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The Effect of Alginate-Chitosan/Quaternary Chitosan Concentration on The Release of Propolis (*Tetragonula* Spp.) in Hydrogel Wound Dressings

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Abstract: The increasing incidence of acute and chronic wounds globally requires the development of effective wound dressings. This study aims to formulate and analyze the release profile of propolis extract (*Tetragonula* spp.), which has potential biological activity for wound healing, from a hydrogel wound dressing (HWD) based on alginate-chitosan/quaternary chitosan biocomposite as a delivery system that can control its release. Propolis extract, which has been tested positive for alkaloids, flavonoids, tannins, and steroids/triterpenoids, was formulated into four HWD formulas (F1-F4) with varying ratios using the solvent casting method. The results of the release test in PBS medium at pH 7.4 at 37°C for 48 hours showed that all formulas produced a gradual release. The results of the One-Way ANOVA test on the final cumulative release showed no statistically significant differences ($p>0.05$). However, descriptively, Formula F2 (4% alginate-8% quaternary chitosan) showed the highest release percentage (99.85%) with the most stable rate, followed by F4 (98.71%), while F3 with high chitosan showed the lowest percentage (86.65%). The best release kinetics analysis followed the Higuchi model ($R^2 \geq 0.95$), indicating that the release mechanism was controlled by diffusion. It was concluded that variations in the alginate and chitosan/quaternary chitosan ratios affected the release pattern and rate, and Formula F2 with a balanced ratio produced an optimal controlled release profile, making it the most ideal candidate for further development as a therapeutic hydrogel wound dressing.

Keywords: Propolis, hydrogel wound dressing, alginate, chitosan, drug release, *Tetragonula* spp.

INTRODUCTION

The incidence of wounds, both acute and chronic, shows an increasing trend every year. A recent study in the United States reported that the prevalence of patients with wounds reached 3.50 per 1,000 population. Globally, the distribution of wound etiology is dominated by surgical or traumatic wounds (48.00%), followed by leg ulcers (28.00%), and pressure ulcers (21.00%) (Susanti & Putri, 2021). To date, an ideal dressing for wounds has not been developed and applied on a large scale that allows for complete self-healing without constant monitoring and care (Markiewicz-Gospodarek et al., 2022).

Wounds are generally treated using paraffin gauze or silicone gauze dressings, which can be combined with hyaluronic acid to accelerate the healing process (Azizza et al., 2024). However, contact dressings such as gauze that has dried out can stick to the wound bed, causing trauma and pain when the dressing is removed (Hawthorne et al., 2021). There are many types of wound dressings, including gauze, hydrofibers, transparent films, and hydrogels. Hydrogel dressings are preferred because they are comfortable and can reduce pain during the healing process. Hydrogels can absorb and retain water during immersion in aqueous solutions due to the hydrophilicity of the polymer chains in the network structure (Tavakoli & Klar, 2020). Research by Saberian et al. (2024) combined alginate-chitosan to produce a hydrogel dressing that showed increased mechanical stability and bioactivity.

Alginate can absorb large amounts of exudate, which is important for healing, can be applied for up to 7 days, is non-sticky, and does not cause skin irritation (Winter, 2022). The advantages of chitosan include being non-toxic, easily biodegradable. polielectronic, and easily interacting with other organic substances (Mustafiah et al., 2018). However, chitosan has limited solubility in water. Therefore, quaternary chitosan is used. In addition, due to the increase in positive charge. quaternary chitosan plays a better role in antibacterial activity than native chitosan (Phonrachom et al., 2023).

Hyaluronic acid is usually extracted from animal sources or produced through microbial fermentation. However, extraction from animal tissues such as the cornea, umbilical cord, chicken combs, and microbial fermentation faces limitations in the availability of raw materials in the future (Azwar et al., 2024). Therefore, alternative wound healing materials with strong potential and sustainable availability are needed. A bee product with

potential for development is propolis, a resin collected from plants to seal gaps in bee hives (Tirtasari et al., 2024). Propolis has various uses, such as anti-inflammatory, antibacterial, anesthetic, wound healing, antifungal, antioxidant, and anticaries (Ramelan & Kustiawan, 2024). Propolis has clear advantages in improving wound healing and has achieved ideal therapeutic effects (Yang et al., 2022).

Based on previous studies, there has been no research on the effectiveness of HWD based on alginate-quaternary chitosan biocomposites and propolis extract, especially in terms of the release profile of propolis as a wound healing agent. Therefore, this study aims to formulate and analyze the propolis release profile in alginate-quaternary chitosan biocomposite-based HWD for wound healing.

METHODS

Propolis Extraction

Performed using the maceration method. The propolis was cleaned and then reduced in size. The propolis was mixed with 70% ethanol 1:3 (w/v) and then blended until homogeneous. The maceration or precipitation process was carried out for 1 day at a room temperature of 25-28°C. The precipitate was filtered, and remaceration was carried out 4 times. The filtrate is then concentrated using a rotary evaporator at 45°C and a rotation speed of 50 rpm (Kara et al., 2022).

