

NANOEMULSION-BASED MOUTHWASH OF ETHYL ACETATE FRACTION OF SERAI WANGI STALK: FORMULATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY TEST

Desy Ayu Irma Permatasari^{1*}, Weri Veranita¹, Novalisa Nindhi Soraya¹,
Eva Kholifah²

¹Department of Pharmacy, Health Sciences Faculty, Universitas Duta Bangsa
Surakarta, Central Java, 57552, Indonesia

²Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Salsabila, Banten, 42171,
Indonesia

*Email Corresponding : desyayu_permatasari@udb.ac.id

Submitted: 27 August 2023 Revised: 7 September 2023 Accepted: 7 September 2023

ABSTRACT

Serai wangi or citronella (*Cymbopogon nardus* L.) has long been used in Indonesia as a component of traditional medicine. A recent study aimed to determine the antibacterial activity of the most active fraction of Citronella stalk extract and to formulate it into a nanoemulsion mouthwash. Fractionation of the ethanolic extract was carried out using liquid-liquid extraction, and it was found that the ethyl acetate fraction (EAF) was the most active. This fraction was then varied at concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78125% for the antibacterial activity test using the disc diffusion test method. To determine the potency of microbial activity, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated. Subsequently, the ethyl acetate fraction was formulated into a nanoemulsion and characterized by measuring the transmittance percentage, droplet size, and polydispersity index (pDI) using a particle size analyzer. The results showed that the ethyl acetate fraction at a concentration of 25% obtained an inhibition zone diameter of 10.67 mm. MIC and MBC values were obtained at a fraction concentration of 6.25%. In addition, characterization of formulae exhibited particle size and pDI as follow 101.6 nm and 0.681 (Formula I); 84.1 nm and 0.609 (Formula II); 108.3 nm and 0.527 (Formula III).

Keywords: Citronella stalk; fractionation; *Streptococcus mutans*; nano-emulsion; mouth wash

INTRODUCTION

Serai wangi, also known as Citronella (*Cymbopogon nardus* L.), has a long history as a component of ancient remedies, especially in Indonesia. Citronella contains alkaloids, terpenoids, saponins, flavonoids, and tannins (Soraya et al. 2016). These constituents have antibacterial activity and could potentially be developed for the eradication of bacterial infections.

One of the serious causes of bacterial infection is tooth decay or dental caries. This refers to the deterioration of the outer layer of the tooth, known as enamel, which results from the production of acids by oral bacteria. This acid can ultimately lead to the formation of cavities in the teeth. Untreated tooth decay can result in discomfort, infection, and tooth loss. *Streptococcus mutans*, which is a gram-positive bacterium, is believed to contribute to

tooth decay. Therefore, there is a need for new antibacterial agents to treat this type of bacterial infection.

Several studies have been conducted on the antibacterial activity of Citronella. [Permatasari et al. \(2022\)](#) investigated pure extracts and fractions from Citronella (*Cymbopogon nardus* L.), namely n-hexane, ethyl acetate, and water fractions, to observe their potential antibacterial activity using preliminary tests. This test used pure extracts at 100% level concentration. The largest inhibition zone (28.2 mm) was obtained for the ethyl acetate fraction (EAF). This result suggested strong antibacterial activity of EAF against *Streptococcus mutans* bacteria ([Permatasari et al., 2022](#)).

One effort to maintain oral hygiene and avoid tooth decay is the use of mouthwash. Marketed mouthwashes contain synthetic chemicals that have detrimental effects if used for a long time. Therefore, it is important to produce a natural mouthwash from Citronella with antibacterial activity from its secondary metabolites ([Soundararajan et al., 2021](#)). Citronella contains alkaloids, terpenoids, saponins, flavonoids, and tannins, which are expected to contribute to antibacterial activity. However, the solubility of alkaloid compounds tends to be low under high particle-size conditions ([Safrida et al., 2020](#)). Thus, it can affect the stability and efficacy of mouthwashes.

Therefore, to increase solubility, nanotechnology can be used ([Juniatik et al., 2017](#)). Nanoemulsification is a method of producing an emulsion with nanosized particles that can stably solubilize oils using surfactants and ultrasonic treatment. The high penetration and surface area of nanoemulsions, resulting from their reduced particle size, can induce strong antimicrobial activity. In addition, nanoemulsions are thermodynamically stable and offer protection against hydrolysis and oxidation, making them widely used in food, cosmetic, and pharmaceutical industries ([Cho et al., 2023](#)). The particle size in nanotechnology is expected to range from 50 nm to 500 nm. A larger surface area of the particles leads to an increase in particle solubility ([Modarres-Gheisari et al., 2019](#)).

