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https://doi.org/10.31925/farmacia.2026.2.10 **ORIGINAL ARTICLE**

DEVELOPMENT AND CHARACTERIZATION OF HONEY/GOTU KOLA/κ-CARRAGEENAN-BASED HYDROGEL FILM WITH THE EVALUATION OF WOUND HEALING ACTIVITY IN EXCISION WOUND RAT MODEL

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Abstract

The high cost of modern wound dressings remains a burden for low-income patients. Formulating honey, gotu kola extract, and κ-carrageenan into a hydrogel film represents a promising, cost-effective alternative, given their sustainability, low cost, and natural abundance. The study aimed to determine the optimal formula for a honey/gotu kola/κ-carrageenan-based hydrogel film and to evaluate its wound-healing activity in a rat excision wound model, along with histopathology and immunohistochemistry. In this study, eight formulations of κ-carrageenan hydrogel were designed using a simple casting method with various honey concentrations (10 - 40%) and different plasticizers, namely sorbitol and glycerol. The formula with the lowest honey content and sorbitol as a plasticizer yielded the most transparent, thinnest film with the highest swelling capacity. Adding 0.5% w/w gotu kola extract still maintained its mechanical characteristics, high swelling capacity, and a preferable active content release profile. It significantly accelerated wound healing by creating unfavorable conditions for bacterial growth, inducing extracellular matrix and collagen type I depositions, and enhancing angiogenesis.

Rezumat

Acest studiu a avut ca obiectiv identificarea unei formule optime pentru un film de hidrogel pe bază de miere, gotu kola și κ-caragenan și evaluarea activității de vindecare a plăgilor într-un model de excizie cutanată la șobolan, coroborat cu analize histopatologice și imunohistochimice. Au fost concepute opt formulări de hidrogel cu κ-caragenan, utilizând metoda simplă de turnare, cu concentrații variate de miere (10 - 40%) și diferiți plastifianți, respectiv sorbitol și glicerol. Formula cu cel mai scăzut conținut de miere și cu sorbitol ca plastifiant a generat filmul cel mai transparent, cel mai subțire și cu cea mai mare capacitate de umflare. Adăugarea extractului de gotu kola în proporție de 0,5% (m/m) a menținut caracteristicile mecanice ale filmului, capacitatea ridicată de umflare și un profil favorabil de eliberare a compușilor activi. Filmul obținut a accelerat semnificativ procesul de vindecare a plăgilor prin crearea unor condiții nefavorabile dezvoltării bacteriene, stimularea depunerii matricei extracelulare și a colagenului de tip I, precum și potențarea angiogenezei.

Keywords: hydrogel film, κ-carrageenan, honey, gotu kola extract, wound healing

Introduction

Wounds remain a global burden, especially chronic and unhealed wounds (1). In 2022, the cost of wound care ranged from $6.04 to $148.65 billion *per* year in the top 10 high-spending countries and continues to rise geographically (2). Modern dressings based on the moist environment principle are extensively applied to treat various wounds (3). A moist wound environment facilitates autolytic debridement, reduces pain and scarring, and activates or enhances the function of biochemical factors and cells, such as collagen and keratinocytes, that play an important role in wound healing (4). The chronic wound needs a long-term application of the dressing. Consequently, it imposes a significant burden on low-income patients (5). Therefore, the development of affordable wound dressings is essential.

A huge number of different types of wound dressings are available on the market, *i.e.*, gauze, transparent films, foam dressings, hydrogels, hydrocolloids, hydro-conductive dressings, medicated wound dressings, and bioactive wound dressings (6). Semi-permeable film dressings are versatile types of wound dressings that offer numerous benefits. They conform well to the contours of the patient's skin and are transparent, allowing for easy monitoring of wounds (7). These dressings also have gas permeability, promoting angiogenesis while effectively blocking the passage of liquids and bacteria (8, 9). Film dressings provide multiple benefits at various stages of wound healing, including contributing to granulation tissue growth, collagen deposition, and converting type III collagen to type I collagen during the remodeling phase (10).

Carrageenan-based dressings are particularly superior for several reasons, including their biocompatibility, biodegradability, and lack of cytotoxicity (11). As a natural polysaccharide, carrageenan offers additional advantages such as availability, reproducibility, and low cost (12). The primary source of carrageenan is κ-carrageenan, derived from red seaweed (*Kappaphycus alvarezii*). This seaweed is mainly farmed in Indonesia, the Philippines, Vietnam, and Malaysia, producing pure κ-carrageenan (13). Cations such as K^+ can efficiently improve the texture properties of κ-carrageenan gels (14). It forms a film when combined with a plasticizer agent such as glycerol or sorbitol. Incorporating natural products with wound-healing properties into the kappa carrageenan-based hydrogel film can further accelerate wound healing. Substances such as honey and medicinal plant extracts, including *Aloe barbadensis*, *Centella asiatica*, and *Curcuma longa*, are known for their wound-healing properties (15, 16).

Honey contains hundreds of components that vary depending on the floral source and other factors (17). Its high sugar concentration reduces water activity and causes osmotic stress in bacteria, preventing infections in wounds (18). It also provides a local cellular energy source and other substances that may enhance cellular nutrition, promoting endothelial cell proliferation (19). The presence of hydroxyl groups in the phenolic compounds influences honey's antioxidant capacity (20). This antioxidant effect can presumably lower neutrophil superoxide production, acting as a free radical scavenger (21). Honey also promotes autolytic debridement, reducing slough in diabetic foot ulcers (22). Among all types of honey, the most well-known honey for healing wounds is Manuka honey (MH), which gained recognition for its antibacterial effect (23). Thus, numerous dressings containing MH are available on the market. Unfortunately, its price is 6 to 25 times higher than that of other honey types (24).

Centella asiatica, or gotu kola, a traditional medicinal herb, is known for its antibacterial, antioxidant, anti-inflammatory, neuroprotective, and wound-healing properties (25). It promotes wound healing in the gastric ulcer rat model through enhanced angiogenesis, likely due to its stimulating effect on collagen I, fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) production (26). Gotu kola has also been shown to reduce inflammatory markers, including IL-1β, IL-6, TNFα, PGE2, COX-2, and LOX, in RAW 264.7 cells and primary murine macrophages (27-30). Furthermore, it can inhibit the biofilm formation of *Streptococcus mutans* (31).

Recent developments in bioactive wound dressings incorporating natural products show great potential for treating chronic wounds (32). In this context, combining a κ-carrageenan-based hydrogel film with honey and *Centella asiatica* (gotu kola) extract represents a promising strategy, particularly for the management of macerated and chronic wounds. The κ-carrageenan matrix offers biocompatibility and high wound exudate absorption, while honey provides antimicrobial activity to inhibit bacterial growth. Simultaneously, *Centella asiatica* extract contributes multi-modal wound-healing actions that can accelerate tissue regeneration.

Materials and Methods

Materials

κ-Carrageenan (purchased from PT Gumindo Perkasa Industri, Indonesia), honey (purchased from Kembang Joyo, Indonesia); *Centella asiatica* extract (PT Sari Alam, Indonesia), pure asiaticoside (purchased from Markherb, Indonesia), sorbitol, potassium chloride (Merck), deionized water (WaterOne™).

Preparations of κ-carrageenan-based hydrogel films with varying honey content and different plasticizer agents

The hydrogel films containing 10%, 20%, 30%, and 40% honey were prepared with different plasticizer agents (glycerol or sorbitol) by the solution-casting method with some modifications (33). Shortly thereafter, 2% w/w of κ-carrageenan was added to deionized water by stirring at 600 rpm; then 4% w/w of 0.5 N potassium chloride and 6% w/w of the plasticizer agent were added. The mixture was stirred continuously for 1 hour at 70 ± 2°C to complete the ionic cross-linking reaction and initiate gelation (34). Furthermore, the honey with different concentrations was added to the mixture at 65 ± 2°C. 35 g of the final solution of fresh hydrogel was then poured into the square silicone mold (10 cm x 10 cm) and dehydrated at 37°C for 24 hours in the dehydrator (LocknLock™).

