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Production, Quality Test, and Activity Evaluation of Methanol Extract and Nanoparticles from *Pluchea indica* (L.) Leaves as Additives in Natural Soap against *Propionibacterium acnes*

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Abstract

Pluchea indica (L.) leaves, both in the form of extracts and silver nanoparticles (AgNPs), are known to exhibit antibacterial activity, but they have not been widely utilized in the health sector, especially in dermatology. This study aims to determine the quality test and antibacterial evaluation of natural soap formulated with methanol extract and nanoparticles of *Pluchea indica* (L.) leaves against *Propionibacterium acnes*. The synthesis of AgNPs was conducted using the reduction method, with $AgNO_3$ as a precursor and methanol extract of *Pluchea indica* (L.) leaves (MEPL) serving as a reducing agent or bioreductor. Natural soap was produced through the cold process method, with a curing period of 3 to 4 weeks. Natural soap, derived from herbal ingredients, was made by combining NaOH, essential oils, and additives such as methanol extract and AgNPs from *Pluchea indica* (L.) leaves. Quality tests were performed to assess moisture content (MC), fatty acid content, and pH levels. Antibacterial activity was assessed utilizing the well-diffusion method, with various sample concentrations ranging from 1.0 g/mL to 0.0625 g/mL. The quality test results for all soap samples met the Indonesian National Standard (SNI) requirements for acidity, free fatty acids, and moisture content. The bar soap without additives exhibited antibacterial activity, with a maximum inhibition zone of 14.26 mm (at 0.25 g/mL) after 15 hours. Natural soap with MEPL demonstrated an inhibition zone of 11.49 mm (at 0.0625 g/mL) after 15 hours, while nanoparticle soap exhibited an inhibition zone of 12.77 mm (at 0.25 g/mL) after 12 hours. In summary, all soap samples showed antibacterial activity, indicating the potential of *Pluchea indica* (L.) leaves as effective antibacterial agents.

1. Introduction

Among the many plants scattered across the archipelago, the majority can be utilized for human survival. However, the utilization of most plants is still limited, often involving only the fruit. The study conducted by Kurniawati *et al.* [1] shows that the most consumed part of the fruit is the flesh, which is found in as many as 25 species, whereas the least consumed is the fruit skin, which is found in only one species. One of the plants that is not used to its full extent is *Pluchea indica* (L.). It belongs to the *Asteraceae* family and contains various secondary metabolites such as flavonoids, tannins, alkaloids, sesquiterpenoids, monoterpenes, glycosides, lignin, and triterpenoids [2].

One of the current technological developments is nanotechnology, which is considered to present significant challenges and opportunities for advancing the life sector [3]. Nanotechnology is a science in modern research related to the synthesis, design, and manipulation of particles that have a size range of 1-1000 nm [4]. The production of AgNPs requires precursor agents and reducing agents. A commonly used precursor agent in nanoparticle synthesis is silver nitrate ($AgNO_3$) [5]. Reducing agents is essential to reduce Ag^+ ions to Ag^0

[6]. Secondary metabolite compounds found in plants, such as phenolic acids, flavonoids, tannins, alkaloids, and terpenoids [7], are proven to play a role in the biosynthesis process of AgNPs [8].

Pluchea indica (L.) plants have the potential as antibacterial agents [9] and their secondary metabolites also have the potential to be bioreductors [10]. However, many people have yet to harness the potential of *Pluchea indica* (L.) plants in the health sector, particularly in dermatology. The ethanol extract of *Pluchea indica* (L.) twigs contains compounds such as phenolic acids, flavonoids, tannins, alkaloids, and terpenoids [7]. Hence, *Pluchea indica* (L.) plants harbor potential bioactive compounds applicable in synthesizing AgNPs.

AgNPs synthesized with plant extracts are typically employed as antibacterials, particularly against skin bacteria. One of the bacteria affecting the skin is *Propionibacterium acnes*, which is a type of normal skin flora that can lead to acne under certain conditions. Antibiotics like erythromycin, clindamycin, and tetracycline can be used against *Propionibacterium acnes* bacteria [11]. Nevertheless, the escalating use of antibiotics can contribute to increased bacterial resistance to these agents [12]. To address bacterial resistance to antibacterial agents, research needs to be directed towards discovering more effective agents that do not induce resistance. One promising approach involves the utilization of AgNPs synthesized from *Pluchea indica* (L.) leaf extracts.

The dosage form of cosmetic products that can be used to maintain skin health and prevent skin infections is soap [13]. Soap is a product produced from the reaction between fatty acids and strong bases that function to wash and clean fat or dirt [14]. Soap is a mixture of sodium or potassium salts of long-chain fatty acids resulting from hydrolysis (saponification) of animal fat with alkali [15]. The quality requirements for solid bath soap by SNI 2016 are to have a maximum moisture content of 15%, a maximum amount of free alkali of 0.1%, free fatty acids of 2.5% and a pH between 8 and 11.

