

FORMULATION AND NANOEMULSION OF NUTMEG ESSENTIAL OIL (*Myristica fragrans* Houtt.) AND SUNSCREEN ACTIVITY TEST USING UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

Nutmeg essential oil (Myristica fragrans Houtt) is a natural ingredient that has potential as a sunscreen. This research aims to formulate and characterize nutmeg essential oil nanoemulsion and test the sunscreen activity of nutmeg essential oil and its nanoemulsion. The essential oil and nanoemulsion formulas were made with 5 variations of essential oil concentrations namely F0 (0%), F1 (1%), F2 (2%), F3 (4%) and F4 (6%). The nanoemulsion characterization includes organoleptic test, pH measurement, nanoemulsion type examination, viscosity measurement, percent transmittance test, particle size distribution measurement, centrifugation test, freez-thaw cycle test. Testing the potential of sunscreen was carried out using a UV-Vis spectrophotometer at a wavelength range of 290-400 nm with ethanol as a blank. The results of the nanoemulsion organoleptic characterization showed a clear and stable appearance. Measurements of pH, type of nanoemulsion, viscosity, percent transmittance, and particle size of nanoemulsion preparations showed good results and were in accordance with the parameters. The nutmeg essential oil nanoemulsion had better SPF, %Te and %Tp values than the essential oil. Nanoemulsion preparations that had the best sunscreen activity in this study were F4 (6%) with an SPF value of 1.538; %Te value of 37.375%; and %Tp value of 80.732%.

ABSTRAK

Minyak atsiri buah pala (*Myristica fragrans* Houtt) merupakan bahan alam yang berpotensi sebagai tabir surya. Penelitian ini bertujuan formulasi dan karakterisasi nanoemulsi minyak atsiri buah pala serta menguji aktivitas tabir surya minyak atsiri buah pala dan nanoemulsinya. Formula minyak atsiri dan nanoemulsinya dibuat dengan 5 variasi konsentrasi minyak atsiri yaitu F0 (0%), F1 (1%), F2 (2%), F3 (4%), dan F4 (6%). Karakterisasi nanoemulsi yang dilakukan meliputi uji organoleptis, pengukuran pH, pemeriksaan tipe nanoemulsi, pengukuran viskositas, uji persen transmitan, pengukuran distribusi ukuran partikel, uji sentrifugasi, uji *freez-thaw cycle*. Pengujian potensi tabir surya dilakukan menggunakan spektrofotometer UV-Vis pada rentang panjang gelombang 290-400 nm dengan etanol sebagai blanko. Hasil karakterisasi organoleptis nanoemulsi menunjukkan penampakan jernih dan stabil. Pengukuran pH, tipe nanoemulsi, viskositas, persen transmitan, dan ukuran partikel sediaan nanoemulsi menunjukkan hasil yang baik dan sesuai dengan parameter. Sediaan nanoemulsi minyak atsiri buah pala memiliki nilai SPF, %Te dan %Tp lebih baik dari minyak atsirinya. Sediaan nanoemulsi yang memiliki aktivitas tabir surya terbaik pada penelitian ini adalah F4 (6%) dengan nilai SPF sebesar 1,538; nilai %Te sebesar 37,375%; dan nilai %Tp sebesar 80,732%.

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INTRODUCTION

The development of time and technology has had many impacts on the earth. One thing that is really felt is that the sun's rays feel hotter and hotter. The negative impact of sunlight is exposure to ultraviolet rays. Human skin that is exposed to ultraviolet light for too long can cause redness, burning, and can even lead to skin cancer (Isfardiyana & Safitri, 2014). One way to protect your skin from ultraviolet rays is to use sunscreen. The ability of a sunscreen to protect the skin by delaying erythema is expressed by the Sun Protection Factor (SPF) (Hassan, *et al.* 2013).

Determination of sunscreen potential is done by determining the SPF value and measuring the percentage of erythema transmission (%_{eT}) and the percentage of pigmentation transmission (%_{pT}). The level of sunscreen ability is divided based on the range of SPF values of the preparation. The divisions are minimum (2-4), medium (4-6), extra (6-8), maximum (8-15), and ultra (<15). Classification of sunscreen potential based on %_{eT} categories of sunblock, extra protection, standard suntan, and fast tanning respectively is <1%; 1-6%; 6-12%; and 10-18%. Meanwhile, the classification of sunscreen potential based on %_{pT} in the categories of sunblock, extra protection, standard suntan and fast tanning respectively is 3-40%; 42-86%; 45-86%, and 45-86% (Pitarisa, *et al.* 2019).

