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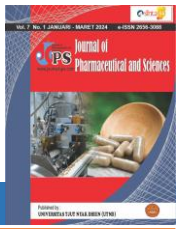

Comparison of the Antibacterial Effectiveness of Peppermint Leaf (*Mentha piperita* L.) Extract and Hand Soap Gel Preparation Against *Staphylococcus aureus* Causing Skin Infections

Perbandingan Efektivitas Antibakteri Ekstrak dan Sediaan Hand Soap Gel Daun Peppermint (*Mentha piperita* L.) Terhadap Bakteri *Staphylococcus aureus* Penyebab Infeksi Kulit

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Abstract

Background: Peppermint leaves (*Mentha piperita* L.) contain various secondary metabolites, including flavonoids, alkaloids, tannins, and steroids, which exhibit antibacterial activity. The development of peppermint extract into a hand soap gel requires evaluation of its physical stability and antibacterial effectiveness. **Aim:** This study aimed to formulate a hand soap gel from peppermint leaf ethanol extract and evaluate its physical characteristics, stability, and antibacterial activity against *Staphylococcus aureus*. **Methods:** Peppermint leaves were extracted by maceration using 70% ethanol. Hand soap gel formulations containing 3%, 6%, and 9% extract concentrations were prepared and evaluated through organoleptic, homogeneity, spreadability, foam height, pH, viscosity, and stability tests under different storage temperatures. Antibacterial activity was determined against *Staphylococcus aureus* using the agar diffusion method. **Results:** The ethanol extract demonstrated antibacterial activity with a maximum inhibition zone of 17.1 mm at 50% concentration. All hand soap gel formulations showed good physical characteristics and stability under various storage temperatures. The antibacterial activity of the formulations increased with extract concentration, producing inhibition zones ranging from 20.8 to 21.1 mm, categorized as very strong activity. Statistical analysis using one-way ANOVA showed significant differences among treatment groups ($p < 0.05$). **Conclusion:** Peppermint leaf ethanol extract can be successfully formulated into a stable hand soap gel preparation with very strong antibacterial activity against *Staphylococcus aureus*.

Keywords: Mentha piperita; Staphylococcus aureus; antibacterial; hand soap gel; physical stability.

Abstrak

Latar Belakang: Daun peppermint (*Mentha piperita* L.) mengandung berbagai metabolit sekunder, seperti flavonoid, alkaloid, tanin, dan steroid, yang memiliki aktivitas antibakteri. Pengembangan ekstrak daun peppermint menjadi sediaan *hand soap gel* memerlukan evaluasi stabilitas fisik serta efektivitas antibakterinya. **Tujuan:** Penelitian ini bertujuan untuk memformulasikan ekstrak etanol daun peppermint ke dalam sediaan *hand soap gel* serta mengevaluasi karakteristik fisik, stabilitas, dan aktivitas antibakterinya terhadap *Staphylococcus aureus*. **Metode:** Ekstrak daun peppermint diperoleh melalui metode maserasi menggunakan etanol 70%. Sediaan *hand soap gel* diformulasikan dengan konsentrasi ekstrak 3%, 6%, dan 9%, kemudian dievaluasi melalui uji organoleptik, homogenitas, daya sebar, tinggi busa, pH, viskositas, dan stabilitas pada berbagai suhu penyimpanan. Aktivitas antibakteri diuji menggunakan metode difusi agar terhadap *Staphylococcus aureus*. **Hasil:** Ekstrak etanol daun peppermint menunjukkan aktivitas antibakteri dengan diameter zona hambat maksimum sebesar 17,1 mm pada konsentrasi 50%. Semua formulasi *hand soap gel* memiliki karakteristik fisik dan stabilitas yang baik dalam berbagai kondisi penyimpanan. Aktivitas antibakteri sediaan meningkat seiring peningkatan konsentrasi ekstrak dengan diameter zona hambat sebesar 20,8–21,1 mm yang tergolong sangat kuat. Analisis statistik menggunakan *One-Way ANOVA* menunjukkan adanya perbedaan signifikan antarkelompok perlakuan ($p < 0,05$). **Kesimpulan:** Ekstrak etanol daun peppermint dapat diformulasikan menjadi sediaan *hand soap gel* yang stabil secara fisik, dengan aktivitas antibakteri yang sangat kuat terhadap *Staphylococcus aureus*.

Kata Kunci: Mentha piperita; Staphylococcus aureus; antibakteri; hand soap gel; stabilitas fisik.

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Introduction

The skin is the most vital part of the body, serving as a barrier that protects the body's components from physical damage and from bacteria, fungi, and viruses. Microorganisms smaller than 0.1 mm are typically invisible to the naked eye [1]. Maintaining clean skin is essential for preventing and minimizing bacterial infections or contamination. *Staphylococcus aureus* is a type of bacterium that frequently adheres to the skin and can cause infections [2]. *Staphylococcus aureus* is a common bacterium found in the environment and is the most prevalent cause of human skin infections. These infections can occur in healthy individuals; however, they are more likely to arise in those with weakened immune systems [3]**.** *Staphylococcus* is also commonly found in the mouths of healthy individuals. Contaminated saliva can serve as a breeding ground for oral microbes. Since saliva frequently comes into direct contact with the body's oral structures, it is essential to maintain oral hygiene.

Back to nature is a lifestyle that embraces the use of natural ingredients to maintain health. Drawing on empirical evidence and personal experience, the utilization of these ingredients offers numerous benefits. This movement has gained global momentum, with an increasing number of individuals opting for natural medicines, which are generally considered safer than their synthetic counterparts.

