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Formulation of Cinnamon Essential Oil *(Cinnamomum burmannii)* and Clove Essential Oil (*Syzygium aromaticum*) Nanoemulsion-Based Liquid Soap

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ABSTRACT

Soap is a cosmetic product that acts as a cleansing agent to prevent the occurrence of skin infections and other bodily diseases caused by bacterial and fungal infections. One of the bacteria that causes skin infection is *Staphylococcus aureus*. The eugenol content in cloves and cinnamaldehyde in cinnamon can inhibit the growth of bacteria. This research aimed to examine a liquid soap formulation based on a nanoemulsion combination of clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum burmannii*) essential oils that met the physicochemical characteristics and determine its activity in inhibiting the growth of *S. aureus* using the paper disk diffusion method. The results suggested that the use of the 2% clove essential oil and 3% cinnamon essential oil exhibited effective antibacterial activity These concentrations were then used to formulate a nanoemulsion with a composition ratio of Tween 80 and PEG 400 as the most optimal surfactant and cosurfactant of 40:10. The nanoemulsion was formulated into a liquid soap with the supplementation of clove and cinnamon essential oils to the liquid soap base for maintaining stability, improving the physical appearance of the soap, and promoting the antibacterial activity of clove and cinnamon essential oils in inhibiting the growth of *S. aureus* bacteria. The resulting liquid soap demonstrated good physicochemical properties and met the requirements.

Keywords: Clove Essential Oil, Cinnamon Essential Oil, Liquid Soap, Antibacterial, Nanoemulsion

INTRODUCTION

The development of effective antibacterial soap formulation is crucial for maintaining skin health and preventing skin-related infections caused by microbial pathogens. The skin, as the body's largest organ and primary barrier against external threats, is frequently exposed to a variety of bacteria, fungi, and viruses. Regular cleansing with antibacterial soap can reduce the microbial load on the skin, thereby lowering the risk of infections and inflammation. Soap is typically prepared using synthetic and natural active ingredients. Soap with natural active ingredients uses extracts from herbal plants or ingredients that are relatively healthier and beneficial for the skin and do not cause any harmful effects (Aris et al., 2021). Soap is formed through a reaction called saponification. Saponification, commonly referred to as the soap-making reaction, refers to the hydrolysis of fats or oils using a base, yielding the main product, fatty acid salts or soap, and the by-product, glycerol (Widyasanti et al., 2016).

Cloves and cinnamon are spices that are also used as traditional medicine by the community at large. These two spices possess antibacterial properties (Radiastuti et al., 2011; Aqmarina et al., 2016). Clove essential oil contains several active compounds in the form of eugenol, tannins, saponins, flavonoids, and alkaloids. Several studies have shown that clove flowers can inhibit the growth of *Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Staphylococcus aureus*, and methicillin-resistant *S. aureus* (MRSA) bacteria (Oshomoh et al., 2015; Huda et al., 2018). The main ingredients in cinnamon essential oil are cinnamaldehyde, eugenol, and coumarin, which act as antibacterial agents (Waty et al., 2018). The use of nanotechnology, especially nanoemulsions, can increase the bioavailability of active ingredients, enhance skin penetration, maintain the stability of the soap, improve the physical appearance of the soap, and increase the antibacterial efficacy (Meliana, 2022).

MATERIALS AND METHODS

Clove essential oil, cinnamon essential oil, *S. aureus* ATCC 25923 bacterial culture, nutrient agar (NA), nutrient broth (NB), methanol, virgin coconut oil (VCO), Tween 80, PEG 400, potassium hydroxide (KOH), sodium carboxymethyl cellulose (CMC-Na), hydroxypropyl methylcellulose (HPMC), sodium lauryl sulfate (SLS), cocamidopropyl betaine (CAPB), propylene glycol, methylparaben, citric acid, and distilled water.

Antibacterial Activity Test

The identification of *S. aureus* ATCC 25923 bacteria was performed by culturing in NB media. *S. aureus* ATCC 25923 bacterial suspension was inoculated by dipping a loop that had been etched into the bacterial culture in NB media and incubating at 37°C for 24 h (Purnamaningsih et al., 2017). Identification was carried out by measuring the absorbance value with a UV-Vis spectrophotometer at a wavelength of 625 nm to determine the concentration of the bacterial suspension. The ideal bacterial suspension has an absorbance level close to that of McFarland standard solutions (0.08–0.10) (Rosmania and Yanti, 2020).

