



## PHYSICOCHEMICAL AND ANTIBACTERIAL CHARACTERIZATION OF HAND SANITIZER LOTION MADE ON BROWN SEAWEED (*Scytosiphon lomentaria*)

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### Abstract

Effective hand hygiene remains essential for reducing the transmission of foodborne and contact pathogens in daily life and in food handling contexts. *Scytosiphon lomentaria* seaweed has antimicrobial activity that can be applied as a hand sanitizer. This study aimed to determine the best addition of *S. lomentaria* to hand sanitizers, based on microbial inhibition and its characteristics. Lotion consisted of four treatments: 0 (control), 20, 30, and 40% *S. lomentaria*. The results showed that *S. lomentaria* powder contained bioactive polyphenol compounds with a total phenolic content of  $117.70 \pm 1.40$  mg GAE / g dry weight and an  $IC_{50}$  value of  $455 \pm 3.4$  ppm, while lotion with the addition of *S. lomentaria* contained polyphenols and saponins. The best treatment was shown by *S. lomentaria* 40% hand sanitizer lotion with *E. coli* inhibition of  $6.30 \pm 0.64$  mm, *S. aureus*  $6.85 \pm 1.12$  mm, hedonic test of color, texture and absorption with a level of like to very like, neutral pH, greenish white color, O/W emulsion type, high adhesive power, homogeneous, and easily absorbed by the skin. This study can serve as an alternative reference for making lotions that provide adequate and safe antimicrobial activity for routine hygiene before eating and food handling.

Keywords: antioxidant, *E. coli* inhibition, hedonic, *S. aureus* inhibition, total phenol

### Karakteristik Fisikokimia dan Antibakteri Losion Pembersih Tangan yang dibuat dari Rumput Laut Cokelat (*Scytosiphon lomentaria*)

#### Abstrak

Kebersihan tangan yang efektif penting untuk mengurangi penularan patogen penyebab penyakit bawaan makanan dan kontak dalam kehidupan sehari-hari serta konteks penanganan makanan. Rumput laut *Scytosiphon lomentaria* memiliki aktivitas antimikrob yang dapat diaplikasikan pada *hand sanitizer*. Penelitian ini bertujuan menentukan penambahan *S. lomentaria* terbaik pada *hand sanitizer* berdasarkan penghambatan mikrob, dan karakteristiknya. Perlakuan losion terdiri atas empat macam, yaitu 0 (kontrol), 20, 30, dan 40% *S. lomentaria*. Hasil menunjukkan serbuk *S. lomentaria* mengandung senyawa bioaktif polifenol dengan total fenolik  $117,70 \pm 1,40$  mg GAE/g berat kering dan nilai  $IC_{50}$   $455 \pm 3,4$  ppm, sedangkan losion dengan penambahan *S. lomentaria* mengandung polifenol dan saponin. Perlakuan terbaik ditunjukkan oleh losion pembersih tangan *S. lomentaria* 40% dengan daya hambat *E. coli* sebesar  $6,30 \pm 0,64$  mm, *S. aureus*  $6,85 \pm 1,12$  mm, uji hedonik warna, tekstur dan daya serap dengan tingkat suka sampai sangat suka, pH netral, warna putih kehijauan, tipe emulsi O/W, daya lekat tinggi, homogen, dan mudah diserap kulit. Penelitian ini dapat menjadi referensi alternatif dalam pembuatan losion yang memberikan aktivitas antimikrob yang memadai dan aman untuk kebersihan rutin sebelum makan dan penanganan makanan.

Kata kunci: antioksidan, daya hambat *E. coli*, daya hambat *S. aureus*, hedonik, total fenol

## INTRODUCTION

Effective hand hygiene remains a foundational public health practice for preventing the transmission of enteric and respiratory pathogens in everyday life and food handling settings. Routine hand decontamination before eating, during food preparation, when handling ready-to-eat products, or after contact with hightouch public surfaces reduces the risk of norovirus, *Salmonella*, pathogenic *Escherichia coli*, and other agents that commonly cause foodborne and contact infections (WHO, 2021; Murray *et al.*, 2022). In many contexts (street food, institutional catering, travel, and informal food vending), convenient, fast-acting hand sanitizers are the most practical means to reduce microbial transfer at the critical preprandial moment when direct hand-to-mouth contact is frequent (Lisitsin *et al.*, 2021; Murray *et al.*, 2022).

Alcohol-based hand rubs (ABHRs) are the current benchmark for rapid, broad-spectrum antisepsis because ethanol and isopropanol denature proteins and disrupt the lipid envelopes of many bacteria and viruses (Jing *et al.*, 2020). However, repeated or frequent use of high-concentration alcohol formulations is associated with adverse dermatologic outcomes, such as xerosis, erythema, increased trans-epidermal water loss, and occupational contact dermatitis, which reduce user compliance and may increase skin colonization by opportunistic bacteria (Kampf, 2018; Narla & Silverberg, 2021).

Concerns about skin tolerance are amplified in groups requiring intensive hand hygiene (healthcare workers, food handlers, caregivers, and parents), and alternative antiseptic chemistries (e.g., quaternary ammonium compounds, chlorhexidine) have limitations, including variable virucidal spectra, potential for irritation or sensitization, environmental persistence, and emerging tolerance or co-selection for antimicrobial resistance (AMR) (Kampf, 2018). These safety and stewardship issues create a pressing need for validated alcohol-free sanitizers that combine broad antimicrobial action with skin-protective and moisturizing properties.

