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Alginate – Gamat Gel Hydrogel Films as the Potential Wound Dressing

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Abstract

Alginate is a biopolymer that is biodegradable, non-antigenic, non-toxic, and has high biocompatibility. Gamat gels of sea cucumber *Stichopus horrens* are used to treat pain, wounds, and other ailments. Victims suffering from burn wounds frequently complain of pain and discomfort while being bandaged. This study aims to analyze the characteristics of alginate as the base biomaterial for hydrogel films and its interaction with gamat gels bioactive components. Antioxidant activity assay was done to the gamat gels using DPPH assay. Hydrogel films were prepared with different alginate concentrations of 1% w/v, 3% w/v, and 5% w/v. Film degradation was analyzed using stereo microscope. Mechanical characteristics were evaluated based on tensile strength, brittleness, elasticity, adhesion capacity, and the ease when handling the films. Hydrolytic stability was evaluated based on swelling capacity and matrix stability. Antibacterial activity assay was carried out using disk diffusion on *Staphylococcus aureus*. Results show that gamat gels have DPPH inhibition capacity of IC_{50} = 2937.5 ppm. The alginate concentrations treatment resulted in films with different morphology. Films with 5% w/v alginate concentration exhibit a rough surface, the better tensile strength, capability to conform to various surfaces, good adhesion (average detachment time of 60 seconds), swelling capacity of 11,96 ± 13,80%, and matrix stability with the average weight loss of 33,26%. FTIR spectroscopy confirmed that alginate was able to bond with Ca^{2+} ions and the gamat gels. Gamat gels show inhibition zone of ≤ 5 mm. Films with 5% w/v alginate concentration were able to release bioactive compounds.

1. Introduction

A wound is defined as damage to biological tissues such as skin, organs, and mucosal membranes. Based on the types of traumas, wounds can take various shapes and conditions with different levels of severity (Eskridge *et al.*, 2012). Burn wounds are a type of injury caused by thermal (fire, liquid, heat, snow), electricity, radiation, laser, and even chemical exposure, where heat causes an increase in capillary permeability that victims would experience loss of blood plasma and extracellular fluids (Tiwari, 2012). World Health Organization reported that 180 thousand deaths are caused by burn injuries each year and Indonesian Ministry of Health noted that there had been 1,3% of burn wounds from cases of injuries in Indonesia in 2018 (Haryono *et al.*, 2021). Victims suffering from burn wounds frequently complain of pain and discomfort while gauze dressings are applicated and changed, meaning that patients go through a combination of pain from the healing process and further tissue damage (Furness *et al.*, 2019).

Sodium alginate ($Na_6H_7O_6$) is a linear polysaccharide derivative of alginic acid comprised of 1,4-β-d-mannuronic (M) and α-L-guluronic (G) acids (Yudiati and Isnansetyo, 2017). Sodium alginate is naturally produced in brown algae as its cell wall building component. Based on a study done by Kelishomi *et al.* (2016), alginate is a biopolymer that is biodegradable, non-antigenic, non-toxic, and has high biocompatibility. These properties boost the role of alginate in industrial activities, mainly because of its mechanical function and flexible forms. Alginate in commonly used as emulsifier, stabilizer, and viscosity controlling agent (Fernando *et al.*, 2018). Biomedical industry is currently developing alginate's functions as wound dressings, bone tissue grafts, and active substance delivery systems for medications in the form of gels,

capsules, films or membranes, hydrogels, microspheres, nanoparticles, etc. (Kothale *et al.*, 2020).

Gamat gel is another term for extract from sea cucumbers *Stichopus horrens* and *Stichopus hermanni*. Gamat gels have been marketed as supplements with medicinal properties to treat gastric ulcer, arthritis, hypertension, and wounds. Sendih and Gunawan (2006) stated that gamat gels have been consumed orally by Indonesian community for its benefit in treating lupus disease, stroke, hypertension, diabetes, rheumatics, and acnes. Secondary metabolites like saponin, tannin, flavonoid, terpenoid, and steroid in sea cucumbers have been reported to possess antimicrobial, antioxidant, antiproliferation, anticoagulant, immunomodulator, and antithrombosis activities (Sutardi *et al.*, 2022). On the other hand, sea cucumber extract also holds the opposite functions of inflammation response activation, triggering cell migration, proliferation, and cell angiogenesis (Sari *et al.*, 2023). These traits hold a potential for gamat gels to be used in wound treatment and healing.

Alginate based wound dressing has an immunostimulant effect that promotes wound healing and upholds blood coagulation. Wang *et al.* (2018) and Hidayati *et al.* (2021) previously explained how in wound treatment, alginate acts as a wound exudate absorbent, macrophages inducer that increases pro-inflammatory cytokines production, as well as to aggregate platelets, fibrinogen, and erythrocytes to the wound surface for wound closing. Nuutila and Eriksson (2021) previously stated that alginate base treatment increases healing quality by maintaining moisture at the wound site, undertaking autolytic debridement, decreasing pain, activating collagen synthesis, and being a source of nutrient for the wound. Based on a study by Ridzwan *et al.* (2003), sea cucumber extracts hold potentials for alternative analgesics to handle pain or thermal injuries. Extract from *Stichopus* sp. previously reported of having antibacterial potential, as well as significant effects in wound healing process (Adam *et al.*, 2022). Alginate and gamat gels may be able to show synergic and favorable functions in each phase of healing process and offer help in treatment for their capability in lessening pain from burn wounds. The aforementioned properties meet the required standards of alternative wound dressings, that an optimal dressing should be able to protect wounds from infections, have high biocompatibility, keep a moist wound environment, and accelerate healing (Schiefer *et al.*, 2022).

Based on a study conducted by Mutia *et al.* (2011), alginate wound dressing with low alginate concentration forms a thin membrane that loses its shape after contact with wet surface. It was stated that by using a higher concentration of 3% instead, with 10% $CaCl_2$ for cross-linking, the membranes came out rigid. Lower alginate concentration was then used for further assays, mixed with common antibiotics, showed that the membranes were able to inhibit bacterial growth. Its results affirm alginate's potential as a drug delivery system. However, said study still offers an opportunity for further development of alginate films. This current research focuses on the influence of alginate concentration in films on their characteristics. Exploration of the film degradation process is done to determine its implication on the characteristics of the film dressings. This study aims to determine the morphological, mechanical, and chemical characteristics of hydrogel films made of alginate and gamat gels, as well as the antioxidant and antibacterial activity of gamat gels and gamat gels encapsulated in alginate hydrogel films.

2. Material and methods

2.1. Materials

Hydrogel films were made using food-grade sodium alginate (Qingdao Bright Moon Seaweed Group Co., Ltd.) and Gold-G Bio Sea Cucumber gamat gel from *Stichopus horrens* (*S. variegatus*) acquired from PT. GnΣ HMP Indonesia.

