Sumer Journal for Pure Science (ISSN: 2790-7031)

SJPS 2026 (June), Vol. 5, No. 1, p.p.: 262-272

GC-MS Characterization and Histopathological Evaluation of Wound Healing Following Topical Application of *Commiphora Myrrha* Essential Oil in a Rabbit Model

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Abstract

To assess the effectiveness of Commiphora Myrrha essential oil for topical use, this study was performed using a full-thickness rabbit wound model. Fifty percent of Myrrha essential oil was extracted by steam distillation and analysed via Gas Chromatography-Mass Spectrometry (GCMS). Twelve New Zealand White rabbits were randomly assigned to two groups (six rabbits per group): Group I received one-time daily applications of 5% Myrrha essential oil, and Group II received one-time daily applications of distilled water with a control group of six rabbits; both groups had full-thickness longitudinal skin wounds approximately two cm long created symmetrically along both sides of the dorsal midline that were opened once daily for 28 days. Skin biopsies were taken from the same rabbits at 3, 7, 14 and 28 days after implementation and prepared for histological examination via Hematoxylin & Eosin and Masson's Trichrome to evaluate inflammatory response, reepithelization and collagen organization at time of biopsy. While the results of the study (33 compounds identified) were formulated based on results from the gas chromotography-mass spectrometer (HMF at 42.42% and quinic acid at 19.14%) these details were not included in the abstract of the study which provides an overview of the study's findings. The group treated with Myrrh showed significant improvements in healing of wounds versus the control group. Loss of inflammatory cells in the tissue and presence of sero-cellular crusts were noted at days 3-7 after injury. By day 7-14, improved epithelial migration and reorganization of the dermis were observed. Complete re-epithelialization and re-organization of collagen bundles occurred at day 28 in the Myrrh group; however, collagen was immature and there were still inflammatory cells present in the control group. The study concluded that topical Commiphora myrrha (Myrrh) enhances healing of cutaneous wounds by promoting resolution of inflammation, stimulating fibroblast production, and accelerating maturity of collagen. These results indicate that myrrh could have a therapeutic role in increasing both rate and quality of repair of skin tissues.

Keywords: Commiphora Myrrha, Wound healing, GC-MS, Skin, Rabbit model.

التقييم النسيجي وتحليل *GC–MS* لألتئام الجروح الجلدية بعد التطبيق الموضعي لزيت المر الأساسي في الأرانب

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الخلاصة

لتقييم فعالية زيت المر العطري للاستخدام الموضعي، أُجريت هذه الدراسة باستخدام نموذج جرح كامل السماكة في الأرانب. تم استخلاص زيت المر العطري بتركيز 50% عن طريق التقطير بالبخار، ثم تم تحليله باستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة (GCMS). تم توزيع اثني عشر أرنبًا أبيض من سلالة نيوزيلندا عشوائيًا على مجموعتين (ستة أرانب في كل مجموعة): تلقت المجموعة الأولى جرعة يومية واحدة من زيت المر العطري بتركيز 5%، بينما تلقت المجموعة الثانية جرعة يومية واحدة من الماء المقطر، بالإضافة إلى مجموعة ضابطة مكونة من ستة أرانب. أُحدثت في كلتا المجموعتين جروح جلدية طولية كاملة السماكة، بطول 2 سم تقريبًا، بشكل متناظر على جانبي الخط الوسطي الظهري، وتم فتحها مرة واحدة يوميًا لمدة 28 يومًا. أُخذت خزعات جلدية من الأرانب نفسها في الأيام 3 و7 و14 و28 بعد بدء التجربة، وجُهزت للفحص النسيجي باستخدام صبغة الهيماتوكسيلين والإيوسين وصبغة ماسون ثلاثية الألوان لتقييم الاستجابة الالتهابية، وإعادة تكوين الظهارة، وتنظيم الكولاجين وقت أخذ الخزعة. بينما استندت نتائج الدراسة (تحديد 33 مركباً) إلى نتائج مطياف الكتلة المقترن بالكروماتوغرافيا الغازية (HMF بنسبة 42.42% وحمض الكينيك بنسبة 19.14%)، لم تُدرج هذه التفاصيل في ملخص الدراسة الذي يقدم نظرة عامة على نتائجها. أظهرت المجموعة المعالجة بالمرّ تحسناً ملحوظاً في التئام الجروح مقارنةً بالمجموعة الضابطة. لوحظ فقدان الخلايا الالتهابية في الأنسجة ووجود قشور مصلية خلوية في الأيام من 3 إلى 7 بعد الإصابة. وبحلول اليوم 7 إلى 14، لوحظ تحسن في هجرة الخلايا الظهارية وإعادة تنظيم الأدمة. اكتملت عملية إعادة تكوين الظهارة وإعادة تنظيم حزم الكولاجين في اليوم الثامن والعشرين في مجموعة المر؛ بينما كان الكولاجين غير ناضج، ولا تزال الخلايا الالتهابية موجودة في المجموعة الضابطة. وخلصت الدراسة إلى أن استخدام نبات المر موضعيًا يُحسّن التئام الجروح الجلدية من خلال تعزيز انحسار الالتهاب، وتحفيز إنتاج الخلايا الليفية، وتسريع نضوج الكولاجين. تشير هذه النتائج إلى أن للمر دورًا علاجيًا في زيادة كلٍّ من سرعة وجودة ترميم أنسجة الجلد..