*Scytosiphon lomentaria*, a brown alga, has demonstrated moderate to strong inhibition zones (9–11 mm) antibacterial activity against Gram-positive *Staphylococcus aureus* (MRSA) and Gram-negative *Pseudomonas aeruginosa* (MDR) (Zakaria *et al.*, 2011; Xu *et al.*, 2017; Silva *et al.*, 2020). The antimicrobial and antiviral potential of brown algae is strongly associated with their high content of secondary metabolites, particularly phlorotannins, which exhibit antiviral effects through the inhibition of viral protein PLpro, modulation of cytokine storms in SARS-CoV-2 infection, and antioxidant mechanisms such as DPPH and ABTS radical scavenging (Maheswari & Babu, 2022b; Tamama, 2021; Hidayat *et al.*, 2020; Catarino *et al.*, 2022; Pradhan *et al.*, 2022). In addition, fucoidans and other sulfated polysaccharides from *S. lomentaria* have shown antimicrobial and anti-inflammatory properties relevant to skin health (Ponce *et al.*, 2019; Gunathilaka, 2023). While lotion formulations are widely used to maintain skin hydration, there is limited availability of hand sanitizer lotions that combine effective antimicrobial activity with skin-protective properties.

Previous studies have highlighted the multifunctional role of seaweed in cosmetics, including antibacterial, moisturizing, photoprotective, and wound-healing properties (Choi, 2013; Poulouse, 2020; Nurjanah *et al.*, 2024; Praselya, 2022). Recent findings have further confirmed the antioxidant and antibacterial activities of *S. lomentaria*, underscoring its potential as a multifunctional bioactive ingredient in skincare formulations (Kavaz *et al.*, 2019; Kwon *et al.*, 2023). Fucoidans and other sulfated polysaccharides isolated from *S. lomentaria* exhibit antimicrobial and anti-inflammatory activities that can support skin health and reduce reliance on harsh preservatives (Ponce *et al.*, 2019).

However, despite the growing evidence of *S. lomentaria*'s biological activity, there is a lack of research exploring its direct application in the formulation of non-alcoholic hand sanitizers that can effectively inhibit microbial growth while preventing dehydration and skin irritation. Existing studies have primarily



focused on the characterization of its extracts or their inclusion in common cosmetic products; however, its specific role in hand sanitizer lotions remains underexplored. This gap highlights the need to investigate the formulation, antimicrobial efficacy, and skin-protective properties of *S. lomentaria*-based hand sanitizer lotions as safer alternatives to conventional alcohol-based hand sanitizers. This study aimed to determine the optimal amount of *S. lomentaria* to be added to hand sanitizers based on microbial inhibition and its characteristics.

## MATERIAL AND METHODS

### Sample Preparation

Fresh *S. lomentaria* was collected from Jolo Sutro Beach, Blitar, placed in plastic bags and cool boxes, transported to the Polytechnic of Marine and Fisheries Sidoarjo Quality Testing Laboratory, and cleaned with running water. The samples were dried at room temperature ( $\pm 26^{\circ}\text{C}$ ) for 5-7 days. The dry samples were cut into  $\pm 0.5$  cm pieces with scissors and blended, while the fresh samples were blended with the addition of distilled water (1:1) as a mixture for hand sanitizer lotion. The dry algal powder was frozen before being used for further testing. Phytochemical tests were performed using the Harborne qualitative method with test parameters of alkaloids, triterpenoids, steroids, flavonoids, saponins, tannins, and polyphenols (Harborne, 1995).

### Method

Algae powder with aquadest (1:1) was tested for microbiological characteristics in the form of antibacterial tests against gram-positive (*Staphylococcus aureus* ATCC 25923) and gram-negative (*Escherichia coli* ATCC 8739) bacterial strains (Kantachumpoo & Chirapart, 2010). Tests on the content of algae powder compounds included phytochemical tests (Harborne, 1995), total phenolic content (Kang *et al.*, 2010) with modifications, free radical scavenging activity ( $\text{IC}_{50}$ ) (Khalaf *et al.*, 2008; Pinteus *et al.*, 2017) with modifications, as well as antibacterial tests against gram-positive (*Staphylococcus aureus* ATCC 25923) and gram-negative (*Escherichia coli* ATCC

8739) bacterial strains (Kantachumpoo & Chirapart, 2010).

The hand sanitizer lotion preparations were divided into four treatment groups: 0 (control), 20% algae, 30% algae, and 40% algae. The lotion preparations for each treatment group were also tested for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Kantachumpoo & Chirapart, 2010), phytochemicals (Harborne, 1995), pH (Sayuti, 2015), adhesion (Pujiastuti & Kristen, 2019), spreadability (Sayuti, 2015), homogeneity (Umarudin *et al.*, 2020), emulsion type (Olejnik & Goscianska, 2023), and hedonic properties (Kim *et al.*, 2017).

### Antibacterial Test

The antibacterial activities of seaweed powder and seaweed lotion were determined using the agar diffusion method. All bacterial strains were cultured on Tryptone Soy Agar at a density of  $1 \times 10^5$  CFU/mL. Samples with different concentrations (undiluted and diluted to 1 mg/mL) were prepared using distilled water. Twenty microliters of each sample solution were placed on a sterile paper disc with a diameter of 6 mm and placed on the surface of the plate inoculated with bacteria. The plates were incubated at  $37^{\circ}\text{C}$  for 24 h, and the diameter of the inhibition zone was measured (in mm). The treatment was repeated three times (Kantachumpoo & Chirapart, 2010).

