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Synthesis, characterization, and sunscreen potential evaluation of citronella oil nanoemulsion (*Cymbopogon Nardus L. Rendle*)

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Abstract

Citronella (*Cymbopogon nardus L. Rendle*) essential oil contains active compounds such as antioxidants and has the potential to be developed as a sunscreen. Nanoemulsion is a water and oil dispersion stabilized by a surfactant layer and has a particle size below 100 nm. This study described the formulation, characterization of citronella oil nanoemulsions, and determination of their activity as a sunscreen through the Sun Protection Factor (SPF) values. The nanoemulsion formulation was made with 3 variations of oil concentration: F1 (1%), F2 (3%), and F3 (5%). The results of the organoleptic test were following the standards which were clear and no phase separation occurred. In addition, the pH value of the 1%, 3%, and 5% prepared nanoemulsions were categorized as safe to be used on the skin. The prepared formulas were categorized as O/W nanoemulsion types. The viscosity values were found to be 12.76, 25.13, and 84.05 cP respectively. Furthermore, the percent transmittance values were 98.4, 98.9, and 99.9%. The particle sizes, respectively, were 9.4 (100%), 10.07 (97%), and 8.96 nm (82.5%). The centrifugation and freeze-thaw cycle tests showed good physical stability. The results of the sunscreen potential test of the 1%, 3%, and 5% citronella oil nanoemulsion showed SPF values of 1.03, 1.13, and 1.14 with %Te values of 77.42, 70.21, and 67.86% respectively, and %Tp values of 95.94, 94.04, and 93.61% respectively. From this research, the citronella oil nanoemulsion were found out to have a higher sunscreen activity than the pure citronella oil.

Keywords: Antioxidant, Citronella essential oil (*Cymbopogon nardus L. Rendle*), nanoemulsion, sunscreen

1. Introduction

Research on nanoemulsion has been developed over the last few decades (Koroleva and Yurtov, 2012). Nanoemulsion is a dispersion of water and oil stabilized by a surfactant and has a particle size below 100 nm (Paquin et al., 2015). The surfactants plays a major role to increase the benefit of preparing the active substances into nanoemulsion. The surfactant supports the mass transfer resistance of the oil release into continuous phase from the dispersed pfase and also supports the particle size reduction leading to a higher activity of the active substances (Agrawal, et al., 2016).

Nanoemulsion has several advantages over emulsion such as good physical stability, large surface area, and high bioavailability. Nanoemulsions has been widely applied in various fields such as pharmacology, food, cosmetics, etc. (Nirmala and Nagarajan, 2017).

The application of nanoemulsions could be applied in essential oils. The essential oils have secondary metabolites and can be obtained from plants (Seow et al., 2014). Essential oil has a high economic value because it has many pharmacological effects (Eden et al., 2018). Citronella essential oil is one of the various essential oils obtained in Indonesia. It can be produced by the distillation of fragrant citronella leaves (*Cymbopogon Nardus L. Rendle*).

The Citronella essential oil has good pharmacological effects such as anticancer, antimicrobial, and antioxidants (Mileva et al., 2014; Wei and Wee, 2013). Citronella oil contains an antioxidant compound that could be utilized as a natural sunscreen. Sunscreen is a substance that can protect the skin from exposure to harmful UV rays (free radicals). The most important component in sunscreen is antioxidant compounds that can reduce free radicals (Gause and Chauhan, 2016).

Although citronella essential oil has been proven to have many pharmacological effects, it still has some drawbacks, such as low physical stability and poor water solubility. These limitations can be handled by modifying citronella oil into nanoemulsions (Nirmala and Nagarajan, 2017). From the research by Sakulu et al. (2009), it was proven that preparing cintronella oil into nanoemulsion help to increase the repellent activity of the substance. From the research by Harni et al (2020), it also proven that preparing citronella oil into nanoemulsion was effective to increase the inhibition towards the pathogen *P. Palmivora.*

In this research, the synthesis and characterization of citronella oil nanoemulsion were carried out to evaluate its sunscreen potency compared to the pure citronella oil's suncreen activity. The nano emulsification method used is the low-energy phase inversion method, Emulsion Inversion Point (EIP). In the EIP method, the W/O macroemulsion is prepared at a constant temperature and then slowly diluted with water. During the dilution process, the system passes through an inversion point where a W/O to O/W phase transformation occurs. At this inversion point, the oil-water interfacial tension is so low that it leads to the formation of a small droplet (Forgiarini et al., 2001).