Indonesia, as an archipelago nation, is home to approximately 20,000 species of medicinal plants, of which around 1,000 have been documented, and roughly 300 are utilised in traditional medicine. One notable plant is peppermint (*Mentha piperita* L.), which is recognized for its empirical health benefits. In Gunungsitoli, many people rely on peppermint to address various health issues, including cholesterol, diabetes, gout, and high blood pressure.

Based on research by [4]**.** Peppermint leaves (*Mentha piperita* L.) exhibit antibacterial activity against several multidrug-resistant (MDR) bacteria, including Escherichia coli, Staphylococcus aureus, Salmonella enterica, and *Candida albicans*. Peppermint has antimicrobial, anti-tumor, and anticancer properties due to its high antioxidant content. Furthermore, peppermint contains essential oils, including menthol, menthone, methyl acetate, and piperitone.

The people of Samosir traditionally use peppermint leaves to treat various ailments, including hypertension and gout. A study [5]**.** demonstrated that the ethanol extract of peppermint leaves in toothpaste effectively inhibits *Streptococcus mutans* bacteria. Another study found that the ethanol extract of peppermint leaves (*Mentha piperita* L.) exhibits strong antimicrobial activity, effectively killing bacteria including *Klebsiella pneumoniae*, *Streptococcus pneumoniae, Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris,* and *Bacillus subtilis.* Hand soap gel was selected for this study due to its numerous benefits. These benefits include excellent spreadability on the skin, minimal interference with physiological functions, as it does not create an impermeable layer or clog pores, a cooling sensation on the skin, ease of rinsing with water, and optimal drug release [6].

Peppermint plants contain phenolic compounds and possess antimicrobial properties. According to a study conducted by Hasibuan [7]**.** Peppermint leaves (*Mentha piperita* L.) comprise primary compounds, which include secondary metabolites such as alkaloids, saponins, tannins, flavonoids, and steroids/triterpenoids. In light of this information, the researchers aim to conduct more comprehensive research by investigating the antibacterial activity of peppermint leaf ethanol extract against Staphylococcus aureus, a bacterium commonly associated with skin infections. Additionally, they plan to formulate a hand soap gel preparation incorporating peppermint leaf ethanol extract, which will subsequently be evaluated. The final step will involve retesting the antibacterial activity of the hand soap gel preparation to confirm that the peppermint leaf ethanol extract retains its antibacterial properties when incorporated into the formulation.

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Research Methods

Materials and Instruments

The materials used in this study consisted of peppermint leaves as the primary raw material, along with several chemical reagents and excipients employed in the extraction process, phytochemical screening, formulation, and evaluation procedures. The solvents and reagents included 70% ethanol, acetaldehyde, sodium lauryl sulfate, methyl paraben, carbopol, glycerin, disodium EDTA, citric acid, triethanolamine (TEA), potassium hydroxide (KOH), lemon oil, nutrient agar (NA), Staphylococcus aureus, pH buffer solutions of 4 and 7, Dragendorff reagent, Mayer reagent, Bouchardat reagent, 1% ferric chloride ($FeCl_3$), magnesium sulfate, amyl alcohol, concentrated sulfuric acid (H_2SO_4), 0.9% sodium chloride (NaCl), hydrochloric acid (HCl), concentrated hydrochloric acid, and barium chloride ($BaCl_2$).

The instruments utilized in this research included standard laboratory glassware, autoclaves, laminar airflow cabinets, ovens, incubators, mortars and pestles, analytical balances, drying cabinets, rotary evaporators, pH meters, viscometers, filter paper, stopwatches, hot plates, magnetic stirrers, microscope slides, water baths, paper disks, and supporting laboratory equipment required for extraction, formulation, and microbiological analysis procedures.

Plant Identification

The identification of peppermint plants collected at Laboratorium Herbarium Medanense (MEDA) is essential for determining their taxonomy. Sampling was conducted purposively. Peppermint leaves were gathered from Jalan Eka Rasmi, Kelurahan Gedung Johor, Kecamatan Medan Johor, North Sumatra. After sorting, 5 kg of wet leaves were uniformly cut and dried at a temperature of 45-50°C. The dried leaves were then ground into powder and stored in a container [8].

Macroscopic Examination

Macroscopic examination of peppermint leaves (*Mentha piperita* L.) was performed organoleptically by observing the morphological characteristics of the leaves, including shape, size, color, texture, and odor. These observations were conducted to confirm the authenticity and physical characteristics of the plant material prior to further analysis.

Determination of Water Content

Water Content determination was carried out by weighing 2 g of powdered simplicia into a porcelain dish, followed by drying in an oven at 105°C until a constant weight was obtained. The sample was subsequently cooled in a desiccator prior to reweighing. The moisture content was calculated as the difference between the sample weights before and after drying [9].

Determination of Water-Soluble Extract Content

A total of 5 g of powdered simplicia was placed in a closed container, followed by the addition of 2.5 mL of chloroform and 97.5 mL of distilled water. The mixture was macerated for 24 hours, with intermittent stirring every 6 hours during the initial extraction period, and then allowed to stand for the remaining 18 hours. After filtration, 20 mL of the filtrate was evaporated to dryness in an oven at 105°C until a constant weight was achieved. The percentage of water-soluble extract content was subsequently calculated [10].

Determination of ethanol-soluble extract content

Five grams of powdered simplicia were macerated with 100 mL of 96% ethanol for 24 hours with periodic stirring during the first 18 hours. The mixture was then filtered rapidly to prevent solvent evaporation. Subsequently, 20 mL of the filtrate was evaporated in a water bath, then dried in an oven at 105°C until a constant weight was obtained. The ethanol-soluble extract content was then calculated as a percentage [8].

