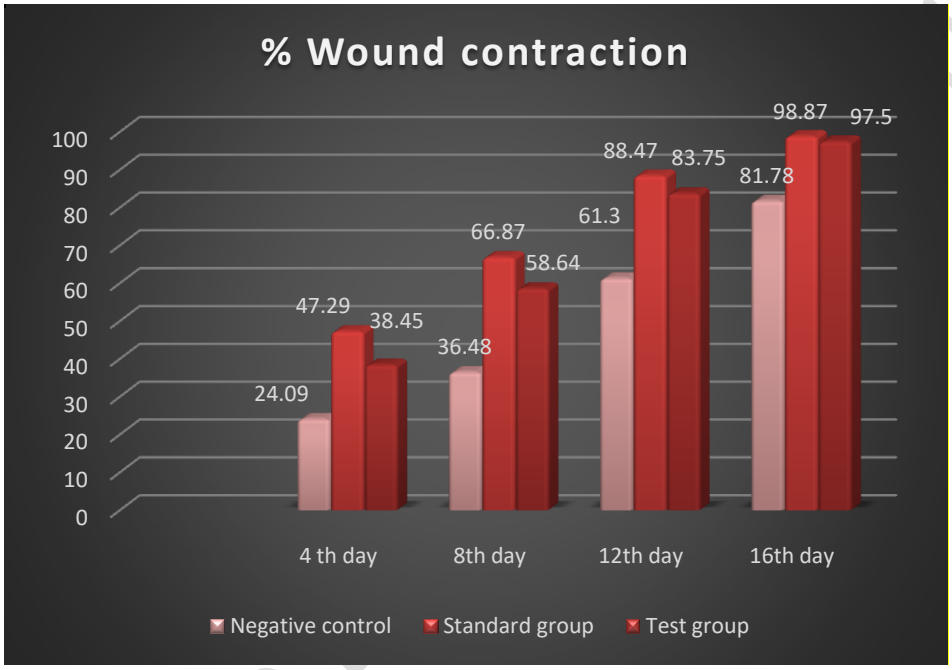


Fig2:- Graphical representation of % wound contraction of all groups.

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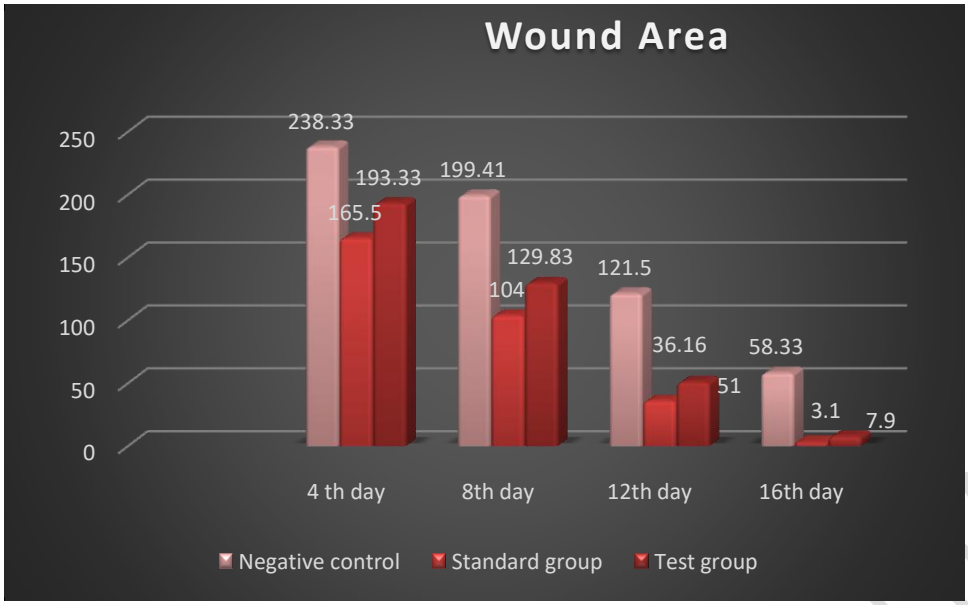


Fig 3:- Graphical representation of wound area of all groups in post wound days.

