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Formulation and assessment of wound-healing potential of a gel formulation containing propolis and green tea extracts

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Abstract

Summary of aims In the quest for restorative healing and a better quality of life, the ancient wisdom of herbal remedies is emerging as a beacon of hope for effective wound management. This study investigated the wound-healing capabilities of a novel gel combining propolis resin (PR) and green tea (GRT) extract, seeking a natural alternative for wound care.

Methods: The PR+GRT gel was formulated and tested for sensory attributes and stability through various methods. Its efficacy was then compared in vivo against a positive control (CICALFATE™) and negative controls (sodium chloride and base gel). On day 9 post-wounding, histopathological analyses were conducted using Hematoxylin and Eosin (H&E) and trichrome staining to evaluate tissue regeneration and collagen deposition.

Results: The PR+GRT gel possessed favorable sensory characteristics and stability. Significantly, it improved wound healing compared to negative controls and performed comparably to CICALFATE™. Histopathological examination highlighted significant differences between treatment groups, particularly in membranous nephropathy, where PR+GRT and CICALFATE™ outperformed the controls.

Conclusions: the PR+GRT gel holds promise as a wound-healing agent, demonstrating effectiveness on par with CICALFATE™. Further research is recommended to explore its synergistic potential and refine histological evaluations for more quantitative analysis. Study limitations include small sample sizes and qualitative histopathological assessments.

Keywords wound healing, propolis, green tea, gel formulation.

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Introduction

Wound healing is a multifaceted biological process characterised by several distinct stages: inflammation, proliferation, and remodeling. Traditional medicine has long embraced a variety of plant-derived compounds to enhance wound repair, and contemporary research is increasingly affirming the efficacy of these age-old practices.¹ When addressing oral discomfort, various therapeutic approaches have been explored. These studies highlight ongoing efforts to find effective treatments for oral ailments.^{2,3} In recent years, there has been a surge in interest surrounding natural substances due to their therapeutic potential.^{4,5}

Among these, propolis (PR)—a resinous compound collected by honeybees—has shown remarkable promise in facilitating wound healing through various mechanisms.^{6,7} Its diverse composition, rich in flavonoids, phenolic acids and terpenes, underpins its antimicrobial properties, effectively combating bacterial and fungal infections that can obstruct the healing process. Furthermore, PR boasts potent antioxidant capabilities that scavenge free radicals, thereby reducing oxidative stress at the wound site, which is vital for effective tissue repair.^{4,6,8-10}

Similarly, green tea (GRT), *Camellia sinensis* L. from the Theaceae family, has garnered recognition for its

wound-healing properties, primarily attributed to its high concentration of polyphenols, especially catechins. Research indicates that these compounds exhibit notable anti-inflammatory effects, mitigating the inflammatory response associated with wound healing and fostering accelerated tissue regeneration. Additionally, GRT promotes collagen synthesis, thereby enhancing wound tensile strength and overall healing efficacy.¹¹⁻¹⁴ Green tea extract (GRT) exerts its potent inflammation-modulatory effects primarily through its rich polyphenol content, especially epigallocatechin gallate (EGCG). The multifaceted mechanisms by which GRT mitigates inflammation involve critical interactions with cellular signaling pathways and molecular targets. A primary mechanism is the inhibition of the nuclear factor-kappa B (NF- κ B) signaling pathway. NF- κ B is a central regulator of inflammatory gene expression. GRT, particularly EGCG, suppresses NF- κ B activation, thereby preventing the transcriptional upregulation of pro-inflammatory mediators such as cytokines (such as TNF- α , IL-1 β , IL-6), chemokines, and inducible enzymes like COX-2 and iNOS. This blockade effectively curtails the inflammatory cascade.^{15,16}

Furthermore, GRT directly reduces the production of key pro-inflammatory cytokines, including IL-1 β , IL-6, and TNF- α , thereby dampening systemic inflammatory responses. Crucially, GRT also demonstrates significant antioxidant

activity. EGCG acts as a potent scavenger of reactive oxygen species (ROS) and enhances endogenous antioxidant defenses.¹⁷ By mitigating oxidative stress, GRT indirectly alleviates inflammation, as ROS can activate pro-inflammatory pathways. These combined actions, alongside potential modulations of other pathways like inflammasome activation, underscore GRT's comprehensive anti-inflammatory potential, making it a promising agent for managing inflammatory conditions.^{18,19}

The synergistic effect realised by combining PR and GRT in a topical formulation may therefore provide a holistic approach to wound care, effectively addressing both infection control and tissue repair (Figure 1).

In parallel, transdermal drug delivery has become a focal point of research as a targeted and efficient therapeutic strategy for various skin and topical conditions. This innovative approach allows localised drug delivery to affected areas, minimising systemic side effects while enhancing treatment effectiveness.¹ This study aims to investigate the potential use of a combined PR and GRT formulation on the wound healing process in an animal model. The outcomes of this research could yield valuable insights into the application of natural substances, paving the way for the development of more effective treatments for wound management.