Antibacterial testing in this study employed the disk diffusion method. Briefly, Glassware such as test tubes, beakers, measuring cylinders, Petri dishes, micropipettes, and other materials were sterilized in an autoclave at 121°C for 15 min. A total of 100 µL of the bacterial suspension was poured into a Petri dish, into which 10 mL of sterilized NA was poured. The Petri dish was then shaken slightly to evenly distribute the suspension and media and allowed to solidify.

Nanoemulsion Formulation

The oil phase used in the optimization process consists of VCO, clove essential oil, and cinnamon essential oil. Meanwhile, the surfactant and cosurfactant used were Tween 80 and PEG 400. The combination of oil, surfactant, cosurfactant, and water was designed to produce a clear and stable solution mixture by using the *Design Expert 13.0* (DOE) software (Table I).

Table I. Design a nanoemulsion formula for a combination of clove oil and cinnamon oil using DOE software

Formula	Oil (mL)	Surfactant (mL)	Cosurfactant (mL)	Water (mL)
1	10	10	40	40
2	10	25	25	40
3	10	40	10	40
4	10	32.5	17.5	40
5	10	17.5	32.5	40

Oil, surfactant, cosurfactant, and water were mixed according to the composition ratio (Table I). The solution was stirred using a magnetic stirrer until homogeneous and then left for 24 h at room temperature to observe for homogeneity or physical stability.

The use of DOE 13.0 software enabled optimization of the proportion of surfactants and cosurfactant in the nanoemulsion formulation. The design used for the nanoemulsion formulation was the *simplex lattice design*. The parameters used to determine the most optimal nanoemulsion formula were percent transmittance, particle size, and particle distribution.

Evaluation of the Nanoemulsion Combination of Clove Oil and Cinnamon Oil

Particle Size and Distribution

The size and distribution of the nanoemulsion particles were tested by diluting 1 mL of the nanoemulsion sample from a combination of clove and cinnamon essential oils into 5 mL of distilled water, which was then analyzed by using the Particle Size Analyzer.

Clarity Percentage

The percentage of clarity was tested by diluting 1 mL of a nanoemulsion sample with a combination of clove and cinnamon essential oils into 5 mL of distilled water. Then, the analysis was performed using a UV-VIS spectrophotometer at a wavelength of 650 nm with distilled water blank and replicated twice. This test was conducted to assess the physical stability and homogeneity of the formulation.

The Formula of the Liquid Soap

Liquid soap formulations are prepared by adding a nanoemulsion combination of clove and cinnamon essential oils into a liquid soap base consisting of KOH, CMC-Na, HPMC, SLS, CAPB, propylene glycol, methylparaben, citric acid, and distilled water.

The soap formula, based on the results of the optimal formula testing for the nanoemulsion liquid soap made from a combination of clove and cinnamon essential oils (Table II).

Table II. Design of the liquid soap formula nanoemulsion combination of clove oil and cinnamon oil

Material Name	Function	Amount (%)
Combination of clove and cinnamon essential oils	Active materials	10
Tween 80 + PEG 400	Surfactant	50
KOH	Emulsifier	2
CMC-Na	*Viscosity agent*	4
HPMC	*Gelling agent*	1.85
SLS	*Foaming agent*	1
CAPB	*Foaming agent*	3
Propylene glycol	Humectant	5
Methyl Paraben	Preservative	0.15
Citric acid	Buffers	1
Distilled water	Solvent	Ad 50 mL

Preparation of the Liquid Soap

The liquid soap preparation method begins with preparing the tools and weighing each material. The nanoemulsion preparation begins by mixing 4 mL of clove oil, 6 mL of cinnamon oil, 40 mL of Tween 80, and 10 mL of PEG 400 in 40 mL of distilled water to prepare the liquid soap base. The concentration of the liquid soap base was increased twice to achieve a 1:1 ratio of the nanoemulsion with the base; the concentration of each material did not change and was in accordance with the expected concentration. After the nanoemulsion and base were prepared, the nanoemulsion was poured into the base gradually and stirred until a homogeneous solution was obtained.

Evaluation of the Liquid Soap

Organoleptic

Organoleptic testing was performed by recording the smell, color, and shape of the soap preparation (National Standardization Agency, 2017).

pH

pH testing was performed using a pH meter; before beginning with the measurement, the pH meter was calibrated using a buffer solution (pH 4 and pH 7). The calibrated tool was then dipped in the liquid soap formula to observe the pH value on the pH meter scale (Zulkarnain et al, 2015).

