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Formulation, Characterization, and Sunscreen Potential Evaluation of Nutmeg Leaf Essential Oil Nanoemulsions (*Myristica fragrans* Houtt.)

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ABSTRACT. Nutmeg leaf essential oil (*Myristica fragrans* Houtt.) is one of the natural ingredients which have antioxidant activity and potential as a sunscreen. The research aims to formulate nutmeg leaf essential oil nanoemulsion, characterize, and determine its potency as a sunscreen. Nutmeg leaf essential oil nanoemulsion was formulated with 1; 3; and 5 mL of oil content with two variations of surfactant. The characterization included organoleptic, pH, viscosity, %T, nanoemulsion type, droplet size, thermodynamic and centrifugation stability tests. Evaluation was conducted using UV-Vis spectrophotometer at a wavelength range of 290-400 nm with ethanol as a blank and non-nano-emulsified as a comparison. The result showed that the nutmeg leaf essential oil nanoemulsion had clear and stable appearance, safe for skin, viscosity values of <200 mPas, %T values of near 100%, nanoemulsions type of o/w, and particles size of <200 nm. The evaluation showed that the sunscreen activities of nutmeg leaf oil nanoemulsion was higher than non-nano-emulsified. The highest sunscreen activity was the formula A nanoemulsion which an oil content of 5 mL and had an SPF value of 1.475; %Te value of 50.571%; and %Tp value of 77.218%. The nanoemulsion sunscreen activity of formula A was categorized as a regular suntan.

Keywords: Erythema, essential oil, nutmeg leaf, pigmentation, sun protection factor

INTRODUCTION

Nanoemulsion is an emulsion that is transparent, translucent, and is a dispersion of oil and water which is stabilized by a film layer of surfactant. Surfactants are important ingredients in nanoemulsion formulations because they can dissolve lipophilic (oil-loving) active ingredients such as Tween 80. Since the use of surfactants in most cases is not sufficient to reduce interfacial tension and water, cosurfactants are needed to help lower the interfacial tension. The addition of cosurfactants in addition to lowering the interfacial tension of oil and water, also increases the fluidity of the interface and possibly increases the entropy of the system (Yuliasari &Hamdan, 2012).

Nanoemulsions have very small droplet sizes, ranging from 5-200 nm (Cinar, 2017) . Nanoemulsions are made by mixing the oil phase and water phase with the help of surfactants and cosurfactants to reduce interfacial tension (Sarmah et al., 2019). Several studies have proven that nanoemulsions can increase effectiveness, prevent creaming and other emulsion damage during storage (Jintapattanakit et al., 2018).

Nanoemulsions are increasingly being used in cosmetic products because they have attractive physical characteristics, such as small droplet size with large interfacial area, transparent and translucent appearance, high solubility capacity, low viscosity, and high kinetic stability because this kind of formula prevent sedimentation and flocculation (Chellapa et al., 2015). Nanoemulsions can be applied to essential oils. Essential oil is one of the secondary metabolites produced by plants (Asbur, 2018).

Essential oils, also known as etheric oils, are volatile oils or volatile oils with different compositions according to the source of production. Essential oils are volatile at room temperature, smell good according to the smell of the plant that produces them and are mostly soluble in organic solvents but insoluble in air. Essential oils are not pure chemical compounds, but consist of a mixture of compounds that have different physical and chemical properties (Guenther, 2006). Nutmeg leaf essential oil can be obtained from distillation of nutmeg leaves.

Nutmeg (*Myristica fragrans* Houtt.) is a plant that grows in almost all tropical countries (Suwarto, 2014). From a morphological point of view, nutmeg is a medium tree. Nutmeg tree trunk upright, woody, single leaf, oval shape, pointed tip and base, shiny green color. The shape of the nutmeg tree, with a beautiful appearance, 10-20 m high, towering up and to the side, the crown of the tree is tapered, pyramidal (conical), oval (cylindrical) and relatively regular branching. The flower is panicle-shaped, coming out of the leaf axils, the male flowers are ball-shaped, yellow. While the seeds are small, oval, red seed coat, brownish black seeds (Hapsoh and Hasanah, 2011).