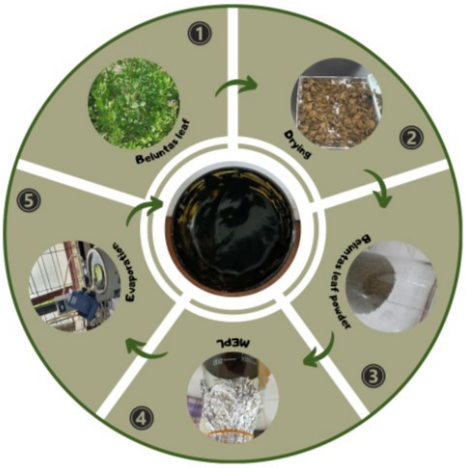

Figure 1. Sample preparation flow chart

Along with the development of chemical and pharmaceutical science and technology, the evolution of cosmetic products has shifted towards natural ingredients as a result of the back-to-nature trend [16]. To enhance soap quality, additional ingredients containing vitamins and various skin-care nutrients are incorporated, along with active ingredients that can reduce pathogenic microorganisms on the skin [17]. While the soap industry currently employs chemicals to improve soap quality, not all additives are safe for sensitive skin. Therefore, additional ingredients from natural sources, such as guava leaves, are being explored as antibacterial agents in soap [18].

Another natural ingredient that has been proven to have potential as an antibacterial against *Propionibacterium acnes* is honey [19]. Additional ingredients can include natural elements from the surrounding environment, such as *Pluchea indica* (L.) leaves, as *Pluchea indica* (L.) leaf extract exhibits antibacterial activity [20]. However, no antibacterial activity test has been conducted on soap with the addition of *Pluchea indica* (L.) extract or with AgNPs against *Propionibacterium acnes* bacteria. Thus, the focus of this research is the production of natural soap with addition of MEPL and AgNPs, along with their antibacterial activity test against *Propionibacterium acnes.*

2. Experimental

2.1. Materials

Pluchea indica (L.) leaves taken from Bangunharjo, Sewon, Bantul. $AgNO_3$, methanol (analytical grade), coconut oil, palm oil, rice bran oil, olive oil, 38% NaOH, 0.1 N KOH, 0.1 N HCl, sterile distilled water, *Propionibacterium acnes* bacteria, nutrient agar (NA) media, muller hinton agar (MHA) media, nutrient brooth (NB) media, 3% clindamycin, Asepso soap, phenolphthalein indicator, ethanol, fragrance, aluminum foil, and McFarland 0.5 solution with a bacterial density of 1.5×10^8 CFU/mL.

2.2. Sample Preparation

Pluchea indica (L.) leaves were cleaned with running water to remove impurities. A total of 1.5 kg of *Pluchea indica* (L.) leaves were oven-dried at 50°C for 24 hours, resulting in 250 g of dried *Pluchea indica* (L.) leaves. The dried leaves were then pulverized using a blender and macerated using methanol solvent. This process involved mixing 250 g of *Pluchea indica* (L.) leaves with 1000 mL of methanol for each maceration. After 24 hours, the solution was filtered using a clean cloth and separated. The remaining filtrate from *Pluchea indica* (L.) leaves underwent two more maceration processes. The overall maceration process yielded 1.4 L of extract, which was then evaporated using a rotary evaporator at 55°C. The resulting evaporation product was stored in a clean, sealed container. The flowchart of the sample preparation process can be seen in Figure 1.

2.3. Phytochemical Screening Test

2.3.1. Flavonoid Test

The Wilstater flavonoid test was conducted by mixing 5 mL of the MEPL with 100 mL of hot water, followed by boiling for 5 minutes and filtration using Whatman No. 1 paper. Subsequently, 0.05 g of magnesium powder and 1 mL of concentrated HCl were added to the filtrate. A red or orange coloration of the solution indicated the presence of flavonoids. Additionally, the flavonoid test was performed using alternative reagents: mixing 5 mL of the extract with 4 mL of 10% NaOH, where the presence of flavonoids was indicated by a yellowish solution.

2.3.2. Tannin Test

The tannin test was conducted by mixing 5 mL of the MEPL with three drops of 10% $FeCl_3$, followed by shaking the solution until homogeneous. The presence of tannins was indicated if the solution turned blackish.

2.3.3. Steroid or Terpenoid Test

The steroid or terpenoid test was performed by mixing 5 mL of the MEPL with three drops of Lieberman-Burchard reagent. The presence of steroids or terpenoids was indicated if the solution turned red, orange, or purple.

2.3.4. Saponin Test

The saponin test was conducted by reacting 5 mL of the MEPL with 10 mL of water and shaking the solution for one minute. Subsequently, two drops of concentrated HCl were added to the mixture. The presence of saponins was indicated if the foam on the surface of the solution remained stable for up to 7 minutes [20].

