Pharmaciana
Vol.12, No.1, March 2022, Page. 106-114
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v12i1.21997

Chemical qualitative analysis and spf value stability of nutmeg seed oil in microemulsions with tween 80 and PEG 400 as surfactants and cosurfactants

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Submitted: 19-10-2021 ***Reviewed: 23-11-2021*** ***Accepted: 30-12-2021***

ABSTRACT

Nutmeg oil contains α-pinene, which can be used as sunscreen. The combination of Tween 80 and PEG 400 can maintain the stability of nutmeg oil microemulsion. This research was a follow-up study that aims to determine the stability of the SPF value and qualitative chemical content of nutmeg seed oil microemulsions (NSM). NSM was made with a nutmeg seed oil concentration of 6.4% and tween 80 and PEG 400 as surfactants and cosurfactants with variations in the ratio of F1 (5: 4), F2 (6: 4), and F3 (7: 4). Nutmeg seed oil and NSM content was analyzed using GC-MS. NSM formula were tested for in vitro SPF value stability by storing NSM in a climatic chamber at 30 °C ± 2 °C with RH 65 % ± 5 % for 4 weeks. The SPF values were calculated every week. GC-MS data were analyzed descriptively and data of SPF value stability were analyzed statistically using one-way ANOVA. The GC-MS results of nutmeg seed oil showed 35 components, including significant compounds, namely α-pinene, sabinene, β-phellandrene and also α-terpinolene. GC-MS results of NSM showed those significant compounds were still detected after being formulated in microemulsion. The results of the sunscreen activity test of NSM before storage were 10.31 ± 0.03 (F1); 10.47 ± 0.07 (F2); 10.45 ± 0.03 (F3) and did not show significant change after storage for 4 weeks ($p > 0.05$). The SPF values of NSM were categorized in maximum activity.

Keywords: Nutmeg Oil, Microemulsion, SPF, Stability, GC-MS

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INTRODUCTION

Exposure to ultraviolet rays from the sun, especially UV B rays can cause cell and tissue damage such as erythema, and aging so that additional protection is needed such as sunscreen which is included in the protective cosmetic class. Sunscreens containing para-amino benzene acid (PABA) are not recommended for long-term use because they quickly brown the skin and are photosensitizers (Hassan et al., 2013). Several natural ingredients are known to have the potential to protect the skin from the adverse effects of UV rays, one of them is nutmeg seed oil, derived from the seeds of the nutmeg plant (Ansory et al., 2019).

Nutmeg contains myristicin, α-pinene, β-pinene, sabinene, and limonene (Ginting et al., 2017; Šojić et al., 2015). Nutmeg seed oil has the ability to protect the skin from ultraviolet rays, especially UV B (Ansory et al., 2019). α-pinene also known to protect skin from UV-A irradiation (Karthikeyan et al., 2018). α-pinene can reduce the inflammation caused by UV rays (Del Prado-Audelo et al., 2021). In order to provide a protective effect against UV radiation, the oil component must be absorbed into the skin (Pagar et al., 2012). Nutmeg seed oil globules are difficult to absorb into the skin, so it needs to be developed into more efficient preparations such as microemulsion.

Microemulsion in drug delivery systems have several advantages: increased solubilization capacity, transparency, relatively stable thermodynamics, and relatively simple manufacturing (Gozali et al., 2015). The globule of the microemulsion is small, namely, 10-140 nm, making the microemulsion have better drug delivery because it can penetrate the skin membrane and is absorbed shorter (Katiyar et al., 2013). Microemulsion, especially using PEG 400 as transdermal delivery can provide the sunscreen effect better than emulsion or cream (Kajbafvala & Salabat, 2021).

Emulsifying agent in the microemulsion can increase stability, penetration, and safety by reducing the interface tension (Wiwiek et al., 2017). Microemulsion of benzophenone-3 for sunscreen can improve UV-blocking activity (Badawi et al., 2014). The combination of Tween 80 and PEG 400 in the nutmeg oil microemulsion stable after six cycles of storage (Shabrina et al., 2021). Based on the above background, it is necessary to continue the research of nutmeg seed oil microemulsion using Tween 80 and PEG 400 to determine the chemical content and SPF value stability.

MATERIALS AND METHOD

Materials

Materials used in this study was nutmeg oil (PT. Nusaroma Essential Indonesia) and other additives were obtained from Multi Kimia Raya such as Tween 80, polyethylene glycol 400 (PEG 400), benzyl alcohol and methanol (p.a). The instruments used in this research were Gas Chromatography-Mass Spectrometry (Shimadzu) and Spectrophotometer (Shimadzu).