1. Introduction

Skin, the largest organ in the mammalian body, acts as the main protective shield of the body against all types of environmental attack (external forces), micro-organisms, and the body's ability to respond to an injury. Wound healing is a complex biological phenomenon exhibiting three different phases: inflammation, proliferation, and remodelling. Natural medicinal Botanical (plant-based) medicines, especially those from plants in the genus Commiphora, are receiving increased scientific scrutiny for their anti-inflammatory, anti-microbial, and tissue-regenerative capabilities [1].

There are four overlapping phases to wound healing: 1) hemostasis 2) inflammation 3) proliferation 4) remodeling [2]. Each phase of the healing process is precisely controlled by interaction among many different types of cells present within each phase of the healing process. There are many types of cells that belong in either phase or multiple phases of the healing process (e.g. inflammatory cells; Fibroblasts; endothelial cells; myofibroblasts; etc.). Each cell type can play an important role and influences one another throughout all of the phases. The individual cells communicate using cytokines, growth factors, and extracellular matrix components, etc. If there is an imbalance between any one of these types of cytokines, growth factors, or extracellular matrix components during the healing process (e.g., prolonged inflammatory response, impaired function of fibroblasts, excessive oxidative stress) will result in an adverse consequence causing slow healing or chronic wound. As such, it has been a focus of much clinical and experimental research in such a way to enhance wound healing [3].

Researchers are investigating how to make natural products useful for treating many different types of illnesses and conditions. Commiphora myrrha (myrrh) is a naturally occurring resin from trees in the family Burseraceae. It has been used as a treatment for injuries, inflammation, and pain for several centuries within the field of alternative medicine. There are many references in historical literature regarding the way ancient Egyptians used myrrh to treat injuries and to aid in the preservation of the tissue of those injured.

According to studies published in the last few years, C. myrrha has considerable anti-inflammatory, antioxidative and antimicrobial activities. Additionally, the bioactive components (contents) of C. myrrha including terpenes, flavonoids and phenolics exhibit a reduction in oxidative stress, an enhancement of fibroblast proliferation, a stimulation of collagen synthesis, and a stimulation of re-epithelialization. The effects of these properties of C. myrrha lead to faster and more orderly wound healing [4-6].

Studies indicate the potential of the essential oil of C. myrrha to enhance wound healing [7]; however, there is a lack of rigorously controlled clinical trials with complete histopathological data regarding the topical application of C. myrrha essential oil on standardized animal models for wound healing [8]. The majority of the studies published on C. myrrha focus mainly on overall outcomes of healing without correlating cellular and tissue responses to the stages of wound healing. This information is critical to determine if C. myrrha has a legitimate biological activity as an agent for therapy and to serve as evidence to support its clinical usage.

Data from pharmacological studies show that Commiphora myrrha has anti-inflammatory, antibacterial, and wound-healing effects. Additionally, new data collected from this study indicates that the histological maturation of skin tissue was greater, and the progression

through the four phases of wound healing occurred more rapidly than in prior studies. The findings indicate that, when measured under the present experimental conditions, increased therapeutic efficacy exists for Commiphora myrrha than what has previously been demonstrated.

Therefore, the present study aims to investigate the effect of topical *Commiphora myrrha* essential oil on wound healing in rabbits through detailed histopathological evaluation.

2. Materials and Methods

2.1 Ethical approval

The Scientific Committee of the College of Science at the University of Sumer approved the study under Approval No. 1529/12/08 on 24 July 2025. The studies conducted with animals were done following the principles of ethical & humane practices implemented by both animal welfare and ethical principles of scientific research..