2.3.5. Alkaloid Test

The alkaloid test was conducted by reacting 10 mL of MEPL with 2 mL chloroform and 2 mL ammonia. Afterward, it was filtered using filter paper. Five drops of concentrated sulfuric acid were added to the filtrate, which was then shaken to form two layers of an immiscible solution. Mayer reagent was added to 2 mL of the top layer of the solution, while Dragendorff reagent was added to the other 2 mL of the top layer. Positive alkaloid content was indicated if the Mayer reagent solution formed a white precipitate and the Dragendorff reagent formed an orange precipitate [21].

2.4. Preparation of AgNPs using *Pluchea indica* (L.) Leaves

Silver nitrate ($AgNO_3$) served as the precursor for producing silver ions. Specifically, 1 mL of 0.3% MEPL was mixed with 90 mL of a 0.5 mM $AgNO_3$ solution. The resulting mixture was stirred using a magnetic stirrer for 15 minutes. Following the mixing process, the solution was incubated in a dark room for 24 hours to facilitate the reaction. Subsequently, the solution was analyzed using a UV-Vis spectrophotometer, PSA (particle size analyzer), and SEM.

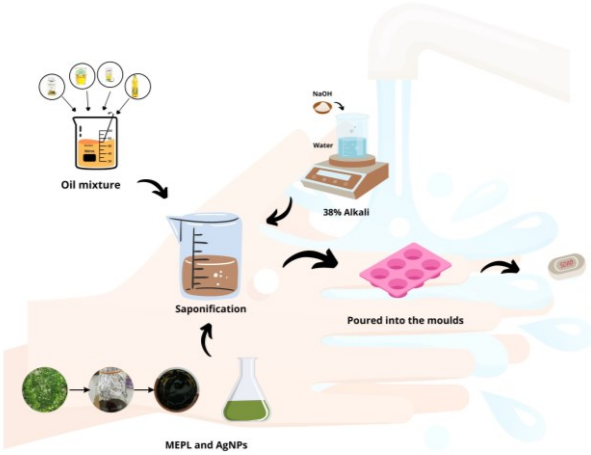

Figure 2. Soap-making flow chart

2.5. Antibacterial Activity Test of AgNPs

The antibacterial activity test of MEPL-AgNPs was conducted using the well-diffusion method. In this procedure, 50 µL of MEPL-AgNPs solution at concentrations of 100%, 50%, 25%, 12.5%, and 6.25% were dispensed into the wells of the agar media. The antibacterial activity was measured by observing the inhibition zone formed on the media, and the sampling process was repeated three times. In the antibacterial test, a 3% positive control of clindamycin was utilized, while distilled water served as the negative control.

2.6. Soap-Making Process

The soap-making process was started by mixing 35 g of palm oil, 30 g of coconut oil, 20 g of olive oil, and 15 g of rice bran oil. Then, 7.5 g of MEPL and MEPL-AgNPs were each added to the mixture. The mixture was combined with 38% NaOH and stirred until it reached trace conditions. The result of the saponification reaction was poured into a mold and left to undergo the curing process, which typically lasted around 3-4 weeks. The curing process is the waiting phase after the soap solidifies, taking approximately 3-4 weeks. The flow chart of the soap-making process can be seen in Figure 2.

2.7. Soap Quality Test

The soap quality test process involved a pH test, moisture analysis, and free fatty acid or free alkali test. The soap's pH was measured using a universal indicator. The moisture analysis was conducted by using a petri dish that was previously oven-dried at 105°C for 30 minutes and then weighed (W_1). Subsequently, 5 g of the soap preparation was weighed and placed in the petri dish, which was then heated in the oven for 1 hour at 105°C (W_2). The procedure was repeated until a constant weight was achieved.

The free fatty acid/free alkali content test was performed by weighing 5 g of the soap sample and placing it into neutral alcohol, followed by boiling it in a water bath for 30 minutes. If the solution turned red, the free alkali content was determined by titrating 0.1 N HCl in alcohol from the burette until the red color disappeared. However, if the solution did not turn red, it was cooled at 70°C and titrated with 0.1 N KOH solution in alcohol until

a consistent color appeared for 15 seconds, after which the fatty acid content was determined.

2.8. Soap Antibacterial Activity Test

Soap samples were made at concentrations of 1.0 g/mL (100%), 0.5 g/mL (50%), 0.25 g/mL (25%), 0.125 g/mL (12.5%) and 0.0625 g/mL (6.25%). A commercially available antibacterial soap was used as a positive control (Asepso soap). The glassware was sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes before the antibacterial activity test. For this natural soap antibacterial test, the MHA selective medium was utilized by dissolving 22.8 g of MHA in 600 mL of distilled water. In addition to the MHA selective medium, other media were prepared, such as NA as a bacterial growth medium and NB as a bacterial enrichment medium.