Preparation of gotu kola/honey/κ-carrageenan-based hydrogel film

The hydrogel film with 10% w/w of honey, and 0.5% and 1% w/w gotu kola extract was made with the same procedure as the previous hydrogel film. The extracts were initially mixed with honey before being added to κ-carrageenan-based hydrogel solutions containing sorbitol as a plasticizer.

Evaluation of dehydrated hydrogel film

The fresh hydrogel film was put in a dehydrator (LocknLock) at 37°C and weighed at 0, 8, 12, and 24 hours until it reached a constant weight (35).

$$\text{Drying shrinkage (\%)} = \sum_{t=0}^{t} \frac{W_t - W_0}{W_0} \times 100\%,$$

W_t represented the hydrogel film's weight at a certain time, and W_0 represented the initial weight of the hydrogel film.

Swelling capacity study

The swelling ability of hydrogel films was measured according to the method of Singh *et al*. (2022)

(33). To perform the evaluation, the hydrogel sample, which had reached a constant weight, was immersed in a PBS (phosphate buffer saline) solution at pH 7.4 ± 0.5. The sample was weighed at various time intervals until it reached equilibrium. The swelling ratio of the hydrogel was calculated using:

$$\text{Swelling ratio (\%)} = \left[\frac{(W_w - W_d)}{W_d}\right] \times 100.$$

Here, W_d represented the initial weight of the hydrogel, and W_w represented the final weight of the hydrogel after it had reached a constant weight.

Film thickness and transparency

The film thickness of the developed formulas was measured at 9 positions using a micrometer, and film transparency was determined by spectrophotometry according to Norajit *et al*. (2010) with some modifications (36). Rectangular samples of the films (9 mm x 35 mm) were cut and placed into the cell spectrophotometer (Beckman Colter DU 720™), and their light transmission was scanned between 400 nm and 800 nm using air as a control.

Mechanical characteristics study (thickness, tensile strength, elongation at break, and elastic modulus)

The mechanical characteristics of the honey/gotu kola/κ-carrageenan-based hydrogel film evaluated include thickness, tensile strength, elongation at break, and elastic modulus, as reported by Jaiswal *et al*. (2019) (37). Three films were cut into rectangular pieces measuring 10 mm x 90 mm using a razor blade. Each film's thickness was measured using a digital micrometer, and the average thickness was calculated. Measurements of tensile strength, elongation at break, and elastic modulus were performed using a universal tensile strength tester (Instron®). The grip distance was set to 50 mm, with a tensile speed of 50 mm/min and a force of 500 N.

In vitro active content release studies

The release of active substances from the hydrogel film was determined by measuring the asiaticoside and reducing sugar content, representing gotu kola extract and honey, respectively. The release of active substances was evaluated using the method outlined by Namazi *et al*. (2016) with some modifications (38). A 500 mg sample of hydrogel film was placed in an Erlenmeyer flask containing 10 mL of PBS. The flask was then incubated at 37°C with a thermostatic shaker set to 50 rpm. At 1, 4, 8, 24, and 32 hours, 1 mL of PBS was collected. After each extraction, an equal volume of PBS at the same temperature was added.

Asiaticoside content at each sampling time and in the extract was measured by LC-MS-MS using a pure asiaticoside standard (39). Meanwhile, the reducing sugar content from honey for each sampling time was determined using the DNSA (3,5-dinitrosalicylic acid) method and measured on a microplate reader at 540 nm (Nanoquant Plate™) (40).

The actual amount of each asiaticoside and reduction sugar of honey released from the hydrogel film were calculated according to the equation:

$$\text{Cumulative release (\%)} = \sum_{t=0}^{t} \frac{M_t}{M_0} \times 100\%,$$

M_t is the cumulative amount of asiaticoside or reducing sugar of honey released at time intervals t, and M_0 is the initial amount of asiaticoside or reducing sugar present in the sample.

Preparations of an animal

10 - 12 weeks old male Wistar rats (200 - 250 g) were received from CV. Kencana Lab Pengembangan Hewan, Indonesia. The rats were acclimatized for 7 days under a 12 h dark/light cycle at 25 ± 2°C and 60-70% relative humidity.

In vivo wound healing study

The study protocol was approved by the Ethics Committee of the School of Pharmacy, Bandung Institute of Technology, Indonesia (KEP/I/2023/XI/H031123SM/PLHM). All experiments were carried out following the animal ethics guidelines of the School Pharmacy Bandung Institute of Technology to minimize the suffering of animals. The wound excision model was made according to the Masson-Mayers *et al*. (2013) method with some modifications. The rat was anesthetized with urethane (625 mg/kg, intraperitoneally). After the removal of the hair on the animal's back, 4 circular excision wounds were made symmetrically on the back of the rat using surgical scissors with a diameter of 13 mm (41). Each wound randomly represents the Untreated Group, the positive Control Group (50 mg of Medihoney™ (a commercial wound gel containing 100% MH), and 2 groups of honey/*Centella* extract/κ-carrageenan based hydrogel film with 10 w/w of honey and 0.5% w/w of *Centella* extract (H10CA0.5) and with 10% w/w of honey and 1% w/w of *Centella* extract (H10CA1) cut 15 mm x 15 mm in size to cover the wound surface. Each wound was treated, except in the Untreated Group, and covered with medical tape (Isopore®) and gauze, then re-covered with medical tape and gauze twice a day for 21 days.

Wound condition was documented daily using a 50-megapixel digital camera at a consistent distance to evaluate wound closure. The wound area was measured by tracing the wound perimeter with a transparent plastic sheet every day and analyzed with ImageJ software. The equation calculated wound closure:

$$\text{Wound closure (\%)} = \left(1 - \frac{\text{open wound area}}{\text{initial wound area}}\right) \times 100.$$

Histopathological study

The animals from 3-, 7-, 14-, and 21-day post-treatment were euthanized in a CO2 chamber, and

the skin tissues were harvested and immediately fixed in 10% (v/v) formalin. Then, the fixed tissue samples were processed, embedded in paraffin, and sectioned to 5 µm thickness. The sections were stained with hematoxylin and eosin (H&E), and Masson's trichrome was then examined under a light microscope (Olympus BX51) to observe the wound healing phase journey after treatment. The photos were obtained using a microscope connected to a digital camera. The collagen index of 21 days tissues was performed by histomorphometry method (42).

Immunohistochemistry study

Immunohistochemical staining for TNF-alpha was performed on paraffin sections from 3 days post-treatment to evaluate the inflammatory phase. Meanwhile, the sections from 7-, 14-, and 21-day post-treatment were stained using immunohistochemistry with CD34 as the marker for angiogenesis.

Statistical analysis

The data were illustrated and analyzed using GraphPad Prism 10.2.3 (403) software. Two-way analysis of variance (ANOVA) and Post hoc comparison tests were done using Tukey's test. Film thickness and collagen index were analyzed by One-way ANOVA. p-values of < 0.05 were considered statistically significant.

Results and Discussion

Determination of optimum honey content and plasticizer agent of hydrogel film

Achieving a high honey content while maintaining good film dressing characteristics required finding an optimum level. Furthermore, selecting an appropriate plasticizer was crucial for improving the film's integrity and water vapor permeability. The subsequent variation in honey and plasticizer content directly influenced the fresh hydrogel's shrinking capacity, with significant differences observed after 8 and 12 hours of dehydration. In formulations using glycerol as the plasticizer (Figure 1A), the shrinkage capacity decreased gradually from 82.76% to 60.79% as the honey content increased from 10% to 40% w/w. A similar trend was observed with sorbitol as the plasticizer, although the decrease was slightly larger, from 84.44% to 59.85% (Figure 1B). Consequently, both the honey content and the type of plasticizer had a significant effect on the resulting film thickness (Figure 1C & Figure 1D).