2.2 Extraction and Characterization of Myrrh Essential Oil

Myrrh essential oil was distilled using steam, as per the British Pharmacopoeia standard (1958), with some improvements made to obtain a greater quantity of essential oils out of the resin. The resin was crushed into very small pieces, with a final particle size of less than 0.5mm. After grinding, the resin was combined together (in terms of total weight) with distilled water at a ratio of 30g powdered resin to 300ml distilled water. After combining, steam distillation was used in a Clevenger type apparatus until a sufficient amount of distillate was produced. The first distillation was then performed again in order to obtain a greater amount of essential oils from the initial distillation process. The distillate collected was extracted using anhydrous ether, dried by adding CaSO4 to remove water, and then concentrated at 37C under vacuo using a rotary evaporator until approximately 50% (v/v) of the volume remained. The essential oil produced from these procedures was a thick, viscous (amber) liquid.

Gas chromatography-mass spectrometry (GC-MS) was used to characterize the chemical composition of essential oil. The GC-MS was comprised of a Shimadzu 15A gas chromatograph and an ADB-5 capillary column (50 m × 0.2 mm i.d., 0.32 mm film thickness). The carrier gas (N2) flowed at 1 mL/min, and the oven temperature was programed from 60 °C (3 min) to 220 °C at 5 °C/min and held at 220 °C for 5 min.

An ointment consisting of the essential oil and petrolatum was made using a concentration of 5% (w/w). A medicinal-grade form of petrolatum was obtained to prepare the 5% (w/w) ointment with sterile essential oil. The 5% (w/w) ointments were then stored at 4 °C until use in a dark container.

2.3 Preparation of Topical Formulations

To prepare myrrha ointment, 5g of *Commiphora Myrrha* was mixed with 95 g of sterile medical-grade petrolatum to obtain a 5% (w/w) concentration. The preparation was slowly mixed under sterile conditions using sterile instruments to ensure the quality of the final product. The final preparations were stored in sterile dark glass containers and kept at 4°C until use.

2.4 Experimental Animals

Twelve healthy New Zealand White rabbits of both sexes, weighing between 1.5 and 2 kg, were used in this study. Animals were housed individually under standard laboratory conditions with controlled temperature and were provided free access to food and water.

The rabbits were randomly allocated into two experimental groups (n = 6 per group):

Group I (Myrrh): wounds treated with 5% *Commiphora Myrrha ointment*.
Group II (Control): wounds treated with sterile distilled water.

2.5 Anesthesia and Wound Induction

Local anesthesia was induced by subcutaneous injection of lidocaine plus epinephrine **(Normon®, 20 mg/mL lidocaine + 0.0125 mg/mL epinephrine solution for injection)** Prior to the production of the wounds, the animal was shaved and disinfected with a 10% povidone-iodine solution, using an aseptic technique to avoid contamination during surgery. A full-thickness, longitudinal skin wound measuring approximately 2 cm in length could be produced. There would be two of these wounds made on either side of an animal's midline. The animal had been surgically prepared to have wounds created in a standardized manner using sterile instruments. Following the application of the topical ointment of cumin, both groups had their wounds bandaged (covered with sterile gauze) and, therefore, neither of them would be able to lick or rub the wound..

2.6 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences [10]. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) post hoc test. Data were expressed as mean ± standard error (SE), and differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1GC-MS Analysis of Commiphora Myrrha Essential Oil

GC-MS analysis of *Commiphora myrrha* 33 different chemical components were identified in the chemical profiling of the essential oil and based on the data presented, it was concluded that the majority of the components are either polar or semi-polar. The two main components were identified as (42.42%) 5-Hydroxymethylfurfural and (19.14%) Quinic acid; however several other compounds that were detected in lesser amounts include 4H-Pyran-4-One derivatives, hexitol derivatives and fatty acid esters compounds, which includes, but are not limited to, Ethyl Palmitate, Ethyl Linoleic acid and Methyl Linoleate, as per Figures 1 through 4 the relative abundance of these identified compounds was set by covering their GC-MS peak area percentages. One possible reason for the relatively large amount of 5-Hydroxymethylfurfural being present within the Essential Oil could be a result of either thermal degradation reaction's that took place during the extraction process and/ or prolonged heating time during the extraction process..