2.2. Methods

Methods used were laboratory experiments and completely randomized design (CRD) in fabricating the hydrogel films, with three treatments and replicated three times. Descriptive analysis was used in degradation analysis, mechanical properties, hydrolytic stability, FTIR analysis, and antibacterial activity assay. DPPH method was used for antioxidant activity assay. ANOVA was used to analyse the films hydrolytic stability.

2.3. Preliminary DPPH (1 1-diphenyl-2-picrylhydrazyl) assay of gamat gel

Gamat gel was tested in different set of concentrations: 1000, 2000, 3000, 4000, 5000, and 6000 ppm. Ascorbic acid was used as antioxidant standard, with concentration values of 4, 12, 20, 28, and 36 ppm (Kumara *et al.*, 2018). All three used distilled water as solvent. The DPPH 0,1 mM solution was prepared in methanol. The procedure followed a previous study by Anis *et al.* (2022) with modifications. Total reaction mixture (RM) was 5 mL with 9:1 ratio between DPPH solution and sample. For blanks, water was added instead of samples. All RMs were then incubated for 45 – 60 minutes. Absorbance was read at 517 nm by Shimadzu UV-Vis Spec. UV-1200. Correction factor was prepared and read beforehand, mixtures made with 9:1 ratio of methanol and each sample (Banerjee *et al.*, 2005). Inhibition percentage formula was used as follows (Sedjati *et al.*, 2017):

$$\% \text{ In} = \frac{A_{blank} - A_{sample}}{A_{blanko}} \times 100$$

A blank = absorbance value methanolic DPPH solution
A sample = absorbance value of RM (- correction factor)

2.4. Hydrogel films preparation

Preparation method refers to Pandima Devi *et al.* (2012) and Fahmy *et al.* (2021) with modifications. Sodium alginate aqueous solutions of 1% w/v, 3% w/v, and 5% w/v concentrations were prepared separately. Each solution was mixed with a stirrer on 70 °C at 300 – 600 rpm for 30 – 60 minutes until it homogenized. Gamat gel was added 0,3 mL into the mixture after the heat was at 0 °C. 1,5 mL glycerol was added after. Each of the hydrogel solution was casted, 25 mL into petri dishes of 9 cm diameter, then was let cool at room temperature (25 °C) for 1 hour, then moved into the refridgerator (4 °C) for 3 – 4 days. 2% CaCl2 aqueous solution was prepared for crosslinking process using the immersion method for 3 – 5 minutes (Azeredo *et al.*, 2012) (Azeredo *et al.*, 2012; Josh dan Reinelt, 2017). Hardened hydrogels that had formed films were then loaded off from the petri dishes and crosslinked with CaCl2, rinsed with distilled water, before being placed in new petri dishes. Crosslinked hydrogel films were let dry in a desiccator for 24 hours.

Table 1. Alginate Concentration Treatment in Hydrogel Film Formulation

Treatment	Gamat Gel (mL)	Glycerol (mL)	Distilled Water (mL)	$CaCl_2$ (g)
Alg 1 g	0.3	1.5	100	2.0
Alg 3 g	0.3	1.5	100	2.0
Alg 5 g	0.3	1.5	100	2.0

2.5. Degradation analysis

Degradation analysis was done to determine the degradation process of hydrogel films and the storing efforts that could be done to prolong the films shelf life. The degradation process of hydrogel films was analysed visually by Olympus LS Stereo Microscope SZ2-ILST. Thermal based test was used to compare the degradation process in different thermal conditions (Pagano *et al.*, 2021; Salama *et al.*, 2018). The analysis was focused on film deformity, for that reason only visual analysis was used (Doh *et al.*, 2020). Hydrogel films were made into 2 groups. First group were kept in Condition A where the films were exposed to changes of temperature from the open air and sunlight. Second group were kept in Condition B, on sterile LAF table under UV light, in covered petri dishes, with room temperature 25 °C. Observations were made 1x24 hours for 7 days. Results were to be expressed as whether or not an occurrence happened that indicates degradation, meaning signs of drying, change of consistency, film shrinkage, change of texture, and other changes seen on the film surface.

2.6. Mechanical properties

Evaluation of the films mechanical properties was done using parameters referring to Pagano *et al.* (2021) with modifications. The parameters include tensile strength, brittleness, elasticity, adhesion capacity, and easiness while handling the films. This was done manually and evaluated with descriptive methods. Tensile strength evaluation followed the working principle of previous study, with modifications so it could be done manually (Peh *et al.*, 2000). Films were made into 2 groups. First group were tested 24 hours after crosslinking process, second group was tested 7 days after. Films were pulled apart multiple times until they showed signs of breakage. Results were to be expressed in the amounts of successful pulls before the films break. Brittleness was evaluated based on the tensile strength result using categorization. Films that broke after 1 – 4 pulls were categorized as weak, 5 – 8 pulls as moderate, and ≥ 9 pulls as strong. Evaluation on elasticity referred to Zhou *et al.* (2022), in which films were tested directly onto volunteers. Films were applicated onto the arm with even, curved, and sharp surfaces, as well as placed in between surfaces. Adhesion capacity assay was done by referring to Chen *et al.* (2022), by expressing adhesion with time. Films were applicated on a surface facing downward and timed for 60 seconds. Time required for the film to detach was observed. Adhesion capacity was then expressed into 3 categories: 1- 29 seconds of detachment time was considered low adhesion, 30 – 59 seconds was considered moderate adhesion, and 60 seconds was to be considered high adhesion. Application difficulty of the films were evaluated based on Peng *et al.* (2022), which stated that wound dressings need to be able to be handled with ease. Score of difficulty were expressed as Easy, Medium, and Difficult.

2.7. Hydrolytic stability

The tests include swelling capacity and matrix stability to determine the films water holding capacity. Swelling capacity was evaluated through swelling test. Each crosslinked hydrogel film was weighted (W1), immersed in distilled water for 1 hour. After immersion, films were wiped with filter paper to rid of excess water, then weighted (W2). After hydration, films were dried at 40 °C for 5 days, reweighted on each day (W3). Swelling test result was expressed as hydration percentage, calculated with the following (Pagano *et al.*, 2021):

$$\% \text{ Hyd} = \frac{W_2 - W_1}{W_2} \times 100$$

While matrix stability was expressed as cumulative weight loss (CWL), and was calculated as following (Drápalová *et al*., 2023):

$$CWL_i\ (\%) = \frac{W_2 - W_3}{W_2} \times 100$$

2.8. FTIR analysis

FTIR analysis was done on sodium alginate solution, gamat gel, and crosslinked alginate-gamat gel film. The analysis was done to characterize the chemical component of each test subject, and to confirm that the crosslinking occurred. FTIR spectra was read using Perkin-Elmer Spectrum, at 4000 – 600 cm^{-1} wavelength, 4 cm^{-1} resolution, 4 times (Li *et al.*, 2016).