Nutmeg leaf essential oil is commonly used as traditional medicine by drinking or smearing it as an external medicine. It is also used as a perfume and cosmetics (Susanti, 2019) . Its antioxidant levels can be used as a natural sunscreen. Sunscreen activity can protect the skin from UV rays exposure (free radicals). The addition of antioxidant compounds into sunscreen preparations is known to reduce the oxidative effects of ROS (*Reactive Oxygen Species*) from UV radiation (Hassan et al., 2013).

In this research, formulation, characterization, and potential testing of nanoemulsion sunscreen activity were carried out compared to non-nanoemulsified of nutmeg leaf essential oil. The low energy method was used to fomulate nanoemulsions and the formation of nanoemulsions is highly depend on environmental conditions such as composition, temperature, and stirring, which then will form a stable conditions (Rao & David, 2012) (Munawiroh et al., 2019).

EXPERIMENTAL SECTION

Tools and Materials

The materials used in this research were nutmeg leaf essential oil (from Deyeuhluhur, Cilacap), surfactant tween 80 (Merck, Germany), propylene glycol p.a (Merck, Germany), methylene blue (Merck, Germany), ethanol p.a (Merck, Germany), and distilled water. This research began with the preparation of nutmeg leaf essential oil nanoemulsion, characterization of nutmeg leaf essential oil nanoemulsion, and followed with the evaluation of nutmeg leaf essential oil nanoemulsion sunscreen pontential.

Nanoemulsion Preparation

Nutmeg leaf essential oil nanoemulsion were prepared in 6 formulas. Nutmeg leaf oil contents were varied at 1, 3, and 5 mL. Surfactant system were added in different amount. Formula A was formed by 20 mL of tween 80 and 15 mL of propylene glycol, while formula B was formed by 25 mL of tween 80 and 10 mL of propylene glycol, as shown as in **Table 1**. The mixture of nutmeg leaf essential oil and surfactant system were homogenized using hotplate magnetic stirrer at speed of 750 rpm at 50 °C for 60 minutes. Then, the distilled water was added gently into the mixture. The mixture was stirred again using hotplate magnetic stirrer for 60 minutes at a speed of 1200 rpm at 50 °C.

Nanoemulsion Characterization

Nanoemulsion prepared from nutmeg leaf essential oil were characterized based on the organoleptic test (Shoviantari et al., 2019), transmittance percentage (Ali & Hussein, 2017). Viscosity test (Gupta et al., 2010), pH test (Ali &Yosipovitch, 2013), nanoemulsion types test , particle size test, thermodynamic and centrifugation stability test .

Organoleptic test was conducted to identify the characteristic of nanoemulsion by observing the color, odor, clarity, and phase separation of the nanoemulsion. The nanoemulsion formula was centrifuged at 10.000 rpm for 30 minutes and its organoleptic was observed. The formula was then stored at low temperature (4 °C), room temperature, and high temperature (40 °C) for 4 weeks, and its organoleptic was observed every 1 week.

pH test was carried out to determine the safety of nanoemulsion for skin. The type of nanoemulsion was evaluated by dripping methylene blue into the nanoemulsion (dye test) to determine the dispersed and the dispersing phase (oil in water or water in oil).

The %T test was conducted using a UV-Vis spectrophotometer with a wavelength of 650 nm to measure the clarity of the formulated nanoemulsion. Viscosity test was measured using an Ostwald viscometer for 3 repetitions. Particle size test was analyzed by Particle Size Analyzer (PSA) to determine the particle size and distribution of the nanoemulsion. Thermodynamic and centrifugation stability test were carried out to determine the stability of the nutmeg essential oil nanoemulsion.

Sunscreen Potential Evaluation

Determination of SPF value

A total of 0.2 mL of each nutmeg leaf essential oil nanoemulsion formula was diluted with ethanol until the volume reached 10 mL. Each nanoemulsion formula was measured its SPF value using a UV-Vis spectrophotometer (Shimadzu 1800) with a wavelength of 290-400 nm with 5 nm intervals and ethanol was used as a blank (Lalus, 2018).