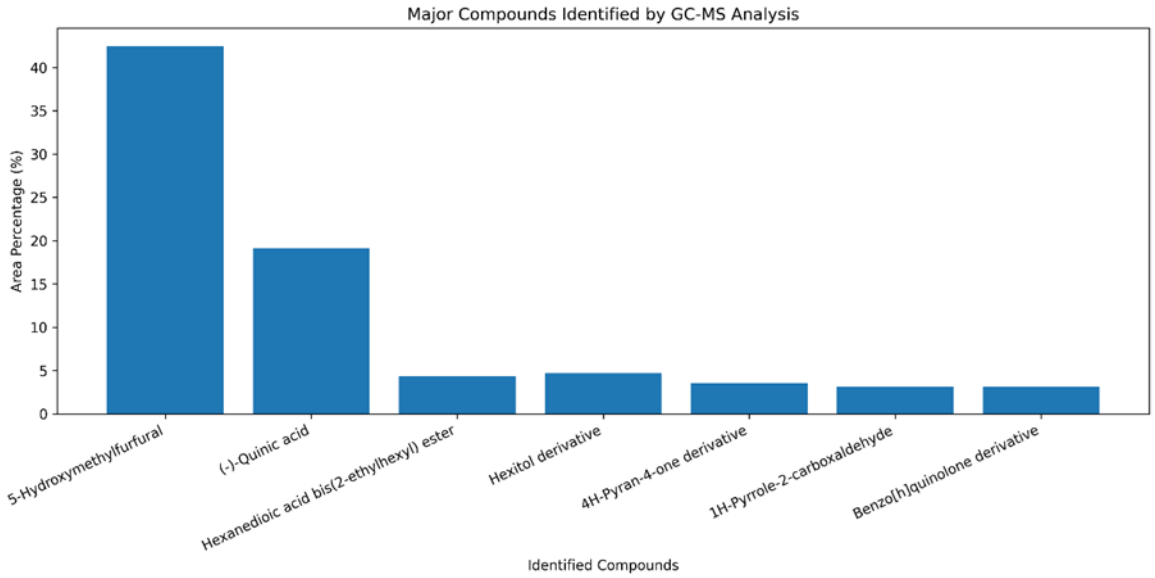

Figure -1 GC-MS analysis showed the major bioactive compounds in the essential oil of *Commiphora Myrrha.*

Reports in the literature have previously reported the antioxidant and anti-inflammatory effects of these constituents [11]. This correlates with earlier pharmacological research [12] that analysed the bioactive properties of Commiphora spp. The overall phytochemical composition observed within this study is similar to previous analyses showing that various classes of compounds are present in Commiphora myrrha, including terpenoids, steroids, and volatile compounds [4].

The use of gas chromatography-mass spectrometry (GCMS) enables you to identify chemical constituent(s) qualitatively and semi-quantitatively only; it does not provide information about the biological activity/therapeutic effects of such identified chemical constituent(s). As a result, any functional interpretations of identified chemical constituent(s) should be cautious, and supported with biological and/or in vivo evidence.

Prior research shows that extracts of Commiphora myrrha, have antioxidant, anti-inflammatory, antimicrobial and wound healing activity. Most of these biological properties have been demonstrated through experimentation and/or by way of in vivo studies as opposed to being proven solely by chemical analysis. It has been suggested that the biological activity is likely due to a synergistic effect of several different phytochemicals [13].

The historical use of myrrh for medicinal purposes has been well established through continued use in traditional practices; however, the validity of this information must also reflect on how well these various forms of traditional medicine are tested scientifically..

3.2 Wound Healing Assessment (Histological Evaluation)

Histological examination of the wound tissues showed that topical application of *Commiphora myrrha* Compared to the control group, they had higher overall quality of care, which included faster rate of healing and greater improvement in re-epithelialization, dermal structure organization, and collagen remodelling during the period of wound healing, as illustrated in Table 1.

In the initial ("acute") inflammatory phase of the healing process, myrrh-treated animals had less infiltration of inflammatory cells and fewer crusts than the untreated control group. The results suggest that the inflammatory response is resolved earlier in the treated wounds than in the controls.

During the proliferative phase (7-14 days), treated subjects exhibited greater migration of epithelial cells, better organization of the dermal layer and faster wound closure than their control counterparts. Treated subjects also demonstrated increased activity of fibroblasts as well as increased deposition of extracellular matrix as shown in Figures 4-7. During the remodeling phase (28 days), collagen fibers from treated subjects demonstrated a more organized pattern of arrangement and a more mature structure than that exhibited by the control group, where there was still evidence of immature structure of collagen and presence of residual inflammatory cells (Figures 8-9)..