2.9. Antibacterial activity assay

Gamat gel and sodium alginate was first tested for saponin content through foam test (Marliana *et al.*, 2005). 0,5 mL of gamat gel and 0,5 g alginate separately was solved in distilled water, shaken for 30 seconds to create stable foam as saponin indicator. Antibacterial assay was then done to gamat gel in different concentrations of 0.3% v/v, 1% v/v, 3% v/v, 5% v/v, and 7% v/v solved in distilled water, as well as the hydrogel film that showed the preferable characteristics from the previous tests. *Staphylococcus aureus* was chosen as the bacteria for the test, as it is one of the causes of wound infections (Alzarea *et al.*, 2022). *S. aureus* was inoculated onto Mueller-Hinton agar plates with 1.5×10^8 CFU/mL turbidity. Test technique referred to Saberian *et al.* (2021) with modifications. Test was carried out using disk diffusion. Paper disks of 8 mm diameter was used for gamat gel immersion, and the hydrogel film was cut with 8 mm blue tip. Gentamicin cream was used as positive control. Incubation period was 24 hours.

2.10. Data analysis

One-Way ANOVA was used to analyze data from hydrolytic stability tests. Descriptive analysis was used for the rest of the results.

3. Results

3.1. Gamat gel DPPH inhibition

Figure 1 shows the increasing DPPH inhibition with increased gamat gel concentration. IC_{50} of gamat gel was obtained to be 2937.5 ppm. Statistical analysis showed P-value <0.05. Ascorbic acid showed to have the IC_{50} value of 20.7 ppm.

3.2. Unloaded hydrogel film

Figure 2 (a) shows that Alg 1% films have smooth and slippery surface, palpable thickness, and the consistency of a jello. Figure 2 (b) shows that Alg 3% films have rough-looking bumpy texture of which didn't spread evenly throughout the surface and creates empty looking patches. Figure 2 (c) shows that Alg 5% films have the same texture but evenly spread and are a tad thicker.

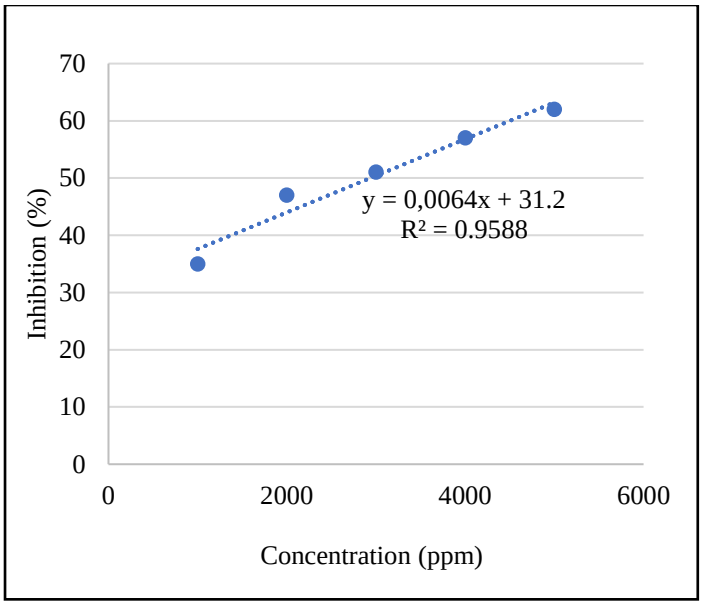


Figure 1. DPPH Inhibition Capacity of Gamat Gel

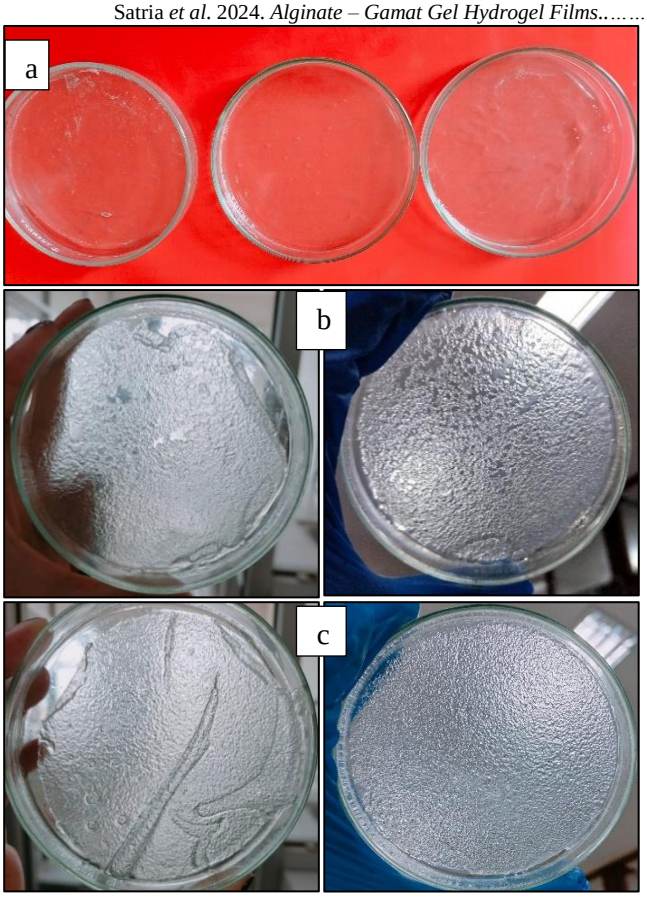
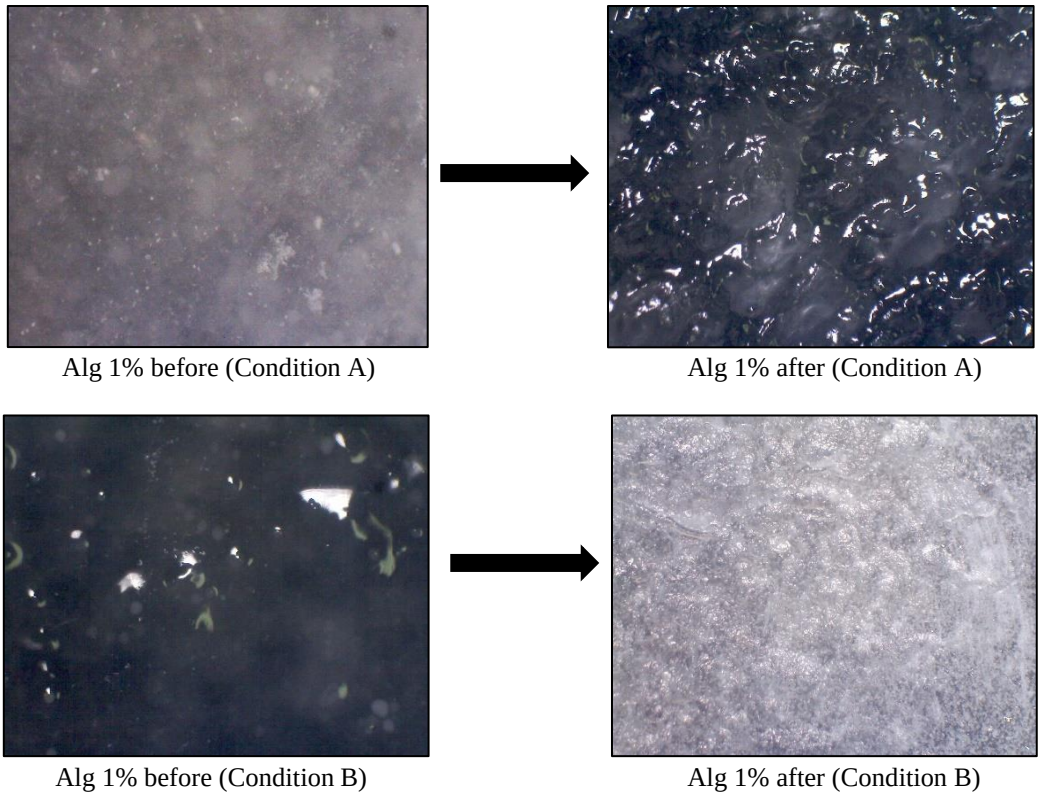


