PHYSICAL EVALUATION OF NANOEMULSION FROM LEMONGRASS STEM (CYMBOPOGON NARDUS L.)

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ABSTRACT

Currently, the use of pharmaceutical preparations based on natural ingredients has begun to be in great demand by the public. This is because the use of synthetic drugs has weaknesses such as being able to cause resistance. Dental caries is one of the most common oral diseases in humans. One of the plants that has the potential to be used as an antibacterial in the oral cavity is Lemongrass (Cymbopogon Nardus L.). The use of emulsion mouthwash in the oral cavity is one of the treatments that is still being carried out. Mouthwash with nano size has advantages such as being able to enter the body more easily. This study aims to determine the physical properties of mouthwash nanoemulsion from ethyl acetate extract of Serai Wangi (Cymbopogon Nardus L.) by evaluating the preparation. The nanoemulsion mouthwash was made with the composition of Palm Oil as the oil phase, 96% ethanol as the cosurfactant, Tween 80 as the surfactant, and phosphate buffer pH 6 as the water phase. Then the mixture was stirred at a speed of 1000 rpm and sonicated and then tested for evaluation of the preparation including pH test, homogeneity test, organoleptic test, and centrifugation test. The results of the analysis of the entire nanoemulsion formula showed that the three formulas I, II and III, it was found that the organoleptic results of the nanoemulsion preparations were in the form of a thick liquid, brownish clear with a distinctive aroma of the extract, the three formulations of the nanoemulsion were homogeneous, there was no separation in the centrifugation test with the pH range of the preparation at 5.65 – 6.59.

KEYWORDS: Nanoemulsion, Mouthwash, Evaluation, Transmitans

INTRODUCTION

Indonesia has many plants that can be used as herbal medicine ingredients. A plant can be used as a source of medicine because it contains secondary metabolites (Pramono and Katno, 2001). One of the plants that can be used as medicine is Lemongrass (Winato, 2019). Lemongrass is a type of herbaceous grass from the order Graminales. Lemongrass has active compounds that can be used for treatment such as antibacterial, antifungal and anti-inflammatory (Maria et al, 2010). This inhibitory ability is due to the content of saponins, flavonoids, polyphenols, alkaloids and essential oils found in fragrant lemongrass leaves (Syamsuhidayat and Hutapea, 1991).

Lemongrass stem (Cymbopogon Nardus L.) are known to have antibacterial activity because they contain saponins, flanovoids and tannins. The results of antibacterial testing showed that the extract and stem fraction of lemongrass stem had antibacterial activity against the growth of Streptococcus mutans bacteria (Mayasari and Sapitri, 2019).

Oral and dental health is important. Healthy mouth and teeth will have an impact on the body of an individual. One of the most common dental problems is dental caries. *Streptococcus mutans* is one of the most common microorganisms found on the surface of the oral cavity. On the surface of the teeth Streptococcus mutans can stick and be able to hydrolyze food debris that is between the teeth. This results in the accumulation of bacteria on tooth enamel and plaque is formed as the initial formation of dental caries. In addition, the presence of plaque can also cause an unpleasant odor in the mouth (Pintauli and Hamidah, 2008).

Nanoemulsions are made in a drug delivery system called the Self-Nanoemulsifying Drug Delivery System (SNEDDS). SNEDDS is a mixture of oils, surfactants, cosurfactants and active substances which when mixed with water will form an oil/water (W/W) nanoemulsion (Sokolov, 2014). The characteristics of nanoemulsions are closely related to physical stability and clarity because they will have an important effect on the size of the resulting particles.

The large size of the globules can cause sedimentation and creaming, so it is necessary to characterize the physical stability and clarity of the resulting nanoemulsion of the ethyl acetate fraction of Serai Wangi (Cymbopogon Nardus) stems.