NA medium was made by dissolving 14.045 g of NA powder into 500 mL of distilled water, while NB medium was prepared by dissolving 1.3 g of NB powder in 100 mL of distilled water. All media were sterilized to prevent interference from other bacterial contaminants during antibacterial test observations. After that, each sample was pipetted into a well and incubated at room temperature for 48 hours. Subsequently, the diameter of the inhibition zone was measured three times every 3 hours over 48 hours for bacteria by calculating the inhibition zone formed in each well using a caliper. This measurement indicated the presence of antibacterial activity against *Propionibacterium acnes* [22].

3. Results and Discussion

The study involved synthesizing AgNPs from methanol extract of *Pluchea indica* (MEPL-AgNPs) and subsequently utilizing these nanoparticles to produce bar soap. Both the MEPL bar soap and the MEPL-AgNPs bar soap were subjected to quality testing and evaluation of their antibacterial properties. *Pluchea indica* (L.) leaf samples were carefully washed and oven-dried at 60°C for 24 hours. The chosen oven temperature of 60°C was aimed to preserve secondary metabolite compounds such as flavonoids, saponins, and tannins that serve as essential reducing agents. According to Wahyuni *et al.* [23], flavonoids remain undamaged under temperatures up to 90°C, saponins are resistant to temperatures up to 70°C, and tannins undergo damage or decomposition at temperatures ranging from 98.89°C to 101.67°C. Following the drying process, the samples were powdered to enhance the surface area, facilitating a more efficient extraction process due to increased contact with the solvent [9].

Maceration was chosen as the extraction method for *Pluchea indica* (L.) leaves due to its simplicity, cost- effectiveness, and utilization of basic tools. The extended contact time with the solvent allows for the binding of active compounds within the sample. The absence of heat in the maceration process is deliberate to avoid damage to secondary metabolites sensitive to heat in *Pluchea indica* (L.) leaves [24]. Remaceration, occurring once every 24 hours, enhances the effectiveness of extraction, maximizing the retrieval of active compounds from *Pluchea indica* (L.) leaf samples.

Upon completing maceration, the macerate underwent filtration and concentration using a rotary evaporator. This process was intended to separate active compounds in *Pluchea indica* (L.) leaves from the methanol solvent. The concentration process continued until the solvent was entirely evaporated, indicated by the cessation of solvent dripping in the condenser, resulting in a macerate with a paste-like consistency. The concentration of the macerate was confirmed by weighing 30 mg of the thick *Pluchea indica* (L.) leaf extract paste, which was then dissolved in 10 mL of methanol, yielding an extract concentration of 30 mg/mL. This 30 mg/mL extract served for the phytochemical screening test and as a reducing agent for silver nanoparticle synthesis.

Phytochemical screening of MEPL (Table 1) revealed the presence of positive flavonoid compounds detected with both HCl reagent and Mg powder, as well as with 10% NaOH reagent. In addition to flavonoids, the MEPL was identified to contain tannins, verified by the 10% $FeCl_3$ reagent and saponins, confirmed through the foam stability test. However, alkaloid compounds in the MEPL were not detected using either Dragendorff or Mayer reagents. Steroid and terpenoid compounds were also absent in the sample, possibly due to their low concentration, leading to non-detection. In contrast, another study mentioned the detection of alkaloids, steroids, and terpenoids in the MEPL [25].

Table 1. Phytochemical screening results of MEPL

Phytochemical Compound	Indicator	Result	Color
Flavonoid			
a. HCl + Mg	Orange/Red	+	Reddish green
b. 10% NaOH	Yellowish	+	Yellowish green
Tanin (10% $FeCl_3$)	Blackish	+	Blackish green
Saponin	Stable foam	+	Foamy
Alkaloid			
a. Dragendorff	Orange precipitant	-	No precipitant
b. Mayer	White precipitant	-	No precipitant
Steroid/Terpenoid	Red/Purple	-	Remains green