Determination of Total Ash Content

Total ash content determination was performed by weighing 3 g of powdered simplicia into a previously ignited and tared silica crucible. The sample was gradually incinerated until all organic matter was completely carbonized and a constant ash weight was obtained. The crucible was then cooled in a desiccator and weighed. The total ash content was calculated based on the remaining inorganic residue [9].

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Determination of Acid-Insoluble Ash Content

The ash obtained from the ash content determination was boiled with 25 ml of dilute HCl for 5 minutes. The acid-insoluble portion was then collected and filtered through ash-free filter paper. This portion was washed with hot water, ignited until a constant weight was achieved, and subsequently weighed. Finally, the acid-insoluble ash content was calculated for the air-dried material [10].

Phytochemical Screening

Phytochemical screening was conducted to identify secondary metabolites in the simplicia sample, including alkaloids, flavonoids, tannins, and saponins, using standard qualitative methods. Alkaloid identification was performed by mixing 0.5 g of powdered simplicia with 1 mL of 2N hydrochloric acid (HCl) and 9 mL of distilled water, followed by heating in a water bath for 2 minutes. The mixture was subsequently cooled and filtered, and the resulting filtrate was treated with Mayer, Dragendorff, and Bouchardat reagents to detect alkaloids. Flavonoid analysis was performed by boiling 1 g of the sample in 100 mL of distilled water for 5 minutes. After filtration, 5 mL of the filtrate was mixed with 2 mL of concentrated HCl, while 0.1 g of magnesium powder was added separately. The appearance of red coloration indicated a positive flavonoid reaction, confirming the presence of flavonoids in the sample. Tannin testing was conducted by adding 1 g of simplicia powder into 10 mL of distilled water, followed by filtration and reheating of the filtrate until a clear solution was obtained. Subsequently, 1–2 drops of 1% ferric chloride ($FeCl_3$) solution were added, and the formation of light green, dark green, or bluish coloration indicated the presence of tannins. Meanwhile, the saponin test was performed by placing 0.5 g of simplicia powder into a test tube containing 10 mL of hot distilled water, then vigorously shaking for approximately 10 minutes until a stable foam 1–10 cm high formed. The persistence of the foam after adding 2 mL of 2N HCl confirmed the presence of saponins in the sample [11].

Extraction

The crude drug powder should be placed in a macerator at a 1:10 ratio and macerated with 70% ethanol solvent. Stir continuously for the first 6 hours, then let it sit for an additional 18 hours. After maceration, separate the liquid using filter paper. Repeat the extraction at least once with the same solvent, using half the volume of solvent used in the initial extraction. Combine all macerated products and evaporate the mixture using a rotator at a temperature of 45-50°C. Yield calculation [11].

Antibacterial Activity Test

The antibacterial activity test was conducted against *Staphylococcus aureus* using the agar diffusion method. Prior to microbiological analysis, all laboratory equipment was sterilized to maintain aseptic conditions. Glassware, including beakers, Erlenmeyer flasks, test tubes, and Petri dishes, was sterilized in a dry-heat oven at 170°C for 1 hour, while inoculating loops and forceps were sterilized by flaming over a spirit lamp. Culture media were sterilized in an autoclave at 121°C for 15 minutes [12]. Nutrient Agar (NA) medium was prepared by dissolving 2.3 g of NA powder in 100 mL of distilled water under continuous stirring on a hot plate, then autoclaving at 121°C for 15 minutes [13]. Slanted agar media were prepared by dispensing 3 mL of sterile NA into sterile test tubes, which were then positioned at an angle of approximately 30–45° until solidified and subsequently stored at 5°C [14].

The bacterial stock culture was prepared using a pure culture of *Staphylococcus aureus* ATCC 12228, which was streaked aseptically onto Nutrient Agar medium in a zigzag pattern under a laminar airflow cabinet near a Bunsen burner flame. The cultures were incubated at 37°C for 24 hours to obtain pure bacterial colonies [16]. For stock culture renewal, bacterial colonies were suspended in 10 mL of 0.9% NaCl solution, then serially diluted by transferring 0.1 mL of the suspension into 9.9 mL of sterile 0.9% NaCl solution. The suspension was homogenized using a vortex mixer until the bacterial concentration reached approximately 10^6 cells/mL [15]. The bacterial inoculum was prepared by suspending one loopful of bacterial culture in 10 mL of 0.9% NaCl solution and adjusting the turbidity to match the McFarland standard equivalent to 10^8 CFU/mL. The suspension was further diluted to obtain a final bacterial concentration of 10^6 CFU/mL [17].

The peppermint leaf ethanol extract was prepared by dissolving 5 g of extract in 10 mL of distilled water to obtain a stock concentration of 500 mg/mL (50%). Serial dilutions were subsequently prepared to obtain concentrations of 400, 300, 200, 100, 90, and 10 mg/mL. The antibacterial activity assay was conducted using the agar diffusion method by pouring 15 mL of sterile Nutrient Agar medium at 45–50°C into sterile Petri dishes, followed by adding 0.1 mL of bacterial inoculum and homogenization. After solidification, sterile

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paper discs previously immersed in the extract solutions for 15 minutes were placed onto the agar surface. The cultures were incubated at 37°C for 24 hours, and the diameters of the inhibition zones formed around the discs were measured with a caliper. Distilled water was used as the negative control, while Clindamycin served as the positive control [16].