Phytochemical Testing of Propolis Extract

The phytochemical testing procedure for propolis extract (*Tetragonula* spp.) as described in the study by Ananta et al. (2024) is as follows:

1. Alkaloid Identification

   A concentrated extract of 0.5 g was dissolved, then 6 mL. of 2 N HCl was added, and heated in a water bath for 2 minutes. The filtrate was divided into three parts, and Dragendorff, Mayer, and Wagner reagents were added. Positive alkaloid results were indicated by orange (Dragendorff), white (Mayer), and brown (Wagner) precipitates.
2. Flavonoid Identification

   0.2 g of thick extract is dissolved in 4 mL of solvent, 0.2 mg of Mg powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol are added, then shaken. A red, yellow, or orange color in the amyl alcohol layer indicates a positive result for flavonoids.
3. Tannin Identification

   For tannin identification, 0.2 g of extract is dissolved in 4 mL of solvent. The filtrate is added with 10% gelatin to observe precipitation or 5%FC for a dark blue or greenish-black color change, both indicating a positive tannin result.
4. Saponin Identification

   0.2 g of concentrated extract is dissolved in 4 mL of solvent in a test tube. The solution is shaken vertically for 10 seconds, then left to stand for 10 minutes. The formation of stable foam 1-10 cm high indicates saponin. For verification. 2 N HCl is added to the filtrate: if the foam does not collapse, the saponin test is positive.
5. Steroid Triterpenoid Identification

   Dissolve 0.2 g of concentrated extract in 4 mL of solvent, then transfer 2 mL of the filtrate to a test tube. Add 1 mL of chloroform to the tube, then add 1 mL of concentrateda.so.to the tube wall. The formation of two layers, red (chloroform) or yellow (H:SO4), indicates a positive result for sterols/triterpenoids.
6. Identification of Quinones

   Dissolve 0.2 g of concentrated extract in 4 mL of solvent, then filter. Transfer 4 mL of the filtrate to a test tube, then add 6 drops of 0.1 N NaOH. The appearance of a red color in the filtrate indicates a positive result for quinone.

Hydrogel Wound Dressing Formulation

Prepared using the solvent casting method. The concentration of ingredients in each formulation is as follows:

Table 1. Hydrogel Wound Dressing Formula

Ingredient Name	Concentration			
	F1	F2	F3	F4
Chitosan	-	-	4% w/v	8% w/v
Quaternary chitosan	4% w/v	8% w/v	-	-
Alginate	8% w/v	4% w/v	8% w/v	4% w/v
Propolis extract	0.5% w/v	0.5% w/v	0.5% w/v	0.5% w/v
Glutaraldehyde	0.005% v/v	0.005% v/v	0.005% v/v	0.005% v/v

First, dissolve chitosan, quaternary chitosan, and alginate in 30°C distilled water at the concentrations specified in the table for each formula. Then, add propolis (0.5% w/v) to the solution and stir at room temperature for 1 hour. A

0.005% v/v glutaraldehyde solution, acting as a cross-linking agent, is added and the mixture is stirred for 5 minutes. The mixture is poured into Petri dishes and dried in an oven at 35°C for 12 hours.

Propolis Release Test

The release of propolis from HWD (diameter 10.0 mm) was tested using the total immersion method in 20 mL of PBS medium pH 7.4 incubated at 37°C. Prior to testing, the propolis content in HWD was determined by dissolving it in PBS for 6 days and measuring its concentration using a UV-Vis spectrophotometer at a wavelength of 297 nm, which was converted using a calibration curve. At the release stage, solution samples were taken at specific time intervals (10, 20, 30, 45 minutes; 1, 2, 4, 8, 30, and 48 hours). Each time a sample was taken, the immersion medium was immediately replaced with fresh PBS in the same volume to maintain sink conditions. The concentration of propolis released at each time point was then analyzed using the same UV-Vis spectrophotometer.

Data Analysis

Descriptive analysis was performed on the phytochemical test results of propolis extract. One-Way ANOVA statistical analysis was performed on the propolis release test, analyzed using IBM SPSS 27.0 statistical software with a 95% confidence level ($p<0.05$). The results of the analysis will be interpreted based on statistical significance and the interpretation will be based on relevant literature.

RESULT AND DISCUSSION

Results of the Propolis Extract Phytochemical Test

The results of the phytochemical test of propolis extract (Tetragonella spp.) are described in the following table:

Table 2. Hydrogel Wound Dressing Formula

Test	Results	Description
Alkaloids	+	Orange precipitate (Dragendorff) and brown precipitate
Flavonoids	+	Orange
Saponin	-	Foam disappears
Tannin	+	Greenish black
Steroids/Titerpenoids	+	Upper phase yellow, lower phase red
Qounine	-	No red color observed

The presence of alkaloids supports the biological activity potential of propolis, where compounds from this group often have pharmacological effects (Maulira et al., 2025). Flavonoids are the main group of compounds in propolis that are responsible for antioxidant, antibacterial, and anti-inflammatory activities (Bankova et al., 2021). Tannins are known to have astringent and antimicrobial properties, which can aid in the wound healing process by coagulating skin proteins (Ananta et al., 2024). The triterpenoid/steroid compound group contributes to the anti-inflammatory and antibacterial properties of propolis (Ananta et al., 2024). The interaction of steroids and triterpenoids with other components such as flavonoids can produce synergistic effects (Hossain et al., 2022).