Methods

Preparation of nutmeg oil microemulsion

The nutmeg oil microemulsion was made by heating water to 30 °C, then Tween 80, PEG 400, and benzyl alcohol were dissolved in distilled water while stirring using a magnetic stirrer at 30 °C until a clear solution was obtained. Nutmeg oil is added into the system until a clear and transparent microemulsion was formed. Stirring was carried out at a speed of 700 rpm for 30 minutes (Shabrina et al., 2021). NSM then stored in climatic chamber for 4 weeks at 30 °C ± 2 °C with RH 65 % ± 5 %. The microemulsion had been tested for globul size using Particle Size Anlyzer with range of 72 nm to 82 nm (Shabrina et al., 2021). The reference formula used according to (Shabrina et al., 2021) can be seen in Table 1.

Table 1. Formula of nutmeg oil microemulsion

Materials	% v/v			Function
	F1	F2	F3	
Nutmeg Oil	6,4	6,4	6,4	Active ingredient
Tween 80	35	40	45	Surfactant
PEG 400	30	30	30	Cosurfactant
Benzyl alcohol	1	1	1	Preservative
Distilled water up to	100	100	100	Solvent

Chemical compound analysis

Determination of the chemical content of nutmeg oil microemulsion was carried out by GC-MS. GC-MS test was carried out before storing samples in climatic chamber for stability test. A total of 1.00µL of nutmeg oil was injected into a GC-MS device operated with a capillary column HP-1 Methyl Siloxane Aglient Tech 30 m x 0.25 mm x 0.25 mm and a hydrogen-synthetic carrier gas, gas flow 0, 50 ml / mm. The results of the detector were compared to the data contained in the Wiley Library.

Determination of correction factor value

Determination of the SPF value requires a correction factor value obtained from the absorbance of sunscreen products whose SPF value is known. Furthermore, the absorbance value is processed using the Mansur equation with the formula (1).

$$SPF = CF \times Total\ (EE \times I \times Absorbance) \quad (1)$$

Note:
SPF : SPF Value on product label
CF : Correction Factor
Total EE x I x Absorbance: Total erythema edema and intensity of UV rays based on spectrophotometer

Determination of sun protection factor value in vitro

The preparation of nutmeg seed oil microemulsion was taken as much as 0.5 ml with each variation of the concentration of tween 80-PEG 400 FI (65%), FII (70%), FIII (75%), then put in a 10 ml volumetric flask and dissolved with methanol pa. The solution was put into a test tube and then homogenized with a vortex (Rantika et al., 2020). The solution was measured by UV spectrophotometer at a wavelength of 290-320 nm using methanol p.a. as blank. The absorbance results were recorded every 5 nm interval and calculated using the Mansur equation so that the SPF value was obtained. The samples for in vitro SPF value stability were analyse every week within 4 weeks of storage.

Data Analysis

Stability data were analyzed using two way-Analysis of Variance. The analysis was followed by Tukey to compare the difference of SPF value between formula and storage time. GC-MS data from nutmeg seed oil and NSM were analyzed descriptively.

RESULT AND DISCUSSION

Nutmeg oil is known to have significant compounds, namely α-pinene (15-26%), β-pinene (13-18%), sabinene (14-29%), β-phellandrene (12-16%), and myristicin (5- 10%) (Evans and Evans, 2009). The GC-MS results of nutmeg oil used showed that there were 35 compounds detected, including the major groups, namely α-pinene, β-phellandrene, sabinene, α-terpinolene, as well as minor components, namely linalool, camphene, α-terpinene, and other resin components. The GC-MS results showed a dominant peak at the retention time of 3.634 minutes which MS detected as α-pinene, 4.473 minutes,

namely sabinene, 5.841 minutes, namely β-phellandrene, and 7.172 minutes as α-terpinolene. The GC of pure nutmeg oil and MS results of α-pinene, β-phellandrene, sabinene, and α-terpinolene can be seen in Figure 1 and Figure 2.