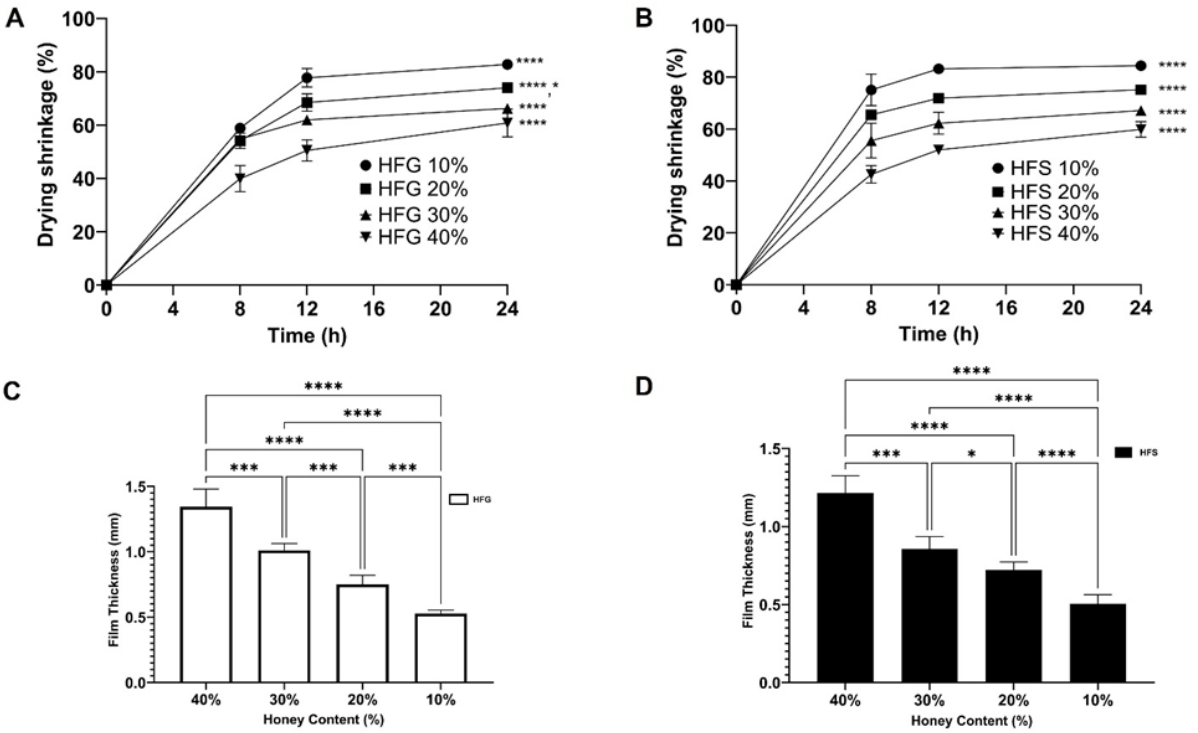

Figure 1.

Drying process of fresh hydrogel and the impact on film thickness. Drying shrinkage of hydrogel film formula with a variation of honey content and glycerol (A) and sorbitol (B) as a plasticizer. The values are given as mean ± standard error of the means (n = 3). The film thickness of the hydrogel film formula with a variation of honey content and glycerol (C) and sorbitol (D) as a plasticizer. The values are given as mean ± standard error (n = 9). HFG: hydrogel film glycerol, HFS: hydrogel film sorbitol. P-values are shown as *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$; ****, $p < 0.00001$. The statistical significance of differences is indicated * *vs*. each group

In evaluating total light transmission to determine film transparency, the results showed that all films exhibited a gradual increase in transmission from 400 nm to 600 nm, followed by a decrease at 800 nm. At each measured wavelength, formulations containing 10-40% w/w honey with glycerol as the plasticizer showed lower light transmission (Figure 2A). Among these, the hydrogel film with 40% w/w honey and glycerol showed the lowest transmission across the tested range, transmitting only 16.44% to 58.83% of light. In contrast, the film containing 10% w/w honey and sorbitol as the plasticizer consistently exhibited the highest light transmission over 400-800 nm, making it the most transparent formulation. This film transmitted 76.7% of light at 400 nm, increasing to 100.67% at 600 nm, before gradually decreasing to 96.93% at 800 nm (Figure 2B).

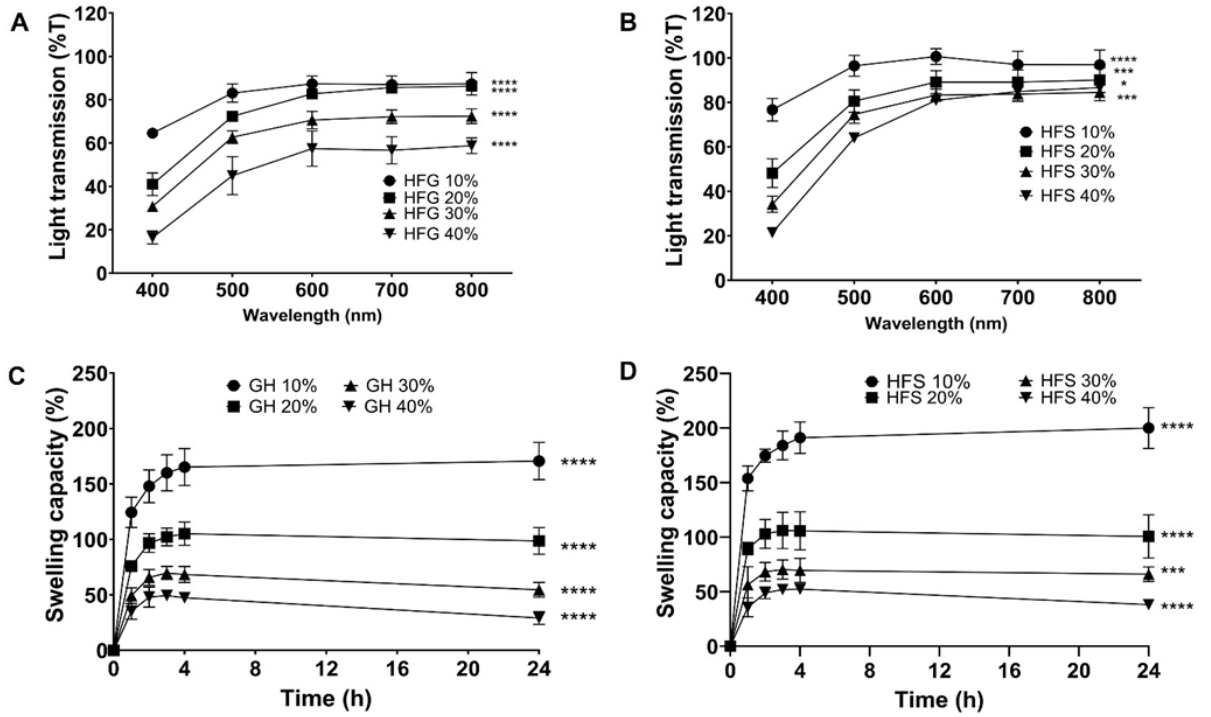

Figure 2.

Evaluation of light transmission and swelling capacity in hydrogel films. Light transmission of hydrogel film formula with variation of honey content and glycerol (A) and sorbitol (B) as plasticizer. SweSwelling capacity of hydrogel film formula with variation of honey content and glycerol (C) and sorbitol (D) as plasticizer. The values are given as mean ± standard error of the means (n = 3).

HFG: hydrogel film glycerol, HFS: hydrogel film sorbitol. P-values are shown as *, $p < 0.05$; **, $p < 0.001$; ***, $p = 0.0002$; ****, $p < 0.0001$. The statistical significance of differences is indicated * *vs*. each group

The evaluation of swelling capacity revealed that all hydrogel films swelled rapidly within the first 4 hours. Formulations containing more than 20% w/w honey reached their maximum swelling during this period, while the others continued to swell slowly for up to 24 hours, indicating that swelling capacity decreased with increasing honey content. The hydrogel film containing 10% w/w honey and sorbitol exhibited the highest swelling capacity, reaching 200.05% of its original weight in PBS (pH = 7.4) within 24 hours. This film consistently swelled significantly more than the formulation with the same honey content but using glycerol as the plasticizer ($p < 0.05$). In contrast, films with higher honey content showed reduced swelling within the first 4 hours and subsequently lost integrity, as evidenced by a decline in mass after 24 hours. For instance, the film containing 40% w/w honey and glycerol demonstrated the lowest swelling capacity, swelling to only 47.45% of its original weight within 4 hours, which further declined to 29.16% after 24 hours (Figure 2C & Figure 2D).