Based on this explanation, this research focused on exploring the antibacterial potential of EAF from Citronella stalks (*Cymbopogon nardus* L.). It originated by examining the most active fraction of Citronella stalks (*Cymbopogon nardus* L.) in inhibiting the growth of *Streptococcus mutans* based on MIC and MBC values. Furthermore, the most active fractions were formulated into a nanoemulsion mouthwash and characterized to ensure the quality of the nanosystem.

RESEARCH METHODS

Equipment and Materials

The materials used were Citronella Stalk extract (*Cymbopogon nardus* L.), 96% ethanol (p.a), chloroform (p.a), anhydrous acetic acid (p.a), concentrated H₂SO₄ (p.a), n-hexane (p.a), ethyl acetate (p.a), distilled water, 0.9% NaCl solution, *S. mutans* bacteria, BHI media (Brain Heart Infusion), MHA media (Mueller Hinton Agar), NB media (Nutrient Broth), blank discs, amoxicillin discs, Tween 80, PEG-400, palm oil, citric acid and phosphate buffers.

The tools used were Black cloth, tin, oven (*Memmert UN30 Oven Lab*), blender (*Cosmos*), sieve No. 40, digital scale (*Durascale*), brown glass bottle, stir bar, filter paper, glass Beaker (*Pyrex*), maceration vessel (*Pyrex*), measuring cup (*Pyrex*), rotary evaporator (*RV 10*), porcelain cup, dropping pipette, water bath (*Memmert*), test tubes (*Pyrex*), milligram scales (*Durascale*), separating funnel (*Pyrex*), erlenmeyer (*Pyrex*), gloves, masks, horn spoons, stir bars, petri dishes (*Normax*), spreader glass, bunsen, loops, cotton wool, aluminum foil, autoclave (*GEA*), micropipette, yellowtip, bluetip, incubator (*Memmert*), Laminar Air Flow (*LAF*), ruler (*Snowpeak*), Magnetic Stirrer (*Thermo Scientific*), Erlenmeyer (*Pyrex*), watch glass, vortex mixer (*DLab MX-S*), centrifuge (*Thermo Scientific*) and Particle Size Analyzer (PSA) (*HORIBA SZ-100*).

Research Methods

1. Plant identification and determination

The identification and determination of Citronella plants were carried out at the Biology Laboratory, Faculty of Applied Science, Universitas Ahmad Dahlan. Based on the research by (Permatasari et al., 2022), an ethanolic extract of Citronella stalks was obtained and used in this study.

2. Fractionation

Fractionation of the ethanolic extract was carried out using liquid-liquid extraction. The concentrated extract dissolved in 100 mL of distilled water and placed in a separatory funnel with the addition of n-hexane (1:1) to attract nonpolar compounds. Subsequently, ethyl acetate was added to the ethanol layer (1:1) to separate the semi-polar and polar compounds in the extract. Polar, semi-polar, and non-polar fractions were further separated and evaporated (Aryantini et al., 2017).

3. Antibacterial activity test

Physiological test of Streptococcus mutans

Gram staining was performed by preparing a bacterial preparation by smearing one dose of bacterial culture on an object glass as thin as possible, drying it, and performing hot fixation. The preparation was then flooded with carbolic violet (Cat Gram A) for 1 minute and the color was rinsed. After that, it was flooded with iodine (Cat Gram B) for 1 minute and rinsed with running water slowly. Bleaching was performed by the addition of 96% alcohol (Gram C stain). Next, the smear was rinsed with running water and dripped with safranin dye (Gram D stain) within 45 seconds until the color appeared. Finally, the preparation was rinsed again with running water and then dried.

Cell morphology and color were observed under a microscope. If the bacteria produces a purplish color, it is considered a gram-positive bacterium. Unless otherwise stated, it was assigned as a gram-negative bacterium and specified by the appearance of a red color. A catalase test was performed to identify bacteria based on their biochemical components. The testing method was to pour one dose of bacteria on an object glass moistened with 3% H₂O₂ solution. Then observe whether gas bubbles form.

Antibacterial activity evaluation

To confirm the most active fraction, antibacterial activity tests were carried out on n-hexane, ethyl acetate, and water fractions from Citronella stalks (*Cymbopogon nardus* L.) at a concentration of 25%. The test steps were as follows: disc paper with a diameter of 6 mm was dipped into each viscous extract and placed into a Petri dish containing agar and *Streptococcus mutans* bacteria. The 25% extract and fraction were prepared by weighing approximately 0.25 mg, then dissolved in 1 mL of DMSO. The diameter of the inhibition formed after incubation for 24 hours at 37°C. A clear zone in the agar Petri dish indicated the absence of bacterial growth (Erlyn, 2016).