Figure 2. Alginat – Gamat Gel hydrogel film with, (a) 1% w/v, (b) 3% w/v, dan (c) 5% w/v alginate concentrations.

3.3. Hydrogel films degradation

Figure 3 and Table 2 show the degradation process of Alg 1% film. Alg 1% under condition A underwent a change in consistency after 6 days of observation, and after 7 days in condition B. Change of consistency simultaneously happened with drying and shrinkage of the film. Alg 1% in condition A went from consistency of jello to mush. Meanwhile, under condition B, the film shrank and became thin to the point of encrustation to the petri dish.

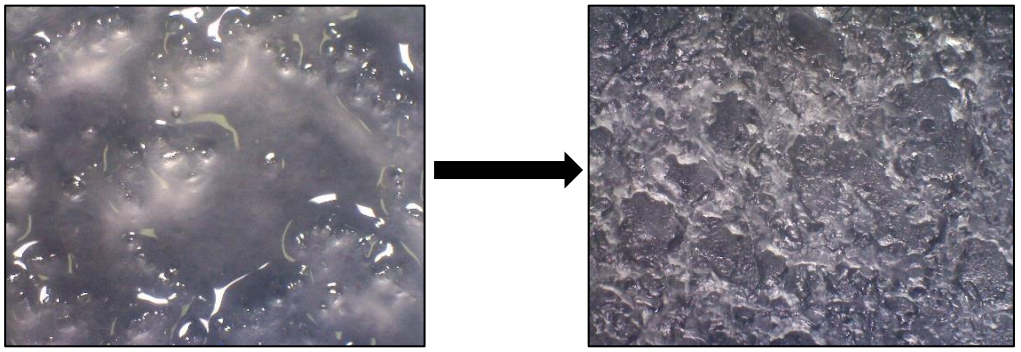


Alg 1% before (Condition A) Alg 1% after (Condition A)

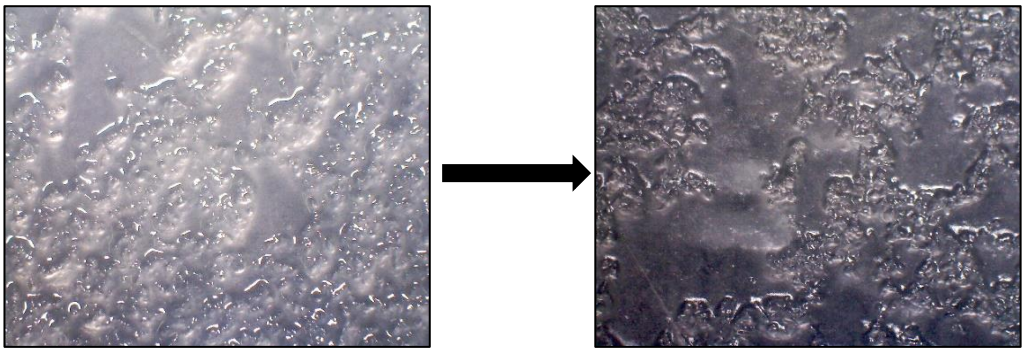
Alg 1% before (Condition B) Alg 1% after (Condition B)

Figure 3. Microscopic changes in hydrogel film of 1% w/v alginate concentration under Condition A and Condition B after 7 days of exposure

Figure 4 and Table 3 show that Alg 3% film under condition A underwent a drastic deformation, the bumpy texture and patches changed because the film dried, the surface came to have holes like it was peeling off itself. Under condition B, the film underwent dryness and shrinkage, and the patches broadened.



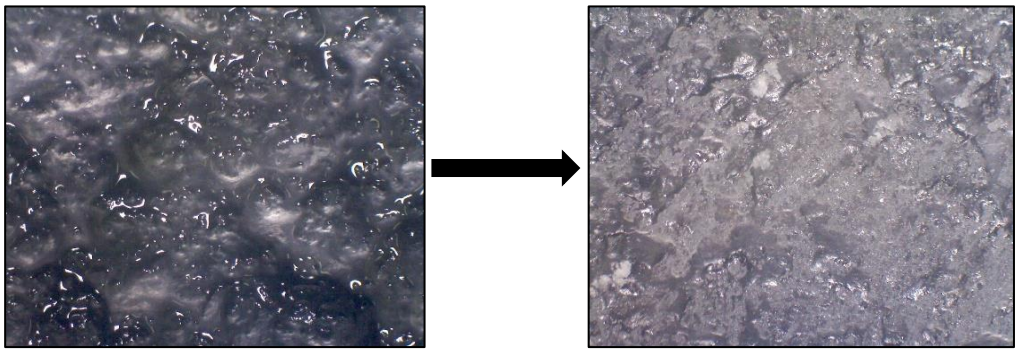
Alg 3% before (Condition A) Alg 3% after (Condition A)



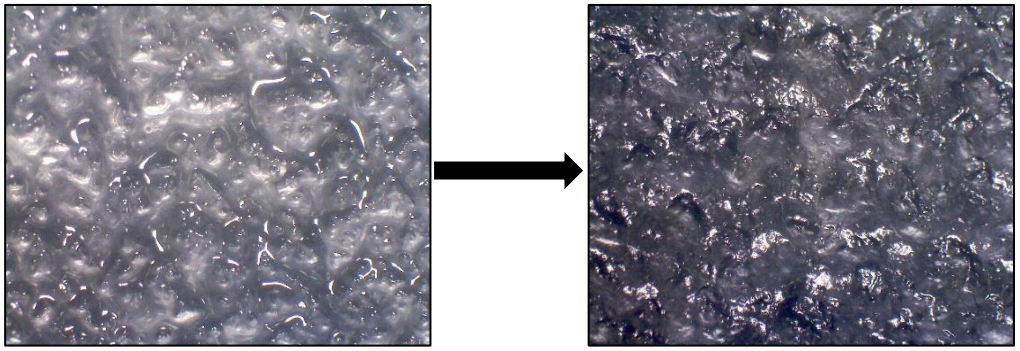
Alg 3% before (Condition B) Alg 3% after (Condition B)

Figure 4. Microscopic changes in hydrogel film of 3% w/v alginate concentration under condition a and condition b after 7 days of exposure.