### Total Phenolic Content

The total phenolic content was measured using the method described by Kang *et al.* (2010), with modifications. The standard solution used was gallic acid at a concentration of 1 mg/mL, and a series of dilutions of various concentrations, namely 40, 30, 20, 10, and 5  $\mu\text{g/mL}$ , were prepared. Next, 1 g of the sample powder was dissolved in 10 mL of distilled water, diluted to a concentration of 200  $\mu\text{g/mL}$ , and made into 2 $\times$  repetitions (duplo). The standard solution and sample extract were taken as much as 10  $\mu\text{L}$  and placed in a 96-well microplate. Then, 50  $\mu\text{L}$  of Follin-Ciocalteau reagent (Merck, Sigma-Aldrich) was added and incubated for 5 min. Next, 40  $\mu\text{L}$  of 7.5%  $\text{Na}_2\text{CO}_3$  was added and incubated for 2 h in

the dark at room temperature. The absorbance was measured using a microplate reader (CORONA SH-1000) at 750 nm. The curve was plotted by plotting the concentration ( $\mu\text{g/mL}$ ) vs. absorbance (nm). The standard curve regression equation was obtained as  $y = ax + b$ ;  $R^2 = c$ , where  $x$  is the concentration and  $y$  is the absorbance. Total phenol is expressed in mg GAE/g dry weight GAE (Gallic Acid Equivalents) per 1 gram of sample powder.

### Free Radical Scavenging Activity ( $\text{IC}_{50}$ ) Test

The DPPH free radical scavenging activity test was performed according to the methods described by Khalaf *et al.* (2008) and Pinteus *et al.* (2017), with several modifications. A total of 50  $\mu\text{L}$  of 0.136 mM DPPH in methanol (25 mg/L) was mixed with 50  $\mu\text{L}$  of powder in distilled water at a concentration of 200  $\mu\text{g/mL}$ . Subsequently, it was stored in a dark room at room temperature for 30 min. Absorbance was measured using a spectrophotometric microplate (ELISA reader) at 517 nm. Vitamin C was used as a comparison to represent the use of commercial/synthetic antioxidants. As a control for the absorbance calculation, 100  $\mu\text{L}$  of distilled water was prepared without the addition of powder and was determined to have 0% absorbance. The percentage of free radical scavenging activity was calculated using the following formula:

$$A = \frac{B - C}{B} \times 100\%$$

Information:

- A = free radical scavenging activity
- B = control absorbance
- C = sample absorbance

Next, a graph was plotted between the sample concentration ( $x$ ) and the percentage of inhibition ( $y$ ). The  $\text{IC}_{50}$  value was calculated using the regression equation.

### pH Test

The pH test was performed by preparing a sample of hand sanitizer gel. The pH meter was cleaned before use, and the on button was pressed. The meter was then dipped into the container containing the sample to be

tested. The numeric scale moves as the pH meter is immersed. The pH value was then recorded and compared to the standard skin pH, which ranges from 4.5 to 7.5. The pH test was performed after the preparation was left to stand for 24 h (Sayuti, 2015).

### Adhesion Test

The adhesion test was performed by placing 0.1 g of the hand sanitizer lotion in the center of a slide and covering it with another slide. A 50 g load was placed on the coverslip for 5 min. The top and bottom of the cover slide were attached to a clamp on the adhesion tester, and the load was released. The time required for both slides to detach from the tester was recorded as the adhesion time of the preparation (Pujiastuti & Kristiani, 2019).

### Spreadability Test

The spreadability test was conducted by placing a 0.5 g sample on a glass slide, with another glass slide placed on top. A 200 g load was applied, and the sample was allowed to stand for 1 min. The resulting spreadability diameter was measured. The resulting spreadability diameter was compared with the standard spreadability diameter, which is 5-7 cm. The test was conducted after the sample was allowed to stand for 124 h (Sayuti, 2015).

### Homogeneity Test

A 0.5 g sample of hand sanitizer was weighed on a transparent watch glass and then weighed. The sample was then observed to determine whether it exhibited a homogeneous composition and no visible coarse particles. The test was conducted after the sample was allowed to stand for 24 h (Umarudin *et al.*, 2020).

### Emulsion Type Test

Emulsion-type testing was performed using a staining method. A small amount of hand sanitizer was placed on a glass slide. One drop of methylene blue was then added, mixed until homogeneous, and observed under a microscope. If the external phase is uniformly colored, the preparation is classified as an oil-in-water (O/W) emulsion (Voigt, 1995).



## Hedonic Test

A hedonic test was conducted to determine the hand sanitizer lotion formulation preferred by the panelists. The hedonic test was conducted by 30 untrained panelists to observe the parameters of color, aroma, texture, and absorption of the lotion. The hedonic test assessment was carried out using a hedonic scale with a range of 1-5, with the following criteria: (1) dislike very much, (2) dislike, (3) normal, (4) like, and (5) like very much (Kim *et al.*, 2017). Each parameter was evaluated by the panelists using descriptive criteria as stated in the hedonic test questionnaire sheet.

## Experimental Design

The formulation of the hand sanitizer lotion was based on the research by Yanuarti *et al.* (2021) with modifications (Table 1).

## Data Analysis

The research design was a completely randomized design (CRD) using the SPSS program. The treatment groups analyzed included normal control (lotion without algae) and lotions with the addition of 20, 30, and 40% algae. The research data were analyzed using parametric analysis in the form of analysis of variance (ANOVA) with a 95%

confidence level and further Duncan's Multiple Range Test (DMRT) with a 95% confidence level to determine the magnitude of the effect between treatments. Hedonic test parameters were analyzed using nonparametric analysis in the form of the Kruskal-Wallis test with a 95% confidence level and the Mann-Whitney U test with a 95% confidence level to determine whether there were significant differences between the treatment groups.