Table 1. Formula of nutmeg leaf essential oil nanoemulsion

	Formula A			Formula B		
	F1	F2	F3	F1	F2	F3
Nutmeg leaf essential oil (v/v)	1	3	5	1	3	5
Tween 80 (v/v)	20	20	20	25	25	25

Propylene glycol (v/v)	15	15	15	10	10	10
Distilled water (v/v)			Add until 100			

SPF value is calculated using equation (1) (Rejeki and Wahyuningsih, 2015).

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

Meanwhile AUC can be defined as area under the curve that can be calculated using equation (2):

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

Description:

A(p) : absorbance at the higher wavelength between two wavelengths

A(p-a) : absorbance at the lower wavelength between two consecutive wavelengths

λ(p) : Wavelength the higher of the two wavelengths

λ(pa) : The wavelength that is lower between the two successive wavelengths.

While the SPF value can be calculated by equation (3) (Rejeki & Wahyuningsih, 2015)

$$\text{Log SPF} = \frac{\sum \text{AUC}}{\lambda n - \lambda 1} \quad (3)$$

λn is the largest wavelength among the wavelengths from 290 nm to 400 nm; wavelength 1 (λ1) is the smallest wavelength (290 nm) (Tahir et al., 2002).

Determination of the percentage of erythema transmission

Percent transmission of erythema (%Te) describes the amount of sunlight that is transmitted after it hits the sunscreen, which can cause skin erythema (skin redness) (Ahmad, 2015). The %Te value is needed to determine the effectiveness of sunscreen against UV-B rays (Widyawati et al., 2019) . The percentage of erythema transmission was determined by measuring the transmission of the diluted nanoemulsion formula using a UV-Vis spectrophotometer (Shimadzu 1800) with wavelength of 292.5-317.5 nm with 5 nm intervals and ethanol as a blank (Lalus, 2018) . The percentage of erythema transmission (Te) is calculated using equation (4) (Lalus, 2018).

$$\%\ \text{Te} = \frac{Ee}{\sum Te} = \frac{\sum Te}{\sum Fe} \quad (4)$$

Description:

Fe = erythema flux value at a certain wavelength

Te = erythema transmittance value

Ee = number of fluxes erythema transmitted by nanoemulsion at a wavelength of 292.5-317.5 nm

Determination of percent transmission of pigmentation

Pigmentation transmission percentage (%Tp) describes the amount of sunlight that is transmitted after it hits the sunscreen, which can cause skin pigmentation (skin becomes darker) (Ahmad, 2015). The percentage value of pigmentation transmission is determined to determine the effectiveness of sunscreen against UV-A rays. a sunscreen is said to have good effectiveness if it has a high SPF value, as well as small %Te and %Tp (Widyawati et al., 2019). The value of percent transmission of pigmentation was determined by measuring the transmission of the diluted nanoemulsion formula using a UV-Vis spectrophotometer (Shimadzu 1800) with a wavelength of 292.5-372.5 nm with 5 nm interval and ethanol as blank (Lalus, 2018) . The percentage of pigmentation transmission (Tp) value is calculated using equation (5) (Lalus, 2018)

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

Description:

Fp = pigmentation flux value at a certain wavelength

Tp = pigmentation transmittance value.

Ep = the amount of pigmentation flux transmitted by the nanoemulsion at a wavelength of 322.5-372.5 nm.

RESULTS AND DISCUSSIONS

Nanoemulsion Preparation

Nanoemulsion formulation of nutmeg essential oil nanoemulsion is categorized as *inspontaneous emulsification* where the energy required is low. The advantages of low energy methods are more effective to form droplets in very small size, not requiring specific equipment, more saving energy, and easy to carried out. Many factors affect the forming of nanoemulsion, such as the composition of the mixture of oil, surfactant-cosurfactant, and water (Handayani et al., 2018). The formulation of nanoemulsions with low energy has drawbacks, such as the result of particle size is less uniform and cannot be applied to a formula with high oil compositions.