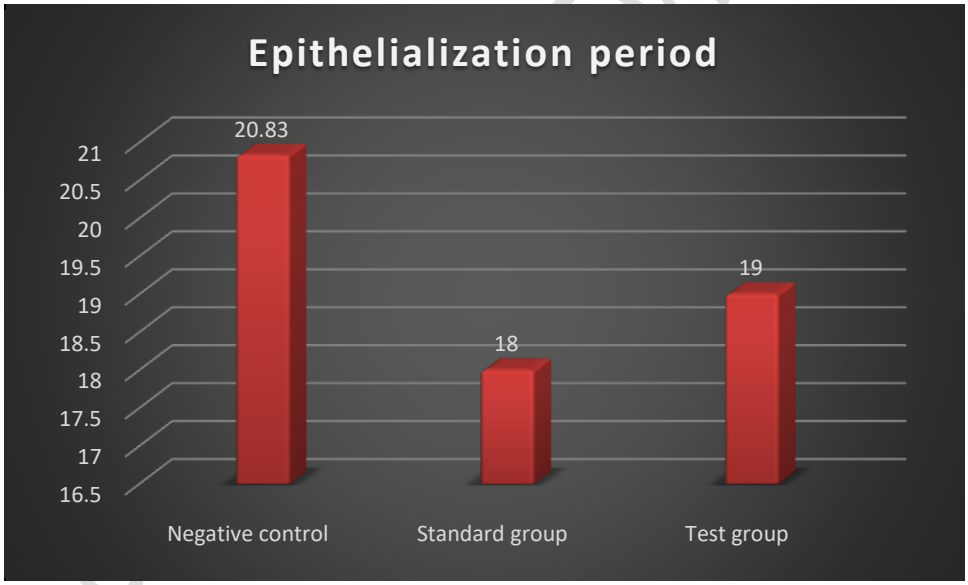


Fig 4:- Graphical representation of Epithelialization period

4| DISCUSSION

Wound healing is a complex and highly regulated biological process that occurs through four overlapping phases: haemostasis, inflammation, proliferation, and remodelling. These stages involve coordinated interactions among specialized cells, extracellular matrix components, and regulatory proteins. Successful wound healing requires the

proper progression through each of these phases. The normal physiology of wound healing depends on controlled levels of reactive oxygen species (ROS) and oxidative stress.

Methanolic extracts demonstrated strong antibacterial and antifungal activity[26], outperforming other solvents and even standard antibiotics[15]. For wound healing studies, topical application of methanolic extract was tested on Wistar rats against a standard drug (framycetinsulphate 1% w/w), showing promising results in enhancing tissue repair through antimicrobial action, collagen synthesis, angiogenesis, and inflammation reduction.

Statistical analysis confirmed that both treatments were effective in promoting wound healing, with values ranging from $P< 0.05$ to $P< 0.01$ compared to the control. However, the standard group demonstrated slightly faster and more pronounced wound contraction, while the test group also showed strong healing potential, particularly evident by day 16 when contraction levels were nearly equivalent. While negative control group shows the minimum wound healing (%) at all-time points,

The results demonstrated that the standard group showed the fastest wound contraction, reaching 98.87% by day 16. The test group treated with methanolic extract of Abhal showed 97.50% wound contraction by day 16, indicating a strong wound-healing effect comparable to the standard drug.

Similarly, the epithelialization period was shortest in the standard group (18 days), followed by the test group (19 days), while the control group required 20.83 days.

These findings suggest that *Abhal* possesses significant wound-healing potential, likely due to the synergistic effects of its phytoconstituents such as phenolics and flavonoids, which exert antioxidant, anti-inflammatory, and antimicrobial activities.

5|CONCLUSION

The present study demonstrates that Abhal (*Juniperuscommunis L.*) possesses significant wound healing potential. Treatment with its methanolic extract resulted in a marked reduction in wound size and a shortened epithelialization period, with efficacy comparable to the standard drug, framycetinsulphate. The observed activity may be attributed to pharmacological properties described in classical Unani literature—anti-

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inflammatory (*Mohallil*), desiccative (*Mujaffif*), and astringent (*Qabiz*)—which collectively reduce inflammation, dry the wound surface, and promote tissue contraction. Additionally, the presence of flavonoids and tannins, known for their antioxidant, antimicrobial, and astringent actions, likely enhanced healing by mitigating oxidative stress, preventing microbial infection, and supporting tissue repair. The extract's antimicrobial effects, previously reported to be comparable to conventional antibiotics such as ampicillin and erythromycin, further reinforce its therapeutic relevance. Overall, these findings highlight Abhal as a promising natural agent for wound management, bridging traditional knowledge with modern pharmacological validation. Future research should focus on conducting dose–response studies to establish the optimal concentration of the methanolic extract of *Juniperuscommunis L.* for topical application, thereby identifying the most effective and safe therapeutic range. Detailed investigations into the mechanism of action, supported by histopathological studies, are needed to confirm tissue-level healing. Efforts should also be directed toward developing advanced pharmaceutical formulations—such as gels, creams, or ointments—that can improve stability, bioavailability, and therapeutic efficacy. In addition, evaluating the extract across diverse wound models, including incision and burn wounds, will provide a more comprehensive understanding of its wound healing potential.

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