Table 1- Percentage of wound Contraction in experimental groups during different healing periods.

Group / Time	Day 3	Day 7	Day 14	Day 28
Myrrh Group	0.75 ± 0.05^{b}	42.86 ± 0.06^{a}	58.65 ± 0.08^{a}	84.21 ± 0.11^{a}
Control Group	0.00 ± 0.00^{c}	8.13 ± 0.11^{c}	23.75 ± 0.14^{c}	40.63 ± 0.15^{c}
LSD (P-value)	0.08 (*P<0.05)	2.15 (**P<0.01)	2.30 (**P<0.01)	3.20 (***P<0.001)

Data are expressed as mean ± SE. Different superscript letters within the same column indicate significant differences at p < 0.05.

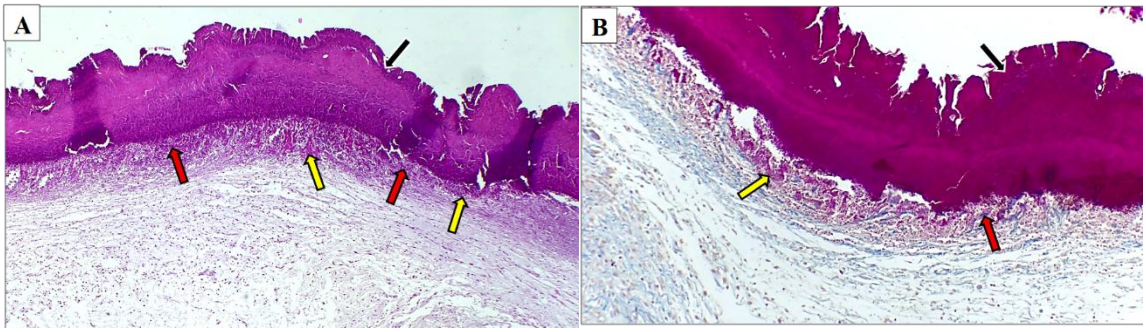

Figure -2 Photomicrograph of skin at three days post-wound induction of the control group rabbit. A and B: A noticeable sero-cellular layer (black arrow) was present and consisted of necrotic debris as well, as well as showing marked amounts of fibrinogenous material within this fibrinogenic layer. The dermis below had large areas of interstitial edema (yellow arrow) that were filled with large amounts of inflammatory cells (red arrow), providing a predominant amount of this fluid being found primarily throughout the wound region. A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

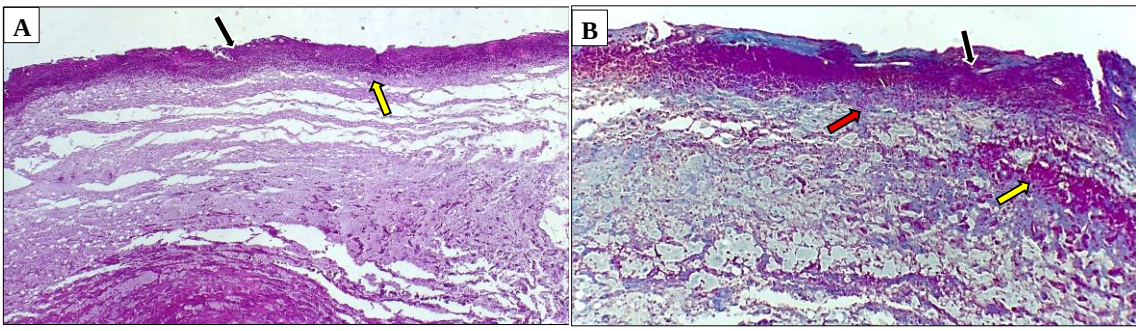

Figure -3 Photomicrograph of skin at three days post-wound induction of Myrrh group rabbit.A and B: The well-defined sero-cellular layer (indicated by the black arrow) is comprised primarily of fibrous material and necrotic debris. It is also thinner than that of the control group. Despite the decrease in the thickness of the superficial layer, the diffused interstitial edema and extensive inflammatory cell infiltration (indicated by the yellow arrow and red arrow, respectively) are observed in the dermal layers beneath the wound.A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