Currently sunscreens are dominated by synthetic chemicals. The use of synthetic compounds can cause side effects on the skin such as irritation with a burning sensation, stinging sensation, and cause allergies. Due to the side effects of synthetic compounds, the development of sunscreen preparations using natural

ingredients is urgently needed. One natural ingredient that has the potential to act as a sunscreen is nutmeg essential oil (*Myristica fragrans* Houtt) (Ansory, *et al.* 2020).

Nutmeg essential oil has the main constituent components including sabinene (41.7), α -pinene (9.4%), β -pinene (7.3%), terpina-4-ol (5.8%), limonene (3.7%), safrole (1.4%) and myristicin (2.7%) (Pal, *et al.* 2011). Myristicin contained in nutmeg essential oil is able to absorb UV B rays. This is because myristicin has a chromophore group (Ansory, *et al.* 2020). The chromophore group in the myristicin compound contained in nutmeg essential oil, namely the C = C bond, is in the benzene ring. Rahmadany *et al* (2021) research shows the potential for microemulsion preparations from nutmeg essential oil as sunscreen. Nutmeg essential oil has the potential as a sunscreen by absorbing UV B rays and restoring skin caused by UV rays (Ansory, *et al.* 2020).

Study and research developments often utilize essential oils to make nanoemulsions. This is done because essential oils have disadvantages, such as low solubility in water. This limitation can be overcome by modifying nutmeg essential oil into a nanoemulsion preparation. Nanoemulsion is the result of a dispersion of oil and water which is stabilized by surfactants and has a size of 10-100 nm (Shoviantari, *et al.* 2019). The advantages of nanoemulsions include good physical stability, good solubility in water, and a large surface area (Nirmala & Nagarajan, 2017). This research aims to formulate and characterize nutmeg essential oil nanoemulsions and test the sunscreen activity and nanoemulsions using UV-Vis spectrophotometry.

METHODS

Tools and materials

The tools used in this research were test tubes, beakers, Erlenmeyer flasks, measuring pipettes, dropper pipettes, measuring cups, fillers, micropipettes, digital pH meters (*Ohaus starter 5000*), ovens, Ostwald viscometers, hot plates, pycnometers, magnetic stirrers, UV-Vis spectrophotometer (*Shimadzu 1800*), and PSA (*particle size analyzer*) Microtac Flex 11.1.0.6.

The materials used in this research were nutmeg essential oil obtained from the refinery industry in Dayeuhluhur Cilacap, surfactant tween 80 p.a (*Merck, Germany*), propylene glycol p.a (*Merck, Germany*), methylene blue (*Merck, Germany*), ethanol p.a (*Merck, Germany*), and distilled water.

Table 1. Nutmeg essential oil nanoemulsion formula

	F0	F1	F2	F3	F4
Nutmeg essential oil (v/v)	0	1	2	4	6
Tween 80 (v/v)	20	20	20	20	20
Propylene glycol (v/v)	15	15	15	15	15
Aquades (v/v)				Add up to 100	

Nanoemulsion Characterization

Organoleptic test

This was done by observing changes in color, aroma, phase separation and clarity for 4 weeks with the same 10 panelists.

pH measurement

This was done using a digital pH meter to determine the nanoemulsion of nutmeg essential oil in the pH range that is acceptable to the skin.

Examination of nanoemulsion type

This is done by adding 1 drop of methylene blue to each nanoemulsion preparation. Methylene blue is a dye that is soluble in water (Ayuningtias, 2017).

Nanoemulsion Formula

The nutmeg essential oil nanoemulsion formula consists of an oil phase and a water phase. The oil phase consists of variations of 0, 1, 2, 4 and 6 mL with the addition of a surfactant system. The surfactant system was prepared from 20 mL Tween 80 and 15 mL propylene glycol. The oil phase was homogenized using a hot plate magnetic stirrer at a speed of 750 rpm at a temperature of 50°C for 60 minutes. Then add the water phase (distilled water) slowly to the oil phase to a volume of 100 mL. The oil phase and water phase were homogenized again for 540 minutes at 50°C with a speed of 1250 rpm. The nutmeg essential oil nanoemulsion formula can be seen in Table 1.