2. Methodology

2.1. Materials

The materials used in this research were citronella oil (*C. nardus* L. Rendle), surfactant tween 80 (MERCK, Germany), propylene glycol, methylene blue (MERCK, Germany), distilled water, and ethanol (MERCK, Germany). The research started with the synthesis of nanoemulsion, followed by the characterization of nanoemulsion, and ended with the sunscreen evaluation of the prepared citronella oil nanoemulsion

2.2. Synthesis of Nanoemulsion

The synthesis of citronella oil nanoemulsion was carried out in three steps, following the formulation in Table 1. The first step was the preparation of the oil phase. The oil phase was a mixture of tween 80 as a surfactant, propylene glycol as a cosurfactant, and citronella essential oil. This oil phase was homogenized using a magnetic stirrer at a speed of 750 rpm at 50 ºC for 30 minutes.

The second step was mixing the oil phase and water phase (distilled water). The distilled water was added slowly into the oil phase. The third step was the homogenization process of both the oil phase and water phase for 30 minutes at 50 ºC with a speed of 1200 rpm using a magnetic stirrer.

Table 1. Citronella oil nanoemulsion formulation.

Formula	Material (%)			
	CO	Tween 80	PG	Distilled water
F1	1	25	10	Add 100%
F2	3	25	10	
F3	5	25	10	

2.2. Characterization of citronella oil nanoemulsion

The characterization of citronella oil nanoemulsion was carried out by conducting an organoleptic test, a pH test (Seibert et al., 2019), a nanoemulsion type test, viscosity value measurement, transmittance percentage test, particle size measurement, centrifugation, and freeze-thaw cycle test (Gurpreet and Singh, 2018).

Organoleptic test was conducted to identify the characteristic of nanoemulsion by observing the color, odor, phases separation, and clarity of the prepared formula. The pH value was measured to identify if the nanoemulsion prepared is safe to be used on the skin. The nanoemulsion type was evaluated by adding a few drops of methylene blue into nanoemulsion to determine the dispersed and the dispersing phase (oil in water or water in oil).

The viscosity of nanoemulsion was measured using ostwald viscometer with 3 times repetitions. Transmittance percentage test was conducted to measure the clarity level of the nanoemulsion as the early stage to determine the droplet size of nanoemulsion. The transmittance percentage test was conducted by using Spectrophotometer UV-Vis (λ 650 nm). Particle size was analyzed by Particle Size Analyzer to ensure the real droplets size formed in the nanoemulsion.

The Centrifugation and freeze-thaw cycle test was conducted to determine the stability of the formula. The formula was centrifuged 10.000 rpm for 30 minutes and its organoleptic was observed. The formula was then storaged in under the temperature of 4 ºC for 24 hours, moved to 25 ºC for 24 hours and then moved to 40 ºC for another 24 hours for the freeze-thaw cycle test. The freeze-thaw cycle was repeated for 3 times.

2.3. Nanoemulsion Sunscreen Potency Test

a) Determination of SPF Value

A total of 0.2 mL of citronella oil and each formula were diluted with ethanol solvent until the volume reached 10 mL (20,000 ppm) (Widyawati et al., 2019). Each formula was measured for its SPF value using a UV-Vis Spectrophotometer (Shimadzu 1800) at a wavelength range of 290-400 nm at 5 nm intervals using ethanol as a blank. The SPF value was calculated by the Equation (1) (Mansur et al., 1986):

$$\text{Log SPF} = \frac{\sum AUC}{\lambda n-\lambda 1}[AUC]_{\lambda p\text{-}A}^{\lambda p} = \frac{A(p\text{-}a)+A(p)}{2}\{\lambda(p)-\lambda(p\text{-}a)\} \quad (1)$$

b) Determination of the Erythemal Transmission

The percentage of erythema transmission was determined by measuring the transmittance of each diluted sample in the wavelength range of 292.5-317.5 nm. The value of %Te can be calculated by the Equation (2) (Schalka et al., 2019):