## RESULTS AND DISCUSSION

### Antibacterial Activity of Lotion and Algae Powder

Antibacterial activity testing was performed using gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria. Antibacterial testing was conducted on seaweed powder and lotion with and without the addition of seaweed. The use of unextracted seaweed powder in lotion production was chosen to obtain a higher yield for the lotion. Furthermore, Lailatussifa *et al.* (2017) stated that the nutritional content and bioactive components of *Sargassum hystrix* brown algae powder were balanced to support the antioxidant activity of the sample and its potential as a natural antioxidant source. *Scytosiphon lomentaria* algae contain various bioactive compounds, such as fucoidan,

Table 1 Formulation of hand sanitizer lotion

Phase	Addition of algae powder (%)			
	0 (control)	20	30	40
<b>Oil</b>				
Olive Oil	14.5	14.5	14.5	14.5
Stearic acid	3.6	3.6	3.6	3.6
Beeswax	3.6	3.6	3.6	3.6
TEA	2.3	2.3	2.3	2.3
<b>Water</b>				
Distilled water	72.7	52.5	42.6	32.7
Nipagin	0.7	0.7	0.7	0.7
Glycerin	1.5	1.5	1.5	1.5
Algae powder	0	20.0	30.0	40.0
Chitosan	1.0	1.0	1.0	1.0
<b>Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>



polyphenols, and phlorotannins, which act as antioxidants, antibacterials, and antivirals (Ponce *et al.*, 2019). The results of the inhibitory zone test for pathogenic bacteria using *Scytosiphon lomentaria* seaweed powder and lotion preparations are presented in Table 2.

The highest inhibition zone for *E. coli* pathogenic bacteria was  $6.33 \pm 1.02$  mm in the 1 mg/mL *S. lomentaria* treatment group (Table 2). The lotion with the addition of *S. lomentaria* algae had the best zone of inhibition against *E. coli*, with a value of  $6.30 \pm 0.64$  mm in the 40% seaweed addition treatment. The higher the concentration of algae powder added to the lotion, the greater the diameter of the inhibition zone against gram-negative pathogenic bacteria. The higher the concentration of algae powder added to the lotion preparation, the greater the diameter of the inhibition zone against *E. coli* gram-negative pathogenic bacteria. Higher concentrations of *S. lomentaria* powder in lotion formulations increased the release of bioactive compounds, such as phlorotannins, fucoidans, and saponins, which diffuse into the agar and act in a concentration-dependent manner by disrupting cell membranes and interfering with bacterial metabolism.

This explains why larger algae powder additions resulted in wider inhibition zones against *E. coli*, as more active molecules exceeded the inhibitory threshold during diffusion (Ayrapetyan *et al.*, 2021; Pradhan *et al.*, 2022). The inhibitory strength of *E. coli* pathogenic bacteria from all groups was classified as moderate for the lotion treatment with the addition of 30% and 40% algae and algae powder, and was classified as low for the lotion treatment without the addition of algae and lotion with the addition of 20% algae. The lotion treatment group with the addition of 30% algae was not significantly different from the lotion with the addition of 40% algae and algae powder, but it was significantly different from the lotion treatment group without the addition of algae and the lotion with the addition of 20% algae.

The strength of antibacterial antibiotics in a sample was as follows: an inhibitory area of  $\geq 20$  mm indicated very strong inhibitory

power, an inhibitory area of 10-20 mm means strong inhibitory power, an inhibitory area of 5-10 mm means moderate inhibitory power, and an inhibitory area of  $\leq 5$  mm indicated weak inhibitory power (Scania & Chasani, 2021). The factors that influence the size of the inhibition area (clear zone) are the sensitivity of the organism, culture medium, incubation conditions, and agar diffusion speed. Agar diffusion is influenced by the concentration of microorganisms, media composition, incubation temperature, and incubation time (Radiena *et al.* 2019). In addition, adding algae to lotion can increase the effectiveness of inhibition against pathogenic bacteria because it contains secondary metabolite compounds (Xu *et al.*, 2017). Key marker compounds in seaweed that have demonstrated antibacterial activity include polyphenols, phlorotannins, sulfated polysaccharides (especially fucoidan), saponins, sterols (such as fucosterol), and flavonoids.

Polyphenols and phlorotannins exert bactericidal and bacteriostatic effects by binding to and precipitating membrane proteins, disrupting cell envelopes, inhibiting key enzymes, and interfering with quorum sensing. Sulfated polysaccharides, such as fucoidan, act through charge-mediated interactions with bacterial surfaces, inhibition of adhesion/biofilm formation, and immunomodulatory effects that reduce pathogen virulence (Pradhan *et al.*, 2022). Saponins function as amphipathic surfactants that increase membrane permeability and promote the leakage of intracellular contents, thereby increasing the potential for bacterial death (Li & Monje-Galvan, 2023). Fucosterol and other terpenoids disrupt membrane integrity and inhibit metabolic enzymes, whereas various flavonoids and smaller polyphenols contribute to additional membrane-targeting, enzyme-inhibiting, and metal-chelating activities that enhance overall antimicrobial potency (Meinita *et al.*, 2021).

Table 2 shows that the zone of inhibition against *S. aureus* was the highest in the 1 mg/mL *S. lomentaria* algae powder treatment group, with a value of  $7.18 \pm 0.63$  mm. The lotion treatment without the addition of algae had the lowest inhibitory power, with a value of

Table 2 Antibacterial activity of *S. lomentaria* algae powder and lotion preparations

Treatment	Pathogenic bacterial inhibition zone	
	<i>Staphylococcus aureus</i> (mm)	<i>Escherichia coli</i> (mm)
Control (without algae)	5.25±2.36 <sup>a</sup>	3.76±0.57 <sup>a</sup>
Lotion with 20% algae	5.32±1.75 <sup>a</sup>	4.12±0.38 <sup>a</sup>
Lotion with 30% algae	6.51±1.41 <sup>a</sup>	5.71±0.83 <sup>b</sup>
Lotion with 40% algae	6.85±1.12 <sup>a</sup>	6.30±0.64 <sup>b</sup>
Algae powder 1 mg/mL	7.18±0.63 <sup>a</sup>	6.33±1.02 <sup>b</sup>

Different letters in the same column indicate significant differences ( $p < 0.05$ ).