Viscosity measurement

The purpose of the measurement is to determine the viscosity level of the nanoemulsion. This is done by inserting 5 mL of nanoemulsion into the Ostwald viscometer then sucking it in using a filler until the sample reaches the upper limit mark of the Ostwald viscometer. The sample drop time is calculated from the upper limit mark to the lower limit mark on the Ostwald viscometer.

Transmittance percent test

This was done using a UV-Vis spectrophotometer at a wavelength of 650 nm and using distilled water as a blank.

Measurement of particle size distribution

The nanoemulsion particle size was measured using a PSA instrument at the Physics Laboratory, Yogyakarta State University. The working principle of this tool is that there is light scattering that occurs due to the shooting of a laser beam that hits the particles in the sample. The scattered light will be read by a photon detector at a certain angle so that it can determine the particle size.

Centrifugation test

This was done by inserting 2 mL of nanoemulsion into an Eppendorf and centrifuging at a speed of 6000 rpm for 30 minutes. Then physical observations were carried out which included phase separation, turbidity and precipitation.

Freeze-thaw cycle test

This was done by storing the nanoemulsion at 4°C for 24 hours, then moving it to 40 °C for 24 hours (1 cycle) and carrying out 3 cycles. Then the changes in color, clarity, aroma, phase separation and precipitation were observed.

Sunscreen Activity Test of Nutmeg Essential Oil and Its Nanoemulsion Preparation of nutmeg essential oil solution

The nutmeg essential oil solution was made with varying concentrations of 1; 2; 4; and 6%. This variation was made by adding ethanol to 1, 2, 4, and 6 mL of nutmeg essential oil to a volume of 10 mL.

Determination of SPF value of nanoemulsion

0.2 mL of each variation of nutmeg essential oil and nanoemulsion was taken and diluted again using ethanol to a volume of 10 mL. Test samples F1, F2, F3, and F4 were obtained with concentrations

of 182 ppm, 364 ppm, 728 ppm, and 1092 ppm respectively. Dilution is carried out to avoid inaccuracies in measuring absorbance values that exceed the measurement range of the instrument due to high sample concentrations. Then the SPF value was measured in duplicate using a UV-Vis Spectrophotometer in the wavelength range 290 - 400 nm with 5 nm intervals. The SPF value is calculated by the equation:

$$\log \text{SPF} = \frac{\Sigma \text{AUC}}{\lambda n - \lambda 1} \quad (1)$$

λn is the largest wavelength between 290 nm to 400 nm; wavelength 1 ($\lambda 1$) is the smallest wavelength (290 nm). Meanwhile, the AUC value is calculated using the equation (Rejeki & Wahyuningsih, 2015).

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

Where $A(p)$ is the amount of absorbance at the higher wavelength between the two wavelengths; $A(p-a)$ is the absorbance value at the lower wavelength between two consecutive wavelengths; $\lambda(p)$ is the higher wavelength value between two wavelengths and $\lambda(p-a)$ is the lower wavelength value between two consecutive wavelengths (Lalus, 2018)

Determination of the percent value of erythema transmission

The percent transmission of erythema was determined by measuring the transmittance of each variation of nutmeg essential oil and its diluted nanoemulsion in the wavelength range 292.5-317.5 nm. The amount of erythema flux transmitted by the sunscreen material (E_e) is calculated using the formula $E_e = \sum T \cdot F_e$. The percent transmission of erythema can be calculated using the equation formula:

$$\% \text{Te} = \frac{E_e}{\sum F_e} \quad (3)$$

Where the $\% \text{Te}$ value is the percent transmission of erythema; F_e is the erythema flux value; and E_e is the amount

of erythema flux transmitted by the nanoemulsion at a wavelength of 292.5-317.5 nm (Lalus, 2018)

Determination of the percent value of pigmentation transmission

The percent transmission of pigmentation is determined by measuring the transmittance of each concentration of diluted nutmeg essential oil in the wavelength range 322.5-372.5 nm. The amount of erythema flux transmitted by the sunscreen material (Ep) is calculated using the formula

$$Ep = \sum T.Fp. \quad (4)$$

The percent transmission of erythema can be calculated using the equation:

$$\%Tp = \frac{Ep}{\sum Fp} \quad (5)$$

Where %Tp is the percent transmission of pigmentation; Fp is the pigmentation flux value; and Ep is the amount of pigmentation flux transmitted by the nanoemulsion at a wavelength of 322.5-372.5 nm (Lalus, 2018)