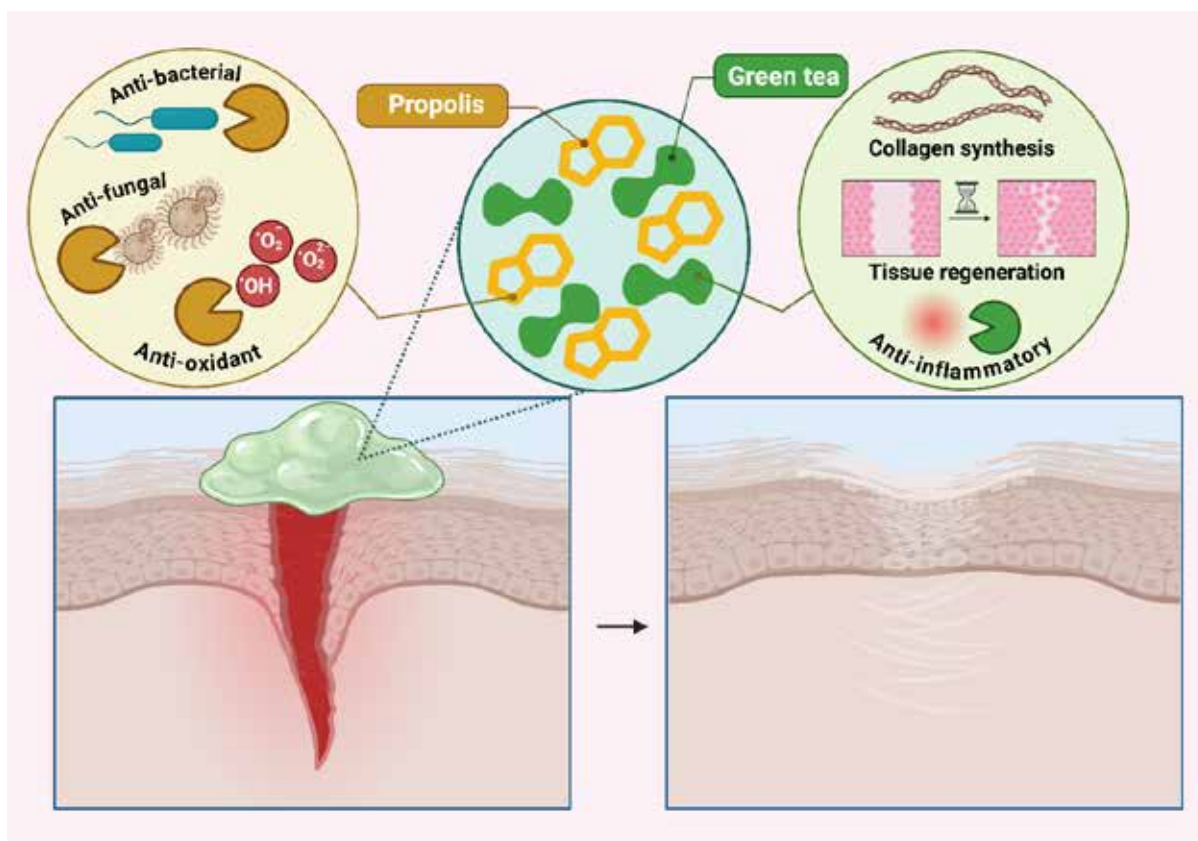


Figure 1: Propolis and green tea, elucidating the potential mechanisms underlying their wound-healing properties. This schematic representation outlines the convergent and divergent molecular targets and pathways, such as modulation of inflammatory mediators, antioxidant defense, and fibroblast proliferation, implicated in the wound-healing actions of propolis and green tea.

Methods

Plant material preparation and phytochemical screening

Plant materials preparation

Raw propolis (PR) was obtained as an unprocessed, resinous extract directly from local beekeepers in Shahdad, Kerman province, Iran. The propolis exhibited a characteristic greenish-brown color, consistent with its presumed botanical origin from mixed botanical origin which are common in the region. Due to its raw, unbranded nature, no further compositional information was available from the supplier. Green tea (GRT) leaves were procured as raw, unbranded material directly from a local market, which was supplied by cultivators in the Rasht region, Gilan province, Iran. The specific cultivar and processing methods prior to acquisition were not provided by the local suppliers. GRT leaves were ground and sieved through a 20-mesh sieve to achieve uniform particle size. PR was coarsely crushed. Both samples underwent phytochemical screening to identify the presence of alkaloids, saponins, flavonoids and terpenoids using established procedures

Phytochemical screening of PR and GRT

The qualitative phytochemical screening of the prepared PR and GRT extracts was conducted to identify the presence of major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenes. All reagents used were of analytical grade. For each test, both a negative control (solvent without extract) and an appropriate positive control (a medicinal plant belonging to the respective phytochemical class) were run in parallel to ensure the specificity and relative intensity of the observed reactions.

a. Alkaloids (Mayer's and Wagner's tests)

Approximately 1mL of the extract solution was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution). The formation of a creamy white precipitate indicated the presence of alkaloids. In parallel, for Wagner's test, another 1mL of the extract was treated with Wagner's reagent (iodine in potassium iodide solution). The appearance of a reddish-brown precipitate confirmed the presence of alkaloids.

b. Flavonoids (Shinoda test, antioxidant assay)

A small portion of the extract (around 2mL) was mixed with a few pieces of magnesium turnings, followed by the dropwise addition of concentrated hydrochloric acid. The development of an orange to red color indicated the presence of flavonoids, while a crimson to magenta color suggested the presence of flavones.

c. Tannins (Ferric Chloride Test):

To 1mL of the extract, 1–2 drops of 5% ferric chloride solution were added. The appearance of a bluish-black or greenish-black coloration indicated the presence of tannins.

d. Saponins (Froth Test):

About 2mL of the extract was vigorously shaken in a test tube for 15 seconds. The persistence of a stable foam (froth) for at least 10 minutes confirmed the presence of saponins. The height of the foam (greater than 1cm) could also be noted.

e. Terpenes (Salkowski Test):

To 1mL of the extract, 0.5mL of chloroform was added, followed by the addition of a few drops of concentrated sulfuric acid carefully along the sides of the test tube. The formation of a reddish-brown color at the interface indicated the presence of terpenes.²⁰

Extraction and total phenolic content determination

Extraction was performed using a 96-hour warm maceration method with 90% ethanol as the solvent. Fresh solvent was added every 24 hours. The combined extracts were concentrated using a rotary evaporator under reduced pressure and subsequently dried in a laboratory oven at temperatures below 50°C. The dried extracts were weighed and stored at -20°C until further analysis. Total phenolic content (TPC) of the PR and GRT extracts was determined using the Folin-Ciocalteu method, employing a gallic acid calibration curve. This method is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds in an alkaline medium, resulting in a blue-colored complex with maximum absorbance at 765nm. TPC is expressed as gallic acid equivalents (GAE). Each sample was analysed in triplicate.²¹

Gel formulation of a combination of PR and GRT

A series of gel formulations combining PR and GRT extracts were meticulously prepared to achieve specific final concentrations of the active compounds. Varying ratios of Carbopol 934, carboxymethyl cellulose (CMC), and hydroxypropyl cellulose (HPC) were initially dispersed in distilled water using a homogeniser (Heidolph, Germany) at a consistent speed of 5000rpm. This dispersion continued until a visually clear and complete dissolution of the gelling agents was achieved.

Following the complete dissolution of the gelling agents, accurately pre-weighed quantities of both PR and GRT extracts were incorporated into the mixture. Specifically, each formulation was designed to contain a final concentration of 3% (w/v) of PR extract and 1% (w/v) of GRT extract. Both PR and GRT extract were standardised to contain phenolic compounds (expressed as gallic acid equivalents) by the Folin-Ciocalteu method. Continuous mixing ensued until the extracts were thoroughly and homogeneously integrated into the solution, ensuring a uniform distribution of the active compounds throughout the gel base. The final volume of each formulation was then adjusted to a predetermined mark with distilled water. For formulations incorporating Carbopol 934, triethanolamine (TEA) was meticulously added dropwise after the homogenisation process to achieve the desired gel consistency and pH, crucial for both stability and application.