The phytochemical profile exhibited by propolis extract (*Tetragonula* spp.) is very promising for the development of health products, particularly in the field of wound care. The combination of flavonoids, tannins, and triterpenoids/steroids forms a strong scientific basis for its antibacterial, anti-inflammatory, and wound healing effects (Hossain et al., 2022).

Results Propolis Release Test Results

The results of the propolis release test from alginate chitosan hydrogel wound dressing preparations can be seen in Figure 1. The test was conducted for 2880 minutes (48 hours) for four different formulas with varying alginate chitosan ratios (F1-F4).

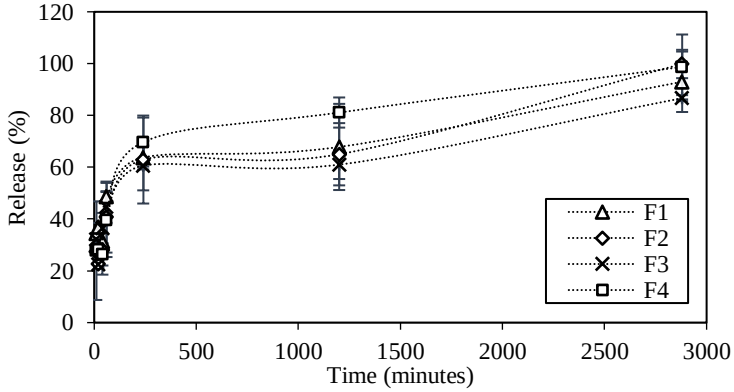

Figure 1. Profile of propolis release from alginate-chitosan hydrogel (F1-F4)

The graph shows that all formulas exhibit a gradual increase in propolis release during the testing period. The

initial phase (0-60 minutes) shows rapid release (burst release), followed by a slow release phase (sustained release) after 240 minutes. The results of the One-Way ANOVA statistical test on the propolis release data showed a significance value (p-value) at 1,200 minutes of 0.147 ($p > 0.05$) or no significant difference in the final cumulative propolis release percentage between the four hydrogel formulas (F1-F4).

The F1 formula with low chitosan concentration showed rapid initial release (34.36% in 10 minutes) due to its looser and more porous gel structure, but leveled off in the final phase (92.99%). In contrast, F3 with high chitosan produced the lowest release (86.65%) due to increased gel density that inhibited diffusion. Formulas F2 and F4, with more balanced ratios, achieved the highest total release (99.85% and 98.71%, respectively) at a stable rate, supported by the formation of an optimal polyelectrolyte complex between the alginate carboxylate group and the chitosan amine group for controlled release (Sato et al., 2020), consistent with the gradual release pattern of propolis in biopolymer systems (Özgen et al., 2025). Thus, the balance of electrostatic interactions () between polymers is key in determining matrix strength and gel permeability.

To understand the mechanism of propolis release from the hydrogel matrix, the release data were tested against drug release kinetics models. Based on linear correlation analysis (R^2 value), the data showed a good fit () with the Higuchi model ($R^2 \geq 0.95$), indicating that propolis release is controlled by diffusion through the hydrogel pores. Previous studies on the release of compounds from polymer films also used the Higuchi model to describe the dominant diffusion mechanism (Riyandari et al., 2018).

However, in the early stages (≤60 minutes), rapid release in F1 and F2 indicates surface release (burst effect), caused by propolis that is not fully incorporated into the polymer matrix and is only adsorbed on the surface. Over a longer period of time, release is controlled by diffusion in a denser gel matrix. The difference in release rates can also be explained based on the physical properties of each formula. Chitosan is cationic, while alginate is anionic. When these two polymers are combined, ionic complexes are formed that affect the porosity, mechanical strength, and swelling capacity of the hydrogel (Sato et al., 2020).

The release profile of propolis is very important in the context of its use as a therapeutic wound dressing. Propolis has been widely recognized in the literature for its antimicrobial, anti-inflammatory, and wound healing activities (El-Sakhawy et al., 2023). Rapid release at the onset is needed to provide immediate antimicrobial effects on open wounds, while slow and sustained release is required to maintain anti-inflammatory activity and tissue regeneration during the healing process (Martinotti & Ranzato, 2015).

CONCLUSION

The release of propolis in each HWD formula did not differ significantly at 1,200 minutes. However, F2, with a total release of nearly 100% and a controlled release rate, was considered the most ideal. This hydrogel is capable of providing a gradual release of propolis over 48 hours, which is suitable for continuous wound care without frequent dressing changes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to this study.

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