Figure 5 and Table 4 show that Alg 5% hydrogel film underwent obvious dryness and shrinkage, indicated from the texture deflation. Under condition B, the changes weren't so obvious. Alg 5% degraded much faster under condition A, while it only showed clear degradation when entering day 7 of observation.



Alg 5% before (Condition A) Alg 5% after (Condition A)



Alg 5% before (Condition B) Alg 5% after (Condition B)

Figure 5. Microscopic changes in hydrogel film of 5% w/v alginate concentration under condition a and condition b after 7 days of exposure

Table 2. Degradation process of hydrogel film of 1% w/v alginate concentration in different conditions in 7 days of exposure

	Texture change		Shrinkage		Consistency change		Broadening of patches		Drying	
Day	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B
1-2	+	+								
3-4	+	+	+	+						+
5-6	+	-	+	-	+	-			+	-
7	+	+	+	+	+	+			+	+

* **Condition A**: exposure to open air and temperature changes, and sunlight. **Condition B**: covered petri dish, cool room temperature (25 °C), and exposure to UV light in a dark room. **Symbol (+)** indicates that an occurrence happened and/or happened again. **Symbol (-)** indicates that an occurrence hadn't happened again yet. **Empty cell** indicates that an occurrence did not happen.

Table 3. Degradation process of hydrogel film of 3% w/v alginate concentration in different conditions in 7 days of exposure

	Texture change		Shrinkage		Consistency change		Broadening of patches		Drying	
Day	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B
1-2	+									
3-4	+		+				+		+	
5-6	+	+	+	+			+	+	+	+
7	+	+	+	+			+	+	+	+

* **Condition A**: exposure to open air and temperature changes, and sunlight. **Condition B**: covered petri dish, cool room temperature (25 °C), and exposure to UV light in a dark room. **Symbol (+)** indicates that an occurrence happened and/or happened again. **Symbol (-)** indicates that an occurrence hadn't happened again yet. **Empty cell** indicates that an occurrence did not happen.

Table 4. Degradation process of hydrogel film of 5% w/v alginate concentration in different conditions in 7 days of exposure

	Texture change		Shrinkage		Consistency change		Broadening of patches		Drying	
Day	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B	Condition A	Condition B
1-2										
3-4	+		+						+	
5-6	+	+	-						+	+
7	+	-	+	+					+	+

* **Condition A**: exposure to open air and temperature changes, and sunlight. **Condition B**: covered petri dish, cool room temperature (25 °C), and exposure to UV light in a dark room. **Symbol (+)** indicates that an occurrence happened and/or happened again. **Symbol (-)** indicates that an occurrence hadn't happened again yet. **Empty cell** indicates that an occurrence did not happen.

3.4. Mechanical properties

Table 5 shows that Alg 5% films had the better tensile strength (9 – 10 pulls before breakage) and is not brittle, even after 7 days.

Figure 6 shows what type of surface the hydrogel films can conform to. Alg 1% could not be applicated onto joints as it was weak to pressure. Alg 3% and Alg 5% conformed to the sharpness of a joint, but the stiffer film structures caused them to form creases. All films were able to conform to curved surfaces.

Table 6 shows the adhesion capacity expressed by the detachment time of the films. Alg 5% film had the better adhesion capacity, reaching the limit of 60 seconds of adhesion time.

Table 7 shows the level of difficulty of the films while handling them, applicating them on and detaching them off the skin surface. Alg 5% film was the easiest to handle.

3.5. Hydrolytic Stability

Table 8 shows that Alg 5% has the highest swelling capacity of 11.96 ± 1.98% from its actual weight. P-value was <0.05, meaning that alginate concentration has a significant influence on the films swelling capacity.

Figure 7 shows that Alg 3% film underwent a cumulative weight loss reaching ≥50% of its hydration weight after 5 days of drying, much faster than the other films. Alg 1% and Alg 5% had the better matrix stability, each undergoing average weight loss of 31.88% and 33.36%. The obtained p-value and R^2 was <0.0001 and 0.9420 respectively.

Table 5. Tensile strength and brittleness of hydrogel films of different alginate concentration

Name	Day 1		Day 7	
	PBB	Brittleness	PBB	Brittleness
Alg 1%	6 – 8	Moderate	1	Weak
Alg 3%	7 – 9	Moderate to strong	4 – 5	Moderate to weak
Alg 5%	9 – 10	Strong	5 – 7	Moderate

*__PBB__: the amount of Pull Before Breakage.

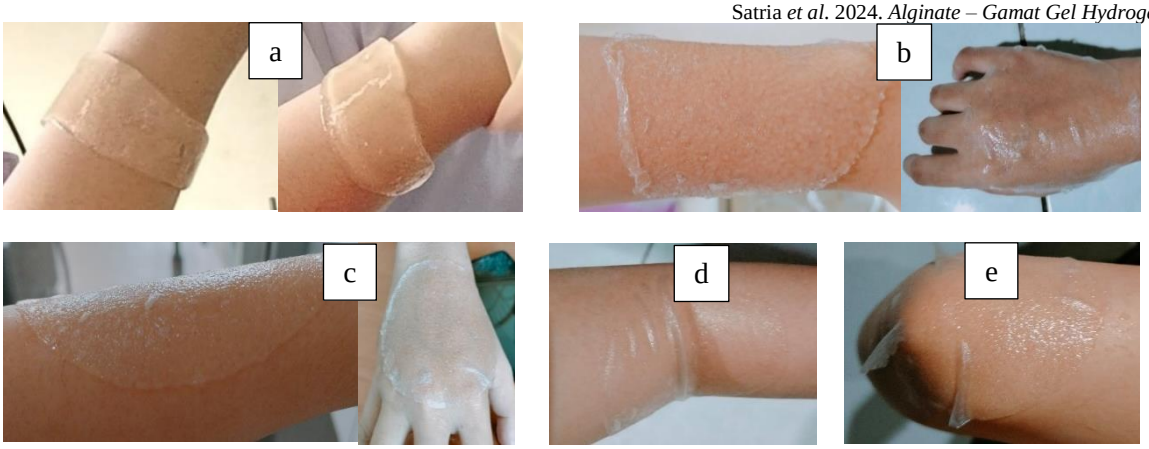


Figure 6. General elasticity of hydrogel films of (a) 1% w/v, (b) 3% w/v, (c) 5% w/v alginate concentration, (d) on the crook of elbow, and (e) on elbow