The precise quantitative composition of the four optimised gel formulations (F1–F4), each consistently containing 3% w/v PR and 1% w/v GRT extracts, is detailed in Table 1.

Physicochemical characterisation of formulated gels

This section details the methodologies employed to evaluate the physicochemical properties of the prepared gel formulations, encompassing assessments of their physical appearance and stability.²²

The physical characteristics of the four formulated gels were initially assessed via visual inspection. This included evaluation of clarity, color, the absence of air bubbles and overall homogeneity. Gel homogeneity was further investigated using microscopic analysis to identify any inconsistencies in the structural matrix.

The consistency and uniformity of the gels were assessed using a finger test. A small aliquot of the formulation was applied between two fingers, followed by gentle compression. This procedure allowed for evaluation of the gel's consistency, uniformity, presence of any palpable particles and the duration of adhesion on the skin surface.

Physical controls

The pH of each gel formulation was determined to assess its stability and potential impact on skin compatibility. One gram of each formulation was dispersed in 10mL of distilled water, and the pH was measured using a calibrated pH meter. Measurements were conducted immediately after preparation (Time Zero) and at the following intervals: 24 hours, 48 hours, 1 week, 2 weeks, 1 month, and 3 months. All measurements were performed in triplicate, and the average value was reported.

The stability of the formulations under centrifugal stress was evaluated to assess their resistance to phase separation. Samples were subjected to centrifugation at 2000rpm for incremental durations: 5, 15, 30, and 60 minutes.

To assess stability at elevated temperatures, 15g of each formulation, prepared 48 hours prior, underwent six cycles of 48 hours at 45°C followed by 48 hours at 4°C. The gels were evaluated for leakage, shrinkage, liquid exudation, color change, bubble formation and particle formation.

To evaluate stability at cold temperatures, 15g of each formulation, also prepared 48 hours prior, were subjected to six cycles of 48 hours at -8°C followed by 48 hours at 25°C. The same evaluations as above were conducted.

Thermal Stability Test: Three samples of each formulation were stored at 4°C, 25°C, and 45°C for stability assessments at 24 hours, 1 week, 1 month, 3 months, and 6 months.

The spreadability of the gels, a key indicator of their application properties, was assessed using a modified two-glass-slide method. A 0.5-gram sample of the gel was placed on the lower glass slide. A second glass slide was then placed on top, with gentle pressure applied to ensure even spreading of the gel. A specified weight (10, 20, 30, 40, 50, and 60 grams) was attached to the end of the upper glass slide. The time required for the slides to separate (with a maximum observation time of 180 seconds) was recorded. Spreadability was calculated using the following formula:

$$\text{Spreadability} = (M * L) / T$$

Where: M=weight tied to the upper slide (20g) and L=Length of the glass (7.5cm).²²⁻²⁴

Wound healing effect

In this study, 24 healthy adult male Wistar rats were used. Animals are randomly divided into four groups of 6. Group A was the negative control and received normal saline; Group B was the positive control and was exposed to CICALFATE™; Group C received gel base, and Group D was treated with gel containing the concentration of 3% PR extract and 1% GRT extract.

Regarding wound creation, the rats were anaesthetised using an i.p. injection of ketamine (50mg/kg) and xylazine hydrochloride (10mg/kg), followed by the creation of a wound as a circular region of approximately 3.14cm² on depilated thoracic areas. The first administration on different groups began one day after wound creation, then every other day. Treatment was done once. Finally, on days 0, 3, 6, and 9, the wound was examined, and a photo of the area was taken. By negatoscope and the software Digimizer, the area and percentage of wound healing were accurately calculated.²⁵

Histopathological assessments

Using ether, the animals were anaesthetised on the last treatment day and then sacrificed. The granulation tissues were excised, fixed in formalin (10%, v/v), and processed for histopathological examinations. The sections (5µm) were stained by Hematoxylin and Eosin (H&E) and Masson's trichrome staining, and evaluated using a routine light microscope (Olympus CX33, Japan). Acute inflammation, chronic inflammation, granulation tissue amount, granulation tissue fibroblast maturation, collagen deposition, epidermal

Table 1. Composition of optimised gel formulations containing PR and GRT extracts.

| Formulation | Carbopol 934 (W/V)% | CMC (W/V)% | GRT extracts (W/V)% | PR extract (W/V)% |
|-------------|---------------------|------------|---------------------|-------------------|
| F1 | 1 | – | 1 | 3 |
| F2 | – | 3 | 1 | 3 |
| F3 | 0.5 | 3 | 1 | 3 |
| F4 | 1 | 3 | 1 | 3 |

regeneration, and neovascularisation were studied in the sections. Histopathological examinations were performed on H&E and Masson's trichrome stained sections. For Masson's trichrome staining, mature collagen fibers stained dark blue, while immature collagen fibers stained light blue. The assessment of various parameters, including collagen deposition, was conducted blindly by an experienced pathologist using a standardised scoring system. For collagen deposition, scores ranged from 1 (scant) to 3 (abundant).⁸

Statistical analysis

Quantitative data were expressed as mean±standard deviation (SD). Statistical analysis with decomposition was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The qualitative data were analysed using the Chi-square test. This experiment was used to compare each experimental condition with and without treatment, using normal saline to calculate the wound area and tissue staining score. P value less than 0.05 was statistically considered significant.

Results

Extraction yield, phytochemical screening and total phenolic content

The extraction yields were calculated at 22.5% for PR and 13.4% for GRT. The qualitative phytochemical screening was performed on both PR and GRT extracts to identify the presence of key secondary metabolites. The results, summarised in Table 2, confirmed the rich phytochemical composition of both extracts.

As presented in Table 2, PR extract demonstrated a strong presence (+++) of flavonoids and a moderate presence (++) of terpenoids. Alkaloids and saponins were found to be

absent (-) in the PR extract. GRT extract, on the other hand, was notably rich in flavonoids and tannins, showing a strong positive response (+++) for tannins and a moderate presence (++) for alkaloids, flavonoids, and saponins. Terpenoids were observed in detectable amounts (+) in the GRT extract. These findings underscore the potential therapeutic benefits of both extracts, attributable to their distinct yet overlapping phytochemical profiles.