Antibacterial activity evaluation in ethyl acetate fraction

The potency of EAF as an antibacterial agent was tested using the disc diffusion test at concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78125%. Paper discs (diameter of 6 mm) that had been dipped in each concentration of EAF, negative control (dimethyl sulfoxide), and positive control (amoxicillin) were placed on the surface of the agar containing the bacterial culture. After being incubated for 24 hours at 37°C, the diameter of the inhibition formed was measured (Erlyn, 2016).

Determination of MIC and MBC

The MIC test samples were a concentration series of EAF (25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78125%). A total of 7 sterile test tubes were prepared and labeled 1-7. The MIC value was determined using 2 mL of Nutrient Broth (NB) medium, which was put into a test tube, after which 1 mL of the test solution was added

to 1 mL of *Streptococcus mutans* bacterial culture. A positive control was made from 2 mL Nutrient Broth (NB) supplemented with 1 mL of 1% amoxicillin and 1 mL of *Streptococcus mutans* bacterial culture. Furthermore, a negative control was prepared from 2 mL of Nutrient Broth (NB) medium supplemented with 1 mL of 1% DMSO and 1 mL of *Streptococcus mutans* bacterial culture. Finally, all samples were incubated for 1 day (24 hours) to observe turbidity as a parameter of MIC (Rollando et al., 2019).

Determination of the MBC value was carried out using one dose of the test solution that was used for the MIC value determination test by streaking it on MHA media that had been poured into a Petri dish.

4. Nanoemulsion formulation

The most active fractions of Citronella at concentrations of 2.5%, 1.25%, and 0.625% were selected to be involved in the formulation. The formula for nanoemulsions mouthwash were provided in Table I.

Table I. Formula for Nanoemulsion Mouthwash

Materials	Formula (%)			Functionality
	FI	FII	FIII	
Ethyl acetate fraction of Citronella stalk	2.5	1.25	0.625	Active agent
Tween 80	35	35	35	surfactant
Ethanol 96%	10	10	10	co-surfactant
PEG-400	15	15	15	co-solvent
Palm oil	5	5	5	oil
Citric acid	0.5	0.5	0.5	pH adjuster
Buffer Phosphate pH 6	ad 100	ad 100	ad 100	solvent

The water phase was prepared by adding EAF from Citronella stalks (25, 12.5, and 6.25 mg) to be dispersed in Tween 80 with the addition of 96% ethanol and PEG-400. Phosphate buffer pH 6 was added and the mixture was stirred at 1000 rpm at 50 °C. In another beaker, the oil phase was prepared by blending palm oil and citric acid with continuous stirring at 1000 rpm. Both the phases were mixed and stirred for 30 minutes at room temperature to obtain a clear nanoemulsion. Furthermore, particle size and polydispersity index tests were performed for characterization.

5. Nanoemulsion characterization

Characterization of the nanoemulsion was carried out in several tests, namely, the % transmittance test using a UV-Vis spectrophotometer. One milliliter of the sample was dissolved in a 100 mL measuring flask using deionized aqua. The percent transmittance of the solution was measured at a wavelength of 650 nm using UV-Vis spectrophotometry. Deionized aqua was used as a blank during testing (Maharani et al., 2021). The particle size was determined using a particle size analyzer (PSA).

Data Analysis

Data analysis in this study was in the form of descriptive with phytochemical screening tests and physiological tests of bacteria described, and conclusions were made. The MIC and MBC values were obtained based on the requirement that the solution from the MIC test is clear or does not have signs of *Streptococcus mutans* bacterial growth. The cup was incubated for 1 day (24 hours). The test parameter for determining the MBC value was the presence or absence of white spots, which are signs of bacterial growth on agar media (Rollando et al., 2019).

The percent transmittance of the nanoemulsion mouthwash was determined using UV-Vis spectrophotometry. The particle size and polydispersity index were determined using a Particle Size Analyzer.

RESULTS AND DISCUSSION

Before antibacterial activity evaluation was carried out on *Streptococcus mutans*, a phytochemical screening was carried out with the aim of determining the presence or absence of saponins, terpenoids, tannins, alkaloids, flavonoids, and steroids. Phytochemical screening showed that Citronella stalk extract contained saponins, terpenoids, tannins, alkaloids, and flavonoids in the absence of steroids. Fractionation of Citronella stalk extract was first used as a solvent, n-hexane, to withdraw non-polar compounds. The insoluble layer in n-hexane was then separated into fractions with ethyl acetate, which is a semi-polar solvent. These fractions can collect active compounds that inhibit bacterial growth, and use water as a polar solvent. Based on the research by (Permatasari et al., 2022), the active fraction of Citronella stalks was obtained, namely, the ethyl acetate fraction (EAF), which was then used in this study.