Viscosity

Measurement of the viscosity of the liquid soap preparations was performed using a Brookfield viscometer (Viscotester VT-04E) by inserting the liquid soap formula into a container with a number adjusted to the number on the rotor (Syamsuddin, 2016). The rotor used was adjusted to a measurable viscosity limit. Then, the viscosity of the liquid soap sample was recorded.

Foam strength and stability

The strength and stability of the foam were tested using a 1-g sample of liquid soap, which was then placed into a tube containing 10 mL of distilled water, which was covered and shaken for approximately 20 s, followed by measuring the height of the foam. The solution was then left for 5 min and the height of the foam reduction was measured.

RESULTS AND DISCUSSION

Antibacterial Activity of Clove Essential Oil and Cinnamon Essential Oil

The antibacterial activity test of clove essential oil and cinnamon essential oil against *S. aureus* ATCC 25923 bacteria was performed by using the disk diffusion method. Clove essential oil (*Syzygium aromaticum* L.) and cinnamon essential oil (i.) show antibacterial activity against S. aureus, which increases with the concentration of clove essential oil and cinnamon essential oil. Based on the results of the antibacterial activity test and the inhibition zones (Figure 1) almost every concentration has a similar inhibition zone (**Supplementary File, Figure 1, Table I-II**). This indicates the presence of antibacterial activity. To clarify, it can be seen from Figure 1 in the supplementary file, which also shows that each tested concentration has its own classification. Therefore, to determine the selected concentrations based on the lowest concentration of clove essential oil that shows antibacterial activity, considering that clove essential oil has a very strong aroma and cinnamon essential oil has strong antibacterial activity. Thus, a concentration of 2% clove essential oil and 3% cinnamon essential oil were used to prepare liquid soap (Purnamaningsih et al., 2017).

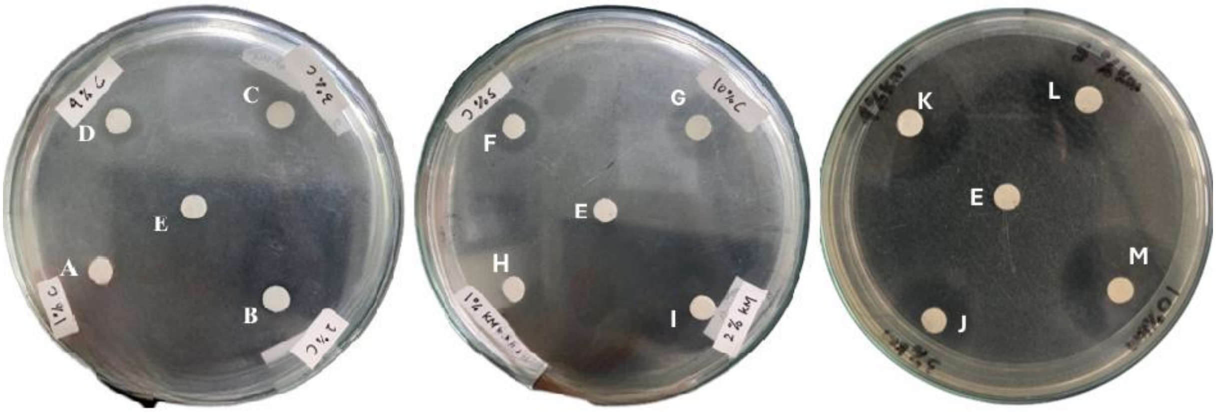

Figure 1. Antibacterial test results from the optimal formula of clove oil and cinnamon oil nanoemulsion soap with various treatment groups and varying concentrations (a) Clove essential oil 1%; (b) Clove essential oil 2%; (c) Clove essential oil 3%; (d) Clove essential oil 4%; (e) Negative control (methanol); (f) Clove essential oil 5%; (g) Clove essential oil 10%; (h) Cinnamon essential oil 1%; (i) Cinnamon essential oil 2%; (j) Cinnamon essential oil 3%; (k) Cinnamon essential oil 4%; (l) Cinnamon essential oil 5%; (m) Cinnamon essential oil 10%

Radiastuti et al. (2011) conducted a study on the antibacterial effectiveness of clove flower essential oil using amoxicillin as a positive control. The results showed that, at a concentration of 4%, clove essential oil demonstrated the ability to inhibit *S. aureus* with an inhibition zone diameter of 17 mm. Based on the research conducted by Aqmarina et al. (2016), cinnamon essential oil contains 61.53% cinnamaldehyde and exhibits antibacterial activity with an inhibition zone diameter of 18.773 ±0.574 mm at a concentration of 0.1%. Meanwhile, the antibacterial activity of clindamycin showed an inhibition zone diameter of 16.586 mm, indicating that cinnamon oil at a concentration of 0.1% already has greater antibacterial activity compared to clindamycin.