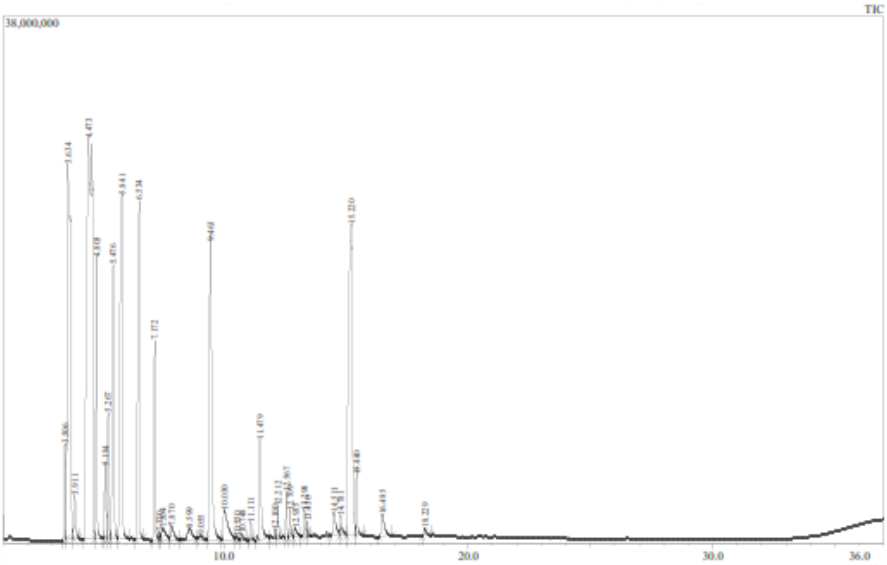

Figure 1. GC result of nutmeg oil (RT 3.634: α-pinene; RT 4.473: sabinene; RT 5.841: β-phellandrene; RT 7.172: α-terpinolene)

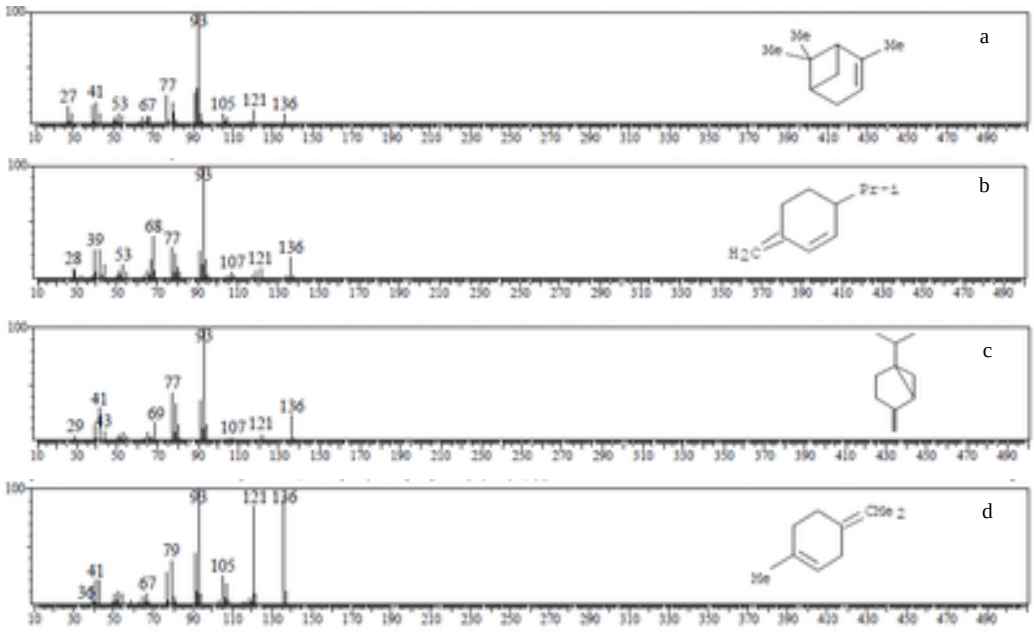

Figure 2. MS result of (a) α-pinene (RT 3.634); (b) β-phellandrene (RT 5.841); sabinene (RT 4.473) and (d) α-terpinolene (7.172)

The GC-MS test results showed that ten components were detected in F1, while 15 components detected F2 and F3. The all formulas show the significant compounds of nutmeg oil, namely α-pinene, β-phellandrene myristicin, and minor compounds, namely camphene. The difference between detected compounds from those formula caused by the additio of surfactant that would enhance and was also favorable for the mass transfer of the analytes (Zhang & Lee, 2013). GC-MS results showed the presence