Characteristics of Nanoemulsion

Organoleptic test

The tests were carried out right after the nanoemulsion was made (week 0) and after 4 weeks (**Table 2**). All nanoemulsion formulas tend to be colorless, have a specific odor, quite clear, and do not undergo phase separation. It can be said that the result of nanoemulsion preparation is good and stable because it has a clear appearance and there is no phase separation (Costa et al., 2012). **Table 2** showed that there was no physical change of the nanoemulsion from week 0 to week 4.

pH test

The nanoemulsion was used on the skin, therefore the pH of the nanoemulsion must be in the range that is safe for the skin, which is 4.5 to 7 and does not cause irritation. If the pH is too acidic it can irritate the skin and if the pH is too alkaline it can make the skin dry (Naibaho et al., 2013). The nanoemulsion preparation has a pH that is safe for the skin (**Table 3**).

Nanoemulsion type examination

Examination of the nanoemulsion type was carried out by adding a few drops of methylene blue to the nanoemulsion. **Figure 1** showed that methylene blue was completely dispersed in the nutmeg leaf oil nanoemulsion. This identified that the nanoemulsion has the type of O/W (*oil in water*). The type of oil-in-water nanoemulsion is formed because the nanoemulsion system has more water phase and the nature of surfactant tween 80 is also a hydrophilic so that the polar head will be more oriented to the water phase. The result, methylene blue can be evenly dispersed in the nanoemulsion (Mardikasari et al., 2016).

Viscosity test

The ideal viscosity value for nanoemulsion preparations is in the range of 1-200 mPas (Gupta et al., 2010). Based on **Table 4,** it showed that the greater the concentration of nutmeg leaf essential oil, the greater viscosity. It was influenced by the higher oil content and the size of the nanoemulsion particles. The larger the size of the nanoemulsion, the greater the fluidity of the nanoemulsion.

Transmittance percentage test

The transmittance value of a nanoemulsion that can be catogorized as good quality should be in the range of 90-100% which shows a clear and transparent appearance (Costa et al., 2012). Based on **Table 5**, it can be seen that the percent transmittance value of nanoemulsions are in the category of good quality nanoemulsions.

Table 2. Results of organoleptic nanoemulsion of nutmeg leaf oil

Parameters	Week 0						Week 4					
	F1		F2		F3		F1		F2		F3	
	A	B	A	B	A	B	A	B	A	B	A	B
Color	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
Scent	F	F	SF	SF	SF	SF	F	F	F	F	F	F
Clarity	C	C	C	C	C	C	C	C	C	C	C	C
Phase separation	I	I	I	I	I	I	I	I	I	I	I	I

Description: CL = Colorless, F = Fragrant, SF = Slightly Fragrant, C = Clear, I = Inseparable, A = Formula A, B = Formula B

Table 3. The results of the pH of the nutmeg leaf oil nanoemulsion

Formula	pH	
	A	B
F1	5.9	5.9
F2	5.9	5.9
F3	5.7	5.9

Description: A = Formula A, B = Formula B

Figure 1. Results of examination of nutmeg leaf oil nanoemulsion

Table 4. The results of the viscosity of the nutmeg leaf oil nanoemulsion

Formula	(mPas)	
	Formula A	Formula B
F1	12.677	11.232
F2	13.152	11.503
F3	14.214	14.714

Description: A = Formula A, B = Formula B

Table 5. The result of percent transmittance of nutmeg leaf oil nanoemulsion

Formula	Transmittance value (%)	
	Formula A	Formula B
F1	99.50	99.56
F2	99.27	98.70
F3	97.47	98.23

Description: A = Formula A, B = Formula B

Particle size test

Based on **Table 6**, it can be seen that the nanoemulsion has a particle size that are less than 200 nm (Cinar, 2017) . Formula 1A has the best homogenity compared to the other formulas. This shows that the concentration of nutmeg leaf essential oil and surfactant affect the homogeneity and size of the nanoemulsion.