The shrinking capacity, transparency, and swelling capacity of film dressing are essential properties, as they govern maximal wound exudate absorption and facilitate the monitoring of wound status (43). Our investigation revealed an inverse relationship between honey content and these key parameters: the dried hydrogel formulated with the lowest honey concentration demonstrated the greatest shrinking, transparency, and swelling. Consistent with these findings, studies on honey-loaded ethyl cellulose/gum tragacanth nanofibers have also shown that higher honey content increases the average fiber diameter (44). High honey content reduced the proportion of hydrogel matrix in the formulation, thereby dimin-

ishing electrostatic repulsion between ionic charges within the polymer network. This alteration impacted both water vapor permeability and hydrogel integrity (45). Additionally, the increased honey content affected mixture viscosity, which influenced the thickness of the dried hydrogel and reduced film clarity (8).

A plasticizer also significantly affects the properties of hydrogel films by improving toughness and imparting flexibility (46). Without it, the films become brittle due to extensive intermolecular forces arising from polymer chain interactions (47). Sorbitol and glycerol are common polysaccharide-based plasticizers for biodegradable and edible films due to their low cost, non-toxicity, and odorless and tasteless properties (48, 49). These agents form hydrogen bonds with the polymer's hydrophilic groups, forming a flexible, pliable hydrogel (50). Based on the optimization evaluation, the hydrogel film formulated with sorbitol as a plasticizer exhibited better properties than the one formulated with glycerol. Sorbitol possesses more hydroxyl groups in its structure than glycerol, which allows for the formation of more junction zones through intermolecular, cross-linking hydrogen bonds between sorbitol and κ-carrageenan. Consequently, this enhanced water structuring influenced water release and strengthened the hydrophobic interactions within the hydrogel (51).

Characteristic and release profile of honey/gotu kola/κ-carrageenan-based hydrogel film

After achieving the optimal honey content and plasticizer, the gotu kola extract was added to the formula. Only a small proportion of gotu kola extract could be added to the hydrogel film formula due to the low clarity of the prevention. The fresh hydrogel, containing 10% w/w honey and sorbitol as a plasticizer, along with 0.5% w/w gotu kola extract, formed a thin, transparent film after 24 hours of dehydration (Figure 3A & 3B). The film could shrink by 47.85% within 4 hours and continued to shrink up to 84.17% after 24 hours. The addition of gotu kola extract did not alter the shrinking ability significantly (Figure 3C). It was also observed in swelling capacity that the gotu kola/honey/κ-carrageenan-based hydrogel film rapidly swelled to almost 200% of its weight within 4 hours and maintained a swelling capacity of 233.27% of its original weight after being immersed in PBS at pH 7.4 for 24 hours (Figure 3D).

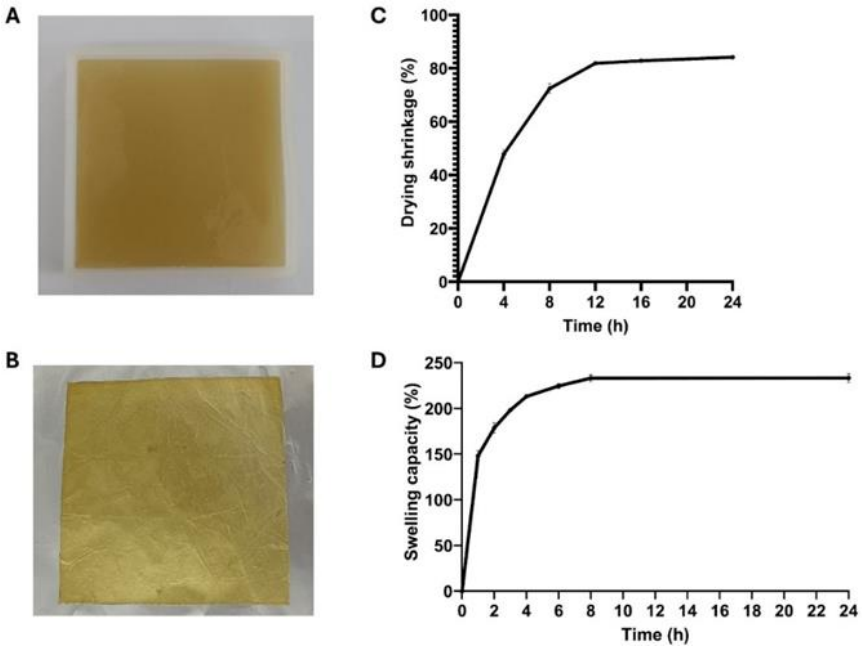

Figure 3.

Characteristic of honey (10% w/w)/ gotu kola (0.5% w/w)//κ-carrageenan-based hydrogel film. Fresh hydrogel (A), dehydrated hydrogel film (B), drying shrinkage capacity for 24 hours (n = 3) (C), swelling capacity for 24 hours (n = 3) (D)

The optimized film formulation exhibited favorable mechanical properties, with a thickness of 0.5 ± 0.1 mm. Its flexibility was confirmed by a low elastic modulus (1.50 N/mm²) and a high elongation at break of up to 136.25% (Table I). Crucially, the incorporation of 0.5% w/w extract into this optimal formulation did not compromise its key performance attributes; the shrinking behavior, film thickness, and swelling capacity remained consistent with the extract-free film. The film also retained sufficient transparency for visual wound monitoring. Furthermore, these mechanical characteristics, namely flexibility and extensi-

bility, are essential for wound-dressing applications, as they allow the dressing to conform to and accommodate body movements without tearing (28, 52).

Table I

Mechanical characteristics of honey/gotu kola/κ-carrageenan-based hydrogel film. Values represent mean ± SD, n = 3

Mechanical characteristic properties	Value
Thickness (mm)	0.5 ± 0.1
Tensile strength (N/mm^2)	2.05 ± 0.16
Elongation at break (%)	136.25 ± 10.23
Elastic modulus (N/mm^2)	1.50 ± 0.092

To ensure therapeutic efficacy, evaluating the release profile of active compounds from the hydrogel film is essential. The film exhibited a rapid initial burst release of asiaticoside (the primary active compound in *Centella asiatica* extract), with 70.8% released into PBS (pH = 7.4) within the first hour. Release continued to a peak of 93.35% at 4 hours, followed by a gradual decline to 74.86% by 32 hours. In comparison, the release of reducing sugars (representing the honey component) followed a different profile. At 4 hours, 82.19% of reducing sugars were detected, rising to a peak of 105.34% at 8 hours, before declining to 67.85% by 32 hours (Figure 4). The value exceeding 100% for reducing sugars may indicate cumulative release exceeding the initial quantification or suggest matrix degradation contributing to the measured sugar content.

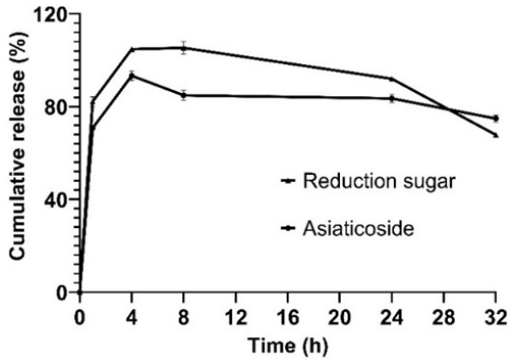

Figure 4.