1. Antibacterial Activity

The identification results of Gram staining of *Streptococcus mutans* bacteria showed a purplish color with a morphological form, namely, coccus. The results of this purple staining indicate that *Streptococcus mutans* bacteria are included in the Gram-positive bacteria which can retain the methyl purple dye from the crystal violet reagent as a test reagent, because Gram-positive bacteria have a simpler cell wall structure compared to Gram-negative bacteria, with the peptidoglycan layer is as many as 40 layers causing this layer to be thicker than Gram negative bacteria.

Biochemical identification using the catalase test showed that no gas bubbles were formed, indicating that the negative *Streptococcus mutans* bacteria have a catalase enzyme that can break down H_2 into O_2 **Figure 1.**

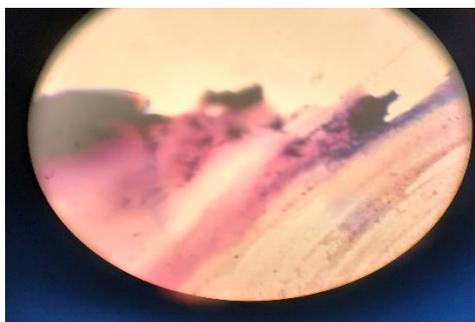


Figure 1. Physiological Test Results of Gram Staining of *Streptococcus Mutans*.

Based on previous studies, the average diameter of the most active inhibition zone was 28.2 mm obtained from EAF with 100% concentration. In this study, the antibacterial activity of the ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction was determined using the disc diffusion method with a concentration of 25 %, and the results obtained from the four sample fractions, which had the most active antibacterial activity, were the ethyl acetate fraction, as shown in **Table II**. This is also in accordance with the preliminary studies that have been carried out previously to determine the potential of the Citronella stalk extract (*Cymbopogon Nardus* L.) (Permatasari et al., 2022).

Table II. Results of Antibacterial Activity Test

Samples	Inhibitory Zone Diameter (mm)			Mean (mm)
	Replicate I	Replicate II	Replicate III	
Extract	8	7.5	7.5	7.67
<i>n</i> -Hexane	8	9	8	8.3
Ethyl Acetate (25%)	9	11	9	9.67
Water	8.5	7	7	7.5
Amoxicillin	16.5	17.5	17	17

The parameters that were measured to determine the antibacterial activity of the Citronella extract and fraction, seen from the clear zone around the disc paper, indicated that there was inhibition of the growth of *Streptococcus mutans* bacteria. The fraction that had the largest inhibition diameter was the Ethyl Acetate Fraction (EAF), with an average diameter of 9.67 mm, then the *n*-hexane fraction had an average inhibition diameter of 8.3 mm, while the water fraction had the smallest inhibition diameter of 7.5 mm. The inhibition diameter of the water fraction was lower than that of the Citronella extract, which was 7.67 mm. This result is in line with the research conducted by (Evangalina et al., 2021), in which the ethyl acetate fraction had a larger inhibition diameter than the other fractions. This is because EAF contains secondary metabolite alkaloids that contribute to its antibacterial activity (Evangalina et al., 2021).

The difference in the formation of inhibitory diameters on bacterial growth between extracts and Citronella fractions is due to differences in the content of active compounds in the extracts and Citronella fractions; therefore, their ability to inhibit the growth of *Streptococcus mutans* bacteria is also different. Thus, when an active substance is separated through fractionation, it is more specific to the active substance contained in the fraction to provide specific biological activity. High antibacterial ability can be seen from the larger inhibitory diameter formed in the antibacterial test; therefore, it can be said that the largest inhibitory diameter has the most active antibacterial activity (Erlyn, 2016).

Further antibacterial activity tests were carried out on the most active fraction of ethyl acetate, which was reduced to 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78125% with 3 replications Table III using the disc diffusion method.

Table III. Test Results of Antibacterial Most Active Fraction Using Disc Diffusion Test Method

Fraction concentration (%)	Inhibitory zone diameter (mm)			Mean (mm)
	Replicate I	Replicate II	Replicate III	
25	10.5	11	10.5	10.67
12.5	9.5	9	9.5	9.17
6.25	9	8.5	8.5	8.67
3.125	8	7.5	7.5	7.67
1.5625	7	7.5	7	7.17
0.781	7	7	6.5	6.83
Amoxicillin	11	11.5	10.5	11

As shown in Figure 2, the 25% concentration had the largest diameter, and the smallest inhibitory diameter was from a concentration of 0.781%. The results of the antibacterial activity tests of the most active fractions are shown in Figure 2. These results showed a clear zone, which indicates the diameter of inhibition for each

concentration. The diameter of the inhibition decreases with a decrease in the concentration of the test solution, which is in accordance with the theory that the diameter of the inhibition decreases in proportion to the decrease in the concentration of the test solution (Erlyn, 2016).