Formulation of Peppermint Leaf Ethanol Extract Hand Soap Gel

Table 1. Formulation of Peppermint Leaf Ethanol Extract Hand Soap Gel (18)

Material Name	Formula (%)				Function
	F0 (%)	F1 (%)	F2 (%)	F3 (%)	
Ekstrak daun peppermint	0	3	6	9	Active ingredients
SLS	1	1	1	1	Surfaktan
Metil Paraben	0.1	0.1	0.1	0.1	Preservative
Carbopol	2	2	2	2	Gelling agent
Gliserin	5	5	5	5	Humektan
Dinatrium-EDTA	0,1	0,1	0,1	0,1	Chelating Agent.
Asam Sitrat	0,5	0,5	0,5	0,5	Buffering agent
Triethanolamine	1	1	1	1	Alkalizing Agent
KOH	1,5	1,5	1,5	1,5	Alkalizing Agent
Minyak Lemon	1	1	1	1	Active ingredient
Aquadest	ad 100	ad 100	ad 100	ad 100	Surfaktan

Hand Wash Formulation

The hand wash formulation was prepared by dispersing Carbopol in 10 mL of hot distilled water to form mixture 1. Separately, glycerin and methyl paraben were mixed until completely dissolved to obtain mixture 2. Subsequently, mixtures 1 and 2 were combined and homogenized, then triethanolamine (TEA) and disodium EDTA were added. Sodium lauryl sulfate was dissolved in 10 mL of hot distilled water and incorporated gradually into the previous mixture under continuous stirring. Citric acid, previously diluted in 5 mL of distilled water, was then added to improve formulation stability. Furthermore, peppermint leaf ethanol extract, peppermint oil, and potassium hydroxide (KOH) were incorporated into the formulation and mixed thoroughly until homogeneous. Finally, distilled water was gradually added to reach a final volume of 100 mL, followed by continuous stirring to ensure a uniform hand wash preparation.

Evaluation of Hand Wash Gel Preparation

The evaluation of the hand wash gel preparation included organoleptic, homogeneity, spreadability, foam height, pH, viscosity, and antibacterial activity tests. Organoleptic evaluation was conducted for four weeks to observe any physical changes in the preparation, including color, odor, and consistency stability during storage [18]. Homogeneity testing was performed by applying the gel preparation onto a glass surface to determine the uniformity of the formulation. A homogeneous preparation was indicated by the absence of coarse particles and the even distribution of the gel matrix [19].

The spreadability test was carried out by placing 0.5 g of gel on a glass plate, followed by covering it with another glass plate and allowing it to stand for 1 minute. Additional loads of 50 g and 100 g were then applied sequentially for another minute, and the spread diameter was measured. A spreadability range of 5–7 cm indicated an acceptable semi-solid consistency [20]. Foam height testing was conducted by weighing 1 g of the sample into a test tube and adding distilled water until the final volume reached 10 mL. The mixture was shaken several times, and the foam height formed was measured immediately. Foam stability was evaluated after the foam stood for 5 minutes, followed by remeasurement of the remaining foam height [21].

The pH test was performed using a calibrated pH meter standardized with buffer solutions at pH 7.00 and pH 4.00. Prior to measurement, the electrode was rinsed with distilled water and dried using tissue paper until the indicator stabilized. The electrode was subsequently immersed in the gel preparation to determine the pH value [22]. Viscosity testing was conducted by placing 100 mL of the hand wash gel into a viscometer container with spindle 6 at a rotational speed of 20 rpm. The viscosity value was recorded directly from the instrument scale [23]. According to SNI 06-2588:2017, the acceptable viscosity range for hand wash gel preparations is 3,000-50,000 cPs [24].

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The antibacterial activity of the hand wash gel formulation was evaluated against Staphylococcus aureus using the agar diffusion method. Approximately 15 mL of sterile Nutrient Agar medium was mixed with 0.1 mL of bacterial suspension and poured aseptically into sterile Petri dishes until solidified. Sterile paper discs previously soaked in the peppermint leaf ethanol extract hand wash gel preparation were then placed onto the agar surface using sterile tweezers. The plates were incubated at 37°C for 24 hours, and the diameter of the clear inhibition zone around the discs was measured with a caliper to assess antibacterial activity.

Data Analysis

The data obtained in this study were processed and statistically analyzed using IBM SPSS Statistics version 25. Prior to hypothesis testing, data normality and homogeneity were assessed using the Kolmogorov–Smirnov test to evaluate the data's distributional characteristics. Subsequently, one-way analysis of variance (ANOVA) was performed to identify significant differences in mean values among the treatment groups. Statistical significance was determined based on the applicable confidence level and probability value (p-value).

Results and Discussion

Results of Macroscopic Examination and Characteristics of Simplified Powder

Macroscopic examination demonstrated that peppermint leaves (*Mentha piperita* L.) possessed an oval morphology with pointed apices, serrated margins, and a slightly rough surface texture. The adaxial surface exhibited a dark green coloration, whereas the abaxial surface appeared lighter green with prominent leaf venation and fine trichomes. These characteristics are consistent with the general morphological description of peppermint leaves reported in herbal pharmacognosy references.

Figure 1. Peppermint Leaf Macroscopic (*Mentha piperita* L.)

Table 2. Results of Examination of the Characteristics of Peppermint Leaf Simplicia

Characteristics	Result (%)	MMI
Water content	9,19%	≤ 10%
Water-soluble extract content	29,86%	≥ 22%
Ethanol-soluble extract content	18,56%	≥ 5%
Ash content	5,69%	≤ 8%
Acid-insoluble ash content	0,2%	≤ 2%

The characterization results demonstrated that all parameters fulfilled the requirements established in the *Materia Medika Indonesia* (MMI). The moisture content of 9.19% indicated that the simplicia had adequate stability during storage, as excessive moisture can accelerate microbial growth and enzymatic degradation, thereby reducing extract quality and shelf life [25]. In addition, the water-soluble extract content reached 29.86%, exceeding the minimum standard requirement of 22%, suggesting the abundance of polar compounds such as flavonoids, tannins, glycosides, and phenolic constituents in peppermint leaves [26].