Table 6. Adhesion capacity of hydrogel films of different alginate concentrations

Name	Detachment time (second)	Adhesion capacity
Alg 1%	25 – 33	Moderate to low
Alg 3%	57 – 60	Moderate to high
Alg 5%	60	High

Table 7. Application difficulty level of hydrogel films of different concentrations

Film	Category
Alg 1%	Tend to be difficult
Alg 3%	Tend to be easy
Alg 5%	Easy

Table 8. Swelling capacity of hydrogel films of different alginate concentrations

Name	Hydration Percentage (%)	*P-value*	F Ratio
Alg 1%	6.01 ± 0.49		
Alg 3%	6.46 ± 1.27	0.04	*F > F crit*
Alg 5%	11.96 ± 1.98		

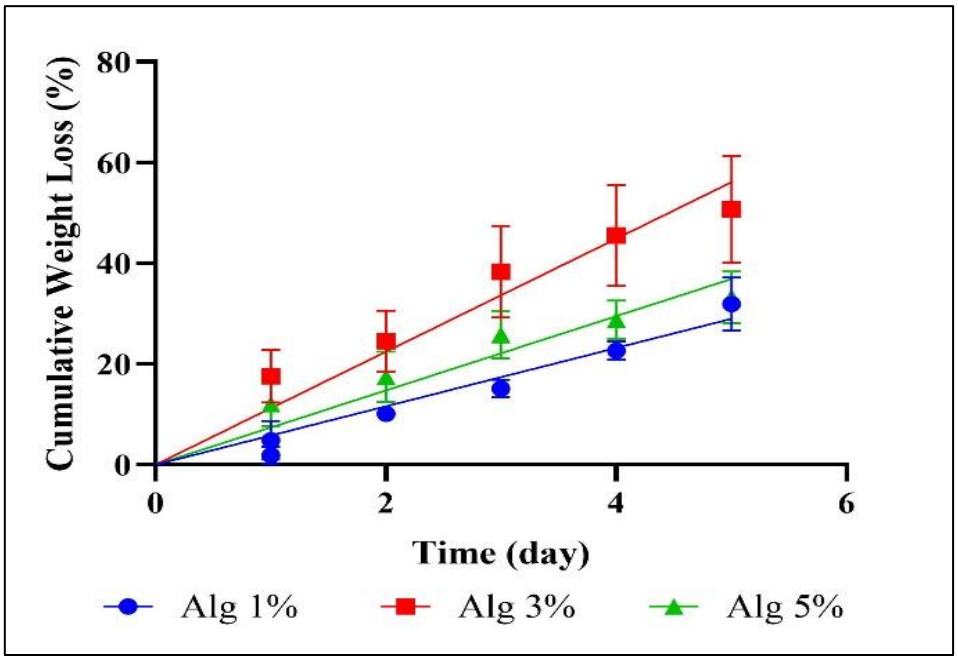


Figure 7. Matrix stability of hydrogel films of different concentrations (P-value <0.05)

3.6. FTIR analysis

Figure 8 shows the spectra of gamat gel (in black), 5% w/v alginate solution pre-crosslink without gamat gel (in red), and Alg 5% film (in blue). Gamat gel spectra shows peaks at 3398, 1646, 1366, 1271, dan 705 cm^{-1} wave numbers. Pre-crosslink alginate spectra shows peaks at 3399, 1646, dan 1388 cm^{-1}. Alg 5% film spectra shows peaks at 3399, 1647, 1413, dan 1027 cm^{-1}.

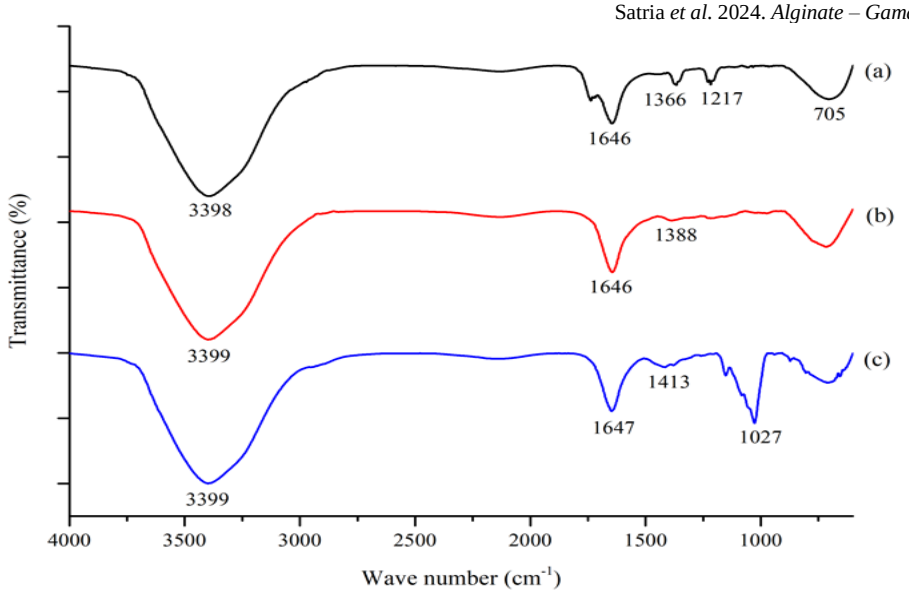


Figure 8. FTIR Spectra of (a) gamat gel, (b) 5% w/v sodium alginate solution pre-crosslinking, and (c) hydrogel film of 5% w/v alginate concentration

3.7. Antibacterial activity

Figure 9 shows the results of foam tests on gamat gel and sodium alginate. Gamat gel managed to form stable foam, while sodium alginate did not, indicating saponin content in gamat gel.

Table 9 and Figure 10 show the positive antibacterial activity of gamat gel and Alg 5% film. Gamat gel in all concentrations, as well as Alg 5%, managed to create inhibition zones against *S. aureus*. Positive control gentamicin inhibited S. aureus by 7,4 – 9,0 mm inhibition zones.

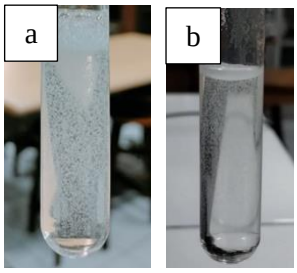


Figure 9. Saponin content in (a) gamat gel and (b) sodium alginate

Table 9. Antibacterial activity of gamat gel and hydrogel film against *Staphylococcus aureus.*

Name	Inhibition Zone (mm)	
	1	2
Alg 5%	≤ 5.0	≤ 5
0,3% v/v Gamat gel	≤ 5	≤ 5
1% v/v Gamat gel	≤ 5	≤ 5
3% v/v Gamat gel	≤ 5	≤ 5
5% v/v Gamat gel	≤ 5	≤ 5
7% v/v Gamat gel	≤ 5	≤ 5
Gentamicin	7,4	9,0

*Data shown had already taken 8 mm diameter of the paper disk into account.

