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 formulate 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. The 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 potential.

**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 varied at 1, 3, and 5 mL. Surfactant system were prepared in 6 formulas. Nutmeg leaf oil contents were distilled water (v/v) 10 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 (Mardikasari et al., 2016), particle size test, thermodynamic and centrifugation stability test (Rahmawanty & Indah Sari, 2021).

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 (dyne 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 viscosity 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 leaf 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

<table>
<thead>
<tr>
<th></th>
<th>Formula A</th>
<th>Formula B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>Nutmeg leaf essential oil (v/v)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Tween 80 (v/v)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Propylene glycol (v/v)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Distilled water (v/v)</td>
<td>Add until 100</td>
<td></td>
</tr>
</tbody>
</table>
The SPF value is calculated using equation (1) (Rejeki and Wahyuningsih, 2015).

$$\log \text{SPF} = \frac{\sum AUC}{\lambda_n - \lambda_1}$$  \hspace{1cm} (1)

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

$$[AUC]_{(p-a)} = \frac{A(p-a) + A(p)}{2} (\lambda(p) - \lambda(p-a))$$  \hspace{1cm} (2)

Description:
- \(A(p)\) : absorbance at the higher wavelength between two wavelengths
- \(A(p-a)\) : absorbance at the lower wavelength between two consecutive wavelengths
- \(\lambda(p)\) : Wavelength the higher of the two wavelengths
- \(\lambda(p-a)\) : The wavelength that is lower between the two successive wavelengths.

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

$$\log \text{SPF} = \frac{\sum AUC}{\lambda_n - \lambda_1}$$  \hspace{1cm} (3)

\(\lambda_n\) is the largest wavelength among the wavelengths from 290 nm to 400 nm; wavelength \(1\) (\(\lambda_1\)) 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 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)

$$% \text{Tp} = \frac{Ep}{\Sigma Fp} = \frac{\Sigma Ee}{\Sigma Fp}$$  \hspace{1cm} (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 categorized 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**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Week 0</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>Color</td>
<td>CL</td>
<td>CL</td>
</tr>
<tr>
<td>Scent</td>
<td>F</td>
<td>SF</td>
</tr>
<tr>
<td>Clarity</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Phase separation</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Formula</th>
<th>pH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>F1</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>F2</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>F3</td>
<td>5.7</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Formula</th>
<th>(mPas)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>F1</td>
<td>12.677</td>
<td>11.232</td>
</tr>
<tr>
<td>F2</td>
<td>13.152</td>
<td>11.503</td>
</tr>
<tr>
<td>F3</td>
<td>14.214</td>
<td>14.714</td>
</tr>
</tbody>
</table>

Description: A = Formula A, B = Formula B

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

<table>
<thead>
<tr>
<th>Formula</th>
<th>Transmittance value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>F1</td>
<td>99.50</td>
</tr>
<tr>
<td>F2</td>
<td>99.27</td>
</tr>
<tr>
<td>F3</td>
<td>97.47</td>
</tr>
</tbody>
</table>

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 homogeneity 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

<table>
<thead>
<tr>
<th>Formula</th>
<th>Volume (%)</th>
<th>Particle size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1.A</td>
<td>100</td>
<td>15.63</td>
<td>0.236</td>
</tr>
<tr>
<td>F1.B</td>
<td>78.7</td>
<td>12.66</td>
<td>1.407</td>
</tr>
<tr>
<td>F2.A</td>
<td>16</td>
<td>16.83</td>
<td>1.260</td>
</tr>
<tr>
<td>F2.B</td>
<td>78.8</td>
<td>13.08</td>
<td>1.576</td>
</tr>
<tr>
<td>F3.A</td>
<td>75.5</td>
<td>15.18</td>
<td>1.639</td>
</tr>
<tr>
<td>F3.B</td>
<td>74.4</td>
<td>14.9</td>
<td>1.777</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Formula</th>
<th>Centrifugation Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1.A</td>
<td>Clear and Homogeneous</td>
</tr>
<tr>
<td>F1.B</td>
<td>Clear and Homogeneous</td>
</tr>
<tr>
<td>F2.A</td>
<td>Clear and Homogeneous</td>
</tr>
<tr>
<td>F2.B</td>
<td>Clear and Homogeneous</td>
</tr>
<tr>
<td>F3.A</td>
<td>Clear and Homogeneous</td>
</tr>
<tr>
<td>F3.B</td>
<td>Clear and Homogeneous</td>
</tr>
</tbody>
</table>

**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](image-url)
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

<table>
<thead>
<tr>
<th></th>
<th>leaf oil</th>
<th>Formula A</th>
<th>Formula B</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPF</td>
<td>1.208</td>
<td>1.321</td>
<td>1.361</td>
</tr>
<tr>
<td>%Te</td>
<td>79.727</td>
<td>69.980</td>
<td>62.391</td>
</tr>
<tr>
<td>%Tp</td>
<td>88.598</td>
<td>70.033</td>
<td>77.218</td>
</tr>
</tbody>
</table>

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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