The ethanol-soluble extract content of 18.56% also exceeded the minimum requirement of 5%, indicating the presence of semi-polar and non-polar bioactive compounds that are soluble in ethanol, including essential oils, terpenoids, and steroids. Ethanol is considered an effective extraction solvent because it can dissolve a broad spectrum of phytochemical constituents with varying polarity [24]. Furthermore, the total ash content of 5.69% and acid-insoluble ash content of 0.20% complied with MMI standards, indicating minimal

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contamination from inorganic impurities such as soil, silica, and metal residues. Low acid-insoluble ash values reflect good handling and processing quality of the simplicia [7].

Phytochemical Screening Results

Table 3. Phytochemical Screening Results of Simplified Peppermint Powder

No.	Secondary Metabolites	Reagents	Results
1	Alkaloids	Mayer, Bouchardat, Dragendorff	Positive (+)
2	Flavonoids	Magnesium powder + concentrated HCl, amyl alcohol	Positive (+)
3	Tannins	$FeCl_3$	Positive (+)
4	Steroids/Triterpenoids	Liebermann–Burchard	Positive (+)
5	Saponins	Hot water shaking test	Positive (+)

(+): contains a group of compounds

The phytochemical screening results confirmed the presence of several secondary metabolites, including alkaloids, flavonoids, tannins, steroids/triterpenoids, and saponins. These findings support previous studies reporting that peppermint leaves are rich in bioactive compounds with antimicrobial potential. Alkaloids exhibit antibacterial activity by interfering with peptidoglycan synthesis in bacterial cell walls, thereby disrupting bacterial structural integrity. Flavonoids can damage bacterial cell membranes by interacting with membrane proteins and phospholipids, thereby increasing membrane permeability and causing cell lysis [24].

Tannins exert antibacterial effects by precipitating proteins and disrupting bacterial enzymes, while saponins can reduce surface tension and damage membrane permeability through their surfactant-like properties. Steroid and triterpenoid compounds may also contribute to antibacterial activity by altering membrane stability and permeability. The synergistic interaction among these secondary metabolites likely accounts for the antibacterial activity of peppermint leaf extract against *Staphylococcus aureus* [24].

Peppermint Leaf Extraction Results

A total of 500 g of peppermint leaf simplicia powder was extracted by maceration with 70% ethanol. Following solvent evaporation on a rotary evaporator at 40–50°C, 87.41 g of thick extract was obtained, yielding 17.48%. This yield is considered satisfactory because it exceeds the minimum acceptable extraction yield of 10% [25]. The relatively high yield obtained indicates that peppermint leaves contain a considerable amount of extractable compounds soluble in hydroethanolic solvent.

The use of 70% ethanol as an extraction solvent was considered appropriate because ethanol-water mixtures possess intermediate polarity, enabling efficient extraction of both polar and semi-polar compounds. Moreover, the maceration method minimizes thermal degradation of thermolabile compounds, such as essential oils and phenolic constituents, thereby preserving the extract's biological activity.

Antibacterial Activity of Peppermint Leaf Ethanol Extract Against *Staphylococcus aureus* Bacteria

The antibacterial activity test demonstrated that the peppermint leaf ethanol extract exhibited concentration-dependent inhibition against *Staphylococcus aureus*. The inhibition zone diameter increased progressively with increasing extract concentration, indicating that higher concentrations contain greater amounts of antibacterial constituents that inhibit bacterial growth. At the lowest concentration (1%), the extract produced an inhibition zone diameter of 6.9 mm, indicating moderate antibacterial activity. In contrast, at the highest concentration (50%), it produced an inhibition zone diameter of 17.1 mm, indicating strong antibacterial activity.

The positive control, clindamycin, produced the largest inhibition zone diameter, 28.6 mm, confirming the sensitivity of Staphylococcus aureus to standard antibiotics. In contrast, the negative control showed no inhibition zone, indicating that the solvent used had no antibacterial effect. The observed antibacterial activity of peppermint extract is strongly associated with the presence of phenolic compounds, flavonoids, alkaloids, tannins, and essential oils such as menthol and menthone, which are known to possess broad-spectrum antimicrobial activity.

Menthol and essential oil components can penetrate bacterial membranes and disrupt membrane integrity, leading to leakage of intracellular contents and bacterial cell death. Flavonoids and tannins may also

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inhibit nucleic acid synthesis and bacterial enzyme activity. The increase in inhibition zone diameter with increasing extract concentration confirms that the antibacterial effect is dose-dependent.

Table 4. Results of the Antibacterial Activity Test of the Extract Against *Staphylococcus aureus*

Concentrations (%)	Inhibition Zone (Mean ± SD)(mm)	Category
1	6.9	Moderate
2	7.3	Moderate
3	7.8	Moderate
4	8.5	Moderate
5	10.4	Strong
6	11.1	Strong
7	11.7	Strong
8	12.6	Strong
9	12.9	Strong
10	13.5	Strong
20	14.5	Strong
30	15.8	Strong
40	16.3	Strong
50	17.1	Strong
Positive Control	28.6	Very Strong
Negative Control	-	-

Note: Inhibition zone categories: <5 mm (weak), 5–10 mm (moderate), 10–20 mm (strong), >20 mm (very strong).