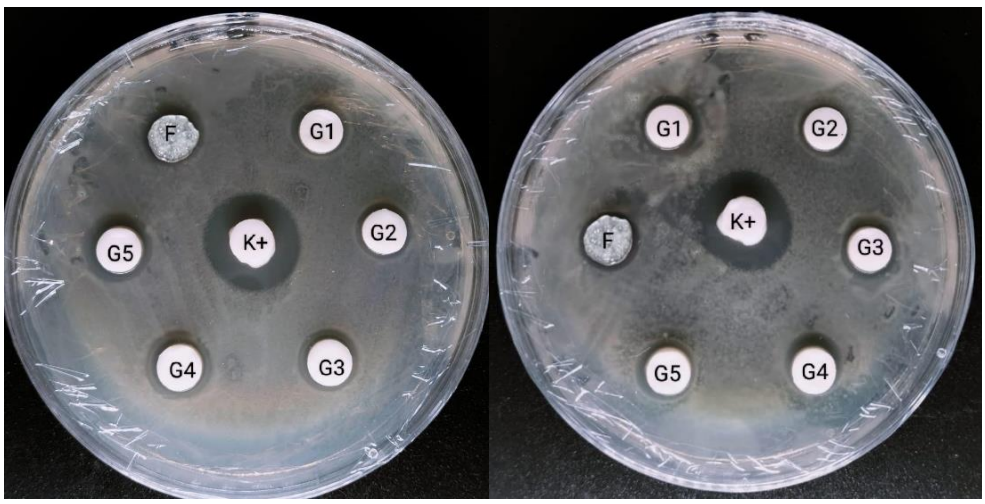


Figure 10. Antibacterial activity of gamat gel and hydrogel film of 5% w/v alginate concentration (K+: gentamicin, F: hydrogel film, G1: 0,3% v/v gamat gel, G2: 1% v/v gamat gel, G3: 3% v/v gamat gel, G4: 5% v/v gamat gel, G5: 7% v/v gamat gel)

4. Discussion

4.1. Antioxidant activity

The gamat gel from Stichopus horrens sea cucumber, produced by PT GnE HMP Indonesia and containing 25% sea cucumber extract, demonstrated very low antioxidant activity with an IC50 value of 2937.5 ppm, equivalent to 20.7 ppm of ascorbic acid. This is better than a previous study of sea cucumber collagen serum, which showed an IC_{50} value of 4045.37 ppm (Stiani *et al.*, 2021). The antioxidant activity in sea cucumbers is influenced by components such as peptides and certain salt compounds, although polyphenols also contribute significantly (Zhong *et al.*, 2007). However, this does not rule out the fact that polyphenols are common antioxidant compounds, and their high content in sea cucumber may uphold its antioxidant function.

Antioxidant assay results can be affected by the concentration of free radicals used. Techniques such as high-pressure processing (HPP) can increase phenolic content without damaging mucopolysaccharides, though they might affect collagen (Fan *et al.*, 2022; Hossain *et al.*, 2022). This study didn't analyze the molecular weight or phenol content, crucial factors in antioxidant capacity. Antioxidants are essential in wound healing, stabilizing ROS levels and reducing oxidative stress. Despite the low antioxidant capacity of gamat gel, further studies on its functional components like collagen and mucopolysaccharides are necessary to enhance its efficacy in burn treatment (Comino-Sanz *et al.*, 2021).

4.2. Hydrogel film

The Alg 1% film had a gelatinous, transparent, jello-like form with a smooth and flat surface, differing from Pereira *et al.* (2013), who produced a thick, dry film with 15% w/w glycerol of alginate weight compared to this study's 1.5% w/v glycerol of distilled water volume. Its morphology resembled Wang et al. (2019), who made 1 mm thick, transparent hydrogels with higher tensile strength due to 6% w/v glycerol. Sericin-based hydrogel films from silk moth caterpillars also had similar appearances (Teramoto et al., 2008). The Alg 3% and Alg 5% films were thinner, sturdier, and had pebbled textures. Alg 3% showed patchiness due to uneven alginate distribution, with no similar previous studies. Alg 5% resembled Duckworth et al. (2020), showing rough textures caused by sodium alginate's self-aggregation (Badita et al., 2020). The cold temperature during film hardening likely caused the pebbled texture, as noted by Bagheri et al. (2019). Rough surfaces in wound dressings enhance cell and protein adhesion, promoting healing (Wang et al., 2019).

4.3. Degradation process

The Alg 1% film degraded completely within seven days, transitioning from a jello-like consistency to a mushy state due to exposure to air, temperature, and sunlight. This degradation process is consistent with findings by Liu *et al.* (2016), where thermal degradation promoted water evaporation from alginate hydrogel films. The change in consistency is likely due to the exchange of Ca^{2+} ions with residual Na^{+} ions. Despite attempts to extend its shelf life, the Alg 1% film did not last longer even when stored in controlled conditions, likely due to factors like sublimation and protein coagulation. In contrast, Alg 3% and Alg 5% films showed minimal changes, suggesting that alginate concentration significantly influences degradation. Efforts to minimize exposure to air and heat extended their shelf life, with Alg 3% and Alg 5% films showing signs of depolymerization and fragmentation slower than Alg 1%, aligning with studies by Doh *et al.* (2020) and Pagano *et al.* (2021).

Mechanically, Alg 1% had moderate tensile strength but was brittle and poorly adhesive, making it unsuitable as a wound dressing. Alg 3% initially had good adhesion and tensile strength, which decreased over time due to uneven molecular aggregation and drying, affecting glycerol's plasticizing effect. Alg 5%, with high tensile strength and elasticity, maintained good adhesion and ease of removal, showing potential as an alternative wound dressing. The semi-moist state of Alg 5% provided better adhesion and ease of removal, essential for treating wounds requiring dead tissue debridement (Najm and Hussein, 2018). Differences in film properties are attributed to alginate concentration, highlighting its importance in achieving desired film characteristics, as supported by studies from Comaposada *et al.* (2015) and Kundu *et al.* (2015).

4.4. Mechanical properties

The ideal dressing should not crumble during handling, conform to the wound's shape, and be easy to remove without aggravating the wound. Tensile strength, the stress that keeps a plane from splitting when stretched (Ascenzi and Bonucci, 1964), and a balance between tensile strength and elasticity are crucial. High film moisture increases elasticity but decreases tensile strength, and vice versa (Eslami *et al.*, 2023). The dressing must also resist external forces during application and removal. The Alg 1% film, despite its moderate tensile strength and good elasticity, was too brittle and had low adhesion, making it unsuitable as a wound dressing. Its heavy weight and slippery surface contributed to its difficulty in handling.