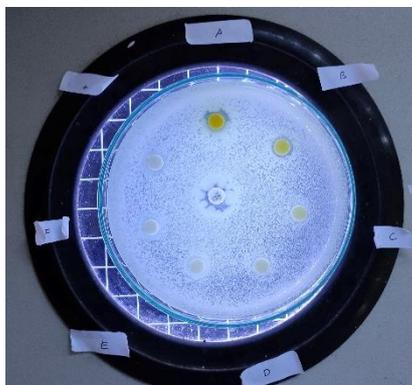


Figure 2. Appearance of Inhibitory Diameter

The MIC value was determined by determining the smallest amount of active substance that had antibacterial activity in the test solution, so that it could inhibit the growth of *Streptococcus mutans*. The MIC and MBC values were determined for the most active fraction of the ethyl acetate fraction. Determination of MIC values is usually carried out using several variations of concentration, in this study it was carried out with variations of concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.5625% and 0.78125%.

The results of the antibacterial test by determining the MIC values at concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78125% are shown in Figure 3. The concentrations of the test solutions were 25%, 12.5 %, 6.25% shown in tubes 2, tube 3, and tube 4 showed a clear respectively, and there were no membranes or white lumps growing. However, at the test solution concentrations of 3.125%, 1.5625%, and 0.78125% shown in tubes 5, 6, and 7 (from left to right), it can be seen that the solution turned cloudy and there was a lump/white coating, which indicates that *Streptococcus mutans* bacteria can still grow in the test solution at this concentration.

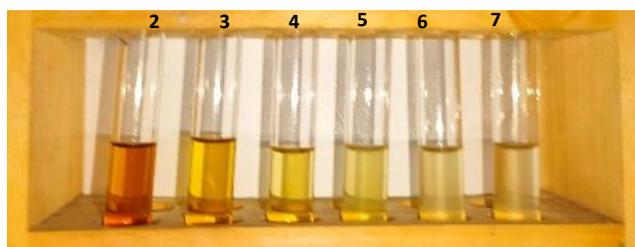


Figure 3. Appearances of Samples in Liquid Dilution Method (from left to right: concentrations of 25%; 12.5%; 6.25%; 3.125%; 1.5625% and 0.78125%, respectively)

From the results obtained, it can be seen that 6.25% is the smallest concentration that can inhibit the growth of *Streptococcus mutans* bacteria. This is characterized by the absence of white biofilm clumps/films in the solution and the color of the solution, which remains clear or does not turn cloudy. Therefore, it can be stated that the ethyl acetate fraction of Citronella stalks has an MIC value of 6.25%, namely in tube 4.

The MBC value was determined on agar media by inoculating the MIC test solution onto agar media, which was carried out on all test solutions. This was done to

reconfirm the three concentrations, 25%, 12.5%, and 6.25%, which are known to be effective in inhibiting the growth of *Streptococcus mutans* bacteria, which are still clear, and there are no signs of bacterial growth, so they can be effective in killing *Streptococcus mutans* bacteria.



Figure 4. Results from Minimum Bactericidal Concentration Test With the Solid Dilution Method

Figure 4 shows that, which was directly observed at concentrations of 25%, 12.5%, and 6.25% in petri dishes containing MHA Agar media and then streaked or inoculated using the test solution at each concentration of the test solution showed that *Streptococcus mutans* bacteria could not grow at that concentration. This was indicated by the fact that the media remained clear. At concentrations of 3.125%, 1.5625%, and 0.78125%, white spots still appeared on the agar medium, indicating that *Streptococcus mutans* bacteria could still grow at this concentration.

The concentration of 6.25% is the MBC value because this concentration is the lowest concentration that kills *Streptococcus mutans*, which is characterized by the inability of bacteria to adapt and grow, as indicated by the clarity of the agar media on the test plate.

The ethyl acetate fraction can kill *Streptococcus mutans*, which is thought to contain alkaloids, which is in accordance with a previous study by (Erlyn, 2016), who found that the main secondary metabolites of the Citronella plant contained alkaloids. Alkaloids, also known as amine groups, are the main compounds found in the ethyl acetate fraction of Citronella plants (Erlyn, 2016). Alkaloids are organic compounds found in plants that have basic properties and can dissolve in alcoholic solvents. The mechanism of antibacterial action of the alkaloid group is by damaging the constituent components of the peptidoglycan protein in bacterial cells, which results in the cell wall layer not being able to form completely so that it can cause death in bacterial cells (Erlyn, 2016).