$$\%Te = \frac{Ee}{\sum Fe} = \frac{\sum T.Fe}{\sum Fe} \quad (2)$$

c) Determination of the Pigmentation transmission

The percentage of pigmentation transmission can be determined by measuring the transmittance of each sample that has been diluted in the wavelength range of 292.5-372.5 nm. The %Tp value can be calculated using the Equation (3) (Schalka et al., 2019):

$$\%Tp = \frac{Ep}{\sum Fp} = \frac{\sum T.Fp}{\sum Fp} \quad (3)$$

3. Results and Discussion

3.1. Citronella Oil Nanoemulsion

The synthesis of citronella oil nanoemulsion was carried out using a low-energy method. The low-energy method is based on phase inversion in the emulsion upon changes in composition or temperature (Koroleva and Yurtov, 2012). The low energy method used in this study is the Emulsion Inversion Point (EIP) method or the composition phase reversal method. The principle of the EIP method is that the W/O macroemulsion is prepared at a constant temperature and then slowly diluted with water. During this dilution process, the system passes through an inversion point where a W/O to O/W phase transformation occurs. At this point of inversion, the oil-water interfacial tension is so low that small droplet sizes can be formed (Forgiarini et al., 2001).

The nanoemulsion obtained by the EIP method was light yellow and clear. In the first synthesis step, the oil phase was made first so that the oil droplets are wrapped by a layer of surfactant and cosurfactant to form micelles. When the water phase was added in the second stage, the surface tension will decrease. This allows the emulsification process to occur spontaneously. In addition, the homogenization process using a stirrer allows the process of forming small oil droplet sizes (Forgiarini et al., 2001).

3.2. Organoleptic Results

The prepared nanoemulsions were obtained in slightly yellow to yellow, slightly fragrant to fragrant. The phases did not separate, and the clarity was very high (Table 2). There was an alteration of the scent characteristic between citronella oil nanoemulsions that were stored for 0 week and 4 weeks. This means that the prepared citronella oil nanoemulsion was relatively less stable, especially in terms of aroma. Changes in the aroma are expected due to the merging of small droplets into larger droplets, causing a change in the density that likely decreases the aroma. In addition, the high clarity of the formulation was found to be unchanged. In this research, tween 80 was used as a surfactant. The use of surfactants provides high clarity and inhibits the phase separation that occurs in the emulsion (Costa et al., 2012).

Table 2. Organoleptic test results on citronella oil nanoemulsion preparation.

Parameters	0 week			4 weeks		
	F1	F2	F3	F1	F2	F3
Color	LY	LY	LY	LY	Y	Y
Scent	LF	LF	F	F	SF	SF
Phase Separation	VI	VI	VI	VI	VI	VI
Clarity	VC	VC	VC	VC	VC	VC

Remarks:
LY : Less Yellow
SF : Slightly Fragrant
VC : Very clear
VI : Very Inseparable

F : Fragrant
Y : Yellow
LF : Less Fragrant

3.3. pH

The pH results obtained from each nanoemulsion varied and decreased until the 3rd week (Table 3). In the citronella oil nanoemulsion F1 (1%), the pH value decreased from 6.5 to 6.3 at weeks 0 to 3. Citronella oil nanoemulsion F2 (3%) has a pH value of 6.3 at week 0 and 6.1 at week 3. Furthermore, the pH value of Citronella oil nanoemulsion F3 (5%) has changed from 6.2 at week 0 to 6.0 at week 3. The obtained pH data was then analyzed using two-way ANOVA. The concentrations (1, 3, and 5%) showed a significant value of 0.001 (p-value <0.05), indicating that the concentration affected the pH obtained by the nanoemulsion.

Table 3. pH measurement results of citronella oil nanoemulsion preparation.

Time	pH		
(Week)	F1	F2	F3
0	6.5	6.3	6.2
1	6.4	6.3	6.1
2	6.3	6.2	6.0

3.4. Nanoemulsion Type

The nanoemulsion type test was conducted by adding methylene blue to each prepared nanoemulsion. Figure 1 displayed that the methylene blue solution completely dissolved in 1%, 3%, and 5% citronella oil nanoemulsion. This result indicates that the type of nanoemulsion formed was oil in water (O/W) type. The O/W type is formed because the nanoemulsion system is made up of water so that methylene blue can dissolve completely (Gurpreet and Singh, 2018).

Figure 1. Result of examination of nanoemulsion type in each sample. (A) before dripping with methylene blue, (B) after dripping with methylene blue.