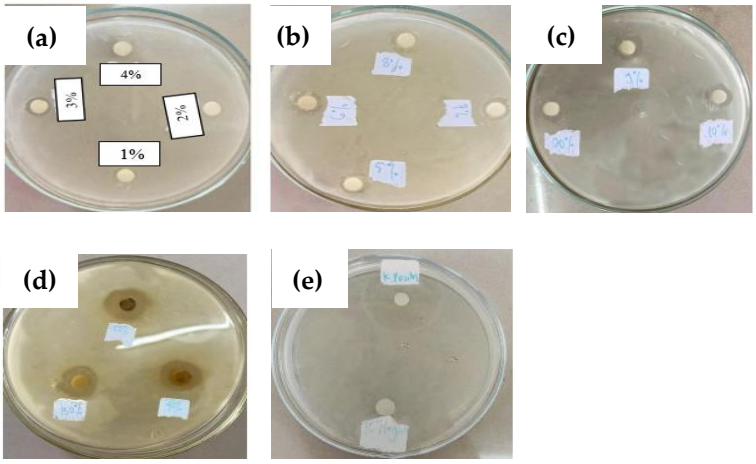

Figure 2. Results of Antibacterial Activity Test of Peppermint Leaf Ethanol Extract

Formulation Results of Peppermint Leaf Ethanol Extract Hand Soap Gel

The peppermint leaf ethanol extract was successfully formulated into a hand soap gel preparation using Carbopol as the gelling agent. Carbopol provided appropriate viscosity and gel consistency, while glycerin functioned as a humectant to maintain skin moisture. Sodium lauryl sulfate (SLS) served as a surfactant and foaming agent; triethanolamine (TEA) and potassium hydroxide acted as alkalizing agents and pH adjusters; methyl paraben functioned as a preservative; and disodium EDTA acted as a chelating agent to improve formulation stability.

The addition of peppermint leaf extract at concentrations of 3%, 6%, and 9% produced gel preparations with acceptable physical characteristics. The gel formulations appeared homogeneous, stable, and aesthetically acceptable for topical application. The incorporation of lemon oil and peppermint aroma also improved consumer acceptability by producing a pleasant fragrance. The final formulation is shown in Figure 3.

Evaluation Results of Hand Soap Gel Preparation

The organoleptic test of the peppermint leaf ethanol extract hand soap gel was performed to evaluate the physical properties of the preparation, including any changes in form, color, and odor. The results are shown in Table 5.

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Figure 3. Ready-made Peppermint Gel with Extra Ethanol

Organoleptic Test Results of Hand Soap Gel Preparation

Organoleptic evaluation revealed that all formulations maintained stable physical appearances throughout the observation period. No phase separation, discoloration, or significant odor changes were observed during storage. The increasing concentration of peppermint extract produced a progressively darker green coloration due to higher levels of plant pigments and other phytochemicals.

The stability of organoleptic properties indicates that the selected formulation components were compatible and maintained formulation integrity during storage. Stable organoleptic characteristics are essential because they influence consumer acceptance and product quality.

Table 5. Organoleptic Test Results of Hand Soap Gel Preparation

Formulation	Consistency	Smell	Color
F0	Thick	-	White
F1	Thick	Peppermint leaves	Brownish-green
F2	Thick	Peppermint leaves	Brownish-green
F3	Thick	Peppermint leaves	Brownish-green

Homogeneity test

The homogeneity test demonstrated that all hand soap gel formulations were uniformly mixed and free from coarse particles. The preparations exhibited even distribution of extract components throughout the gel matrix, indicating successful dispersion of active ingredients and excipients. Homogeneous formulations are important to ensure consistent delivery of active compounds and uniform antibacterial effectiveness during application (Figure 4).

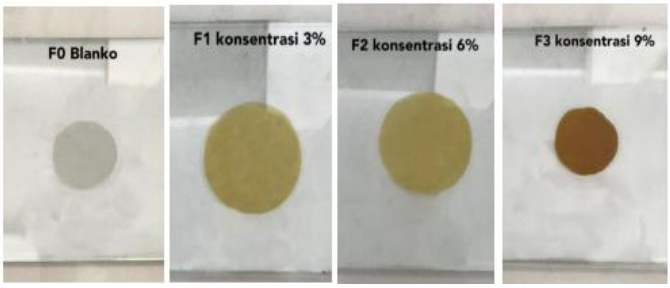

Figure 4. Homogeneity of Hand Soap Gel Preparation

Spreadability Test

The spreadability test showed that all formulations fulfilled the acceptable spreadability requirement of 5–7 cm. Adequate spreadability is important because it affects ease of application and uniform distribution of the gel on the skin surface. The results indicated that increasing extract concentration did not significantly alter gel spreadability, suggesting that the gel matrix maintained appropriate rheological characteristics.

The observed spreadability values indicate that the gel formulations possessed suitable consistency for topical use. Excessively high viscosity may reduce spreadability, whereas overly low viscosity can decrease product retention on the skin. Therefore, the obtained spreadability values demonstrate a balanced consistency suitable for hand soap gel preparations.

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Table 6. Spreadability Evaluation Results of Peppermint Leaf Ethanol Extract Gel

Formulation	Spread Diameter Without Load (cm)	Spread Diameter with 50 g Load (cm)	Spread Diameter with 100 g Load (cm)	Requirement (5–7 cm)
F0	5.0	5.3	5.7	Meets Requirement
F1	5.0	5.2	5.3	Meets Requirement
F2	5.2	5.2	5.3	Meets Requirement
F3	5.0	5.3	5.5	Meets Requirement

Viscosity Test

The viscosity values of all formulations were within the acceptable range established by SNI standards for gel preparations (3,000–50,000 cPs). The increase in viscosity observed in formulations containing peppermint extract may be attributed to interactions between phytochemical compounds and the Carbopol gel matrix.