Asiaticoside of gotu kola extract and reduction sugar of honey released from the hydrogel film (n = 3)

Asiaticoside was chosen to represent the release of gotu kola extract, while the reducing sugars of honey served as the marker for honey. Asiaticoside is one of the three main triterpenes found in the plant, alongside madecassoside and madecassic acid (53). A previous study demonstrated significant wound healing activity of 0.2% w/w asiaticoside in topical formulations in both normal and delayed-type wound models (54). Meanwhile, the high sugar concentration in honey reduces water activity and induces osmotic stress in bacteria, thereby helping prevent wound infections (18). Asiaticoside release was faster and more extensive from the H10CA0.5 film compared to a previous study, in which 91% of asiaticoside was released from an alginate film over 12 hours (55). The H10CA0.5 film achieved a comparable release level in just 4 hours. Interestingly, the apparent concentration of reducing sugars detected in the medium exceeded the total honey sugar content loaded into the film. This anomaly suggests that the assay detected additional reducing substances beyond the honey's reducing sugars, likely originating from the κ-carrageenan matrix itself, a brittle sulfated galactan polymer (56). Furthermore, the release of active content from the H10CA0.5 film was more prominent than in other κ-carrageenan hydrogel films from some previous studies. Wang *et al*. (2021) found that the release of cinnamon essential oil reached only 20% after 48 hours (57). Meanwhile, Postolović *et al*. (2022) found that the release of active content from curcumin extract was less than 30% released in 8 hours and reached 87.64% in 24 hours (58).

Wound healing activity in the excision wound rat model

In vivo wound-healing activity of the hydrogel film groups was evaluated based on wound closure and the macroscopic appearance of the wounds. All wounds showed contraction during the treatment days at different velocities.

On the first day of the treatment, the wound of the honey/*gotu kola*/κ-carrageenan-based hydrogel film with 0.5% w/w and 1% w/w gotu kola extract (H10CA0.5 and H10Ca1) Groups started to contract and reached wound closure of 2.74% and 3.18%, respectively. In contrast, the wound areas of the commercial wound gel had 100% of MH, and the untreated groups increased, reaching -10.81% and -16.39% of wound closure, respectively. These wounds began to contract on the fourth day. The wound contraction in the hydrogel film groups was consistently superior throughout the treatment period and showed a statistically significant difference (two-way ANOVA, $P < 0.0001$). As a result, the H10CA1 wound Group started to fully close by day 14, while the H10CA0.5 wound Group fully closed by day 16. All animals in these groups achieved 100% wound closure by day 17. There were no significant differences in wound closure between the H10CA1 and H10CA0.5 groups ($P = 0.8812$). The wounds in the commercial wound gel group began to close by day 21, whereas none of the wounds in the Untreated Group reached 100% closure at the end of the study. Statistical analysis showed a significant difference in wound closure between the two groups ($P < 0.0001$) (Figure 5). A noticeable wound contraction in the hydrogel groups might be due to the gotu kola extract. Similar results were found in several studies that used gotu kola extract and fraction for the evaluation of wound healing activity in incision, excision, and partial burn wound models (63-65).

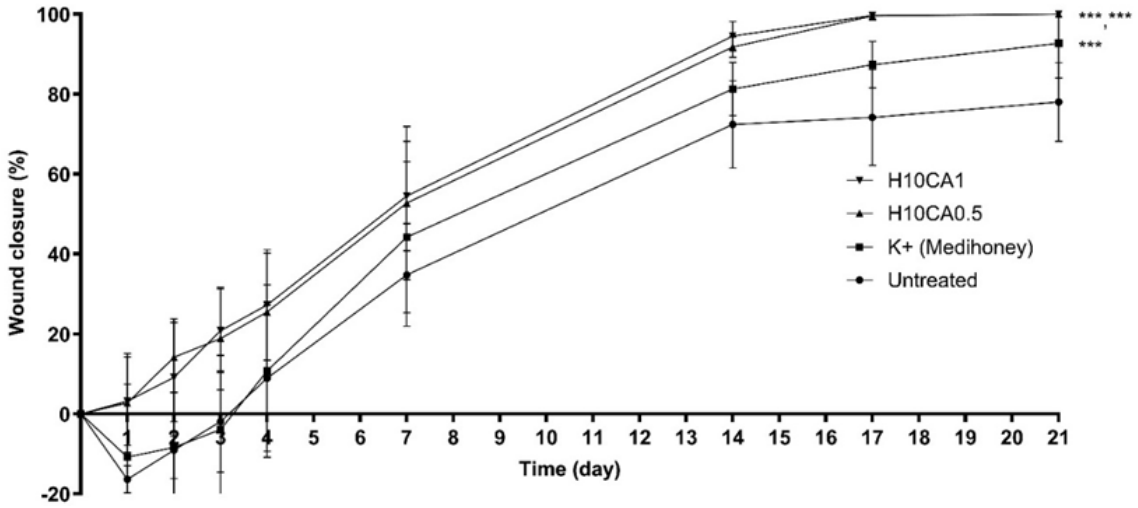

Figure 5.
Representative graph of wound closure of each group during the time course (n = 6). All values are given as mean ± standard error of the means.
The p-values are shown as ***, P < 0.001. The statistical significance of differences is indicated * *vs*. the untreated groups

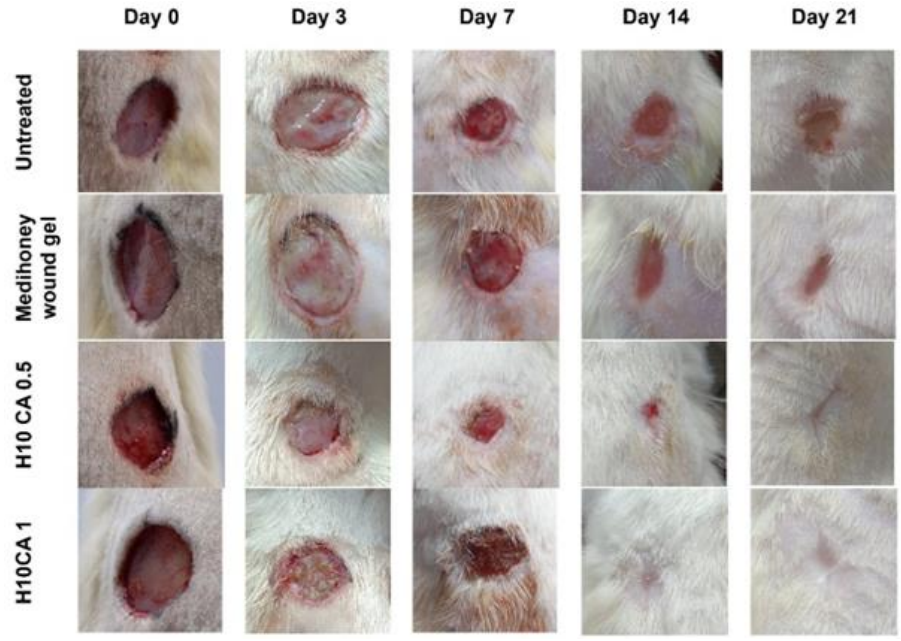

Figure 6.
Macroscopic appearance of the wounds in each group at 0, 3, 7, 14 and 21 days after treatment

The macroscopic appearance of the wounds varied among the groups. On day 3, the wounds in the H10CA0.5 and H10CA1 Groups were smaller compared to the other groups, indicating the start of wound contraction. Additionally, wound exudates predominantly covered the wound surface in the untreated and commercial wound gel groups, suggesting the inflammation phase was still ongoing (Figure 6). The wound exudates in these groups had diminished by day 7 and were replaced by granulation tissue, indicating the proliferation phase had begun. Consequently, the wounds were smaller, but those in the hydrogel film groups showed better conditions. This trend continued throughout the treatment period. Scar tissue had fully formed on day 14 in the H10CA1 Group, although there was still a wound spot in the H10CA0.5 Group. Meanwhile, the wounds in the untreated and commercial wound gel groups remained open. By day 21, the remodeling phase continued. As a result, the scar tissue in the H10CA1 and H10CA0.5 Groups became thicker, but the epithelialization of H10CA1 was more noticeable (Figure 6). The application of H10CA0.5 and H10CA1 films significantly accelerated wound healing, as evidenced by the MH Group. The effect was due to the high absorbance of the wound exudate by the κ-carrageenan hydrogel component, creating a dry, clean wound surface (59). Meanwhile,

the rapid release of honey during wound exudation led to hyperosmotic and low-pH conditions in the wound bed (60). These actions created highly unfavorable conditions that prevented bacterial growth. In the absence of bacterial infections, the role of pro-inflammatory neutrophils and macrophages will be minimal, thereby accelerating the end of the inflammatory phase and allowing the proliferation phase to begin more quickly (61, 62).