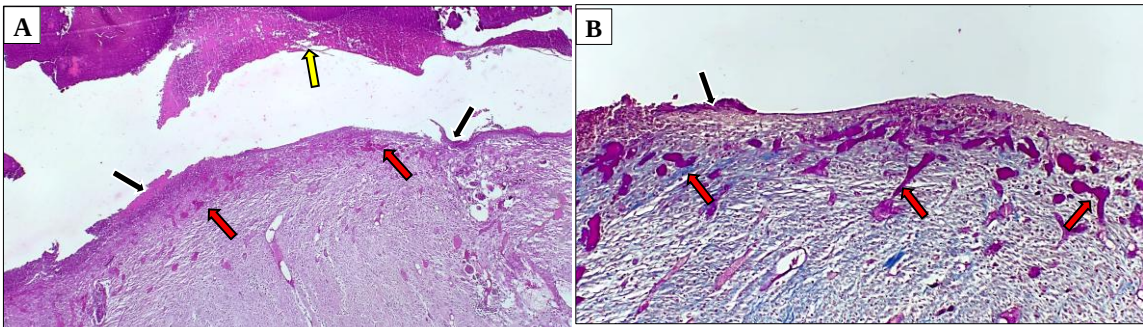

Figure -4 Photomicrograph of skin at seven days post-wound induction of the control group rabbit. A and B: The incomplete re-epithelialization (representing an incomplete epithelial surface) observed at the black arrowed sites indicates limited migration of epithelium inward from the wound edges. The wound base was still covered by a persistent sero-cellular membrane (yellow arrow) and exhibited marked interstitial edema (red arrow) in the underlying dermis. The Treatment group demonstrated a significantly greater degree of epithelial proliferation and a better organized dermal repair compared to the Control group, indicating a faster change from inflammatory to proliferative stages. A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

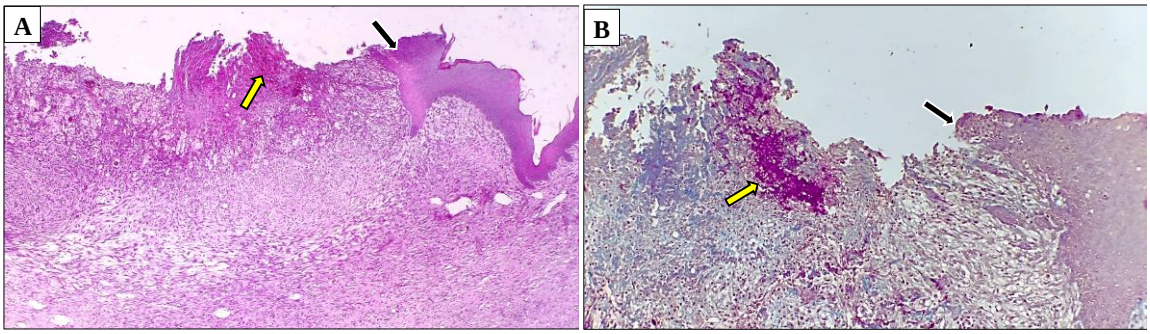

Figure -5 Photomicrograph of skin at seven days post-wound induction of the Myrrh group rabbit. A and B: The layer of sero-cells has been entirely removed and replaced by the first stages of re-epithelialization (black arrow), where there is a much greater extension to the center of the wound than in the control, which had only a slight extension. The dermis underneath the wound has become moderately edematous (yellow arrow) and shows no sign of an inflammatory infiltrate; the acute inflammatory phase has resolved. A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