Based on the previous study, it is conclude that the active fraction of lemongrass (Cymbopogon Nardus) stem is the ethyl acetate fraction. Therefore, in this study, the lemongrass (Cymbopogon Nardus) stem extract was developed into a nanoemulsion formulation using tween 80 as a surfactant and palm oil as the oil phase.

RESEARCH METHOD

Tools and Material

The material used in this study was the ethyl acetate fraction of lemongrass stem (Cymbopogon Nardus L.) The additives used in this study were tween 80, 96% ethanol, PEG – 400, palm oil, citric acid and phosphate buffer. The tools used in this study was magnetic stirrer (*Thermo Scientific*), beaker glass (*Pyrex*), erlenmeyer glass (*Pyrex*), watch glass and vortex mixer (*DLab MX-S*), centrifuge (*Thermo Scientific*).

Formulation of lemongrass stem (Cymbopogon Nardus L.) Nanoemulsion

Variations in addition of ethyl acetate extract of citronella stem (2.5%; 1.25% and 0.625%) were suspended in palm oil, with a magnetic stirrer, then added with ethanol and finally PEG-400 was added and stirred with a magnetic stirrer (1000 rpm). The mixture was then added with buffer phospat pH 6, and stirred homogeneously at a speed of 1000 rpm for 30 minutes until a nanoemulsion was formed, then the physical evaluation of the preparation was carried out. Homogenization in this formulation was carried out for 30 minutes at 1000 rpm using a magnetic stirrer in each phase. Then, the formed nanoemulsions was evaluate by organoleptic test, homogeneity evaluation, pH evaluation and centrifugatin evaluation.

Organoleptic Testing

The organoleptic test was carried out by observing the nanoemulsion preparations that were formed visually, namely observations on the color, shape and aroma of the formulations I, II, and III.

Homogeneity Evaluation

The preparation is done by applying a gel sample to the object glass or other suitable transparent material, the preparation should be shows a homogeneous arrangement and no visible grains rude (Wasiaturrahmah, 2018).

pH Evaluation

The pH measurement was carried out using a pH-meter. At first the electrode was calibrated with a standard buffer of pH 4 and pH 7. The calibration process was completed when the pH value indicated on the screen was in accordance with the standard buffer pH value and was stable, then the electrode was immersed in the preparation. The pH value that appears on the screen is then recorded. Measurements are carried out at room temperature (DepKes RI, 2014).

Centrifugation Evaluation (mechanical test)

Preparations are included in centrifugation tube then put it in the centrifuge at a speed of 3800 rpm for 30 minutes. Physical appearance of the preparation before and after experiment compared visually then in observe whether there are signs phase separation or other change (Jufri, M, 2020).

RESULT AND DISCUSSION

The formulation was made by adding the active fraction of Lemongrass Stem with a concentration of 2.5% and 1.25% and 0.625%. From the results of the tests that have been carried out, it was found that the higher the concentration of the extract, the more concentrated the formed nanoemulsion. The characteristics of nanoemulsions are closely related to physical stability and clarity because they will have an important effect on the size of the resulting particles.

Furthermore, an evaluation of the nanoemulsions mouthwash was carried out which included organoleptic examination, homogeneity test, nanoemulsion pH test, and centrifugation test that been described below here.

1. Organoleptic Test

The results of organoleptic testing of nanoemulsion preparations can be seen in the following table.

Table 1. Organoleptic of Nanoemulsion Formula

Nanoemulsion Formula	Color		Shape	Aroma
Formula I	Clear, Color	Brownish	Viscous liquid	distinctive aroma of lemongrass extract
Formula II	Clear, Color	Brownish	Viscous liquid	distinctive aroma of lemongrass extract
Formula III	Clear, Yellow	Brownish	Viscous liquid	distinctive aroma of lemongrass extract

The nanoemulsion is in the form of a thick liquid with a distinctive aroma of lemongrass extract, with a different color gradient for each concentration. The brownish color results from the color of the lemongrass extract used.