Histopathology and immunohistochemistry findings

Histological examination supported the macroscopic evaluation and explained how the hydrogel film stimulates the wound-healing process at the tissue level. Furthermore, immunohistochemistry allowed visualization in situ of the specific tissue factor involved in various stages of wound healing. High expression of TNF-α marked the inflammatory phase, and high expression of CD34 marked the proliferation and remodeling phase (61). The histology and immunohistochemistry results supported the mechanism discussed above. On the third day, tissues stained with H&E showed differences in the granulation process among the groups. The H10CA0.5 and H10CA1 Groups showed a thicker layer of granulation tissue than the Untreated Group. The wound tissue treated with commercial wound gel also showed more granulation than the untreated wound (Figure 7A). Masson's trichrome-stained wound tissue on days 7, 14, and 21 after treatment showed notable differences (Figure 7A). After 7 days, collagen deposition, indicated by green areas, was not apparent in any group. Collagen deposition began on day 14 and substantially increased by day 21. The densest collagen tissue was observed in the group treated with hydrogel film containing 1% w/w gotu kola extract, followed by the group with 0.5% w/w gotu kola extract and the commercial wound gel group. In contrast, the Untreated Group showed minimal collagen tissue. Histomorphometry analysis of the collagen index on day 21 revealed significant differences ($P < 0.05$) among the groups (Figure 7B).

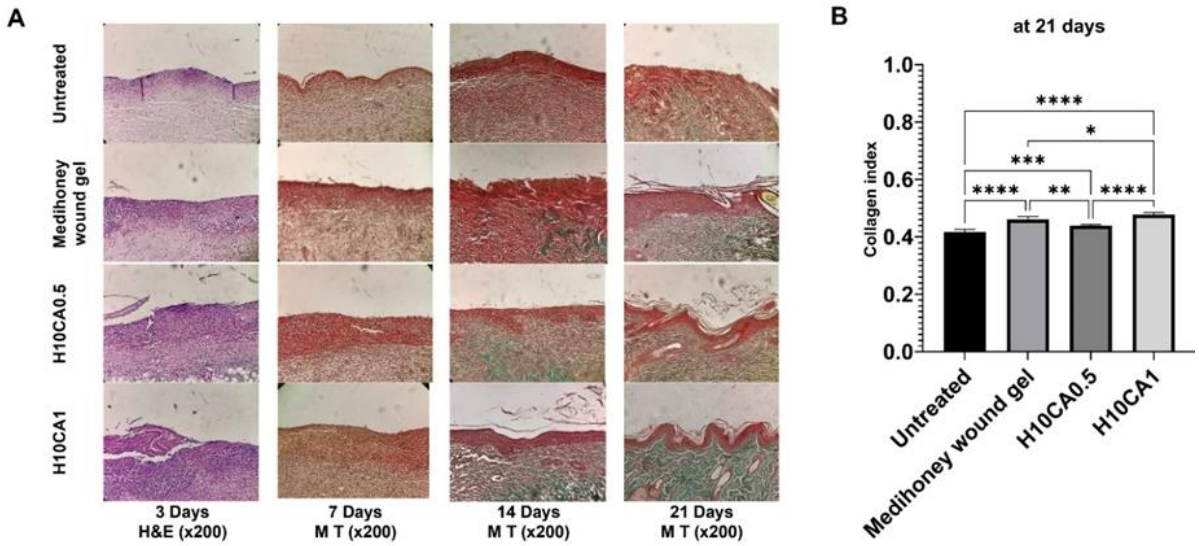

Figure 7.

Photomicrograph of wound tissue during treatment days. (A) H&E staining at 3 days (deep purple areas refer to granulation tissue) and Masson's trichrome staining at 7,14 and 21 days after treatment (green areas refer to collagen deposition). (B) Quantification of collagen density by histomorphometry at 21 days after treatment (n = 6)

*P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.00005

From immunohistochemistry evaluation, the results showed that on the third day of treatment, TNF-α expression was highest in the untreated wound, followed by the commercial gel-treated wound (Figure 8). Meanwhile, wounds treated with H10CA0.5 and H10CA1 showed low expression of the marker, indicating reduced inflammatory activity. Additionally, the relative expression of CD34 was lowest in untreated wounds, followed by wounds treated with commercial wound gel. After 7 days of treatment, the wound tissues treated with H10CA0.5 and H10CA1 films already showed expression of the vascular endothelial factor, indicating the onset of the proliferation phase. In contrast, the marker was still not expressed in untreated wound tissues or in those treated with commercial wound gel, indicating a predominantly inflammatory phase. On the 14th and 21st days of treatment, the relative CD34 expression level increased in each group, indicating enhanced angiogenesis activity (Figure 8). The highest density of relative CD34 expression was observed in wound tissues treated with H10CA1 film, followed by those treated with H10CA0.5 film, indicating high angiogenesis activi-

ty. The wound displayed thick granulation tissue, a high density of collagen type I, and high CD34 expression. These factors indicate high proliferation, collagen deposition, and angiogenesis. Simultaneously, the release of gotu kola extract into the wound bed promotes wound healing (66). The active constituents of *Centella asiatica* – pentacyclic triterpenes such as asiaticoside, madecoside, madecassic acid, and asiatic acid – contribute to this effect (67). Asiaticoside induces cell cycle progression, proliferation, and collagen synthesis (68). Asiatic acid stimulates glycosaminoglycan synthesis and extracellular matrix accumulation in rats and possesses anti-inflammatory properties (69, 70). Madecoside enhances collagen I synthesis and exhibits anti-inflammatory activity (71).

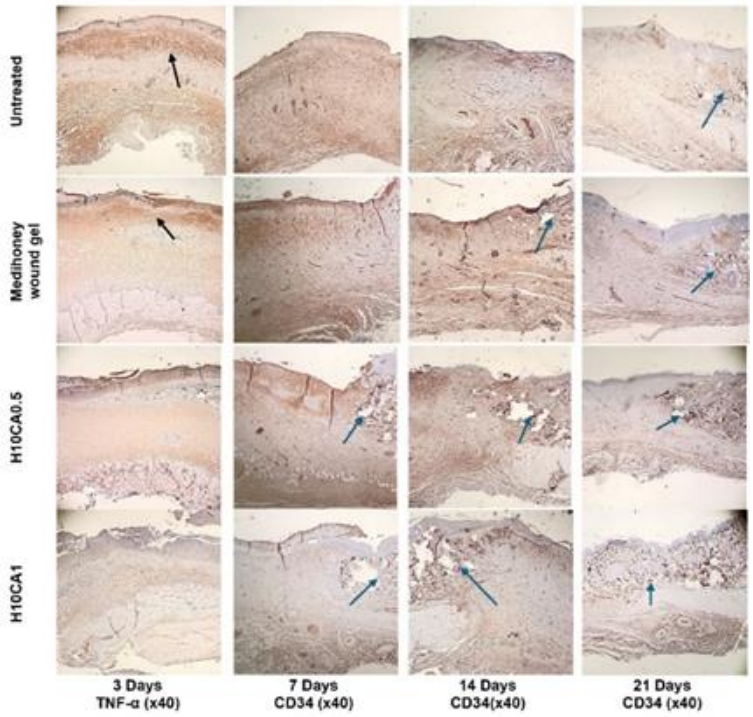

Figure 8.
TNF-α expression (black arrow) on day 3 of treatment and vascular endothelial factor (CD34) expression (blue arrow) on day 7, 14, and 21 of treatment

Conclusions

The concentration of honey and the type of plasticizer significantly influenced the properties of the dried hydrogel film. Formulations with the lowest honey concentration and sorbitol as the plasticizer exhibited the highest drying shrinkage and swelling capacity, attributable to a greater hydrogel proportion and increased hydrogen bonding. Importantly, the incorporation of 0.5% w/w *Centella asiatica* extract did not significantly alter these film characteristics, preserving good transparency. The resulting film facilitated the rapid release of its active components, with over 90% of asiaticoside and most of the honey's reducing sugars being released from the κ-carrageenan matrix within 4 hours.