Based on **Figure 2** and **Figure 3**, it can be seen that the nanoemulsions prepared has more than one peak, except for F1A. Formula F1A has a homogeneous particle size distribution with a polydispersity index (PDI) value close to 0, this indicates that the particle size dispersion is homogeneous. A polydispersity index value of more than 0.7 indicates high heterogeneity and has a very wide size distribution. The smaller value of the polydispersity index, the more uniform the particle size. The big size difference between the particles will affect the particle characteristic (Prihantini et al., 2019)

Table 6. Particle size of nutmeg leaf essential oil nanoemulsion

Formula	Volume (%)	particle size (nm)	PDI
F1.A	100	15.63	0.236
F1.B	78.7	12.66	1.407
	21.3	80.5	
F2.A	16	16.83	1.260
	19.5	154.3	
F2.B	78.8	13.08	1.576
	21.2	169.2	
F3.A	75.5	15.18	1.639
	24.5	161.3	
F3.B	74.4	14.9	1.777
	24.6	232.8	

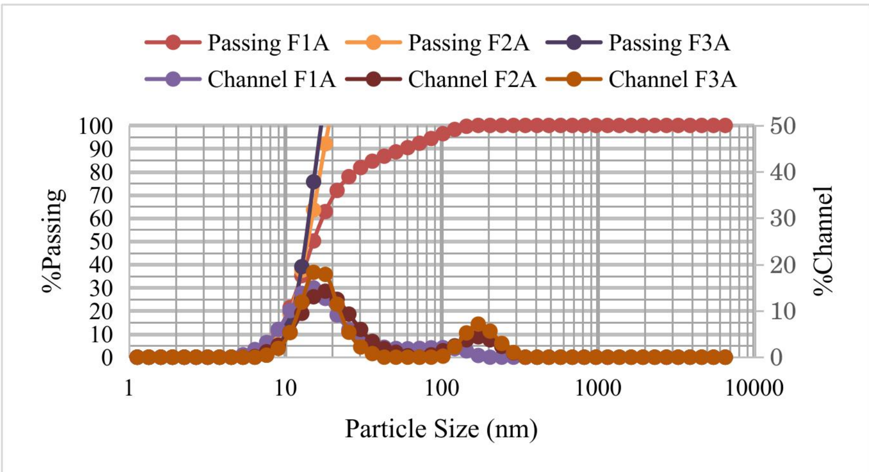

Figure 2. Distribution graph of nutmeg leaf oil nanoemulsion formula A

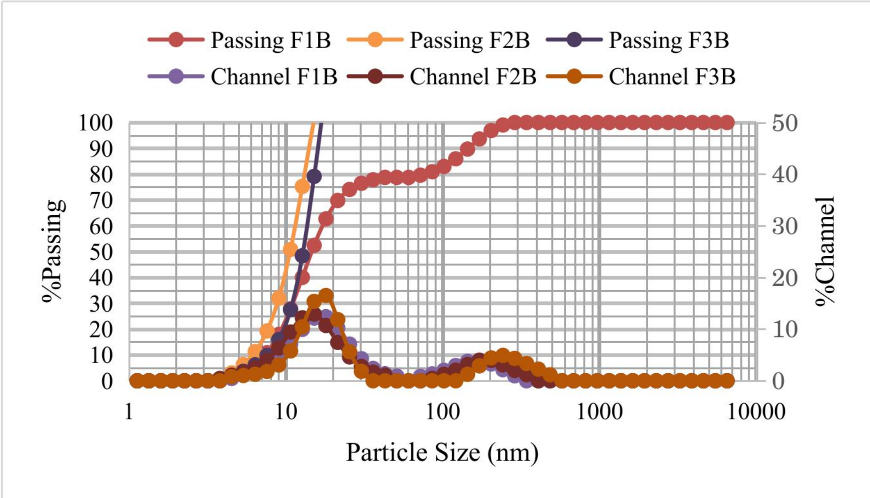

Figure 3. Distribution graph of nutmeg leaf oil nanoemulsion Formula B

Table 7. The results of the centrifugation test of nutmeg leaf essential oil nanoemulsion

Formula	Centrifugation Test
F1.A	Clear and Homogeneous
F1.B	Clear and Homogeneous
F2.A	Clear and Homogeneous
F2.B	Clear and Homogeneous
F3.A	Clear and Homogeneous
F3.B	Clear and Homogeneous

Centrifugation test

Based on **Table 7**, it can be seen that after centrifugation there was no physical change, this indicates that the nanoemulsion was quite stable when given the kinetics energy.