The mechanism of dissolution of methylene blue in the water is solvation. Methylene blue ionizes in water into anions and cations, then the ions are surrounded by water molecules according to the induced dipole force. The type of oil in water (O/W) nanoemulsion is as expected because it is safe when used topically (Koroleva and Yurtov, 2012).

3.5. Viscosity

Based on Table 4, it shows that the greater concentration of citronella oil in the prepared nanoemulsion will reduce the volume of water, thereby increasing their viscosity value. An increase in the amount of oil causes more dispersed droplets so that the friction between the droplets is higher and increases the viscosity. An increase in the amount of oil also causes an increase in the density of a particle. When the density of the particles is large, the mass will be heavier.

Table 4. The viscosity test results of citronella oil nanoemulsion preparation.

No	Sample	Viscosity (cP)
1.	F1	12.76
2.	F2	25.13
3.	F2	84.05

The greater the particle density, the more the fluid flow is hampered. This increases the viscosity value.In addition, viscosity is influenced by the cohesive force between molecules, namely the force of the attraction between similar molecules. The increase in the amount of oil causes the cohesive force in the system to be large. This is what causes the viscosity to increase (Giancoli, 2009).

3.6. Transmittance Percentage

A good nanoemulsion formula will have clear visuals with transmittance> 90% (Costa et al., 2012). The highest transmittance value was found in 1% citronella oil nanoemulsion (Table 5) with more water composition. Therefore, it is expected to have a higher clarity level.

Table 5. %T measurement results of citronella oil nanoemulsion.

No.	Sample	% T
1.	F1	99.9
2.	F2	98.9
3.	F3	98.4

3.7. Particle Size

Table 6 indicates that the three nanoemulsion formulations have been included in the size range below 100 nm (Gupta et al., 2016). The highest level of homogeneity was owned by 1% citronella oil nanoemulsion, followed by 3% and 5% citronella oil nanoemulsions (Figure 2). A higher oil concentration in the prepared nanoemulsions results in a less homogeneous formulation because it might need a longer time and faster stirring to homogenize the mixtures. Meanwhile, in this study, the homogenization time and stirrer speed were made the same for the three formulas.

Table 6. Size distribution of citronella oil nano-emulsion.

Formula	Particle Size	PDI
F1	9.4 nm (100%)	0.0815
F2	10.07 nm (97%) 5530 nm (3%)	0.0122
F3	8.96 nm (82.5%) 216.8 nm (17.5%)	2.5030

Table 7. Centrifugation test results for citronella oil nanoemulsion.

After centrifugation	Sample		
	F1	F2	F3
Turbidity	No	No	No
Phase separation	No	No	No
Precipitation	No	No	No

The optimum formula was found in citronella oil nanoemulsion with a particle size of 9.4 nm. The particle size of less than 90 nm is known to increase the stability of nanoemulsion preparations against sedimentation (Gupta et al., 2016).

The polydispersity index value serves as an indicator of a homogeneous particle size distribution. A low polydispersity index value indicates that the dispersion system is more stable in the long term (Gao et al., 2008). A polydispersity index of <0.5 indicates a fairly uniform particle size (monodisperse). The results obtained in this study indicate that 1% and 3% citronella oil nanoemulsions are included in the monodisperse category because they have a polydispersity index value of <0.5. Meanwhile, 5% citronella oil nanoemulsion has a very high polydispersity index value of 2.503. This shows that 5% citronella oil nanoemulsion is still heterodisperse and unstable.

3.8. Centrifugation

The results of the centrifugation test (Table 7) showed that after the centrifugation test on each nanoemulsion preparation, there was no change in terms of turbidity, phase separation, and precipitation. The synthesized citronella oil nanoemulsion preparation has a good level of physical stability (Gurpreet and Singh, 2018).