Adequate viscosity is important for maintaining product stability, preventing phase separation, and improving residence time on the skin surface. The relatively stable viscosity values obtained in this study indicate that the formulation components were compatible and maintained gel consistency throughout storage.

Table 7. Viscosity Test Results of Hand Soap Gel Preparation

Formulation of Hand Soap Gel	Viscosity Test Parameter (cps)
F0	3432
F1	4600
F2	4600
F3	4600

Foam Height Test

All formulations met the foam height requirement per SNI standards, indicating good foaming capability and stability. Foam formation is primarily influenced by the presence of surfactants such as sodium lauryl sulfate. The higher foam height observed in formulations containing peppermint extract may be attributed to saponins, which possess natural surfactant properties.

Foam stability is an important parameter for hand soap products because it influences consumer perception of cleansing ability. Stable foam formation observed in this study indicates that the formulations possessed satisfactory cleansing characteristics.

Table 8. Foam Height Test

Preparation Formulation	Foam Height (T)	Foam Height After 5 Minutes	Description
F0	49,5mm	32,7mm	Meets Requirement
F1	61,9mm	19,8mm	Meets Requirement
F2	76,1mm	56,5mm	Meets Requirement
F3	78,6mm	49,8mm	Meets Requirement

Stability Testing of Hand Soap Gel

Stability testing conducted under room-temperature, high-temperature, and low-temperature storage conditions demonstrated that all formulations remained physically stable throughout the observation period. No significant changes in color, odor, consistency, or phase separation were observed. The absence of instability phenomena suggests that the formulation components were physically and chemically compatible. The use of Carbopol, glycerin, EDTA, and appropriate pH adjustment helped maintain gel stability during storage. Stable formulations are essential to ensure consistent product quality, efficacy, and safety throughout shelf life.

Results of the Stability Testing of Hand Soap Gel Formulations

The results of the 14-week stability test of the peppermint leaf ethanol extract hand soap gel formulation showed that storage at room temperature (28 ± 2 °C), high temperature (40 ± 2 °C), and low temperature (4 ± 2 °C) did not result in any changes in color, odor, or consistency; observations were made once a week. Thus,

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it can be concluded that the formulation exhibits good physical stability under room-temperature storage conditions. The stability test results are shown in Figures 5, 6, and 7.

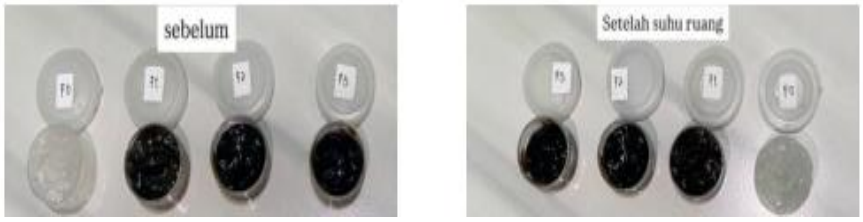

Figure 5. Room-temperature hand soap gel Formulation

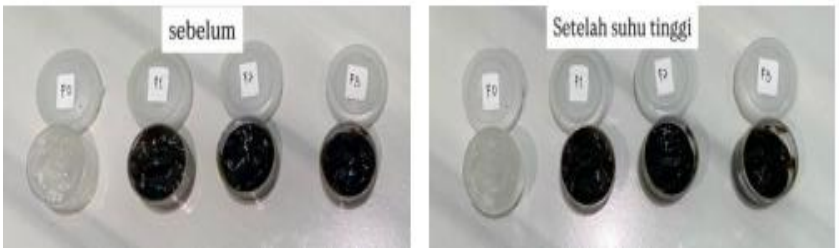

Figure 6. High- Temperature Hand Soap Gel Formulation

Figure 7. Low-Temperature Hand Soap Gel.

pH Determination

pH measurements were taken over 4 weeks at room temperature and recorded every week. The data showing the effect of storage duration on the pH of the peppermint leaf ethanol extract hand soap gel formulation are presented in Table 9.

Table 9. Results of the pH Stability Test for Hand Soap Gel Formulations

Storage Period (week)	pH value			
	F0	F1	F2	F3
1	5,99	4,91	5,78	6,50
2	5,92	4,91	5,62	6,40
3	5,87	4,85	5,60	6,34
4	5,81	4,83	5,50	6,31

The pH values in Table 9 indicate that all formulations were within the acceptable range of 4.5–6.5, consistent with the skin's physiological pH. Maintaining the formulation within this pH range is essential to reduce the risk of skin irritation associated with overly acidic preparations and to prevent skin dryness resulting from excessively alkaline conditions.

Viscosity Test Results

Viscosity measurements at room temperature were conducted over 4 weeks, with readings recorded weekly. The viscosity measurement data for the peppermint leaf ethanol extract Hand Soap Gel formulation are shown in Table 10.

Based on the viscosity test results, all formulas fell within the range of 3,000–50,000 cPs, which complies with the Indonesian National Standard (SNI 16-4399-1996) for gel preparations. Consequently, all formulas

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met the required viscosity criteria for topical dosage forms, specifically gel soaps, and demonstrated appropriate, stable thickness throughout the observation period.