Direct testing in comparison with the positive control could not be conducted in this study owing to time constraints and the unavailability of a proper location for testing.

Particle size

Based on the results of the ANOVA analysis, the model probability value for the particle size was 0.0001, indicating a significant difference from the formula tested because it has a model probability value of <0.05. For this particle size parameter, the lack of fit probability value was not readable or was declared as an error. This could be because the data obtained, and its replication were considered almost perfect or that there were no differences. The resulting R-square value was 0.9999. Meanwhile, the Predicted R2 value was 0.9999 and the Adjusted R2 value was 0.9999. There was no difference between the Predicted R2 value and the Adjusted R2 value. The proportion of Tween 80 and PEG 400, which have the best particle size, was 40:10 (Figure 2 and Figure 3). The greater the proportion of Tween 80 used, the smaller the particle size. This value was directly proportional to the level of clarity; the smaller the particle size of a nanoemulsion, the better the level of clarity produced. According to Rina et al. (2017), nanoemulsion is a very small emulsion preparation with droplet sizes ranging from 5 to 200 nm (Patel and Patel, 2013).

Polidispersity index

Based on the results of the ANOVA analysis, the model probability value of the particle distribution was 0.0001, suggesting the derivation of significant differences from the formulas tested. Meanwhile, the probability value of the lack of fit was 0.1368. The lack of fit value of the particle distribution indicated no significant difference from the model because it had a probability value >0.05. The resulting R-square value was 0.9589. Meanwhile, the Predicted R2 value was 0.9177 and the Adjusted R2 value was 0.9476. The Predicted R2 value was in accordance with the Adjusted R2 value because it had a difference of <0.2.

Optimization of Formula Nanoemulsion Combination of Clove and Cinnamon Essential Oils