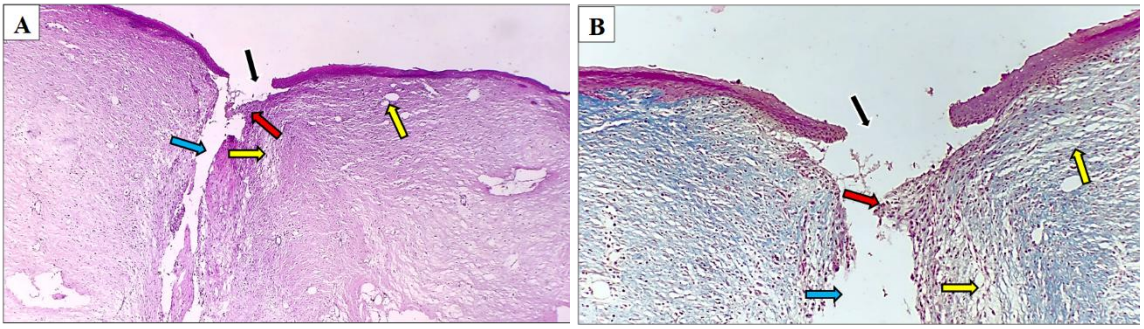

Figure -6 Photomicrograph of skin at fourteen days post-wound induction of the control group rabbit. A and B: Evidence of wound contraction was noted, although no significant amount of epithelial skin covering had formed at the time of evaluation (black arrow). There remained a significant longitudinal dermal cleft or fissure (blue arrow) within the wound bed, indicating that there was incomplete mesenchymal fusion. In addition, the granulation tissue surrounding the wound bed contained inflammatory cells (red arrow), and the collagen fibers were thin and loosely arranged (yellow arrow) when compared to the consolidated, filled dermal architecture of the Treatment group.: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

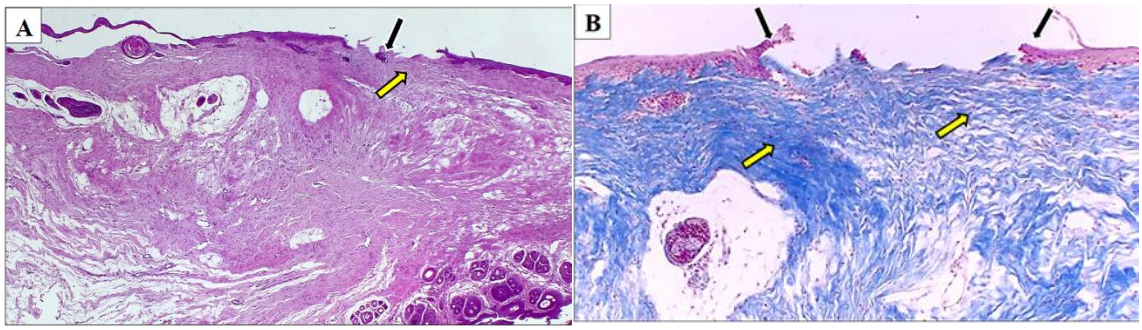

Figure -7 Photomicrograph of skin at fourteen days post-wound induction of the Myrrh group rabbit. A and B: The area of the wound has contracted significantly (as indicated by the black arrow), but the surface is not yet entirely closed (healing of the skin). The area of dermis is formed completely and has an organized, mature collagen structure (indicated by the yellow arrow), whereas the control case (untreated) still has an extensive longitudinal fissure on the surface of the repair. The area has healed completely without any inflammation; however, the control group continues to show evidence of inflammation. A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

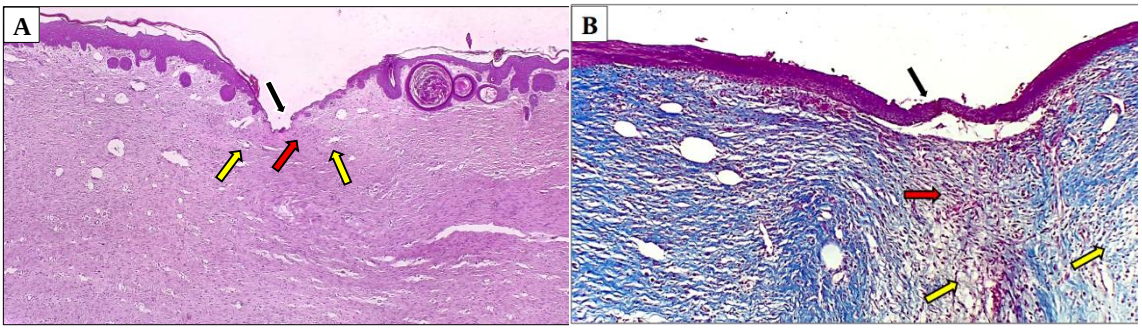

Figure - 8 Photomicrograph of skin at twenty-eight days post-wound induction of control group rabbit. A and B: Epidermal continuity had completely healed (black arrow), but dermal repair remained very immature. The wound had much inflammatory cell infiltration (red arrow) showing that there were still many inflammatory cells present and should have been resolved by that time, indicating a delay to complete restoring the wound in the inflammatory phase. Additionally, the collagen type I fibers (yellow arrow) were synthesizing loosely, not compacted, indicating that they lacked the usual dense, twisted appearance found in fully matured/ remodeled tissues. Therefore, although the epidermis is completely closed, the structural quality of the area is poorer compared to the Treatment group. A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