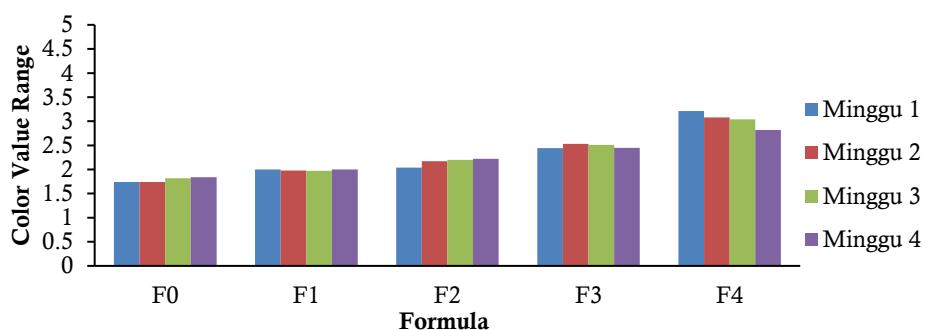


Figure 1. Graph of color organoleptic test results

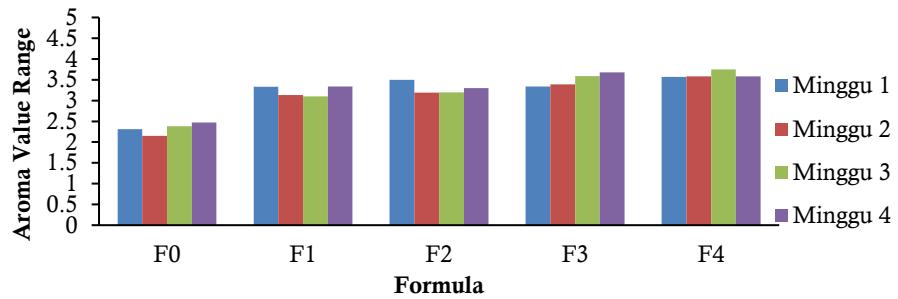


Figure 2. Chart aroma organoleptic test results

RESULT AND DISCUSSION

Nanoemulsion Formulation

The nutmeg essential oil nanoemulsion formulation was made using a surfactant system. Use of Tween 80 as a surfactant and propylene glycol as a cosurfactant. Surfactants are organic compounds that function to reduce surface tension. The amphiphilic nature of surfactants will bind polar solutions (water phase) in the hydrophilic part and bind non-polar solutions (oil phase) in the lipophilic part, thus uniting the two phases (Viriya & Lestari, 2015).

Nanoemulsion Characterization

Organoleptic Test

Based on Figures 1, 2, 3 and 4, the organoleptic test results for aroma, color, clarity and phase separation show a good range. This shows that the nutmeg essential oil nanoemulsion preparation has good stability.