The total phenolic content (TPC) was determined using a gallic acid calibration curve. The results indicated that the total phenolic content was 5.4g and 6.89g of gallic acid equivalent per 100g of dried extract for PR and GRT, respectively (Figure 2).

Physicochemical characteristics of formulated gels

The consistency of the formulations (F1, F2, F3, F4) was assessed using a finger test, which revealed that all four formulations exhibited a clear and uniform texture with excellent spreadability. Stability tests, including centrifugation at 25°C for 60 minutes, indicated that none of the formulations displayed signs of instability.

Table 2. The results of phytochemical studies for Propolis and Green tea extract

| Phytochemical group | Propolis extract | Green tea extract |
|---------------------|------------------|-------------------|
| Alkaloids | - | ++ |
| Flavonoids | +++ | ++ |
| Saponins | - | ++ |
| Tannins | ++ | +++ |
| Terpenoids | ++ | + |

- indicates no detectable result and + to +++ indicates relative intensity of positive response compared to control

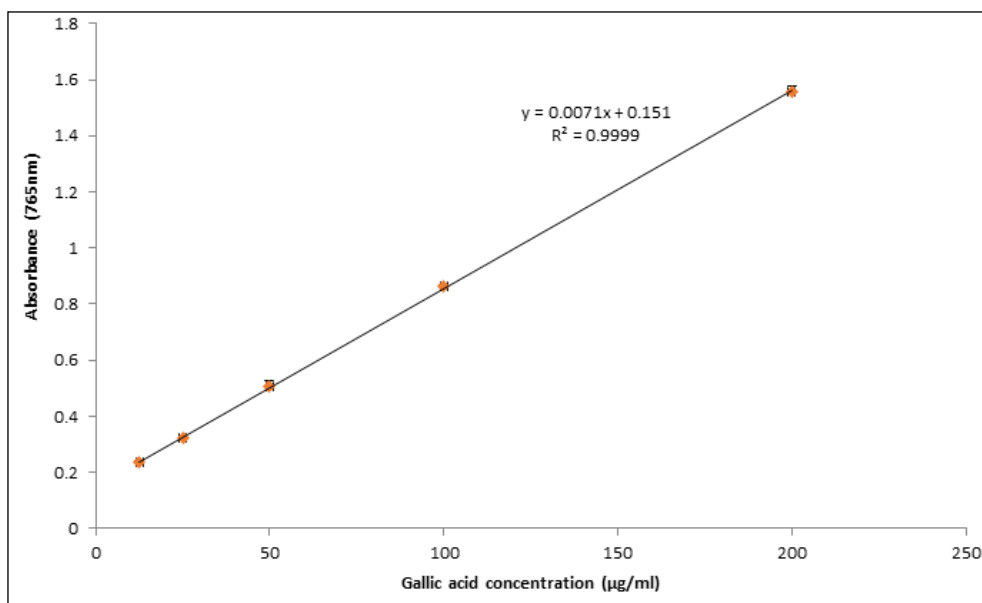


Figure 2 Gallic acid calibration curve measured at 765nm (mean±SD of three independent experiments). This calibration curve demonstrates the linear relationship between gallic acid concentration and absorbance at 765nm, serving as a standard for quantifying total phenolic content in unknown samples

The pH values of formulations (F1-F4) were measured at various time intervals, revealing a narrow range of acceptable pH fluctuations. Moreover, no notable changes in swelling, color, sedimentation, wrinkling, air bubbles, consistency, or solid particles were detected in any of the formulations (F1-F4) following thermal cycling between 4°C and 45°C. All formulations maintained stability without visible changes after undergoing six freeze-thaw cycles (-8°C to 25°C).

Thermal stability was evaluated at 4°C, 25°C, and 45°C, with significant alterations observed after one month at 45°C. Spreadability tests revealed variability among the formulations, with Formulation 1 showing the highest level of consistency.

Drug release study

For the drug release study, absorbance-concentration curves were generated for the GRT extract ($\lambda_{\text{max}}=278\text{nm}$) and the PR extract ($\lambda_{\text{max}}=298\text{nm}$). The analysis of these curves revealed that the λ_{max} of the two extracts is distinctly different. Based on the release kinetics obtained from the Franz diffusion cell studies, formulation F1 exhibited an optimal release rate and was subsequently selected for further investigation (Figure 3).

Wound area assessment

The wound area changes were meticulously monitored and compared across four distinct groups: the NaCl control group, the base control group, the PR+GRT gel treatment group, and the CICALFATE™ positive control group. The results indicated that the wound area decreased over time in all groups, demonstrating a progressive healing trend. Notably, the PR+GRT gel treatment group showed slightly

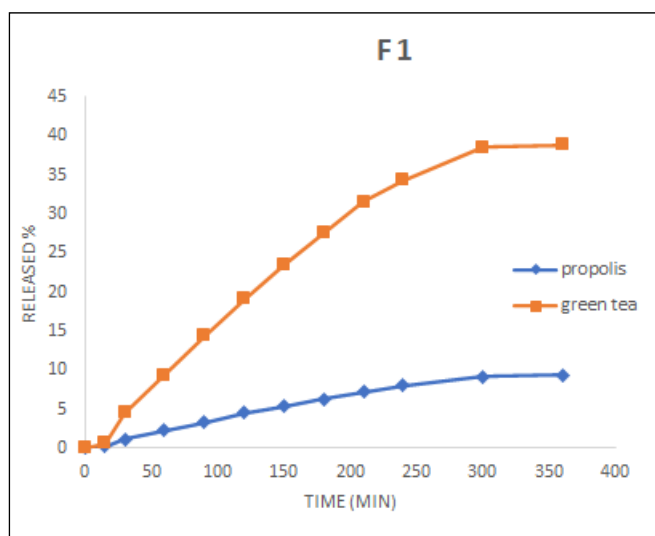


Figure 3. Release kinetics of PR and GRT extract from the F1 formulation obtained from the Franz diffusion cell studies. This figure illustrates the *in vitro* release profiles of both active compounds, PR and GRT extract, from the optimised F1 formulation over time. These kinetics provide crucial insights into the controlled and sustained delivery potential of the formulation, which is vital for its intended therapeutic efficacy.

more favorable outcomes on days 1 and 3, suggesting a potentially enhanced healing response. However, statistical analysis revealed no significant differences in wound healing percentages among the groups at specific time points, including days 0, 3, 6, and 9. This suggests that while the PR+GRT gel treatment may have exhibited a marginally improved healing trajectory, the overall wound healing rates were relatively comparable across all groups. Further analysis and investigation may be necessary to fully elucidate the effects of the PR+GRT gel treatment on wound healing outcomes (Figure 4).