2. Nanoemulsion Formulation and Characterization

In (Fahdi et al., 2022), it was found that Citronella mouthwash preparations could inhibit the growth of the *Streptococcus mutans* bacteria, with variations in the concentration of Citronella extract of 15% with inhibition zone value of 25.05 mm (Fahdi et al., 2022). Based on these previous studies, nanoemulsion mouthwash could potentially be developed as an alternative therapy for oral candidiasis/dental caries (Juniatik et al., 2017).

Therefore, in this study using, 2,5%; 1,25% and 0,625% respectively for nanoemulsion formulations were prepared using EAF of Citronella stalks. The formed nanoemulsion provides a clear visualization and has a clear brown color on FI and FII and a clear yellow color on FIII, as shown in **Table IV**. Characterization was then carried out, including the % transmittance test with a UV-Vis spectrophotometer and a

nanoparticle size test with a Particle Size Analyzer. Nanoemulsion characterization was carried out using the parameter of percent transmittance, which was measured using a UV-Vis spectrophotometer at λ 650 nm with aquadest as the blank. The results of the % transmittance test for the nanoemulsion preparations are shown in **Table IV**.

Table IV. Physical Appearance of Nanoemulsion Along with % Transmittance Results

No	Formula	% Transmittance	Visualization
1	FI (2.5%)	99,676	 Clear
2	FII (1.25%)	99,974	 clear
3	FIII (0.625%)	100,009	 Clear

Based on **Table IV**, it can be seen that the results of the % transmittance indicate that the more solute from the most active fraction of Citronella added to the formula, the more concentrated the resulting color will be, so that a lower percent transmittance value will be obtained. Based on this research, it was found that Formulation III had the highest % transmittance, where the active fraction of Citronella dissolved in the preparation was also the lowest compared to Formulations I and II.

According to (Singh et al., 2020), a good nanoemulsion will have a clear visual appearance with a high transmittance value. This clear and transparent appearance of the formulation can be obtained from the formulation with the highest transmittance percentage value, which is in the range of 90% -100%. Based on the data obtained in this study, it is shown that the three formulas have entered the range of 90% -100% so that they meet the requirements for the percent transmittance test. Therefore, further testing can be carried out to determine the size of nanoparticles using a Particle Size Analyzer (PSA).

The next characteristic of the nanoemulsion to be determined is the size of the nanoparticles, which is described by the Z-average/droplet size distribution of the nanoemulsion and the Polydispersity Index (pdI) test. The droplet size of the nanoemulsion was determined with a Particle Size Analyzer (PSA-HORIBA SZ-100) using the dynamic light scattering method with a scattering angle of 90°. Based on this test, data will be obtained regarding the size of the droplets formed on the nanoemulsion, which are expected to be within the nano-/ nano-size range. With this DLS technique, tests using PSA can be applied to measure the size and size distribution of particles and

molecules that are dispersed or dissolved in a solution (Singh et al., 2020). The results of the nanoemulsion droplet size tests on the nanoemulsion preparations are shown in Table V. Figure 5 shows the droplet size peak, which indicates the size of the nanoemulsion.

Table V. Droplet Size and Polydispersity Index Results

No	Formulation (% w/w)	Droplet size (nm)	pDI
1	FI (2,5%)	101.6	0.681
2	FII (1,25%)	84.1	0.609
3	FIII (0,625%)	108.3	0.527