The Alg 3% film initially had good adhesion and high tensile strength, but these properties decreased over time, making the film brittle. This brittleness resulted from uneven molecular aggregation due to low alginate concentration and glycerol leaching out, reducing the film's flexibility (Paixão *et al.*, 2019). In contrast, Alg 5% maintained a combination of high tensile strength and elasticity. Its adhesion capacity and ease of removal were due to the semi-moist film, which prevented significant drying. Alg 5% demonstrated potential as an alternative wound dressing, especially for debridement, due to its good mechanical characteristics. The concentration of sodium alginate is crucial for mechanical resistance, as supported by studies (Comaposada *et al.*, 2015; Kundu *et al.*, 2015).

4.5. Hydrolytic stability

Wound dressings are ideal if they can absorb exudate and maintain a moist environment. Pagano *et al.* (2021) demonstrated that alginate films could absorb exudate exceeding 50% of their initial weight. The Alg 5% film exhibited the highest absorption with a hydration percentage of 11.96 ± 1.98%. The discrepancy in results is likely due to different testing methods; this study used a semi-wet film in the swelling test without drying it first, as dry films are deemed less suitable for further use. Alg 1% film, despite its high water content, failed as an effective wound dressing due to its inability to absorb much water and its heavy weight, leading to poor adhesion. The low alginate concentration in Alg 1% resulted in unstable matrix and rapid transformation to mush, indicating low matrix stability and easy disintegration, which is unsuitable for wound dressings (Obagi *et al.*, 2019).

Alg 3% also lacked sufficient matrix stability to serve as a viable wound dressing, as it couldn't provide consistent moisture and allowed absorbed exudate to return to the wound surface. Conversely, Alg 5% displayed better absorbency and matrix stability due to the higher alginate concentration, which locked in water content more

effectively. Sodium alginate's hydrophilic nature forms hydrogen bonds with water molecules, enhancing stability (Hou and Wu, 2019). However, alginate hydrogels have limited long-term stability since the water-alginate bond is reversible and susceptible to environmental and physiological factors. The ionic crosslinking process, particularly with Ca^{2+}, aims to stabilize the alginate gel, but Alg 1% still showed limitations as it could disintegrate during ion exchanges in the environment (Lee and Mooney, 2012). While the release of Ca^{2+} ions can aid haemostasis in wounds (Subramaniam *et al.*, 2021), it is a disadvantage for Alg 1% if it alters film consistency.

4.6. FTIR analysis

Gamat gel from Stichopus horrens contains 25% sea cucumber extract along with active components such as amino acids, collagen, saponins, mucopolysaccharides, fucoidan, glucosamine, and chondroitin. Noteworthy transmission peaks are at wave numbers 3398, 1646, 1366, 1217, and 705 cm^{-1}, indicating the presence of collagen amide groups. These peaks correspond to N-H bond stretching, C=O bond stretching, N-H bending, C-H stretching, and C-O-S stretching, respectively, demonstrating successful collagen extraction and preservation (Hidayati *et al.*, 2021; Zhang *et al.*, 2009). Additionally, the 1535-1334 cm^{-1} range identifies amide II groups, confirming fucoidan and glucosamine content. This spectrum aligns with FTIR analysis of other sea cucumber collagens, indicating the presence of functional groups like OH alcohol, C-H alkane, C=C alkene, and various amide, ester, carboxyl, and ketone groups (Song *et al.*, 2018; Zhu *et al.*, 2012).

The crosslinking stage between sodium alginate and $CaCl_2$ is crucial for forming the hydrogel's physical structure. Calcium chloride acts as a coagulant, strengthening the sodium alginate gel by binding with the carboxyl groups in the alginate, creating a stable "egg box" structure that can absorb exudates (Makarova *et al.*, 2023). Pre-crosslinked sodium alginate without gamat gel shows peaks at 3399, 1646, and 1388 cm^{-1}, corresponding to OH, asymmetric COO^-, and symmetric COO^- stretching. Crosslinking shifts these peaks due to interactions between Ca^{2+} ions and alginate carboxyl groups. In the Alg 5% hydrogel film, the addition of gamat gel and subsequent peak shifts indicate the formation of intermolecular bonds between alginate, gamat gel, and Ca^{2+} ions, reinforcing a stable hydrogel structure (Badita *et al.*, 2020; Pongjanyakul and Puttipipatkhachorn, 2007). This aligns with previous studies on alginate crosslinking and its interactions with divalent cations (Li *et al.*, 2016).

4.7 Antibacterial activity

Antibacterial features in hydrogel-based wound dressings help prevent infection in burn wounds, ensuring uninterrupted healing. Gamat gel, which tested positive for saponin compounds, and Alg 5% hydrogel film demonstrated antibacterial activity against Staphylococcus aureus. This aligns with previous research showing that Stichopus horrens extract has antibacterial capacity against S. aureus with a clear zone of 7.35 mm at a concentration of 625 ppm (Rasyid et al., 2018). The antibacterial activity of gamat gel and Alg 5% film suggests that the bioactive components in S. horrens, such as fucoidan, mucopolysaccharide, and collagen, contribute to this effect.

Previous studies indicated that methanol extracts of S. horrens did not show significant antibacterial activity, likely due to methanol's inability to isolate bioactive components effectively (Mohaved et al., 2020). In contrast, the gamat gel used in this study contains a variety of bioactive compounds, as confirmed by FTIR readings. The presence of collagen and fucoidan in the extract shows that while collagen may promote bacterial growth, the polysaccharide fraction, including chondroitin sulfate and fucoidan, can inhibit it (Ibrahim et al., 2018). Alg 5% film's bacterial inhibition capacity suggests that the appropriate alginate concentration in the film allows for the release of encapsulated bioactive compounds, supporting findings that alginate does not lock bioactive compounds, and higher bioactive content results in a larger inhibition zone (Saberian et al., 2021).

5. Conclusions

Based on chemical, morphological, and mechanical characteristics of hydrogel film made from alginate and gamat gel, the 5% w/v alginate concentration treatment is best compared to the other treatments. Gamat gel has the DPPH inhibition capacity of IC_{50} value of 2937.5 ppm, and in the concentration of 0.3 – 7% w/v, it has some antibacterial activity. The films, specifically Alg 5%, showed potentials to be an alternative wound dressing.

Ethics approval

No permits were required.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

S., K. E.: Conceptualization, Data curation, Formal analysis, Investigation, Software, Visualization, Roles/Writing - original draft. S., K. E., S. S., and Y. E**:** Funding acquisition**,** Methodology**,** Project administration**,** Resources**,** Supervision**,** Validation, Writing - review & editing

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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