Sunscreen potential evaluation (SPF)

The SPF value testing was conducted to determine how long nanoemulsion is able protect the skin. The SPF value was carried out by diluting the nanoemulsion in ethanol and then measured using a UV-Vis spectrophotometer with a wavelength of 290-400 nm with 5 nm intervals. Ethanol was used as a blank. Non-nanoemulsified nutmeg leaf essential oil was also measured for its SPF value as a comparison with the same oil content of 1, 3, and 5 mL. The test results can be seen in **Table 8** and **Figure 4**.

Based on the results, it shows that the nutmeg leaf essential oil nanoemulsion has a higher SPF value than the nutmeg leaf essential oil without being nanoemulsified. The SPF value increased with the addition of nutmeg leaf oil. Formula A has a higher SPF value than formula B, this shows that the tween ratio of formula A (tween ratio 80: PEG 20:15) is more effective in increasing sunscreen activity than formula B (tween ratio 80: PEG 25:10).

Erythemal transmission (%Te) value evaluation

The test of the erythema transmission value was carried out at a wavelength of 292.5-317.5 nm. The results of the %Te test can be seen in **Table 8** and **Figure 5**. Based on **Figure 5**, it can be seen that the %Te value of nutmeg leaf essential oil nanoemulsion is lower than non-nanoemulsified nutmeg leaf essential oil. This shows that more UV rays are transmitted after hitting the nutmeg leaf essential oil without being formed in nanoemulsion than when it hits the nutmeg leaf essential oil in the form of nanoemulsion.

Pigmentation transmission (%Tp) value evaluation

The %Tp value was tested at a wavelength of 322.5-372.5 nm. The results of the %Tp test can be seen in **Table 8** and **Figure 6**. Based on **Figure 6**, it can be seen that the nutmeg leaf essential oil nanoemulsion has a lower %Tp value than non-nanoemulsified nutmeg leaf essential oil. This shows that the UV light transmitted after hitting the nutmeg leaf essential oil nanoemulsion is less than that of the nutmeg leaf essential oil without being formed as a nanoemulsion. A sunscreen is said to have good effectiveness if it has a high SPF value, and a small %Te and a small %Tp (Widyawati et al., 2019).

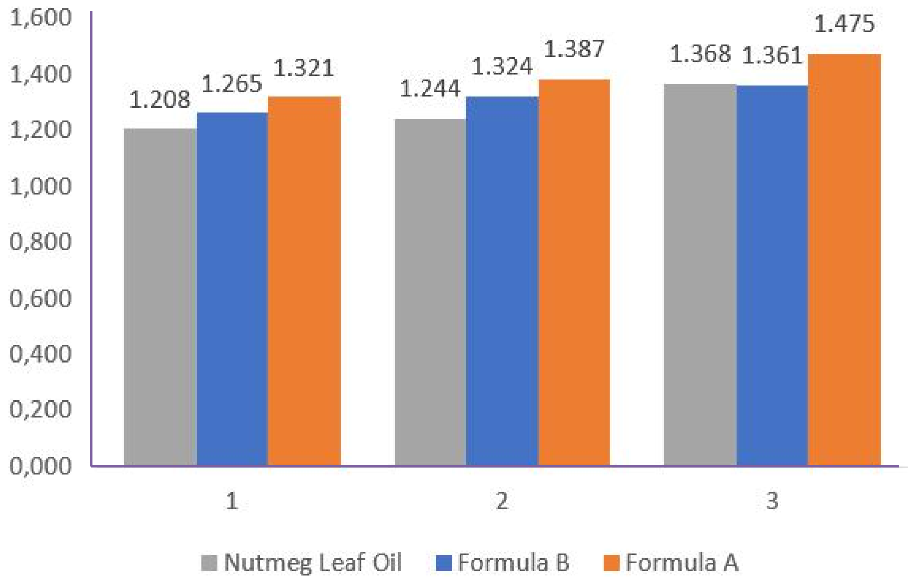

Figure 4. The SPF value of nutmeg leaf essential oil and nanoemulsion of nutmeg leaf essential oil