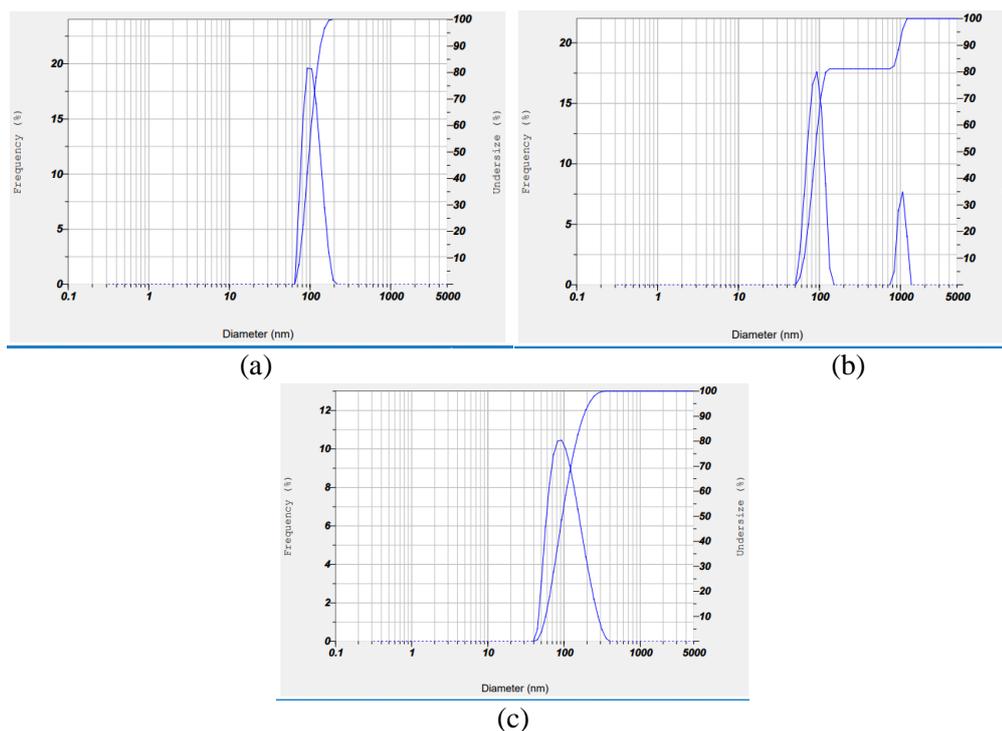


Figure 5. Droplet Size Test Results for (a) Formula I, (b) Formula II, and (c) Formula III.

In addition, a Polydispersity Index (pDI) test was carried out to determine the size of the distribution of molecular mass in a particular sample. The closer the value is to zero, the better is the distribution. A Polydispersity Index (pDI) test was carried out to determine the distribution of the nanoemulsion particles. The pDI (Polydispersity Index) was determined using a Particle Size Analyzer (PSA). The results of the nanoemulsion droplet size test on the nanoemulsion preparations are shown in Table V, along with the droplet size.

Therefore, it can be concluded that formulations made with concentrations of 2.5, 1.25, and 0.625% of the active ethyl acetate fraction of Citronella stalks (*Cymbopogon nardus* L.) can be used to prepare nanoemulsions. can be one of the mouthwashes of nano size, so that it can specifically provide therapeutic effects. The results of this nanoemulsion preparation have characteristics that match the nano size, as evidenced by the droplet size test, which results in the size of each concentration corresponding to the nano size and based on the polydispersity index, which has a value between 0.08-0.7. Thus, the formula has entered the range of the middle value of a good polydispersity index, where this value is in the upper range, which means that the distribution algorithm will operate the best.

To produce good formula stability in pharmaceutical preparations, it can be seen from the pDI value that the lower the pDI value, the more stable the formulation, so that the good pDI value is smaller. If the PDI value is greater, it indicates that the product has a non-uniform particle size, so that the formula can flocculate quickly and form more quickly (Yazgan, 2020).

The limitation of this study is that the preparation evaluation test was not carried out, including physical stability, so it cannot be known with certainty related to the stability of the formed preparations.

CONCLUSION

From the research that has been done it is concluded that the ethyl acetate fraction of Citronella stalks (*Cymbopogon nardus* L.) with an average diameter of the inhibition zone of each concentration variation is 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.781% respectively 10.67 mm, 9.17 mm, 8.67 mm, 7.67 mm, 7.17 mm and 6.83 mm. The MIC and MBC values of the most active fraction (Ethyl Acetate Fraction) of Citronella stalks (*Cymbopogon nardus* L.) were obtained at a concentration of 6.25%. Obtained nanoemulsion mouthwash preparations with nano particle sizes and pDI values were 101.6 nm; 0.681 for Formula I, then 84.1 nm; 0.609 for Formula II, then 108.3 nm; 0.527 for Formula III. Thus, in future research, it will be possible to test the antibacterial activity of nanoemulsion mouthwash preparations against *Streptococcus mutans* and to optimize the conditions in the preparation formulation.

ACKNOWLEDGMENT

This research was fully funded by the National Competitive Program Funding provided by the Ministry of Education, Culture, Research, and Technology under the 2022 Beginner Lecturer Research scheme. We thank LPPM Universitas Duta Bangsa for providing the opportunity to participate in this program.