Applying the honey/gotu kola/κ-carrageenan hydrogel film to a wound excision model twice daily for 21 days significantly accelerated wound healing through multimodal actions. The hydrogel film effectively absorbed wound exudate, maintaining a dry and clean wound surface. This property, combined with honey's antimicrobial effects, created an unfavorable environment for bacterial growth, thereby accelerating the resolution of the inflammatory phase. Meanwhile, gotu kola extract promoted proliferation and remodeling by stimulating extracellular matrix accumulation, enhancing angiogenesis, and increasing collagen deposition.

To support its future clinical application, studies on shelf-life, dermal toxicity, and wound healing efficacy in chronic and/or infected wound models should be conducted.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

The study protocol was approved by the Ethic Committee of School of Pharmacy, Bandung Institute of Technology, Indonesia (approval number: KEP/I/2023/XI/H031123SM/PLHM).

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Mahmoudi M, Gould LJ. Opportunities and challenges of the management of chronic wounds: a multidisciplinary viewpoint. Chronic Wound Care Manag Res. 2020;7:27-36.
2. Queen D, Harding K. Estimating the cost of wounds both nationally and regionally within the top 10 highest spenders. Int Wound J. 2024;21(2):e14709.
3. Ahmad N. *In vitro* and *in vivo* characterization methods for evaluation of modern wound dressings. Pharmaceutics. 2022;15(1):42.
4. Nuutila K, Eriksson E. Moist wound healing with commonly available dressings. Adv wound care. 2021;10(12):685-698.
5. Kapp S, Santamaria N. The financial and quality-of-life cost to patients living with a chronic wound in the community. Int Wound J. 2017;14(6):1108-1119.
6. Laurano R, Boffito M, Ciardelli G, Chiono V. Wound dressing products: A translational investigation from the bench to the market. Eng Regen. 2022;3(2):182-200.
7. Kuddushi M, Shah AA, Ayranci C, Zhang X. Recent advances in novel materials and techniques for developing transparent wound dressings. J Mater Chem B. 2023;11(27):6201-6224.
8. Amruth P, Akshay P, Rosemol Jacob M. Developmental prospects of carrageenan-based wound dressing films: Unveiling techno-functional properties and freeze-drying technology for the development of absorbent films—A review. Int J Biol Macromol. 2024;276(1):133668.
9. Dhivya S, Padma VV, Santhini E. Wound dressings–a review. BioMedicine (Taipei). 2015;5(4):22.
10. Ellis S, Lin EJ, Tartar D. Immunology of wound healing. Curr Dermatol Rep. 2018;7(4):350-358.
11. Neamtu B, Barbu A, Negrea MO, Berghea-Neamțu CȘ, Popescu D, Zăhan M, et al. Carrageenan-based compounds as wound healing materials. Int J Mol Sci. 2022;23(16):9117.
12. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny RJ. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur J Pharm Biopharm. 2004;57(1):19-34.
13. Rupert R, Rodrigues KF, Thien VY, Yong WT. Carrageenan from *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae): metabolism, structure, production, and application. Front Plant Sci. 2022;13:859635.
14. Wang Y, Yuan C, Cui B, Liu Y. Influence of cations on texture, compressive elastic modulus, sol-gel transition and freeze-thaw properties of kappa-carrageenan gel. Carbohydr Polym. 2018;202:530-535.
15. Albahri G, Badran A, Hijazi A, Daou A, Baydoun E, Nasser M, et al. The Therapeutic Wound Healing Bioactivities of Various Medicinal Plants. Life (Basel). 2023;13(2):317.
16. Naskar A, Chatterjee K, Roy K, Majie A, Nair AB, Shinu P, et al. Mechanistic roles of different varieties of honey on wound healing: Recent update. J Pharmacol Pharmacother. 2024;15(1):5-18.
17. Al-Kafaween MA, Alwahsh M, Mohd Hilmi AB, Abulebdah DH. Physicochemical characteristics and bioactive compounds of different types of honey and their biological and therapeutic properties: A comprehensive review. Antibiotics (Basel). 2023;12(2):337.
18. Combarros-Fuertes P, Fresno JM, Estevinho MM, Sousa-Pimenta M, Tornadijo ME, Estevinho LM. Honey: another alternative in the fight against antibiotic-resistant bacteria?. Antibiotics (Basel). 2020;9(11):774.
19. Scepankova H, Combarros-Fuertes P, Fresno JM, Tornadijo ME, Dias MS, Pinto CA, et al. Role of honey in advanced wound care. Molecules. 2021;26(16):4784.
20. Oryan A, Alemzadeh E, Moshiri A. Biological properties and therapeutic activities of honey in wound healing: A narrative review and meta-analysis. J Tissue Viability. 2016;25(2):98-118.
21. Saikaly SK, Khachemoune A. Honey and wound healing: an update. Am J Clin Dermatol. 2017;18(2):237-251.
22. Shukla VK, Srivastava V. Honey debridement. In: Téot L, Meaume S, Akita S, Del Marmol V, Probst S, editors. Skin Necrosis. Switzerland: Springer Nature; 2024.p.399-404.
23. El-Senduny FF, Hegazi NM, Abd Elghani GE, Farag MA. Manuka honey, a unique mono-floral honey. A comprehensive review of its bioactives, metabolism, action mechanisms, and therapeutic merits. Food Biosci. 2021;42:101038.
24. Hegazi NM, Elghani GE, Farag MA. The superfood Manuka honey, a comprehensive review of its analysis and authenticity approaches. J Food Sci Technol. 2022;59(7):2527-2534.
25. Arribas-López E, Zand N, Ojo O, Snowden MJ, Kochhar T. A systematic review of the effect of *Centella asiatica* on wound healing. Int J Environ Res Public Health. 2022;19(6):3266.
26. Cheng CL, Guo JS, Luk J, Koo MW. The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. Life Sci. 2004;74(18):2237-2249.
27. Park JH, Choi JY, Son DJ, Park EK, Song MJ, Hellström M, et al. Anti-inflammatory effect of ti-