5.25 ± 2.36 mm. The inhibitory power of the lotion, as indicated by the diameter of the clear zone, increased with increasing algal concentration. However, the strength of the antibacterial activity in all treatment samples was classified as moderate, with a clear zone diameter of 5-10 mm. None of the treatment groups showed any significant differences in the inhibitory power against the pathogenic bacteria *Staphylococcus aureus*.

The increase in the inhibitory power of lotions with the addition of algae is related to their bioactive content, which influences their bioactivity. The oxidative potential of hydroxyl groups can change bacterial cell membranes, inhibiting bacterial growth (Bogolitsyn *et al.*, 2019). The inhibition zone for lotions containing algae or algae powder against gram-positive bacteria (*S. aureus*) was higher than that against gram-negative bacteria (*E. coli*). This is due to the physical differences in the ability of gram-positive and gram-negative bacteria to grow on simplicia. These physical differences include the morphological structure of bacterial cells and their composition within the cells (Sahgal *et al.*, 2011). Gram-negative bacteria are surrounded by an outer membrane with a high lipopolysaccharide (LPS) content. This membrane allows gram-negative bacteria to resist several antibiotics, whereas gram-positive bacteria have cell walls that are relatively polar compared to the cell walls of gram-negative bacteria, making gram-positive bacteria more easily penetrated by antimicrobial compounds.

In addition, gram-positive bacteria contain high levels of nucleic acids.

Antibacterials derived from phenolic compounds can denature proteins and nucleic acids, irreparably damaging bacterial cell membranes. Bacterial growth inhibition occurs due to interference by active compounds in the extract or simplicia (Sameeh *et al.*, 2016). Increasing the concentration of the extract or simplicia is needed to kill microbial cells rather than to inhibit their growth (Ibrahim *et al.*, 2013).

### Phytochemical Screening

Phytochemical analysis was carried out based on the Harbourne (1995) method, which includes tests for alkaloids, triterpenoids, steroids, flavonoids, saponins, tannins, and polyphenols. Phytochemical tests were performed on the lotion preparations to determine the content of the ingredients from the extract that remained after being made into a lotion (Badriyah *et al.*, 2023). The dry powder of *S. lomentaria*, as a result of research, contains polyphenol and tannin compounds. This was indicated by a blue solution when the Folin–Ciocalteu phenol reagent Hi-LR was added. Polyphenols and tannins function as antioxidants related to the mechanism of capturing free radicals (hydroxyl groups (-OH) in phenolic rings can donate hydrogen atoms or electrons to free radicals, thereby neutralizing them. In addition, polyphenols can bind transition metal ions, such as iron (Fe<sup>2+</sup>) and copper (Cu<sup>2+</sup>), and modulate the activity of antioxidant enzymes (Parcheta *et al.*, 2021). Polyphenols and tannins can interact with lipids in bacterial cell membranes, causing damage to the membrane integrity and bacterial death (Zhong *et al.*,

2023). Meanwhile, lotions with the addition of 20%, 30%, and 40% *S. lomentaria* contained polyphenolic compounds, tannins, and saponins. The saponin content was indicated by the formation of stable foam when water and HCl reagent were added. Saponin is a natural glycoside compound with surfactant properties, which means it can form foam and has cleaning properties (Rai *et al.*, 2021). The addition of algae to lotions can contain polyphenol and tannin compounds. Compounds with potential antioxidant and antibacterial properties can be predicted from the phenolic, alkaloid, and flavonoid groups, which are polar compounds (Mehmood *et al.*, 2022).

### Total Phenolic Content

*S. lomentaria* algae powder was analyzed to determine the amount of phenolic compounds in the algae. The activity of phenolic compounds is determined by the number of hydroxyl groups on the benzene ring (Dhianawaty & Ruslin, 2015). The total phenolic content was analyzed using the Folin-Ciocalteu reagent and gallic acid as a reference.

The average total phenolic content at a concentration of 200 ppm (200 µg/mL or 0.2 mg/mL) was  $117.70 \pm 1.40$  mg GAE/g dry basis. The total phenol content of *S. lomentaria* powder is relatively higher compared to the total phenol content of several other types and species of brown algae, such as *S. muticum* ( $230.8 \pm 17.1$  mg GAE/100 g dry basis) (Farvin and Jacobsen, 2013), *Padina pavonica* ( $1.076 \pm 0.87$  g GAE/100 g extract) (Khaled *et al.*, 2012), *Codium vermilara* ( $16.72 \pm 0.065$  mg GAE/g extract) (Pinteus *et al.*, 2017), *S. vulgare* ( $7.09$  g GAE/100 g extract) (Plaza *et al.*, 2010), *S. thunbergii* ( $11.5$  g GAE/100 g extract) (Luo *et al.*, 2010), and *S. hystrix* ( $11.43$  g GAE/100 g dry basis) (Lailatussifa, 2017). However, the total phenolic content of *S. lomentaria* powder in this study was lower than that of *S. polycystum* ( $59.30$  g GAE/100 g extract) (Lailatussifa and Pereira, 2022), *Fucus spiralis* ( $397.23 \pm 0.02$  mg GAE/g extract), *Bifurcaria bifurcata* ( $129.17 \pm 0.002$  mg GAE/g extract) (Pinteus *et al.*, 2017), and *S. kjelmanianum* ( $16.3$  g GAE/100 g extract).