Histopathological scores on day 9 post-wounding (H&E and trichrome staining)

Histopathological assessments on day 9 post-wounding revealed significant differences ($p\text{-value}=0.030$) in histopathological scores between the CICALFATE™ and PR+GRT gel groups compared to the NaCl control group (Figure 5). This indicates markedly improved tissue structure and wound healing in the treatment groups, with both CICALFATE™ and PR+GRT gel demonstrating significantly better healing outcomes than the control (Figure 5). Qualitative analysis of pathological data further supports these findings, suggesting a measurable impact of these treatments on a specific pathological condition. While other analysed parameters showed no significant differences (data not shown), this qualitative analysis, crucial for understanding treatment effectiveness on specific pathological markers, highlights the influence of different treatments on tissue repair and regeneration at a cellular level. To enhance understanding, cross-referencing these qualitative results with quantitative data and additional histopathological scores (inflammation, fibrosis, epithelialisation) directly related to wound healing processes would be beneficial. Figure 5 displays representative Masson's trichrome stained sections, illustrating the differences in collagen deposition among the treatment groups. As shown in Figure 5, the groups treated with CICALFATE™ and PR+GRT exhibited significantly higher deposition of mature collagen (dark blue staining) compared to the NaCl control group, consistent with the quantitative histopathological scores.

The H&E and trichrome staining results provide insight into tissue regeneration and collagen deposition. The treatment groups showed some level of effectiveness in both tissue and collagen regeneration (Figure 6).

Representative microscopic images illustrate the morphological features of cutaneous wounds in various experimental groups. Sections were stained with H&E for general tissue architecture and Masson's Trichrome (MT) to delineate collagen deposition. All images are presented at $\times 100$ magnification, with a corresponding scale bar denoting 200micrometers. Within the H&E stained sections, prominent red arrows highlight areas indicative of active angiogenesis and the formation of new capillary structures, crucial for efficient wound healing.

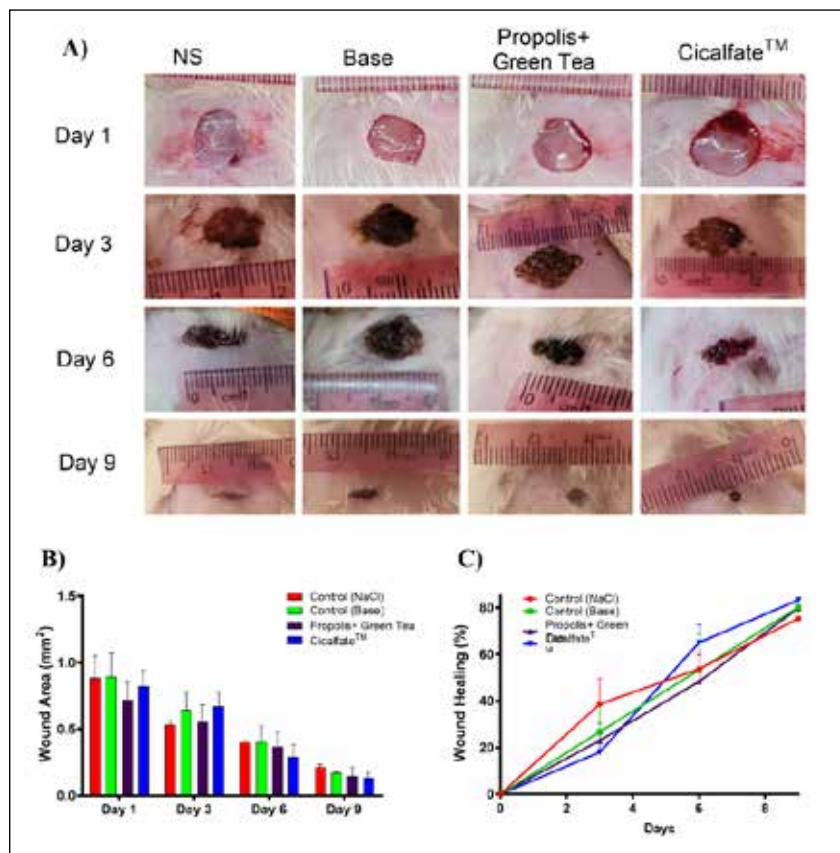


Figure 4: Comprehensive analysis of wound healing progression during the treatment period across different groups. (A) visually depicts the daily changes in wound size and the overall closure process, illustrating macroscopic healing, (B) quantifies the wound area in millimeters squared over time, providing precise measurements of contraction, and (C) presents the calculated percentage of wound healing, serving as a key indicator of treatment efficacy. Together, these metrics provide a holistic view of the therapeutic effects of various interventions on wound repair.

Discussion

This study investigated the potential of a gel formulation containing PR+GRT extract as a wound healing agent, comparing its efficacy to a positive control (CICALFATE™) and two negative controls (NaCl and a base gel). Our findings contribute to the growing body of research exploring natural extracts for wound management.

The extraction process revealed a significant difference in yield between PR and GRT, with values of 22.5% and 13.4%, respectively. This discrepancy likely stems from the differing composition of the raw materials, as PR is a complex resin while GRT originates from dried plant material. Phytochemical analysis further supported these compositional differences, indicating the presence of distinct classes of compounds in each extract, such as steroids in GRT. The standardisation of phenolic compounds provides a basis for future studies aimed at quantifying active components in these extracts.²⁶

The favorable sensory attributes (uniformity and good spreadability) and stability observed during initial evaluations of the formulations, especially after adding triethanolamine as a thickening agent, suggest a promising potential for a dermatological formulation.²⁷ These tests (centrifuge, pH, thermal cycling, freeze-thaw) confirmed the formulations'

stability and resilience, except for minor discoloration at higher temperatures (45°C), which will require careful consideration in storage conditions. Based on the release profile (Figure 3), formulation F1 was selected for further studies as it exhibited an optimal release profile and the highest level of consistency.