trated extract of *Centella asiatica* in phthalic anhydride-induced allergic dermatitis animal model. Int J Mol Sci., 2017;18(4):738.
28. Cho YC, Vuong HL, Ha J, Lee S, Park J, Wibow AE, et al. Inhibition of inflammatory responses by *Centella asiatica via* suppression of IRAK1-TAK1 in mouse macrophages. Am J Chin Med. 2020;48(5):1103-1120.
29. Hafiz ZZ, Amin MA, Johari James RM, Teh LK, Salleh MZ, et al. Inhibitory effects of raw-extract *Centella asiatica* (RECA) on acetylcholinesterase, inflammations, and oxidative stress activities *via in vitro* and *in vivo*. Molecules. 2020;25(4):892.
30. Feng X, Huang D, Lin D, Zhu L, Zhang M, Chen Y, et al. Effects of asiaticoside treatment on the survival of random skin flaps in rats. J Invest Surg. 2021;34(1):107-117.
31. Chonsut P, Romyasamit C, Konyanee A, Niyomtham N, Goodla L, Mordmuang A. Potential activities of *Centella asiatica* leaf extract against pathogenic bacteria-associated biofilms and its anti-inflammatory effects. Adv Pharmacol Pharm Sci. 2024;2024(1):5959077.
32. Firoozbahr M, Kingshott P, Palombo EA, Zaferanloo B. Recent advances in using natural antibacterial additives in bioactive wound dressings. Pharmaceutics. 2023;15(2):644.
33. Singh BN, Nallakumarasamy A, Sinha S, Rastogi A, Mallick SP, Divakar S, et al. Generation of hybrid tissue engineered construct through embedding autologous chondrocyte loaded platelet rich plasma/alginate-based hydrogel in porous scaffold for cartilage regeneration. Int J Biol Macromol. 2022;203:389-405.
34. Mammarella EJ, Rubiolo AC. Crosslinking kinetics of cation-hydrocolloid gels. Chem Eng J. 2003;94(1):73-77.
35. Huang X, Xu L, Yu X, Li Y, Huang Z, Xu R, et al. Near-infrared light-responsive multifunctional hydrogel releasing peptide-functionalized gold nanorods sequentially for diabetic wound healing. J Colloid Interface Sci. 2023;639:369-384.
36. Pereira R, Carvalho A, Vaz DC, Gil MH, Mendes A, Bártolo P. Development of novel alginate-based hydrogel films for wound healing applications. Int J Biol Macromol. 2013;52:221-230.
37. Jaiswal L, Shankar S, Rhim JW. Carrageenan-based functional hydrogel film reinforced with sulfur nanoparticles and grapefruit seed extract for wound healing application. Carbohydr Polym. 2019;224:115191.
38. Namazi H, Rakhshaei R, Hamishehkar H, Kafil HS. Antibiotic loaded carboxymethylcellulose/MCM-41 nanocomposite hydrogel films as potential wound dressing. Int J Biol Macromol. 2016;85:327-334.
39. Liu SJ, Liu ZX, Ju WZ, Chen ZY, Xiong NN, Tan HS. Determination of asiaticoside in rat plasma by LC–MS–MS and its application to pharmacokinetics studies. Chroma. 2010;72:821-825.
40. Ayala JR, Montero G, Coronado MA, García C, Curiel-Alvarez MA, León JA, et al. Characterization of orange peel waste and valorization to obtain reducing sugars. Molecules. 2021;26(5):1348.
41. Masson-Meyers D, Enwemeka CS, Bumah V, Andrade T, Frade MA. Topical treatment with *Copaifera langsdorffii* oleoresin improves wound healing in rats. Int J Phytomed. 2013;5(3):378-386.
42. Zhai Q, Zhou F, Ibrahim MM, Zhao J, Liu X, Wu J, et al. An immune-competent rat split thickness skin graft model: useful tools to develop new therapies to improve skin graft survival. Am J Transl Res. 2018;10(6):1600-1610.
43. Tavakoli S, Klar AS. Advanced hydrogels as wound dressings. Biomolecule*s*, 2020;10(8):1169.
44. Ghorbani M, Ramezani S, Rashidi MR. Fabrication of honey-loaded ethylcellulose/gum tragacanth nanofibers as an effective antibacterial wound dressing. Colloids Surf A Physicochem Eng Asp. 2021;621:126615.
45. Fouda MM, El-Aassar MR, El Fawal GF, Hafez EE, Masry SH, Abdel-Megeed A. k-Carrageenan/poly vinyl pyrrolidone/polyethylene glycol/silver nanoparticles film for biomedical application. Int J Biol Macromol. 2015;74:179-184.
46. Mekonnen T, Mussone P, Khalil H, Bressler D. Progress in bio-based plastics and plasticizing modifications. J Mater Chem A. 2013;1(43):13379-13398.
47. Tang X, Alavi S, Herald TJ. Effects of plasticizers on the structure and properties of starch–clay nanocomposite films. Carbohydr Polym. 2008;74(3):552-558.
48. Islam HB, Susan MA, Imran AB. Effects of plasticizers and clays on the physical, chemical, mechanical, thermal, and morphological properties of potato starch-based nanocomposite films. ACS Omega. 2020;5(28):17543-17552.
49. Fu J, Alee M, Yang M, Liu H, Li Y, Li Z, et al. Synergizing multi-plasticizers for a starch-based edible film. Foods. 2022;11(20):3254.
50. Dong Y, Li Y, Ma Z, Rao Z, Zheng X, Tang K, et al. Effect of polyol plasticizers on properties and microstructure of soluble soybean polysaccharide edible films. Food Packag Shelf Life. 2023;35:101023.
51. Stenner R, Matubayasi N, Shimizu S. Gelation of carrageenan: Effects of sugars and polyols. Food Hydrocoll. 2016;54:284-292.
52. Arno MC, Inam M, Weems AC, Li Z, Binch AL, Platt CI, et al. Exploiting the role of nanoparticle shape in enhancing hydrogel adhesive and mechanical properties. Nat Commun. 2020;11(1):1420.
53. James JT, Dubery IA. Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. Molecules. 2009;14(10):3922-3941.
54. Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK, Dhawan BN. *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. J Ethnopharmacol. 1999;65(1):1-11.
55. Sikareepaisan P, Ruktanonchai U, Supaphol P. Preparation and characterization of asiaticoside-loaded alginate films and their potential for use as effectual wound dressings. Carbohydr Polym. 2011;83(4):1457-1469.
56. Campo VL, Kawano DF, da Silva Jr DB, Carvalho I. Carrageenans: Biological properties, chemical modifications and structural analysis–A review. Carbohydr Polym. 2009;77(2):167-180.

57. Wang Y, Yuan C, Liu Y, Cui B. Fabrication of kappa–carrageenan hydrogels with cinnamon essential oil/hydroxypropyl–β–cyclodextrin composite: Evaluation of physicochemical properties, release kinetics and antimicrobial activity. Int J Biol Macromol. 2021;170:593-601.
58. Postolović K, Ljujić B, Kovačević MM, Đorđević S, Nikolić S, Živanović S, et al. Optimization, characterization, and evaluation of carrageenan/alginate/poloxamer/curcumin hydrogel film as a functional wound dressing material. Mater Today Commun. 2022;31:103528.
59. Shi L, Liu X, Wang W, Jiang L, Wang S. A self-pumping dressing for draining excessive biofluid around wounds. Adv Mater. 2019;31(5):e1804187.
60. Molan PC, Rhodes T. Honey: a biologic wound dressing. Wounds. 2015;27(6):141-151.
61. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci. 2016;73(20):3861-3885.
62. Phillipson M, Kubes P. The healing power of neutrophils. Trends Immunol. 2019;40(7):635-647.
63. Shetty BS, Udupa SL, Udupa AL, Somayaji SN. Effect of *Centella asiatica* L (Umbelliferae) on normal and dexamethasone-suppressed wound healing in Wistar Albino rats. Int J Low Extrem Wounds. 2006;5(3):137-143.
64. Somboonwong J, Kankaisre M, Tantisira B, Tantisira MH. Wound healing activities of different extracts of *Centella asiatica* in incision and burn wound models: an experimental animal study. BMC Complement Altern Med. 2012;12:103.
65. Azis HA, Taher M, Ahmed AS, Sulaiman WM, Susanti D, Chowdhury SR, et al. *In vitro* and *In vivo* wound healing studies of methanolic fraction of *Centella asiatica* extract. S Afr J Bot. 2017;108:163-174.
66. Yao CH, Yeh JY, Chen YS, Li MH, Huang CH. Wound-healing effect of electrospun gelatin nanofibres containing *Centella asiatica* extract in a rat model. J Tissue Eng Regen Med. 2017;11(3):905-915.
67. Rachpirom M, Pichayakorn W, Puttarak P. Preparation, development, and scale-up of standardized pentacyclic triterpenoid-rich extract from *Centella asiatica* (L.) Urb. and study of its wound healing activity. Heliyon. 2023;9(7):e17087.
68. Lu L, Ying K, Wei S, Fang Y, Liu Y, Lin H, et al. Asiaticoside induction for cell-cycle progression, proliferation and collagen synthesis in human dermal fibroblasts. Int J Dermatol. 2004;43(11):801-807.
69. Maquart FX, Chastang F, Simeon A, Birembaut P, Gillery P, Wegrowski Y. Triterpenes from *Centella asiatica* stimulate extracellular matrix accumulation in rat experimental wounds. Eur J Dermatol.1999;9(4):289-296.
70. Diniz LR, Calado LL, Duarte AB, de Sousa DP. *Centella asiatica* and its metabolite asiatic acid: wound healing effects and therapeutic potential. Metabolites. 2023;13(2):276.
71. Liu M, Dai Y, Li Y, Luo Y, Huang F, Gong Z, et al. Madecassoside isolated from Centella asiatica herbs facilitate burn wound healing in mice. Planta Med. 2008;74(8):809-815.