The preparation is in the form of a thick liquid with a distinctive aroma of lemongrass extract, with a different color gradient for each concentration. The brownish color results from the color of the lemongrass extract used. Based on Figure 1, the visual characterization of the nanoemulsion looks clear and transparent. However, the greater the amount of ethyl acetate fraction incorporated into the oil phase, the more concentrated the nanoemulsion formed. This means that there is a limit to the amount of ethyl acetate fraction that can be incorporated into the nanoemulsion system. The filling capacity of the ethyl acetate fraction in the nanoemulsion may depend on its solubility in the system used (a mixture of oil, surfactant and cosurfactant).

2. pH Evaluation

The results of pH evaluation of nanoemulsion preparations can be seen in the following table.

Table 2. pH test of nanoemulsion preparation

Nanoemulsion Formulation	Formula I	Formula II	Formula III
pH	5.65	5.90	6.59

The results of the pH test using Formula I, II, III showed that the results for each preparation were in the range of 5.65 – 6.59. From the test results, it was found that the more extracts added to the preparation, the pH of the resulting nanoemulsion preparation decreased or became more acidic. This is because the active substances in citronella stems contain several flavonoid and phenolic compounds according to research by Hendrik, 2013. Secondary metabolites of flavonoids and phenolics have many hydroxyl

groups (-OH) which are the main contributors to the acidic nature of a compound. However, the pH of the nanoemulsion preparations produced in this study was still in the vulnerable pH range of mouthwash preparations, namely 5-7 (Hidayanto, A., 2017).

3. Homogeneity test

The results of homogeneity test of nanoemulsion preparations can be seen in the following table.

Table 3. Homogeneity test result

Formula	Formula I	Formula II	Formula III
Homogeneity	Homogeneous	Homogeneous	Homogeneous

The preparation is applied to the slide and then covered with another slide and then the homogeneity of the preparation is observed. Based on the results of the homogeneity test, it showed that all preparations were homogeneous. The preparation is said to be homogeneous if the preparation is evenly distributed, the surface is smooth and there are no visible particles or lumps visually. Homogeneity in the preparation is needed so that the active substance is evenly distributed so that the same dose and efficacy are obtained in every use.

Picture 1. Nanoemulsion Formula in the object glass for homogeneity test

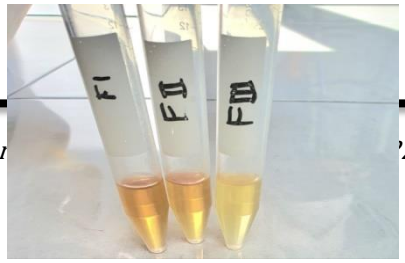
4. Centrifugation test

The results of centrifugation test of nanoemulsion preparations can be seen in the following table.

Table 4. The result of centrifugation test

Nanoemulsion Formulation	Precipitation	Phase separation	Turbidity
Formula I	(-)	(-)	(-)
Formula II	(-)	(-)	(-)
Formula III	(-)	(-)	(-)

Information : (-): No separation
(+): Separation occurs



Picture 2. Centrifugation test result

The centrifugation test was made with a one-time measurement. The preparation was put into a centrifugation tube and then centrifuged at a speed of 3800 for 30 minutes. From the test results on each formula showed no precipitation, phase separation, and turbidity. In nanoemulsion preparations, the surfactant used is tween 80. The surfactant will form a film layer on the droplet surface which can prevent the incorporation of droplets in the dispersion medium. So there is no separation in the dosage phase.

CONCLUSION

From the results of the evaluation of the nanoemulsion preparations in the three formulas 1, II and III, it was found that the organoleptic results of the nanoemulsion preparations were in the form of a thick liquid, brownish clear with a distinctive aroma of the extract, the three formulations of the nanoemulsion were homogeneous, there was no separation in the centrifugation test with the pH range of the preparation at 5.65 – 6.59. So that further evaluation can be carried out to see the droplet particle size of the formed nanoemulsion.

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