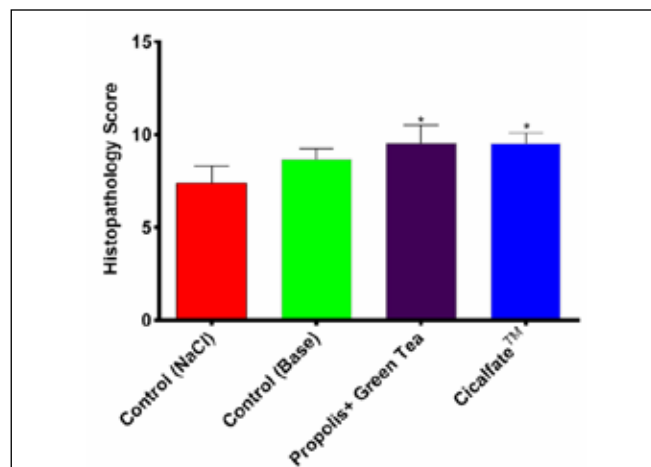


Figure 5: Comparison of histopathological parameters and collagen deposition at Day 9 post-wounding. Representative MT stained sections from Control NaCl, Control Gel Base, PR+GRT and CICALFATE™ groups, illustrating differences in tissue regeneration and collagen deposition.

Given this, further understanding of the controlled release of bioactive components will be critical for designing future formulations with specific therapeutic outcomes.

The study's findings indicate a positive effect of the PR and GRT extract formulation 1 on wound healing. The results suggest that the treatment group (PR+GRT gel) demonstrated a notable improvement in wound healing, with an effect comparable to that of the positive control group (CICALFATE™) and significantly better than that of the two other control groups. These results are consistent with the existing literature, which supports the use of flavonoids and phenolic compounds in wound healing due to their anti-inflammatory, angiogenic, and fibroblast-stimulating properties.^{28,29}

The histopathological findings on day 9 post-wounding point to significant differences between the treatment groups. Specifically, the membranous nephropathy parameter showed

a statistically significant difference ($p=0.030$), with the groups treated with PR+GRT and CICALFATE™ presenting improved scores compared to the control groups. This finding suggests that the active components in these treatments may influence cellular-level recovery. The qualitative pathology data, while limited to descriptive categories, further supports the above observation. While the membranous nephropathy showed statistically significant differences across groups, other parameters did not show the same extent of variation. This suggests a potentially targeted effect of the herbal formulation on particular aspects of tissue healing. However, the lack of specific scoring criteria and visual data for histopathology means the interpretation of these results is limited.

The results from H&E and trichrome staining further elucidate tissue regeneration and collagen deposition. Treatment groups exhibited a degree of effectiveness in both tissue and collagen healing. H&E staining, a commonly used

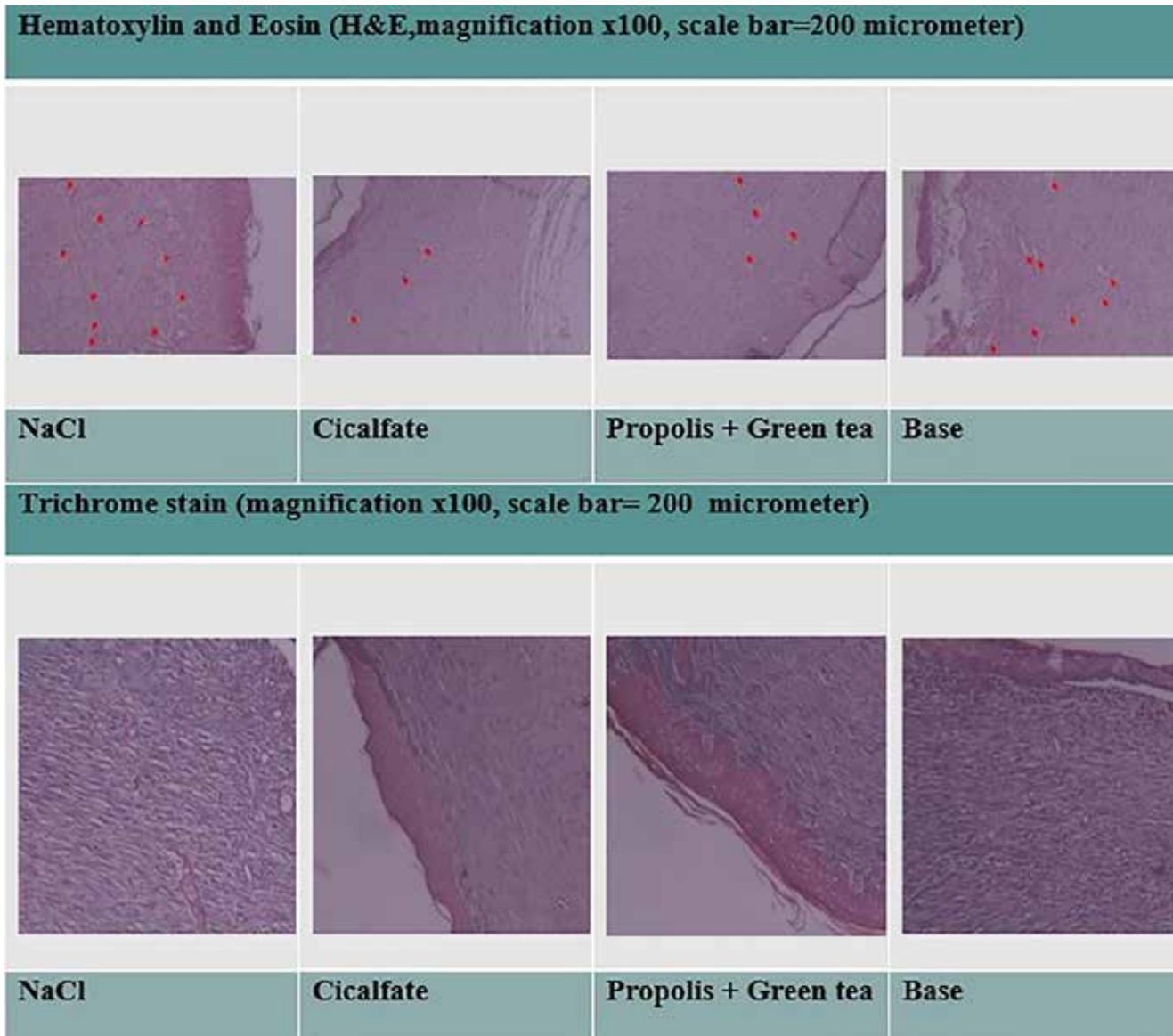


Figure 6. Histopathological analysis of wound tissues across the treatment cohorts.

technique in histopathology, facilitates the visualisation of cellular architecture, inflammatory markers, and overall tissue organisation. The analysis of cross-sectional images is crucial for understanding the efficacy of each treatment in promoting tissue repair and regeneration.¹ This systematic approach allows us to draw meaningful conclusions regarding the therapeutic benefits of the various treatments evaluated in this study.