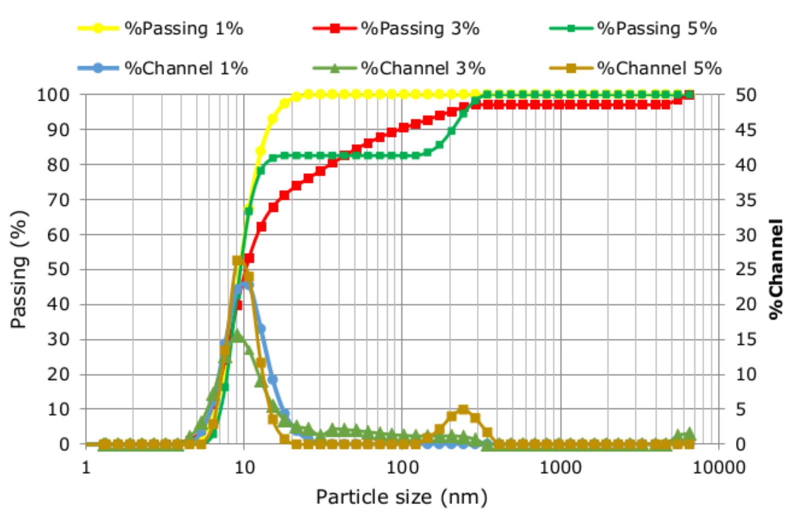

Figure 2. Nanoemulsion particle size distribution.

3.8. Freeze-thaw Cycle

The results of the freeze-thaw cycle test (Table 8) were observed by physical observation whether there was a change in terms of organoleptic, clarity, phase separation, and precipitation. The synthesized citronella oil nanoemulsion showed a good level of stability because there were no changes in terms of organoleptic, clarity, phase separation, and precipitation after the freeze-thaw cycle test. This indicates that the prepared nanoemulsions are stable to temperature changes. This stability is proposed due to the oil in water being protected by a surfactant layer that forms micelles. Micelle will prevent droplets from combining with the dispersing medium (Gurpreet & Singh, 2018).

Table 8. Freeze-thaw cycle of nanoemulsion.

Parameters	Before			After		
	F1	F2	F2	F1	F2	F3
Color	LY	LY	LY	LY	LY	LY
Clarity	C	C	C	C	C	C
Scent	DS	DS	DS	DS	DS	DS
Phases Separation	N	N	N	N	N	N
Precipitation	N	N	N	N	N	N

Remarks:
LY = Light Yellow
DS = Distinctive smell
N = None
C = Clear

3.9. Sunscreen Potential Evaluation

a. SPF value

The SPF value was tested by dissolving the citronella oil nanoemulsion into ethanol and measuring its absorbance using a UV-Vis spectrophotometer with a wavelength range of 290-400 nm. In order to compare the SPF value of the nanoemulsion of citronella oil and the SPF value of pure citronella oil, the measurement of the SPF value was also carried out for pure citronella oil dissolved in ethanol with the same concentration as 1%, 3%, and 5%. The results of the measurement of the SPF value can be seen in Table 9 and Figure 3.

Based on the results, the SPF values of the citronella oil nanoemulsion with an oil concentration of 1%, 3%, and 5% were obtained to be 1.03, 1.13, and 1.14 respectively. The SPF value of pure citronella oil with the same concentration was 1.01, 1.02, and 1.03. The results showed that the SPF values of the prepared nanoemulsion were higher than the citronella oil. The SPF value increased along with the increase in the concentration of citronella oil so that the SPF value could be increased by adding the concentration of citronella oil into the nanoemulsion.

Table 9. SPF, %Te, and %Tp values of nano-emulsion and citronella oil.

Sample	CO			CO Nanoemulsion		
	SPF	%Te	%Tp	SPF	%Te	%Tp
F1	1.01	93.79	98.41	1.03	77.42	95.94
F2	1.02	89.73	96.29	1.13	70.21	94.04
F3	1.03	83.58	94.84	1.14	67.86	93.61

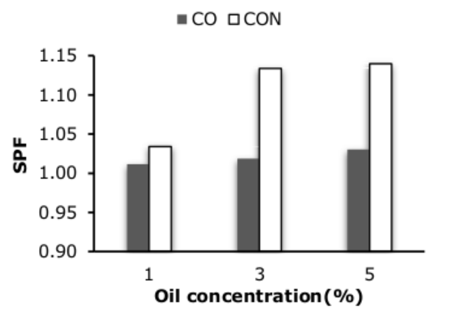

Figure 3. SPF value of citronella oil (CO) and citronella oil nanoemulsion (CON).

b. %Te value

The test of the erythema transmission value (%Te) was tested at a wavelength of 292.5–317.5 nm. The results of the measurement of the %Te value can be seen in Figure 4. According to the tests, the %Te values of the citronella oil nanoemulsion formula with a concentration of 1, 3, and 5% were 77.42, 70.21, and 67.86% respectively. The %Te values of the pure citronella oil with the same oil concentration were obtained to be 93.79, 89.73, and 83.58%.