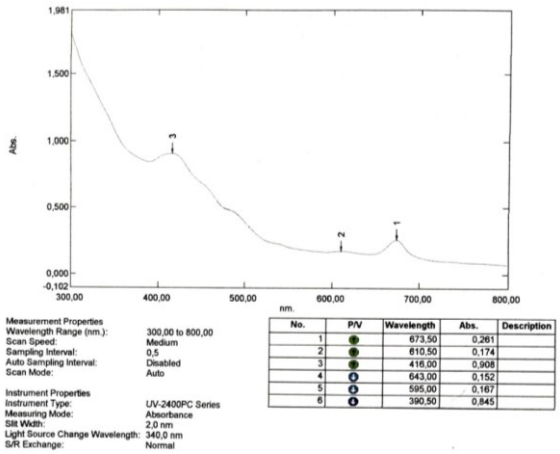

Figure 3. UV-Vis spectrum of MEPL-AgNPs

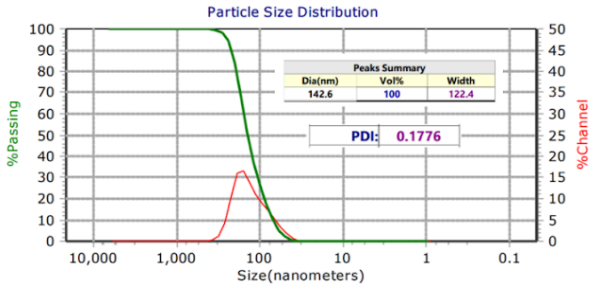

Figure 4. PSA spectrum of MEPL-AgNPs

The results of the synthesized MEPL-AgNPs revealed an incubation time of 24 hours, an $AgNO_3$ solution concentration of 0.5 mM, and no incorporation of a stabilizer. The synthesized MEPL-AgNPs exhibited a peak wavelength of 416 nm, with an absorbance value of 0.908, and a uniform spherical shape with a size of 142.6 nm. The spectrum of UV-Vis spectrophotometer and PSA results for MEPL-AgNPs can be seen in Figures 3 and 4.

In the synthesis of AgNPs using plant extract, MEPL-AgNPs are formed through the reduction reactions of Ag^+ ions. Hydroxyl (−OH) groups present in secondary metabolite compounds play a pivotal role in reducing silver ions into AgNPs [26]. These functional groups actively donate electrons to Ag^+ ions, facilitating the generation of AgNPs. Phenolic compounds derived from secondary metabolites function as bioreducing agents, transforming Ag^+ ions into Ag^0 nanoparticles capped by the extract's active compound. The scheme of bioreduction of $AgNO_3$ by phenolic compound into MEPL-AgNPs can be seen in Figure 5 [27].

The characteristics of the MEPL-AgNPs formed can be observed in the results of SEM characterization, as depicted in Figure 6. The magnification of the MEPL-AgNPs ranges from 300 to 30,000× with an acceleration voltage of 15 kV. SEM analysis of each image reveals that the generated particle sizes exhibit a lack of pores, nearly identical shapes, and a uniform particle distribution. The diverse or uneven sizes observed can be attributed to aggregation effects or the tendency of particles to aggregate, resulting in non-uniform particle sizes [28, 29]. The absence of detectable silver (Ag) elements in the sample may be due to suboptimal magnification, excessively high acceleration voltage, and improper determination of testing points during the analysis, leading to the undetected presence of silver elements.

The diffusion method is one of the methods used for testing antibacterial activity. In this method, the antibacterial activity is assessed based on the ability of antimicrobial substances (samples) to diffuse into agar media that has been previously inoculated with test bacteria. The determination of antibacterial activity occurs through the observation of an inhibition zone on the agar medium [30]. Three diffusion methods, namely the disc, ditch, and hole/cup methods, are commonly employed to evaluate antibacterial activity.