There was a strong correlation between high phenolic levels and antibacterial activity. Phenolic compounds, particularly phlorotannins in brown seaweeds, exert antimicrobial effects through mechanisms such as disruption of bacterial cell membranes, protein precipitation, inhibition of enzymatic activity, and interference with the quorum sensing pathways. Studies have consistently reported that extracts with higher total phenolic content (TPC) display stronger antibacterial activity, as phenolics enhance both bactericidal and bacteriostatic effects against Gram-positive and Gram-negative bacteria (Cotas *et al.*, 2020). The high or low total phenolic content is influenced by intrinsic factors (sample type and species, sampling location, and sample age) and extrinsic factors (temperature, climate, depth, salinity, tidal zone, and tidal cycle) (Lann *et al.*, 2012).

### DPPH (1,1-diphenyl 2-picrylhydrazyl) Free Radical Scavenging Activity

The  $IC_{50}$  value is the substrate concentration that reduces DPPH activity by 50%. This parameter is defined as the amount of antioxidant required to reduce the DPPH absorbance by 50% of the initial absorbance (Mishra *et al.*, 2012). The  $IC_{50}$  values of the dry powder of *S. lomentaria* and standard vitamin C are presented in Table 3.

The  $IC_{50}$  value of *S. lomentaria* dry powder was  $0.455 \pm 0.004$  mg/mL or  $455 \pm 3.40$  ppm. This value is higher than the  $IC_{50}$  value of *S. polycystum* phlorotannin extract ( $1.20 \pm 0.01$  mg/mL), *S. polycystum* polyphenol extract ( $1.27 \pm 0.01$  mg/mL) (Sianipar & Gunardi, 2023), *S. filipendula* polysaccharide sulfate (1,000 ppm) (Costa *et al.*, 2011), *Fucus vesiculosus* polysaccharide sulfate (800 ppm) (Suresh *et al.*, 2013), and *S. plagiophyllum* polysaccharide sulfate (700 ppm) (Suresh *et al.*, 2013). The  $IC_{50}$  value of *S. lomentaria* dry powder was  $0.33 \pm 0.03$  mg/mL, which was lower than the  $IC_{50}$  value of *S. hystrix* extract (Maheswari and Babu, 2022a).

The  $IC_{50}$  value of *S. lomentaria* dry powder was significantly different from that of the vitamin C standard with a confidence level of 95% (Table 4). The  $IC_{50}$  value of *S.*



Table 3 IC<sub>50</sub> values of *S. lomentaria* powder and vitamin C

Sample	IC <sub>50</sub> Value (mg/mL)	IC <sub>50</sub> Value (ppm)
Vitamin C	0.013±0.002 <sup>a</sup>	13.10±1.30 <sup>a</sup>
<i>S. lomentaria</i> dry powder	0.455±0.004 <sup>b</sup>	455±3.4 <sup>b</sup>

Different letters in the same column indicate significant differences ( $p < 0.05$ ).

Table 4 Physicochemical characteristics of lotion

Treatment group	pH value	Adhesive strength(second)	Spread power (mm)	Homogeneity
0% algae	7.4±0.14 <sup>a</sup>	15.01±4.54 <sup>a</sup>	50.34±0.46 <sup>a</sup>	Homogeneous, finely clotted, slightly thick
20% algae	7.26±0.10 <sup>ab</sup>	23.14±2.33 <sup>b</sup>	63.22±1.71 <sup>c</sup>	Homogeneous, easy to spread
30% algae	7.14±0.10 <sup>b</sup>	45.53±3.74 <sup>c</sup>	55.57±0.96 <sup>b</sup>	Homogeneous, slightly thick, easy to spread
40% algae	6.94±0.10 <sup>c</sup>	105.84±6.03 <sup>d</sup>	52.20±2.00 <sup>a</sup>	Homogeneous, slightly thick, easy to spread

Different letters in the same column indicate significant differences ( $p < 0.05$ ).

*lomentaria* dry powder was lower than that of standard vitamin C, with a value of  $0.013 \pm 0.002$  mg/ml ( $13.10 \pm 1.30$  ppm). The IC<sub>50</sub> value is classified as strong if it ranges between 50 and 100 ppm (Ramadhan *et al.*, 2022). Suresh *et al.* (2013) stated that brown algae with an IC<sub>50</sub> value of 1000 ppm is reactive and has the potential to be used as an anticancer agent in vitro. The high or low IC<sub>50</sub> value of a sample is influenced by several factors, including the solvent used, the amount of dissolved bioactive content, the sample harvest season, the sampling location, and the type of sample species (Maheswari & Babu, 2022a).

## pH

The pH of the lotion was determined to ensure that it could be used as a topical preparation without causing skin irritation. Table 4 lists the physicochemical characteristics of the lotion, including the pH test results.

The higher the concentration of algae added to the lotion, the lower was the pH of the lotion. This is consistent with the study by Ramdani *et al.* (2021), who stated that the addition of red algae (*Eucheuma cottonii*) to

lotion preparations reduced the pH value of the lotion to 6.67. To prevent skin irritation, an ideal lotion preparation has a pH value of 4.5–8.0.

## Adhesive Strength

Adhesive strength is included in the physicochemical characteristics of the lotion, as shown in Table 4. The results showed that the adhesive strength values were significantly different among the treatment groups ( $p < 0.05$ ) (Table 4). The lowest adhesive force was found in the normal control/lotion base treatment group without the addition of algae, with a value of  $15.01 \pm 4.54$ . The adhesion value increased as the concentration of algae in the lotion preparation increased. The minimum standard value for good adhesion to the lotion is more than four seconds (Salsabila *et al.*, 2021).

The higher the adhesive power, the better the lotion's resistance to the skin, and hence, the better the protective ability of the lotion (Oktaviasari and Zulkarnain, 2017). The higher the adhesive force, the longer it takes for the two glass objects to separate;

therefore, the better the adhesive power of the lotion preparation, the longer the preparation will stick to the skin, and the longer the active substance in the lotion will be in contact with the skin (Wong *et al.*, 2023). When producing lotion preparations, the higher the mixing temperature and the longer the stirring time, the higher the adhesive power (Hiola *et al.*, 2018).