The findings presented in this study, as visualised in Figure 1, highlight the significant antioxidant and antimicrobial potential inherent in PR and GRT. These observations are consistent with a substantial body of scientific literature that has extensively documented these bioactivities. For instance, numerous studies have confirmed the potent free radical scavenging capabilities of compounds found in both PR and GRT, attributing these effects to phenolic compounds, flavonoids, and catechins.³⁰⁻³⁵ Furthermore, the antimicrobial efficacy of PR against a broad spectrum of bacteria and fungi, alongside the antibacterial and antiviral properties of GRT catechins, has been well-established.^{36,37} Our work aims to contextualise these known properties within a novel formulation.

We can conclude that these findings demonstrate the gel's capacity to not only support healing but also to promote proper remodeling of the wound bed through collagen deposition, which is essential for preventing fibrosis and ensuring functional recovery.

Conclusions

In conclusion, while this study demonstrates the potential of a PR and GRT extract gel for wound healing, particularly in improving membranous nephropathy, the results suggest that its performance is comparable to that of a standard pharmaceutical treatment. The study results underscore the need for further research, which should include a more in-depth evaluation of the synergistic potential of natural extracts combined with traditional agents in wound management. This will potentially lead to improved therapeutic outcomes.

Limitations

While this study presents promising results regarding the wound healing potential of the PR and GRT extract gel, several limitations should be acknowledged. Firstly, the sample size may not have been large enough to generalise the findings across a broader population or various wound types. Additionally, the lack of established scoring criteria for histopathological evaluations limits the depth of analysis regarding tissue changes and the progression of healing. Future studies should incorporate more comprehensive scoring systems and visual data to assess the therapeutic effects of the treatment better. Moreover, extended duration for follow-up assessments could provide insights into the long-term efficacy and safety of the gel formulation. Exploring the synergistic effects of combining natural extracts with traditional pharmacological agents could further enhance

therapeutic outcomes and provide a more comprehensive approach to wound management. A limitation of the current study is that the wound healing experiments were conducted under sterile conditions. In clinical practice, many acute and chronic wounds are complicated by bacterial colonisation or active infection, which can significantly impair the healing process and potentially compromise the efficacy of therapeutic agents. Our PR + GRT gel's primary mechanisms of action are related to tissue regeneration and modulation of the wound microenvironment. Therefore, future research should explore the performance of the gel in infected wound models and investigate strategies for its optimisation, potentially through the incorporation of antimicrobial agents, to ensure its effectiveness in a broader range of clinical scenarios requiring concurrent infection control.

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Conflict of interest

The authors declare no conflicts of interests.

Ethics statement

This research was approved by the ethics committee in medical research at Kerman University of Medical Sciences, Iran, with the license number IR.KMU.REC.1397.358.

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Author contribution

Authors substantially contributed to the conception, design, data acquisition, analysis, and interpretation of this work. They were involved in drafting and critically reviewing the manuscript for important intellectual content, and all provided final approval for publication, agreeing to be accountable for all aspects of the work.

References

- Goncalves RV, Freitas MB, Esposito D. Cellular and molecular mechanisms of oxidative stress in wound healing. *Oxid Med Cell Longev.* 2022;2022:9785094.
- Rezvaninezhad RS, Navabi N, Atai Z, Shahravan A. The effect Co2 laser on reducing pain associated with aphthous stomatitis. *J Babol Univ Med Sci.* 2016;18(10):20–25.
- Ataei Rahmati Z, Abedini G, Rezvaninejad R, Ansari M. Clinical evaluation of the anesthetic effect of diphenhydramine in quince seed mucilage base. *J Kerman Univ Med Sci.* 2016;23:321–333.
- Dekebo A, Geba C, Bisrat D, Jeong JB, Jung C. Wound healing, anti-inflammatory and anti-oxidant activities, and chemical composition of Korean propolis from different sources. *Int J Mol Sci.* 2024;25(21):11352.
- Iosageanu A, Mihai E, Seciu-Grama AM, Utoiu E, Gaspar-Pintiliecu A, Gatea F, et al. In vitro wound-healing potential of phenolic and polysaccharide extracts of Aloe vera gel. *J Funct Biomater.* 2024;15(9):266.
- El-Sakhawy M, Salama A, Tohamy HS. Applications of propolis-based materials in wound healing. *Arch Dermatol Res.* 2023;316(1):61.