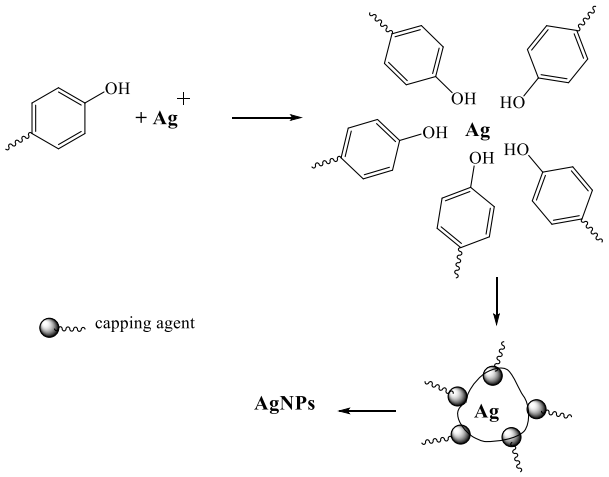

Figure 5. Reduction of $AgNO_3$ to MEPL-AgNPs by phenolic compound

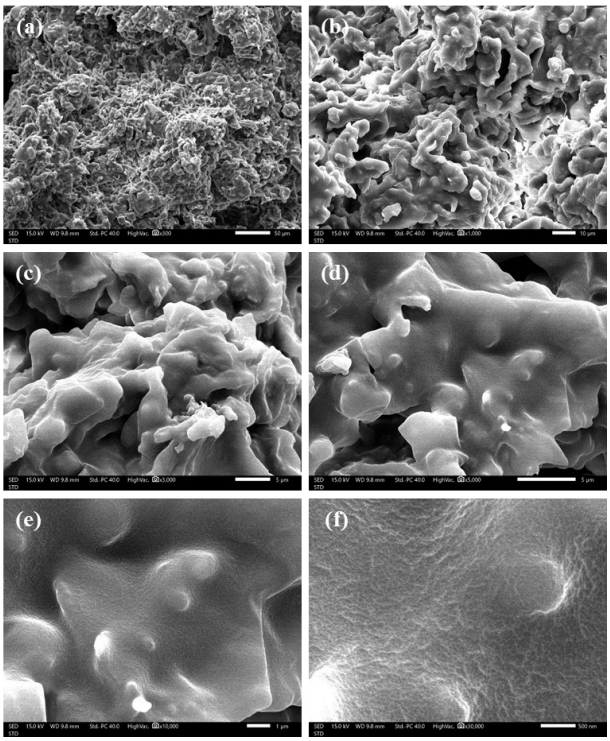

Figure 6. SEM images of MEPL-AgNPs with magnification of (a) 300×, (b) 1.000×, (c) 3.000×, (d) 5.000×, (e) 10.000×, and (f) 30.000×

Table 2. Average diameter of inhibition zone of MEPL-AgNPs at 24 hours of incubation time

MEPL-AgNPs sample	Average diameter of inhibition zone diameter (mm)
A (100%)	9.94
B (50%)	7.94
C (25%)	5.91
D (12.5%)	0
E (6.25%)	0
Asepso soap (+)	42.91
Distilled water (-)	0

In the present study, the antibacterial activity against *Propionibacterium acnes* bacteria was evaluated using the pitting diffusion method. The test samples used to impede bacterial growth included MEPL-AgNPs, distilled water as a negative control, and Asepso soap as a positive control. AgNPs were prepared in six concentration variations. Antibacterial activity was characterized by the formation of a clear zone or zone of inhibition around the wells containing the test samples. The inhibition zone is a translucent area signifying the inhibition of bacterial growth in that environment. The observation and measurement of the zone of bacterial inhibition were conducted at three-hour intervals for 48 hours by measuring the diameter of the inhibition zone. The inhibition MEPL-AgNPs against *Propionibacterium acnes* bacteria with five types of concentrations can be seen in Table 2.

The inhibitory effect of MEPL-AgNPs against *Propionibacterium acnes* was observed to commence at hour 12. The average amount of inhibition formed at each concentration of MEPL-AgNPs between hours 12 to 48. The antibacterial activity test results indicated that the nanoparticles were effective in inhibiting the growth of *Propionibacterium acnes* bacteria. The diameter of the inhibition zone at each concentration, on average, produced a smaller size compared to the positive control inhibition zone and a larger size compared to the negative control inhibition zone.

At 18 hours of incubation time, the most potent antibacterial activity of MEPL-AgNPs was observed at a concentration of 100%, yielding an inhibition zone diameter of 10.94 mm. This sample exhibited strong efficacy in inhibiting the growth of *Propionibacterium acnes* bacteria. Conversely, the MEPL-AgNPs with the least antibacterial activity were those with a concentration of 25% at 42 hours of incubation time. This sample displayed an inhibition zone diameter of 4.95 mm, indicating a weak antibacterial effect [31].

The minimum inhibitory concentration (MIC) is the smallest concentration of antibacterial substances that can still inhibit bacterial growth after incubation for 24 hours, with no growth of bacterial colonies. This can be determined by observing the number of bacterial colonies that experience inhibition in growth through the magnitude of the inhibition zone in the sample [32]. MIC is often used as a technique to determine the minimum concentration of antibacterial substances needed to inhibit the growth of a bacterium. This information can later be used for effective dose determination to control an infection against bacteria [31]. Figure 7 shows that MEPL-AgNPs at a concentration of 25% have the ability to inhibit the growth of *Propionibacterium acnes*. This is supported by the MIC value of 25% and an inhibition zone diameter of 5.91 mm.

When measuring the pH of MEPL-AgNPs bar soap, the recorded pH was 9.36. In contrast, the pH of plain soap was measured at 9.05. The standard pH range for soap typically falls between 9 and 11, with measurements within this range considered relatively safe for the skin [33]. The pH value of the soap-containing nanoparticles remains relatively stable due to the consistent use of a 38% NaOH concentration, resulting in only minor fluctuations in pH. It is crucial to maintain a balanced pH in soap formulations, as excessively acidic pH levels can lead to skin irritation, while overly basic pH levels can cause skin dryness and scaliness. In the measurement of MEPL-AgNPs bar soap and plain soap as a control, the results of moisture content in percent mass fraction were 4.94 and 3.95, respectively, ensuring that the soap preparation complies with the Indonesian National Standard for bar soap.