### Spreadability Test

The spreadability test measures the area where the lotion is spread to determine the spreadability of the lotion or emulsion when applied to the skin (Dina *et al.*, 2017). The spreadability test results are presented in Table 4. The higher the concentration of algae added to the lotion preparation, the lower the value of the lotion's spreadability, because algae can act as a thickener in the lotion (Lopez-Hortas *et al.*, 2021). This is in accordance with the research by Ramdani *et al.* (2021), who reported that the addition of 2% (w/w) *E. cottonii* algae to the lotion preparation can reduce the spreadability value of the lotion to  $5.16 \pm 0.03$  cm. However, all the treatment groups complied with the standard spreadability of semisolid materials of 5-7 cm (Apriliani *et al.*, 2021). The spreadability of a lotion is influenced by several factors, including the mixing temperature, mixing time (Hiola *et al.*, 2018), type of active ingredient formulation, viscosity, and adhesive power (Nurjanah *et al.*, 2020).

### Homogeneity Test

The results of the homogeneity test for the lotion are presented in Table 4. Table 4 shows the results of the homogeneity test for each treatment group. All treatment groups were classified as homogeneous, and no solid lumps were formed that were visible when the lotion was in contact with a glass object. The emulsifier, namely TEA and stearic acid, which makes the emulsion between the oil phase and the water phase mix properly, is responsible for the good homogeneity of the lotion. The presence of air bubbles in the lotion was caused by the use of glycerin during the preparation of the water phase of the lotion (Apriliani *et al.*, 2021). The homogeneity of

a preparation is influenced by the presence of emulsifiers (Moravkova and Filip, 2013). In addition, the mixing temperature and stirring time influence the homogeneity of the preparation. Stirring time can expand the contact area by increasing the stirring speed, thereby increasing the homogeneity of the mixture (Hiola *et al.*, 2018).

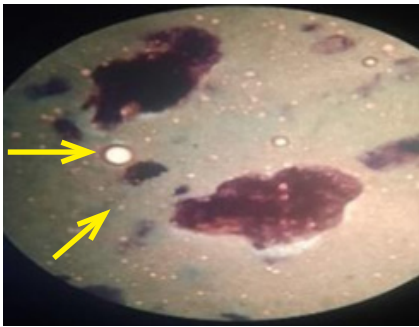
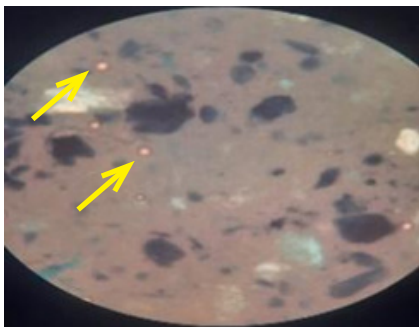
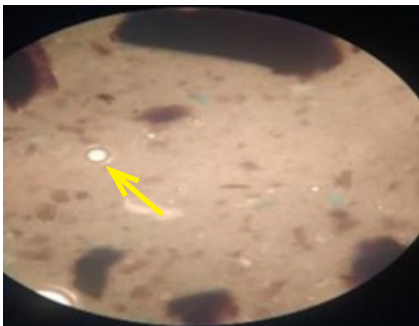
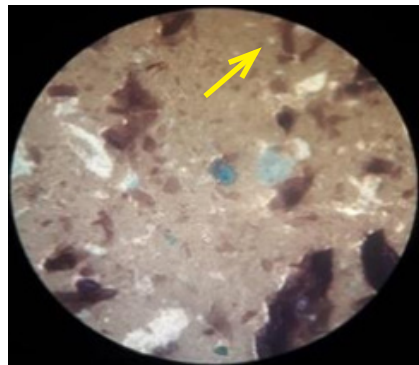
### Emulsion Type

An emulsion is a biphasic system in which one immiscible liquid is dispersed as droplets in another immiscible liquid. The principal types of emulsions are oil-in-water (O/W) and water-in-oil (W/O), and the selected type strongly determines the behavior of topical products (Wu & McClements, 2015). Determining the emulsion type is essential because the continuous phase governs key product attributes, such as skin feel, spreadability, hydration performance, and preservative efficacy, which directly affect lotion functionality and consumer acceptance (Nour, 2018). Simple diagnostic tests, such as microscopic observation, are routinely used to verify phase continuity and droplet distribution and to indicate whether thickeners or emulsifiers (e.g., seaweed polysaccharides) are required to improve stability and reduce visible oil globules (Abraham *et al.*, 2024). The emulsion types of all the lotion treatment groups are listed in Table 5.

Table 5 shows that all treatment groups were included in the oil-in-water (O/W) emulsion type. O/W lotions contain more than 31% water (Guzmán *et al.*, 2022). The results showed that the water phase, as an external phase, was colored with methylene blue, whereas the oil phase, as an internal phase, was not. The solubility of methylene blue is soluble in water, allowing it to create a color that spreads in the water phase (Pujiastuti & Kristen, 2019). Increasing the concentration of algae added to the lotion preparation did not affect the type of lotion emulsion but affected the texture of the oil in water. The higher the concentration of algae added to the lotion preparation, the more evenly the oil was dispersed on the surface of the lotion, resulting in fewer oil bubbles in the emulsion. This is related to the ability of algae



Table 5 Emulsion type of lotion

Treatment Group	Picture	Emulsion Type
0% algae		O/W
20% algae		O/W
30% algae		O/W
40% algae		O/W

to act as thickeners and emulsifiers in lotion preparations (Fernando *et al.*, 2019).