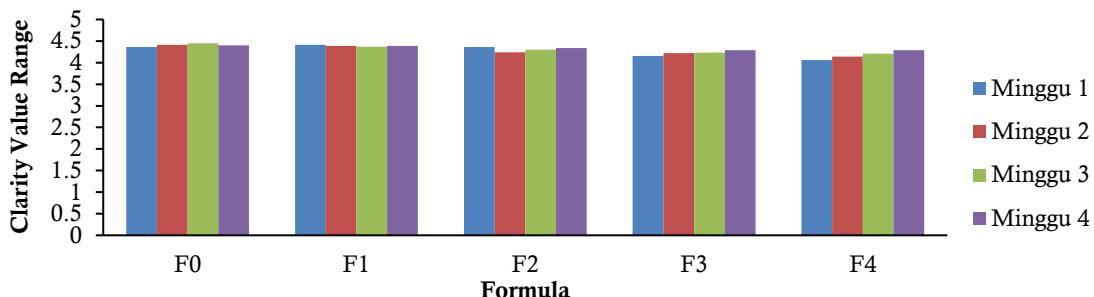


Figure 3. Graph of color organoleptic test results

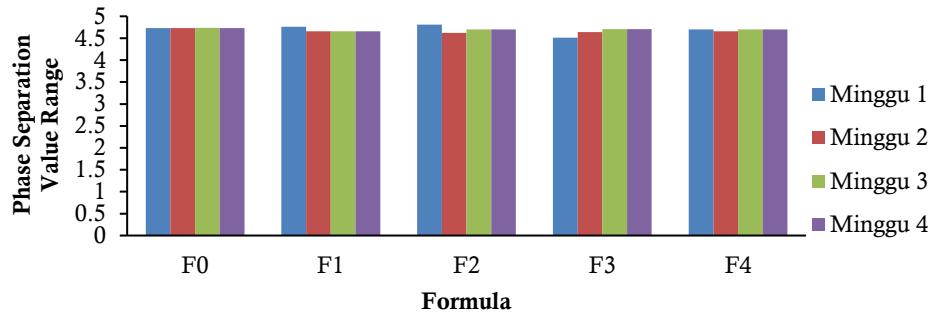


Figure 4. Chart organoleptic test results separation phase

Table 2. Description of organoleptic test data

Range Mark	Information			
	Color	Clarity	Aroma	Phase Separation
0.0 – 2.0	Colorless	Murky	Not Fragrant	Separate
2.1 – 4.0	A bit yellow	Clear	Fragrant	Not Separating
4.1 – 5.0	Yellow	Very clear	Very Fragrant	Very Undivided

pH measurement

According to Permata *et al* (2023), the pH standard for human skin is 4.6-6.5, because if the pH is too acidic it can irritate the skin and if it is too alkaline it

can cause scaly skin. Based on Figure 5, all nutmeg essential oil nanoemulsion formulas show a value range of 5-6. This shows that all formulas have a safe pH and do not cause irritation to the skin.

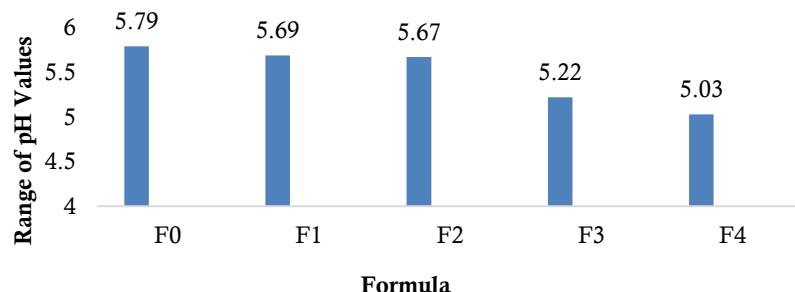


Figure 5. nanoemulsion pH measurement results

Examination of nanoemulsion type

Based on Figure 6, it shows that methylene blue dissolves completely in the nanoemulsion. According to Kunarto & Singh (2018) methylene blue which

dissolves completely in nanoemulsion shows the oil in water (o/w) type. This happens because the nanoemulsion formula contains more water than the oil phase.



Figure 6. Results of nanoemulsion type examination

Viscosity measurement

The viscosity measurement results in Figure 7 show that the greater the concentration of nutmeg essential oil, the greater the viscosity value of the

nanoemulsion. According to Kunarto *et al* (2020), this is because the more particles that dissolve, the higher the friction between the particles and increase the viscosity.

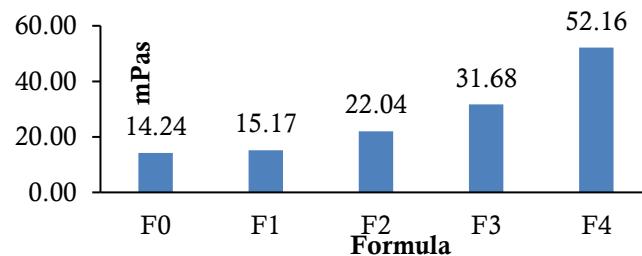


Figure 7. Graph of nanoemulsion viscosity measurement results

Transmittance percent test

A percent transmittance value close to 100% indicates that the nanoemulsion has clear and transparent properties (Ali &

Hussein, 2017). Based on Figure 8, all nanoemulsion formulations show good clarity.