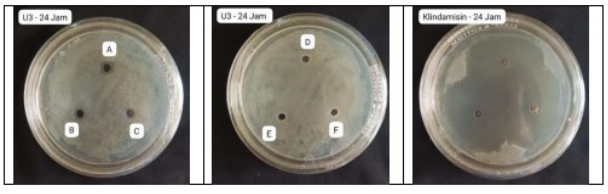

Figure 7. Inhibition zones of MEPL-AgNPs bar soap after 24 hours incubation

Table 3. Soap quality test

No.	Parameter	Analysis result			Quality standard
		Plain soap	MEPL bar soap	MEPL-AgNPs bar soap	
1.	pH	9.05	9.18	9.36	6.0-11.0
2.	Free fatty acids (%mass fraction)	0.088	0.0861	0.0574	Max 2.5 %
3.	Moisture content (%mass fraction)	3.95	3.78	4.94	Max 23%
4.	Soap presentation				-

According to the Indonesian National Standard [34], the maximum moisture content in bar soap preparations is 23%. Excessive moisture content in soap preparations can lead to easy shrinkage and discomfort during use. Moisture content significantly influences soap hardness; higher moisture content results in softer soap, while lower moisture content contributes to harder soap [35]. In the measurement of MEPL-AgNPs bar soap and plain soap, the results of free fatty acids in percent mass fraction were 0.0574% and 0.088%, respectively. These findings align with the Indonesian National Standard parameters, where the maximum acceptable level of free fatty acids in soap is 2.5%. For a detailed presentation of the soap quality test results, refer to Table 3.

Based on the antibacterial test of natural soap (Table 4), MEPL bar soap exhibits antibacterial activity, evident from the presence of inhibition zones. The inhibition zone is characterized by a translucent area surrounding the well. The well-diffusion method was chosen for the antibacterial activity test as it is deemed more effective than the disc diffusion method. In this study, the tested samples included MEPL bar soap and plain soap, both with concentrations of 100%, 50%, 25%, 12.5%, and 6.25%. Asepso antibacterial soap served as the positive control, while distilled water was used as the negative control, considering that *Propionibacterium acnes* bacteria thrive in contact with distilled water [36]. Asepso antiseptic soap was chosen as the positive control due to its documented antibacterial activity, which is evident from the formation of an inhibition zone.

The antibacterial activity observed is influenced by the compounds present in *Pluchea indica* (L.) leaf extract. *Pluchea indica* (L.) possesses metabolite compounds such as alkaloids, flavonoids, tannins, and essential oils [37]. The antibacterial mechanism of flavonoids involves damaging microbial membranes, and these compounds can also interfere with peptidoglycan transpeptidase activity, disrupting cell wall formation and leading to lysis [38]. The categorization of the inhibition zone is based on strength, with criteria including very strong, strong, medium, and weak categories. Criteria for the antibacterial activity strength are defined by inhibition zone diameters: <5 mm as weak, 5-10 mm as moderate, 10-20 mm as strong, and >20 mm as very strong [31].

The antibacterial activity of MEPL bar soap that inhibits bacterial growth is 0.0625 g/mL, resulting in an inhibition zone of 11.49 mm at the 15^{th} hour. In comparison, plain soap exhibits bacterial growth inhibition at a concentration of 0.0625 g/mL, with an inhibition zone of 11.97 mm at the 15^{th} hour.

Surprisingly, antibacterial activity of MEPL bar soap is lower than that of plain soap. This discrepancy could be attributed to the combination of antibacterial substances present in bar soap formulations with additives. The formulation of MEPL bar soap includes olive oil, rice bran oil, coconut oil, and palm oil as the oil mixture. The known antibacterial properties of olive oil and coconut oil, combined with *Pluchea indica* (L.) leaf extract, may interact in a way that weakens their individual effects, resulting in a lower inhibition zone compared to plain soap.

The formation of surfactants in soap can also enhance antibacterial activity. Surfactants, or surface-active agents, are chemical compounds with both hydrophobic (oil and fat affinity) and hydrophilic (water affinity) parts. In soap, surfactants work by reducing the surface tension between oil and water, allowing oils and dirt to dissolve in water and be rinsed away. The antibacterial mechanism of surfactin can be summarized as follows: (1) disrupting the cell membrane of pathogenic bacteria, leading to membrane disintegration or an imbalance in osmotic pressure; (2) hindering the protein synthesis of pathogenic bacteria, thereby impeding cell reproduction; (3) suppressing the enzyme activity of pathogenic bacteria, influencing normal cell metabolism [39].