7. Machado Velho JC, França TA, Malagutti-Ferreira MJ, Albuquerque ER, Livero F, Soares MR, et al. Use of propolis for skin wound healing: systematic review and meta-analysis. *Arch Dermatol Res.* 2023;315(4):943–955.
8. Abu-Ahmed H, Abdel-Wahed R, Elkammar M, El-Neweshy M. Evaluation of the effectiveness of propolis compared with honey on second intention wound healing in the equine. *Middle-East J Scientific Res.* 2013;14(10):12928.
9. Gupta P, Singh A, Singh N, Ali F, Tyagi A, Shanmugam SK. Healing potential of propolis extract-Passiflora edulis seed oil emulgel against excisional wound: biochemical, histopathological, and cytokines level evidence. *Assay Drug Dev Technol.* 2022;20(7):300–316.
10. Jongjitaree S, Koontongkaew S, Niyomtham N, Yingyongnarongkul BE, Utispan K. The oral wound healing potential of Thai propolis based on its antioxidant activity and stimulation of oral fibroblast migration and proliferation. *Evid Based Complement Alternat Med.* 2022;2022:3503164.
11. Chen G, He L, Zhang P, Zhang J, Mei X, Wang D, et al. Encapsulation of green tea polyphenol nanospheres in PVA/alginate hydrogel for promoting wound healing of diabetic rats by regulating PI3K/AKT pathway. *Mater Sci Eng C Mater Biol Appl.* 2020;110:110686.
12. Dehzad MJ, Ghalandari H, Nouri M, Makhtoomi M, Askarpour M. Effects of green tea supplementation on antioxidant status and inflammatory markers in adults: a grade-assessed systematic review and dose-response meta-analysis of randomised controlled trials. *J Nutritional Sci.* 2025;14:e25.
13. Park SY, Lee HU, Lee YC, Kim GH, Park EC, Han SH, et al. Wound healing potential of antibacterial microneedles loaded with green tea extracts. *Mater Sci Eng C Mater Biol Appl.* 2014;42:757–762.
14. Xu FW, Lv YL, Zhong YF, Xue YN, Wang Y, Zhang LY, et al. Beneficial effects of green tea EGCG on skin wound healing: a comprehensive review. *Molecules.* 2021;26(20): 6123.
15. Capasso L, De Masi L, Sirignano C, Maresca V, Basile A, Nebbioso A, et al. Epigallocatechin gallate (EGCG): pharmacological properties, biological activities and therapeutic potential. *Molecules.* 2025;30(3):654.
16. Mokra D, Joskova M, Mokry J. Therapeutic effects of green tea polyphenol (–)-Epigallocatechin-3-Gallate (EGCG) in relation to molecular pathways controlling inflammation, oxidative stress, and apoptosis. *Int J Mol Sci.* 2022;24(1):340.
17. Li S, Wang Z, Liu G, Chen M. Neurodegenerative diseases and catechins: (–)-epigallocatechin-3-gallate is a modulator of chronic neuroinflammation and oxidative stress. *Front Nutr.* 2024;(11):1425839.
18. Mahmoud A, Wilkinson F, Sandhu M, Lightfoot A. The interplay of oxidative stress and inflammation: mechanistic insights and therapeutic potential of antioxidants. *Oxid Med Cell Longev.* 2021;2021:9851914.
19. Bellanti F, Coda ARD, Trecca MI, Lo Buglio A, Serviddio G, Vendemiale G. Redox imbalance in inflammation: the interplay of oxidative and reductive stress. *Antioxidants.* 2025;14(6):656.
20. Rameshk M, Shariffar F, Mehrabani M, Pardakhty A, Farsinejad A, Mehrabani M. Proliferation and in vitro wound healing effects of the microniosomes containing Narcissus tazetta L. bulb extract on primary human fibroblasts (HDFs). *Daru.* 2018;26(1):31–42.
21. Sarhadynejad Z, Shariffar F, Pardakhty A, Nematollahi MH, Sattaie-Mokhtari S, Mandegary A. Pharmacological safety evaluation of a traditional herbal medicine “Zereshk-e-Saghir” and assessment of its hepatoprotective effects on carbon tetrachloride induced hepatic damage in rats. *J Ethnopharmacol.* 2016;190:387–395.
22. Ran F, Mu K, Liu G, Liu Y, et al. Preparation, characterization, and wound healing promotion of hydrogels containing Glucosyloxybenzyl 2-Isobutylmalates extract from *Bletilla striata* (Thunb.) Reichb.f. *Int J Mol Sci.* 2024;25(19): 10563.
23. Aslani A, Ghannadi A, Najafi H. Design, formulation and evaluation of a mucoadhesive gel from *Quercus brantii* L. and *coriandrum sativum* L. as periodontal drug delivery. *Adv Biomed Res.* 2013;2:21.
24. Brown MB, Traynor MJ, Martin GP, Akomeah FK. Transdermal drug delivery systems: skin perturbation devices. *Methods Mol Biol.* 2008;437:119–139.
25. Keshavarzi A, Montaseri H, Akrami R, Moradi Sarvestani H, Khosravi F, Foolad S, et al. Therapeutic efficacy of Great Plantain (*Plantago major* L.) in the treatment of second-degree burn wounds: a case-control study. *Int J Clin Pract.* 2022;2022:4923277.
26. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. *Molecules.* 2023;28(4):1845.
27. Nazar M, Hasan M, Maghfira M, Mustiqillah S, Nada CE, Syahril S, et al. Formulation of peel-off mask loaded with blue emissive carbon dots from *Hylocereus polyrhizus* peels as a UV-blocking active agent. *Results in Engineering.* 2025;27:105848.
28. Zulkefli N, Che Zahari CNM, Sayuti NH, Kamarudin AA, Saad N, Hamezah HS, et al. Flavonoids as potential wound-healing molecules: emphasis on pathways perspective. *Int J Mol Sci.* 2023;24(5): 4607.
29. Subramanian S, Duraipandian C, Alsayari A, Ramachawolran G, Wong LS, Sekar M, et al. Wound healing properties of a new formulated flavonoid-rich fraction from *Dodonaea viscosa* Jacq. leaves extract. *Front Pharmacol.* 2023;14:1096905.
30. Cora M, Üreyen Esertaş ÜZ, Kara Y, Kolaylı S. Antioxidant, antimicrobial, antiviral, and antiproliferative properties of Turkish propolis sample. *European Food Res Technology.* 2025;251(1):123–133.
31. Nichitoi MM, Josceanu AM, Isopescu RD, Isopencu GO, Geana E-I, Ciucure CT, et al. Polyphenolics profile effects upon the antioxidant and antimicrobial activity of propolis extracts. *Scientific Reports.* 2021;11(1):20113.
32. Pyrgioti E, Graikou K, Cheilari A, Chinou I. Assessment of antioxidant and antimicrobial properties of selected Greek propolis samples (North East Aegean Region Islands). *Molecules.* 2022;27(23):8198.
33. Gabr GA, Hassan HMM, Seshadri VD, Hassan NMM. Comparative study of phenolic profile, antioxidant and antimicrobial activities of aqueous extract of white and green tea. *Z Naturforsch C J Biosci.* 2022;77(11,12):483–492.
34. Rha CS, Jeong HW, Park S, Lee S, Jung YS, Kim DO. Antioxidative, anti-inflammatory, and anticancer effects of purified flavonol glycosides and aglycones in green tea. *Antioxidants (Basel).* 2019;8(8):278.
35. Tsai TH, Tsai TH, Chien YC, Lee CW, Tsai PJ. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. *Food Chem.* 2008;110(4):859–864.
36. Rana A, Malik A, Sobti RC. Anti-bacterial properties of propolis: a comprehensive review. *Current Microbiology.* 2025;82(10):479.
37. Borozaan A, Popescu S, Emilian M, Ciulca A, Moldovan C, Gergen I. Comparative study on the antimicrobial activity of propolis, catechin, quercetin and gallic acid. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* 2023;51:12826.