REFERENCES

- Aryantini, D., Sari, F., & Juleha. (2017). Uji Aktivitas Antibakteri Fraksi Aktif Terstandar Flavonoid dari Daun Belimbing Wuluh (*Averrhoa bilimbi* L.). *Jurnal Wiyata*, 4, 143–150. <https://doi.org/P-ISSN2355-6498>
- Cho, M. Y., Lee, E. S., Jung, H. I., & Kim, B. Il. (2023). Anti-biofilm activity of a novel nanoemulsion containing Curcuma xanthorrhiza oil. *Journal of Dentistry*, 137(August), 104647. <https://doi.org/10.1016/j.jdent.2023.104647>
- Erlyn, P. (2016). Efektivitas Antibakteri Fraksi Aktif Serai (*Cymbopogon citratus*) terhadap Bakteri *Streptococcus mutans*. *Syifa 'MEDIKA*, 6(2), 111–125.
- Evangelina, I. A., Syafitri, F. U., Mardiaty, E., & Laviana, A. (2021). Daya antibakteri fraksi etil asetat daun kemangi (*Ocimum basilicum*) terhadap *Streptococcus mutans* ATCC 25175 pada clear retainer secara in vitro. Antibacterial potential of the ethyl acetate fraction of basil (*Ocimum basilicum*) leaves on Streptoco. *Padjadjaran Journal of Dental Researchers and Students*, 5(2), 97. <https://doi.org/10.24198/pjdrs.v5i2.28065>
- Fahdi, F., Syahdabri, H., Herviani, S., Kesehatan, I., Husada, D., Tua, D., & 77, B. N. (2022). *Formulasi Obat Kumur Ekstrak Daun Sereh (Cymbopogon citratus) Terhadap Pertumbuhan Bakteri Streptococcus mutans*. 5(1), 231–236.
- Juniatik, M., Khoirunnissa, H., Martien, R., & Utami, S. (2017). Formulation of Nanoemulsion Mouthwash Combination of Lemongrass Oil (*Cymbopogon citratus*) and Kaffir Lime Oil (*Citrus hystrix*) against *Candida Albicans* ATCC 10231. *Trad. Med. J.*, 22(1), 7–15.
- Maharani, N., Aisiyah, S., & Purwaningsih, D. (2021). Formulasi Mouthwash Ekstrak Kulit Buah Nanas (*Ananas comosus* (L.) Merr.) dengan Variasi Konsentrasi Gliserin sebagai Antibakteri terhadap *Streptococcus mutans* ATCC 25175. *Journal of Pharmacy*, 10(2), 8–19. <https://doi.org/2656-8950>

- Modarres-Gheisari, S. M. M., Gavagsaz-Ghoachani, R., Malaki, M., Safarpour, P., & Zandi, M. (2019). Ultrasonic nano-emulsification – A review. *Ultrasonics Sonochemistry*, 52(October 2018), 88–105. <https://doi.org/10.1016/j.ultsonch.2018.11.005>
- Permatasari, D. A. I., Veranita, W., Novalisa, & Soraya, N. (2022). Uji Potensi Ekstrak Etanol dan Fraksi N Heksan-Etil Asetat-Air dari Batang Serai Wangi (*Cymbopogon nardus* L.) terhadap Bakteri *Streptococcus mutans*. *Parapemikir : Jurnal Ilmiah Farmasi*, 11(1), 116–122.
- Rollando, R., Prasetyo, Y. S. A., & Rehmadata Sitepu. (2019). Uji Antimikroba Minyak Atsiri Masoyi (*Massoia aromatica*) terhadap Bakteri *Streptococcus mutans*. *Majalah Farmasi Dan Farmakologi*, 23(2), 52–57. <https://doi.org/10.20956/mff.v23i2.6585>
- Safrida, S., Khairil, K., Artika, W., & Rinaldi, R. (2020). Pandanus amaryllifolius Roxb. leaf extract prepared by nanoemulsion technique as a natural mouthwash. *Journal of Physics: Conference Series*, 1460(1). <https://doi.org/10.1088/1742-6596/1460/1/012050>
- Singh, S., Virmani, T., & Kohli, K. (2020). Nanoemulsion System for Improvement of Raspberry Ketone Oral Bioavailability. *Indo Global Journal of Pharmaceutical Sciences*, 10(01), 33–42. <https://doi.org/10.35652/igjps.2020.10105>
- Soraya, C., Sunnati, & Maulina, V. (2016). Efek Antibakteri Ekstrak Batang Serai (*Cymbopogon citratus*) Terhadap Pertumbuhan *Enterococcus Faecalis*. *Cakradonya Dent J*, 8(2), 69–78.
- Soundararajan, V., Kandasamy, V., & Subramani, P. (2021). Antibiofilm, antioxidant and larvicidal activity of formulated nanoemulsion from *Ocimum tenuiflorum*. *Materials Today: Proceedings*, 45, 3438–3443. <https://doi.org/10.1016/j.matpr.2020.12.932>
- Yazgan, H. (2020). Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. *Lwt*, 130(May), 109669. <https://doi.org/10.1016/j.lwt.2020.109669>