Table 4. Comparison of soaps' inhibition zone diameter

Sample	Concentration (g/mL)	Inhibition zone diameter (mm)								
		3 h	6 h	9 h	12 h	15 h	18 h	21 h	24 h	48 h
MEPL-AgNPs bar soap	1.0000	0	0	0	8.14	7.46	7.21	8.49	7.49	3.32
	0.5000	0	0	0	8.4	9.85	10.01	10.74	10.53	8.69
	0.2500	0	0	0	12.77	10.43	10.47	11.27	10.73	9.09
	0.1250	0	0	0	11.79	10.22	10.01	10.93	10.71	8.52
	0.0625	0	0	0	8.31	6.8	5.3	5.3	6.84	0
MEPL bar soap	1.0000	0	0	0	9.35	9.13	7.26	6.6	8.99	9.35
	0.5000	0	0	0	10.075	7.9	6.31	6.3	5.76	10.075
	0.2500	0	0	0	10.51	7.03	7.11	5.9	6.66	10.51
	0.1250	0	0	0	10.43	9.57	11.21	8.71	8.82	10.43
	0.0625	0	0	0	7.89	11.49	8.35	9.95	8.25	7.89
Plain soap	1.0000	0	0	0	9.74	10.57	10.07	9.72	9.87	8.3
	0.5000	0	0	0	9.8	11.78	11.8	11.08	10.08	8.8
	0.2500	0	0	0	11.91	14.26	13.45	13.43	13.72	10.03
	0.1250	0	0	0	12.24	13.56	12.62	12.54	13.11	10.08
	0.0625	0	0	0	10.47	11.97	10.83	10.06	10.48	8.67
Asepso soap (+)		0	0	0	12.33	11.33	11.05	10.62	11.68	11.33
Distilled water (-)		0	0	0	0	0	0	0	0	0

The efficacy of MEPL bar soap can be attributed to the presence of secondary metabolite compounds with antibacterial potential, including flavonoids, alkaloids, saponins, tannins, and steroids. Additionally, *Pluchea indica* (L.) contains essential oils that further contribute to its antibacterial properties [40]. The results of the antibacterial activity test for bar soap with silver nanoparticle additives from MEPL at various concentrations indicate that all soap samples exhibit antibacterial activity. Observations were conducted every 3 hours for 48 hours. The minimum concentration capable of inhibiting bacterial growth is the 0.0625 g/mL sample, resulting in an inhibition zone of 8.13 mm for nanoparticle soap at the 12th hour, categorized as having moderate antibacterial properties.

The optimum concentration for inhibiting bacterial growth is the 0.25 g/mL sample, producing an inhibition zone of 12.77 mm at the 12th hour, categorized as a strong antibacterial agent. The antibacterial activity test on the control, plain soap, revealed a minimum concentration capable of inhibiting bacterial growth, namely a sample of 0.0625 g/mL with an inhibition zone of 11.97 mm at the 15th hour, categorized as having strong antibacterial properties. The optimum concentration for inhibiting bacterial growth is the 0.25 g/mL sample, resulting in an inhibition zone of 14.26 mm at the 15th hour, categorized as a strong antibacterial agent.

Asepso soap, as a positive control, exhibited the largest inhibition zone of 12.33 mm. This is attributed to chloroxylenol in Asepso soap, which contains properties that are capable of killing bacteria, damaging bacterial cell walls, and causing the inactivation of bacterial enzymes. In contrast, distilled water, serving as a negative control, did not exhibit any inhibition zone around the well area. This absence of inhibition is because distilled water lacks antibacterial activity.

The limited antibacterial activity of MEPL-AgNPs may stem from the thickness of the peptidoglycan layer in the tested bacteria. *Propionibacterium acnes*, a gram-positive anaerobic bacteria predominant in acne lesions [41], reacts differently to AgNPs. Gram-positive bacteria, with thicker cell walls due to the peptidoglycan layer, exhibit greater resistance to AgNPs compared to gram-negative bacteria [42].

The antibacterial activity demonstrated by MEPL-AgNPs bar soap additives is surprisingly lower than that of plain soap. This could be due to a combination of antibacterial substances in the bar soap preparation with additives, coupled with the possibility that the amount of AgNPs used is insufficient. MEPL-AgNPs bar soap and MEPL bar soap possess antibacterial activity with the expectation of a synergistic effect. However, the combination seems to have a diminishing effect on each other, resulting in lower antibacterial activity compared to plain soap. Antibacterial combinations can yield additive, synergistic, or antagonistic effects. Additive effects are equivalent to single antibacterials, synergistic effects are greater, and antagonistic effects are smaller [43].

When comparing natural soap samples, the highest antibacterial activity of 14.26 mm at hour 15, with a strong category, was found in the sample of soap without the

addition of additive substances from the leaves of the *Pluchea indica* (L.). Therefore, the addition of additive substances such as AgNPs or *Pluchea indica* (L.) leaf extract is less effective for natural soap.

4. Conclusion

The quality of all soap samples has met the Indonesian National Standard (SNI) criteria concerning acidity, free fatty acids, and moisture content. Plain soap exhibits notable antibacterial activity, achieving a maximum inhibition zone of 14.26 mm at hour 15. In comparison, MEPL bar soap demonstrates an inhibition of 11.21 mm at hour 18, and MEPL-AgNPs bar soap registers an inhibition of 12.77 mm at hour 12. Notably, plain soap emerges as the soap variant with the highest level of antibacterial activity. Therefore, the addition of MEPL or MEPL-AgNPs as additives to the soap is ineffective in enhancing the antibacterial and antifungal activities of the soap.

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