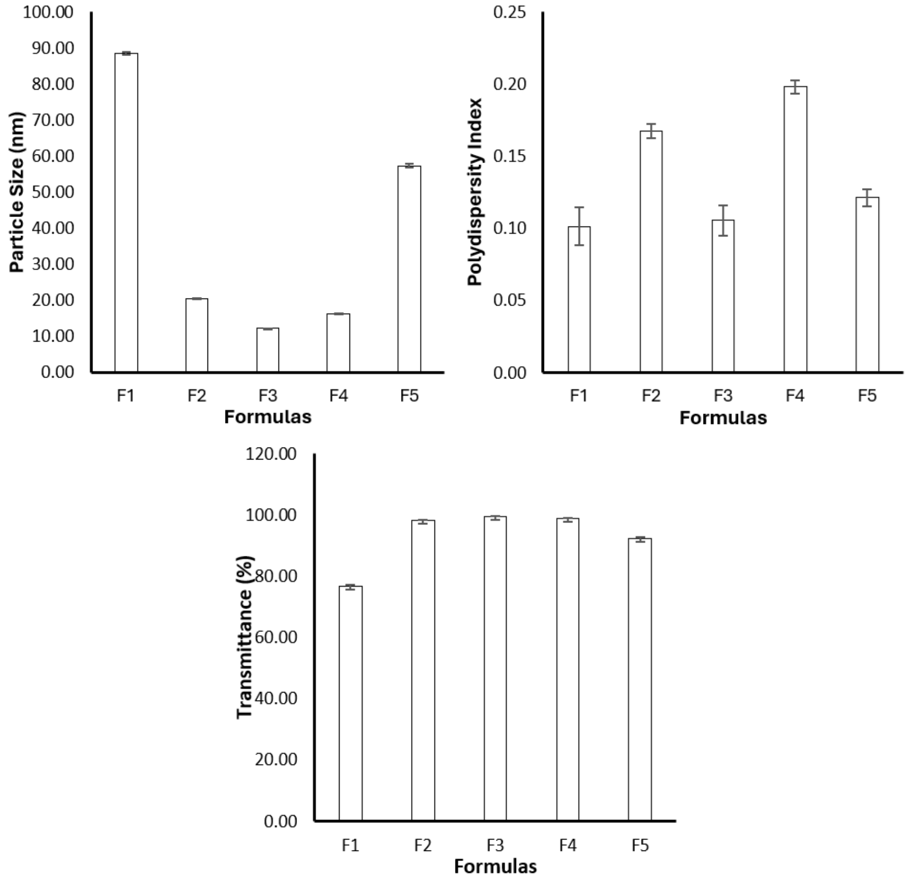

Figure 2. The results of the physical evaluation of the nanoemulsion combination of clove and cinnamon essential oils based on the particle size test; polydispersity index test; and transmittance test have five formulas: FI = PEG 400 40%; Tween 80 10%, FII = PEG 400 25%; Tween 80 25%, FIII = PEG 400 10%; Tween 80 40% FIV = PEG 400 17.5%; Tween 80 32.5%, FV= PEG 400 32.5%; Tween 80 17.5%.

The particle size distribution of the nanoemulsion formulation combining clove and cinnamon essential oils ranged from 0.088 to 0.203 (Figure 2 and Figure 3). This shows that all nanoemulsion formulas produced have a uniform particle size distribution of <0.5. The particle size distribution is stated to be uniform so that it can be absorbed at a relatively equal and fast rate, thereby increasing its bioavailability (Balakumar et al., 2013; Einien et al., 2012).

Clarity percentage

Based on the results of the ANOVA analysis, the model probability value was 0.0001. This finding suggests a significant difference from the formula tested because it has a model probability value of <0.05. Meanwhile, the probability value of the lack of fit was 0.4963. The lack of fit value of percent transmittance shows no significant difference from the model because it has a probability value of >0.05. The resulting R-square value was 0.9992. Meanwhile, the Predicted R2 value was 0.9984 and the Adjusted R2 value was 0.9990. The Predicted R2 value was in accordance with the Adjusted R2 value because it had a difference of <0.2.

The ratio between Tween 80 and PEG 400, which has the best percentage transmittance, was 40:10 (Figure 2 and Figure 3). The greater the proportion of Tween 80 used, the better the clarity of the resulting nanoemulsion. A nanoemulsion has a good level of clarity if it has a percent transmittance value that is close to 100% or >90% (Sagar et al., 2014). Then, the selected criteria were used to determine the optimum value expressed by the desirability value.

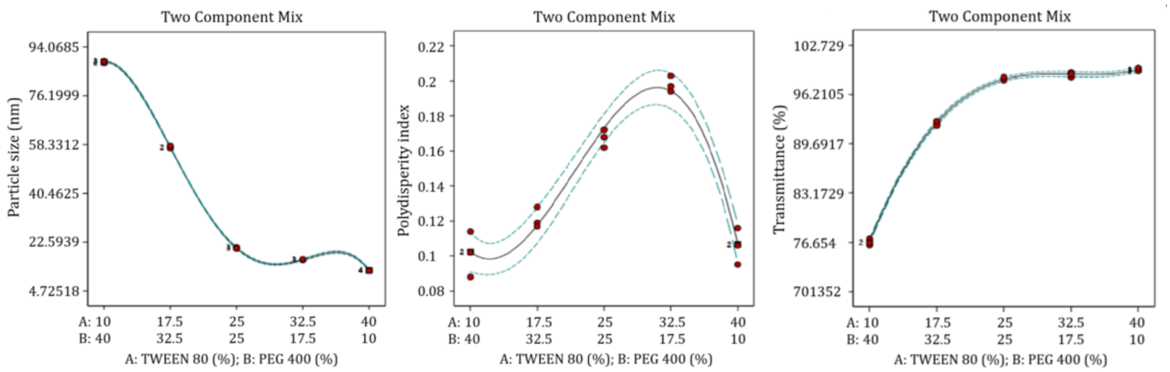

Figure 3. Graph depicting the relationship between the proportion of Tween 80 and PEG 400 on the particle size, polydispersity index, and transmittance.

Based on the test results, two solutions were obtained with the highest desirability value, namely 0.997 with a Tween 80 concentration of 40% and PEG 400 of 10% (Supplementary Table III).

The results of the clarity test or percent transmittance (% transmittance) in the nanoemulsion formulations were used to assess the physical stability and homogeneity of the emulsion. Emulsions with nano-sized particles appeared clearer or had a high transmittance value. In contrast, if the particle size is large, it could cause the emulsion to become cloudy (Indalifiany et al., 2021).