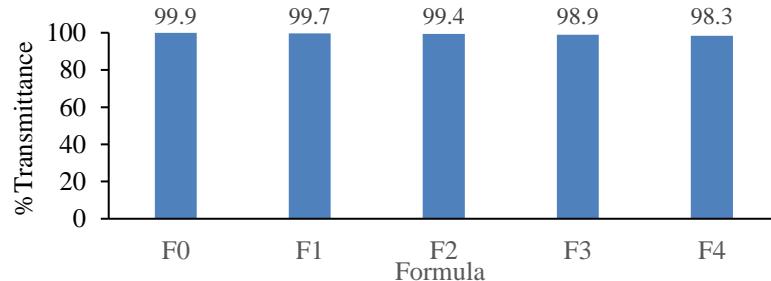


Figure 8. Graph of nanoemulsion percent transmittance test results

Measurement of particle size distribution

Based on Table 2, all nutmeg essential oil nanoemulsion formulas show a particle size of <10 nm. This shows that all formulas fall in the size range of 10-200 nm (Devarajan & Ravichandran, 2011). The polydispersity index (PDI) is a value that functions as an indicator of homogeneous (monodisperse) particle size

distribution. A PDI value >0.5 indicates a fairly homogeneous particle size, while a PDI value <0.5 indicates particles that have a high level of homogeneity (heterodisperse) (Avadi, *et al.* 2010). The results obtained show that F1 is in the monodisperse category, while F2, F3, and F4 are in the heterodisperse category.

Table 3. Results of measurements of nanoemulsion particle size distribution

Formulas	Particle Size (nm)	Volume (%)	PDI
F1	13,89	82,2	0,427
	93,5	17,8	
F2	16,46	74,5	1,625
	172,2	25,5	
F3	19,37	71,6	1,374
	188,5	28,4	
F4	19,57	70,2	1,235
	256,8	29,8	

Centrifugation test**Table 4. Nanoemulsion centrifugation test results**

Observation After Centrifugation	Nutmeg Oil Nanoemulsion Sample				
	F0	F1	F2	F3	F4
Turbidity	NA	NA	NA	NA	NA
Phase Separation	NA	NA	NA	NA	NA
Precipitation	NA	NA	NA	NA	NA

NA: Not Avail

Stable nanoemulsions can be observed without phase separation (Budiputra, *et al.* 2014). Based on Table 2, the results of the centrifugation test for the nanoemulsion preparation show that there was no change in terms of turbidity, phase separation and precipitation. This shows that the nanoemulsion preparation has a good level of stability.

Freeze-thaw cycle test

The results of the freeze-thaw cycle test were physically observed to see if there were any changes in terms of organoleptic, phase separation and precipitation. Based on Table 4, it is proven that the nanoemulsion preparation of nutmeg essential oil that has been made shows a good level of stability because there is no change in terms of organoleptics, clarity, phase separation and precipitation after the freeze-thaw cycle test.

Table 5. Nanoemulsion freeze-thaw cycle test results

C = Pengamatan Organoleptis	Sebelum Uji Freeze-Thaw Cycle					Setelah Uji Freeze-Thaw Cycle				
	F0	F1	F2	F3	F4	F0	F1	F2	F3	F4
- Color	C	C	C	C	C	C	C	C	C	C
- Clarity	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
- Aroma	DS	DS	DS	DS	DS	DS	DS	DS	DS	DS
- Phase Separation	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
- Precipitation	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Colorless; C1 = Clear; DS = Distinctive Scent; NA = Not Avail

Test of the sunscreen potential activity**Test the SPF value**

SPF value testing was carried out to determine the time span when nanoemulsion can protect the skin. The

SPF value according to Figure 9 shows that the nanoemulsion preparation of nutmeg essential oil is greater than that of nutmeg essential oil. This is because nanoemulsion has a smaller droplet size. The small

droplet size will expand the surface of the absorption area, so that the nanoemulsion absorbs more ultraviolet light (Arianto & Cindy, 2019). The SPF value of nutmeg

essential oil and its nanoemulsion cannot be categorized as sunscreen because it is in the range 1-1.5 (Pitarisa, *et al.* 2019).