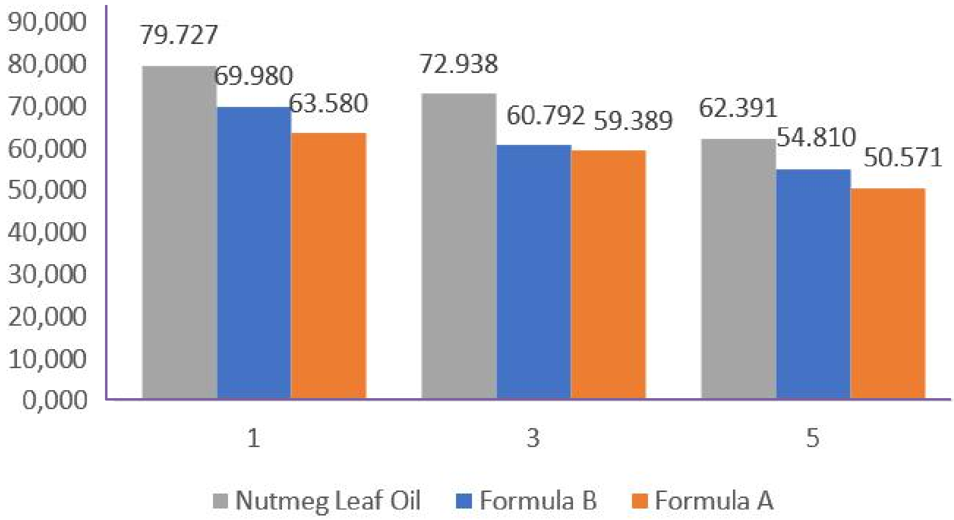

Figure 5. The value of %Te of nutmeg leaf essential oil and nanoemulsion of nutmeg leaf essential oil.

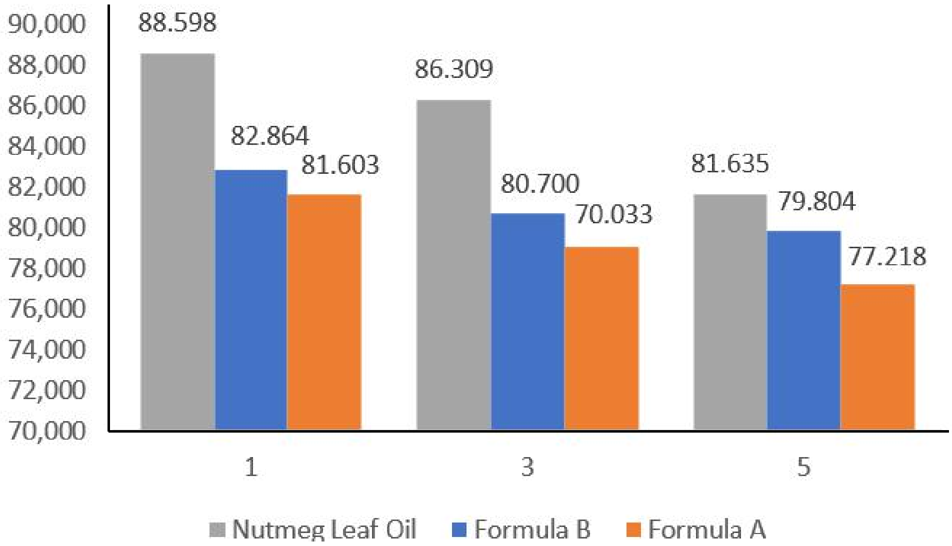

Figure 6. Value of %Tp nutmeg leaf essential oil and nutmeg leaf essential oil nanoemulsion

Table 8. Value of SPF, %Te, and %Tp of nutmeg leaf essential oil nanoemulsion

	leaf oil			Formula A			Formula B		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
SPF	1.208	1.244	1.368	1.321	1.387	1.475	1.265	1.324	1.361
%Te	79.727	72.938	62.391	63.580	59.389	50.571	69.980	60.792	54.810
%Tp	88.598	86.309	81.635	81.603	79.033	77.218	82.864	80.700	79.804

CONCLUSIONS

Nutmeg leaf essential oil nanoemulsions have a good organoleptic character, a suitable pH for skin and oil-in-water nanoemulsion type. Nutmeg leaf essential oil nanoemulsion has a better sunscreen activity than non-nanoemulsified nutmeg leaf oil. The surfactant ratio applied to formula A is better than the surfactant ratio applied formula B because it can increase sunscreen activity more than formula B.

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