Evaluation of the Nanoemulsion Liquid Soap Combination of Clove and Cinnamon Essential Oils

Organoleptic

The physical appearance of the nanoemulsion liquid soap combination of clove and cinnamon essential oils on Figure 2 in this supplement file. The resulting soap formula has good homogeneity and a smooth texture, with no lumps, sediment, or color separation (National Standardization Agency, 2017). This liquid soap has a light yellow or yellowish-white color. Nanoemulsion liquid soap, a combination of clove and cinnamon essential oils, produces a distinctive aroma of clove and cinnamon. This demonstrates that the liquid soap tested in this research meets the necessary organoleptic requirements.

pH

Based on the test results, the pH values of the nanoemulsion liquid soap with a combination of clove and cinnamon essential oils were 9.86; 9.73; 9.75, indicating that the liquid soap produced had met the pH value based on the SNI standards, namely 8 to 11 (National Standardization Agency, 2017).

Viscosity

The test results for the nanoemulsion liquid soap showed that the mix of cinnamon and clove essential oils met the needs, which were between 400 and 4,000 m.Pas with spindle 4 (Sari et al., 2019). Three times, the nanoemulsion liquid soap with clove and cinnamon essential oils was tested for viscosity. The results are shown below: 1,734 mPas, 1,730 mPas, and 1,704 mPas. The average viscosity for the nanoemulsion liquid soap combination of clove and cinnamon essential oils is 1,722.67 mPas.

Foam strength and stability

Foam power and stability testing aimed to determine the ability of a nanoemulsion liquid soap with a combination of clove and cinnamon essential oils to produce and maintain foam for a certain period of time. The foam produced in this nanoemulsion liquid soap is attributable to SLS, CAPB, and a nanoemulsion formula containing surfactants and cosurfactants in the form of Tween 80 and PEG 400. The requirement from the test is >60% (Chasani et al., 2022; Hambali et al., 2019; Yuan et al., 2014).

Based on the test the foam produced by the nanoemulsion liquid soap with a combination of clove and cinnamon essential oils was stable as it met the standard, namely >60% of the requirement (Rinaldi et al., 2021) (Supplementary Table IV).

Stability Test

The stability test of the optimum soap formula was performed over 30 days in a closed environment away from direct sunlight. This test aimed to evaluate the short-term effects that might occur when the product is stored outside its ideal conditions and to determine the appropriate storage conditions for the soap formulation. The parameters analyzed during this test included the visual appearance, pH, and viscosity of the optimum soap formula, both before and after 30 days of storage.

It can be seen (Supplementary Table V) that the visual of the optimal formula of clove oil and cinnamon oil nanoemulsion soap is marked with "V," which means that there are no phase boundaries in the soap preparation, which shows that it is physically mixed homogeneously over time. In addition, the average pH value of the soap decreased over 4 weeks of storage, from 5.196 ± 0.047 to 5.100 ± 0.050. Based on the t-Test statistical results, no significant difference ($p > 0.05$) was recorded between that before and after storage, with a p-value of 0.111. Therefore, no difference was noted in the pH value before and after storage, indicating that the soap formula was stable.

In addition to pH, viscosity testing was conducted using an Ostwald viscometer to determine the thickness of the soap formulation, which could affect its viscosity, spreadability, and stability during storage. As seen in Table 5 in the supplement file, the viscosity value of the soap is consistent with the supporting theory, which states that the ideal viscosity for soap ranges from 500 to 2,500 (Haliza et al., 2020; Septiyanti et al., 2019; Vellayanti, 2020). Thus, soap has a viscosity that meets the specification for serum formulations. As seen in Supplementary Table V, there was a decrease in viscosity over the 30-day storage period, although it was statistically insignificant ($p > 0.05$), as shown by $p = 0.109$.

CONCLUSION

Antibacterial liquid soap prepared in this study employed active ingredients in the form of a combination of 2% clove essential oil and 3% cinnamon essential oil. The liquid soap was formulated by applying nanoemulsion technology, using the parameters of % transmittance, particle size, and particle distribution to obtain the optimal combination of Tween 80 and PEG 400 as surfactant and cosurfactant (40:10). The nanoemulsion liquid soap preparation with a combination of clove and cinnamon essential oils demonstrated good test results for organoleptic physicochemical properties, pH, viscosity, power and foam stability, and met the standard requirements for a liquid soap.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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