Based on Figure 4, it can be seen that with the same oil content, the %Te value of citronella oil in nanoemulsion form is always lower than that of pure citronella oil. This result indicates that the amount of UV light transmitted after hitting the pure citronella oil is higher than the amount of UV light transmitted after hitting the nanoemulsion. Based on the measurement of the %Te value, it is also proven that by making citronella oil in the nanoemulsion form, it will increase the effectiveness of sun protection.

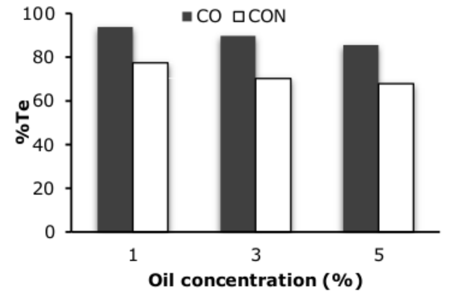

Figure 4. %Te values of citronella oil (CO) and citronella oil nanoemulsion (CON).

c. %Tp value determination

The pigmentation transmission percentage (%Tp) assay was carried out at a wavelength of 322–317.5 nm. The measurement of the %Tp values is displayed in Figure 5.

The %Tp values obtained from 1, 3, and 5% citronella oil nanoemulsion were 95.94, 94.04, and 93.61% respectively. The %Tp values of the pure citronella oil with the same concentration were calculated to be 98.41, 96.29, and 94.84%. Figure 5 indicates that with the same oil content, the %Tp values of citronella oil in nanoemulsion are always lower than that of pure citronella oil. Therefore, based on the measurement of the %Tp value, it is also confirmed that citronella oil in the form of nanoemulsion will increase the effectiveness of sun protection.

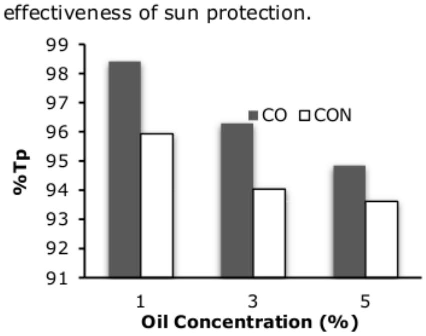

Figure 5. %Tp values of citronella oil (CO) and citronella oil nanoemulsion (CON)

In general, based on the measurement results of SPF, %Te, and %Tp values, citronella oil nanoemulsions with an oil concentration of 1, 3, and 5% do not show good sunscreen activity. However, their sunscreen properties are better, with higher SPF, %Te, and %Tp values, than the pure citronella oil with the same oil concentration. This finding is because the nanoemulsion has a smaller globule size that creates the wider UV absorption area.

4. Conclusion

Citronella oil nanoemulsions were formulated into 3 formulations with different concentrations, namely 1%, 3%, and 5%. The ratio of tween 80 and propylene glycol was 25:10. The characteristics of the prepared citronella oil nanoemulsion have a distinctive organoleptic visualization, an acceptable pH value to the skin (6.0-6.5) with nanoemulsions type of O/W. The nanoemulsion viscosity for the formulations of 1%, 3%, and 5% were 12.76, 25.13, and 84.05 cP, while the percent transmittance of the nanoemulsion with the respective concentrations were 98.4, 98.9, and 99.9%. The particle size distribution of 1%, 3%, and 5% nanoemulsions were 9.4 nm (100%), 10.07 nm (97%), and 8.96 nm (82.5%) respectively. Furthermore, the citronella oil nanoemulsions showed good physical stability when tested by centrifugation and freeze-thaw cycle tests. Analysis of the potential as a sunscreen of the 1%, 3%, and 5% citronella oil nanoemulsion showed the SPF values of 1.03, 1.13, and 1.14 respectively, the %Te value of 77.42, 70.21, and 67.86%, while the %Tp value of 95.94, 94.04, and 93.61%. The prepared nanoemulsions was proven to have the higher sunscreen activity compared to the pure citronella oil's sunscreen activity with the same concentration. The more effective ratio of the oil, surfactant, and water could beexplored in the next research to get formula with the best stability and activity.

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