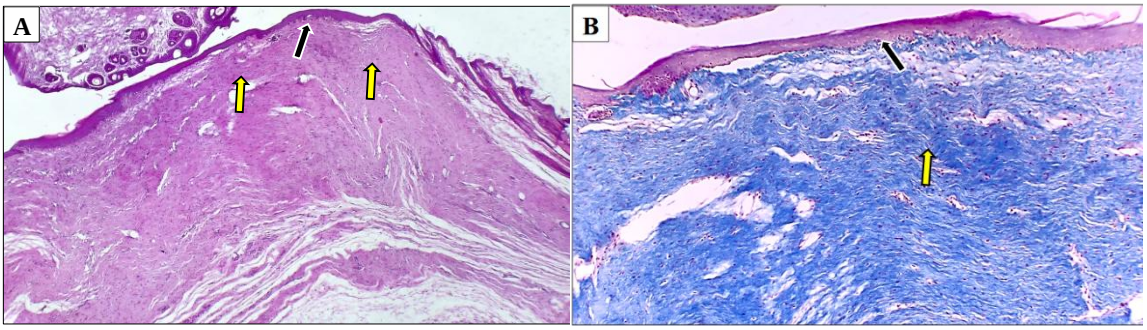

Figure -9 Photomicrograph of skin at twenty-eight days post-wound induction of Myrrh group rabbit. A and B: The wound healed completely with full epithelial surface closure (black arrow) and evenly ordered collagen fibers (yellow arrow) in the dermis. Interestingly, in the treatment group, there were no inflammatory cells evident, suggesting complete resolution of the inflammatory phase. The control group, however, showed significant ongoing inflammatory cell infiltration, indicating that the inflammatory phase had a delayed transition to the remodeling phase of repair. A: H&E and B: Masson Trichrome Stain. A: 40x and B: 100x.

Histological results from this study indicate that using Commiphora myrrha topically may benefit the various stages of healing a wound, especially the inflammatory and proliferative stages. Evidence for this comes from the decreased number of inflammatory cells in the treated wounds, improved organization of the tissue in the treated wounds, and the level of maturity of collagen in the treated wounds.

These findings partially concur with [14,15,16] findings in regard of improved histological/histomorphometric parameters due to myrrh treatment compared to untreated controls; specifically, there was an increased degree of healing during early stages post injury.

The presence of Commiphora myrrha in the current research indicates its positive effect on wound healing is due to it's anti inflammatory action as well as tissue regeneration qualities. These similarly beneficial effects have been seen by Al-Mobeeriek and others. Al-Mobeeriek published a report using myrrh for the treatment of oral mucosal ulcers, comparing it with tetracycline and chlorhexidine antiseptic mouthwashes, in clinical practice.

Numerous bioactive compounds can be found in Commiphora myrrha, and it has been proven to possess antioxidant and antimicrobial properties based on prior studies. The aforementioned studies that provided supporting information regarding these attributes are not limited to the use of chemical profilers..

During the proliferative phase (7-14 days), improved epithelial migration and earlier wound closure were observed in the treated group compared to controls. Histological findings indicated better dermal organization; however, these observations are descriptive and require further quantitative morphometric and biochemical validation.

In the remodeling phase (28 days), the myrrh-treated group showed mature and well-organized collagen; the control group was still exhibiting immature collagen with residual inflammatory cells. Similar findings have been reported in studies investigating natural resins, which demonstrated improved collagen maturation and enhanced tissue strength. The antimicrobial properties of *Commiphora myrrha* may also contribute indirectly to improved wound healing by reducing microbial load at the wound site, thereby limiting prolonged inflammation and secondary infection, as reported by [4, 17] have found similar results evaluating some of the same natural resins as Myrrh; enhanced collagen maturation and increased tensile strength of the healed tissues were reported for these treatments.

Overall, the present study suggests that *Commiphora myrrha* may enhance histological features of wound healing, particularly inflammation resolution and tissue remodeling. However, GC-MS analysis provides only chemical identification and does not confirm biological mechanisms. Therefore, further in vivo, biochemical, and quantitative morphometric studies are required to validate these findings [18].

4. Conclusion

Commiphora Myrrha essential oil significantly enhances wound contraction and closure compared with untreated controls. In conclusion, *Commiphora Myrrha* essential oil is a promising natural option for promoting skin wound healing, especially in resource-limited settings. The present GC-MS analysis confirms the chemical richness of Commiphora myrrha essential oil. However, further in vivo, biochemical, and clinical studies are required to determine the biological relevance and therapeutic potential of the identified compounds.

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