Table 10. Results of the viscosity test for the peppermint leaf ethanol extract hand soap gel formulation

Storage Period (week)	Viscosity Value			
	F0	F1	F2	F3
1	8250	11250	14000	27500
2	7050	10500	12750	27500
3	6000	10000	12750	24000
4	6000	10000	11500	24000

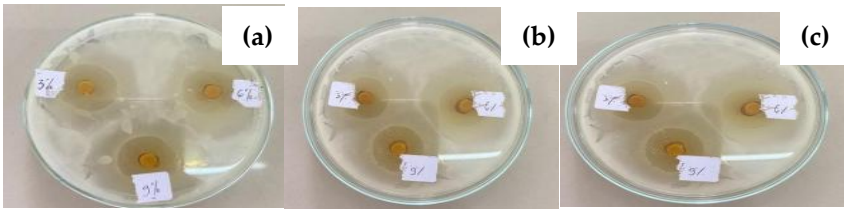
Antibacterial Inhibition Zone Diameter Results of Peppermint Leaf Ethanol Extract Hand Soap Gel Preparation

The antibacterial activity test results for Hand Soap Gel preparations containing 3%, 6%, and 9% ethanol extract of peppermint leaves are presented in Table 11.

Table 11. Results of Antibacterial Activity Test of Peppermint Leaf Ethanol Extract Hand Soap Gel

Concentration	Repetition 1(mm)	Repetition 2(mm)	Repetition 3(mm)	X±SD
3%	19,8	20,4	22,2	20.8±1,25
6%	18,6	21,3	23,1	21.0±1,40
9%	19,5	22,2	21,5	21.1±2,26
K (+)	26.4	26.9	30.3	27.9±2,12
K (-)	-	-	-	-

(+) = *Hand Soap Bi Wash*
(-) = *Base Gel Without Extract*



Repetition 1 Repetition 2 Repetition 3

Figure 8. Antibacterial Activity Test Results Hand Soap Gel.

The antibacterial activity test demonstrated that hand soap gel formulations containing 3%, 6%, and 9% peppermint leaf ethanol extract exhibited inhibitory activity against *Staphylococcus aureus*, as evidenced by clear inhibition zones surrounding the paper discs. The largest inhibition zone was observed in the 9% formulation, measuring 21.1 mm, followed by the 6% and 3% formulations with inhibition zones of 21.0 mm and 20.8 mm, respectively. Based on the inhibition zone classification, all formulations exhibited very strong antibacterial activity. The positive control, a commercial antibacterial hand soap (Hand Soap Bi Wash), produced an inhibition zone diameter of 27.9 mm. In contrast, the negative control showed no inhibitory effect, indicating that the gel base alone exhibited no antibacterial activity.

The antibacterial effectiveness observed in this study was higher than that reported by Karimi [27], who demonstrated that methanolic root extract inhibited the growth of *Staphylococcus aureus* with an inhibition zone diameter of 15.0 mm at a concentration of 200 mg/mL. The stronger inhibitory activity observed in the present study suggests that formulating peppermint extract as a hand soap gel may enhance the diffusion and release of antibacterial compounds into the agar medium. In addition, another study [28] developed a herbal hand sanitizer gel containing peppermint leaf extract, Azadirachta indica extract, and Aloe vera extract, which produced inhibition zones of 31–32 mm against *Staphylococcus aureus*, approaching the effectiveness of commercial antibacterial products.

Similarly, another investigation [29] reported that a non-alcoholic hand sanitizer formulation containing mint leaves, *Aloe vera*, and lime exhibited antibacterial activity against *Staphylococcus aureus* with an inhibition zone diameter of 16.375 mm at a concentration of 100%. Compared with previous studies involving Lamiaceae plants, the peppermint hand soap gel formulation evaluated in the present study demonstrated relatively high

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antibacterial effectiveness, particularly compared with single crude extract preparations. These findings indicate that the formulation system, gel stability, and interactions between active compounds and excipients may enhance the antibacterial potential of peppermint leaf extract against *Staphylococcus aureus*.

Data Analysis

The statistical analysis demonstrated that all datasets were normally distributed and homogeneous, as indicated by the Kolmogorov–Smirnov and homogeneity tests, with p-values greater than 0.05. One-way ANOVA analysis revealed significant differences among treatment groups ($p < 0.05$), indicating that the peppermint extract concentration significantly influenced antibacterial activity.

Subsequent Tukey post-hoc analysis demonstrated significant differences between the positive control and all gel formulations. However, no statistically significant differences were observed among the 3%, 6%, and 9% gel formulations. These findings suggest that although increasing extract concentration slightly increased the inhibition zone diameter, the antibacterial effects among the tested formulations were relatively comparable. Overall, the findings of this study demonstrate that peppermint leaf ethanol extract can be successfully formulated into a stable hand soap gel preparation with strong antibacterial activity against *Staphylococcus aureus*. The formulation exhibited satisfactory physical characteristics, stability, and antibacterial efficacy, indicating its potential for use as a natural antibacterial cleansing product.

Conclusions

Peppermint leaf ethanol extract (*Mentha piperita* L.) was successfully formulated into a hand soap gel preparation that fulfilled the required physical evaluation parameters, including organoleptic properties, homogeneity, spreadability, foam height, pH, viscosity, and stability. The hand soap gel formulations containing 3%, 6%, and 9% extract concentrations demonstrated very strong antibacterial activity against *Staphylococcus aureus*, with inhibition zone diameters ranging from 20.8 to 21.1 mm. Statistical analysis using One-Way ANOVA indicated significant differences among treatment groups ($p < 0.05$). These findings suggest that peppermint leaf ethanol extract has potential for development as a natural antibacterial hand-cleansing preparation.

Conflict of Interest

The authors declare no conflict of interest.

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