### Hedonic Test

Hedonic tests were performed on color, aroma, texture, and absorption parameters. The hedonic scale ranged from 1 to 5. Scale

value 1 = really do not like it; 2 = do not like; 3 = neutral/normal; 4 = like; and 5 = really like it (Joy & Rani, 2013). The results showed a significant difference in the hedonic value of lotion color preference for each treatment group (Figure 1).

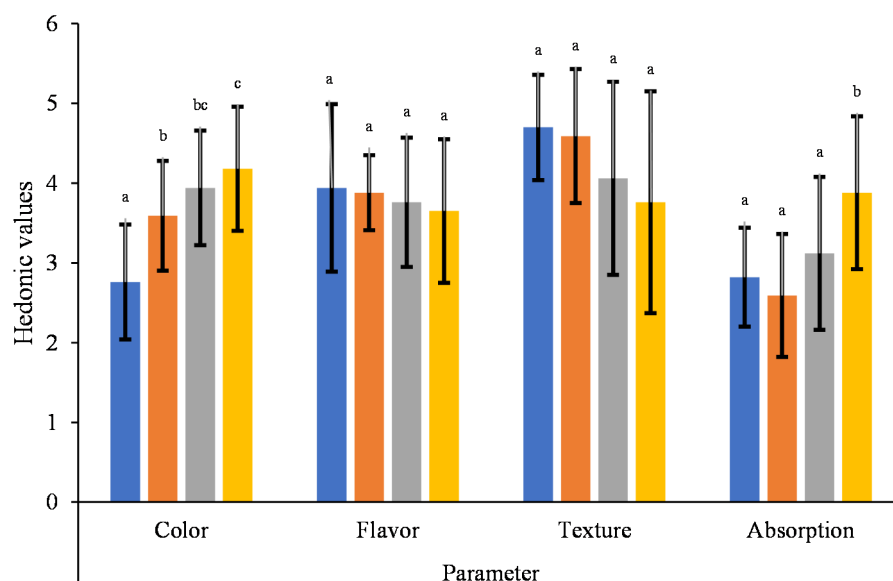


Figure 1 Hedonic test of hand sanitizer lotion for normal control (lotion without algae) (■), lotion with added algae 20% (■), lotion with added algae 30% (■) and lotion with added algae 40% (■)

The color of the lotion with the addition of 40% algae was the most preferred by the panelists, with a liking level of  $4.18 \pm 0.78$ . The clearer the color of the lotion, the more the panelists preferred it. The lotion with 40% algae added showed a matcha green color. The sample that was least liked by the panelists was the normal control treatment (lotion without the addition of seaweed), with a value of  $2.76 \pm 0.72$ , indicating a dislike towards neutral. The higher the concentration of algae added to the lotion, the more intense the color of the lotion, and the more liked it was by the panelists. This is in accordance with the research by Pratama *et al.* (2020), who stated that increasing the concentration of *Kappaphycus alvarezii* algae to jicama (2:1) in lotion preparations was preferred by panelists with a neutral preference level of  $3.1 \pm 0.58$  compared to samples without the addition of algae and samples with the addition of a higher concentration of algae. The color formed during preparation is influenced by the color of the constituent ingredients (Paakki *et al.*, 2018).

The level of liking for the aroma of the lotion ranged from neutral/usual to liking, with a value range of  $3.65 \pm 0.90$  to  $3.94 \pm 1.05$ . The highest aroma hedonic value was found

in the lotion base sample without the addition of algae. The higher the concentration of algae added to the lotion preparation, the more the fragrant smell of olive oil mixed with the fresh aroma of the algae was reduced. In contrast to the research of Sirait *et al.* (2022), which stated that the addition of 1% *Caulerpa racemosa* algae extract to hand cream preparations without adding other active ingredients was preferred by panelists with a very like level (hedonic value  $5.00 \pm 1.673$ ). However, the test results are in line with the research by Pratama *et al.* (2020), who stated that increasing the concentration of *Kappaphycus alvarezii* algae in lotion preparations did not affect the level of panelists' liking for the lotion aroma, with hedonic values ranging from 3.13-3.55 with neutral levels to liking.

All treatment groups had hedonic texture values ranging from neutral to like to like to very like (3.76-4.70), with a quality value from slightly soft to soft. The base lotion treatment group without the addition of algae was the most preferred by the panelists, with the hedonic quality of a soft texture. Adding algae to the lotion created a slightly rough and thick texture. This is in line with the research by Pratama *et al.* (2020), which states that increasing the concentration of algae in lotion





preparations reduces the panelists' level of preference because algae can make the lotion chewier, which the panelists dislike.

The highest hedonic value of lotion absorption was found in the lotion treatment with the addition of 40% algae, amounting to  $3.88 \pm 0.96$ , with the quality characteristic of high absorption. The hedonic value of the absorption capacity of the lotion with the addition of 40% algae was significantly different from that of all other treatment groups. The addition of algae to the preparation functions as a moisturizer, increasing skin hydration and absorption capacity (Nejad *et al.*, 2020).

## CONCLUSION

The addition of 40% *S. lomentaria* powder to lotion preparations can be used as a hand sanitizer with moderate bacterial level inhibition. It contains secondary bioactive compounds in the form of polyphenols and saponins as antioxidants, with an  $IC_{50}$  value of *S. lomentaria* powder of  $455 \pm 3.4$  ppm and a total phenolic content of  $117.70 \pm 1.40$  mg GAE/g dry weight, respectively. The *S. lomentaria* hand sanitizer lotion was an O/W emulsion and was well accepted by the panelists based on the hedonic test. This research can be an alternative reference for making non-alcoholic hand sanitizer lotions that are safe for the skin.

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