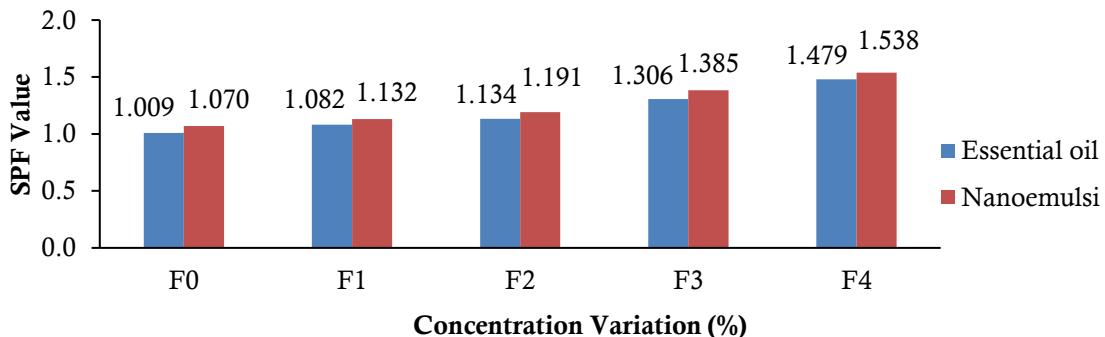


Figure 9. Graph of test results for SPF nanoemulsion and nutmeg essential oil

Test the percent value of erythema transmission

The percent erythema transmission value (%Te) is needed to determine the effectiveness of sunscreen against UV-B rays (Widyastuti, *et al.* 2016). Based on Figure 10%Te of nutmeg essential oil and

its nanoemulsion is outside the range of sunscreen potential because the %Te value is above 18% (Pitarisa, *et al.* 2019). This shows that nutmeg essential oil and its nanoemulsion do not have protection against UV-B rays.

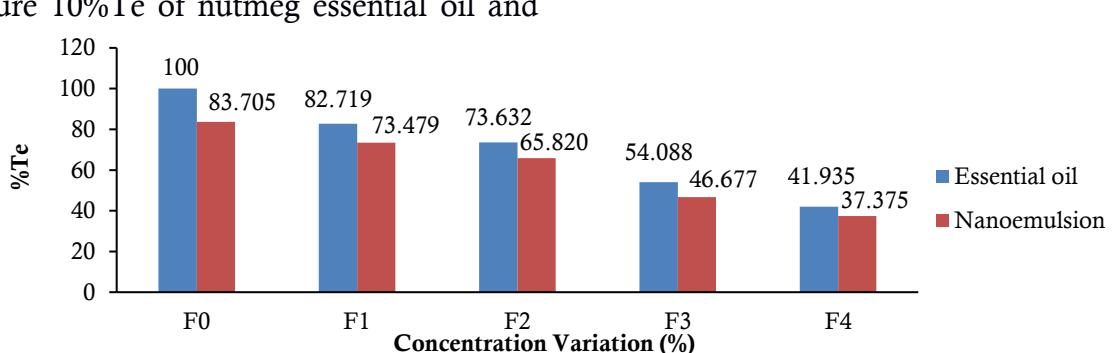


Figure 10. Graph of %Te test results for nanoemulsion and nutmeg essential oil

Test the percent value of pigmentation transmission

The percentage transmission value of pigmentation (%Tp) is needed to determine the effectiveness of sunscreen against UV-A rays. Based on Figure 11, the %Tp of nutmeg essential oil and its

nanoemulsion shows that only the F4 concentration falls into the range of fast tanning classification. Meanwhile, F0, F1, F2, and F3 are not included in the classification because they have %Tp values above 86% (Pitarisa, *et al.* 2019).

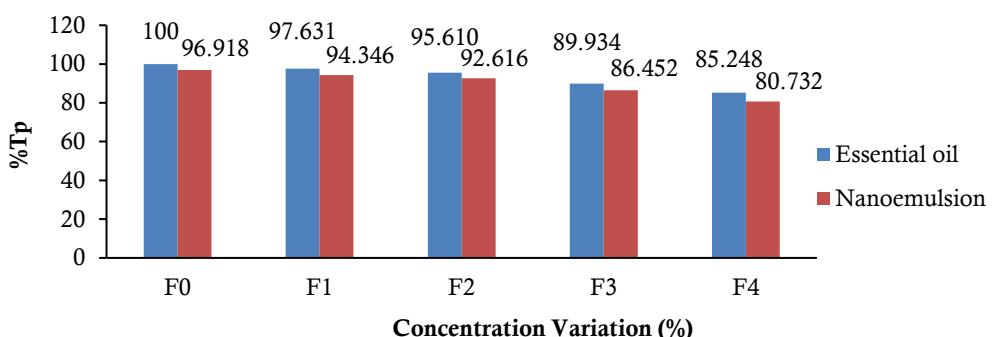


Figure 11 Graph of %Tp test results for nanoemulsion and nutmeg essential oil

CONCLUSION

Based on research results, nutmeg essential oil nanoemulsion has good physical stability with a dominant droplet size of <10 nm. Meanwhile, for sunscreen test results, nutmeg essential oil has a smaller SPF value than the nanoemulsion. In addition, both nutmeg essential oil and nanoemulsion have low activity as a sunscreen.

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