of dominant peaks derived from α-pinene compounds with retention times of 3.567 (F1), 3.589 (F2), and 3.539 (F3) minutes, respectively. Other peaks shown are β-phellandrene at 4.30 (F1), 4.298 (F2) and 4.296 (F3) minutes, respectively. The retention time were relatively shifting because the The α-terpinolene component was only detected in F3 at 7,113 minutes due to the high amount of PEG 400. The α-terpinolene are included in unsaturated tertiary alcohols which attached to a saturated carbon atom and have higher solubility in water and PEG rather than other sesquiterpenes or monoterpenes (Kim et al., 2009). In this research, PEG 400 as cosurfactant had role to maintain the nutmeg oil contain in microemulsion due to the formation of hydrogen bond that would prevent precipitation or crystallization of the component (Chen et al., 2012). The role of cosurfactants is to overcome the repulsion of the same phase so that the permeability between water and oil can be increased to form a microemulsion and the active substance can be trapped between those two combined phases (Yadav et al., 2018). Tween 80 when combined with PEG 400 can also maximize loading capacity so that the drug could be well maintained in the microemulsion system. (Nazar et al., 2009). The GC test results of the NSM F1, F2, and F3 can be seen in Figure 3.

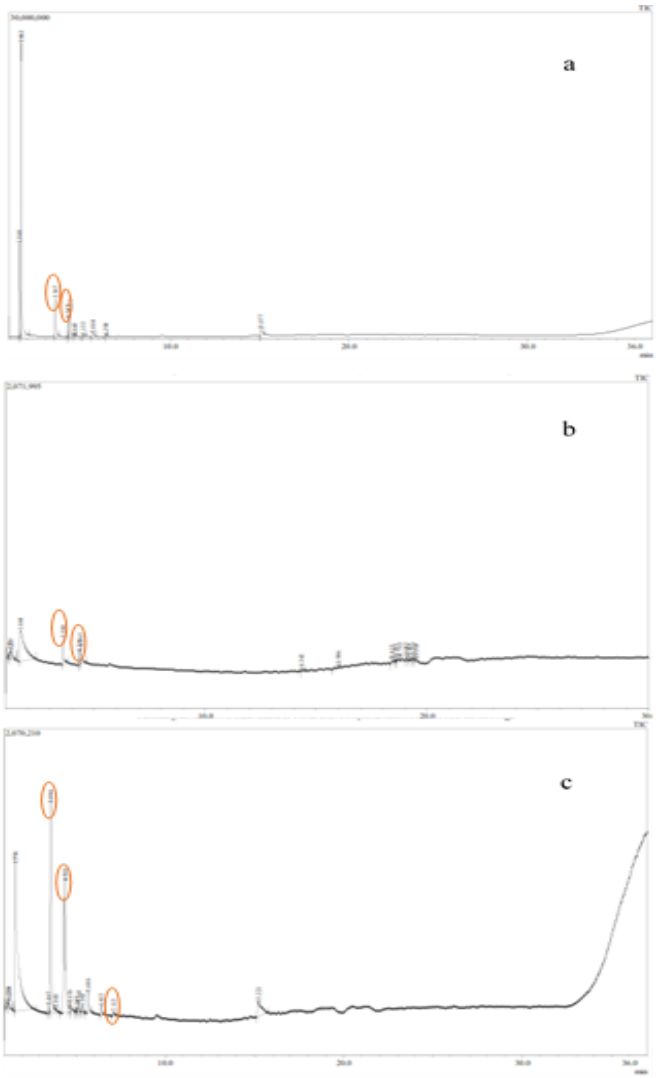

Figure 3. GC-MS result of NM F1 (a), F2 (b) and F3 (c)

The SPF value in vitro was calculated using correction factor. The product used for correction factor determination were Parasol SPF 25+. The used of this product was because of the similarity of the form as the Parasol was a liquid sunscreen with a clear appearance. Absorbance data from the preparation of parasol cooling mist sun SPF 25+ PA++ can be seen in the Table 2.

Table 2. Determination of correction factor value for parasol cooling mist sun SPF 25+

Λ	Abs	EE x I	(EE x I) x Abs
290 nm	3.719	0.015	0.056
295 nm	3.735	0.082	0.305
300 nm	3.768	0.287	1.083
305 nm	3.708	0.328	1.215
310 nm	3.710	0.186	0.692
315 nm	3.830	0.084	0.321
320 nm	3.863	0.018	0.070
$\sum_{290}^{320}$ EE x I x Abs			3.742

Calculation of correction factor value:

$$25 = CF \times 3{,}742$$

$$CF = \frac{25}{3.742} = 6.681$$

Determination of the CF (Correction Factor) value of the Parasol cooling mist sun SPF 25+ product was 6.68, where this value was used to determine the SPF value of the nutmeg oil microemulsion. Several factors that influence the determination of the CF value include the uncertain methods to evaluate sunscreen products, the solvents used were different from the evaluated sunscreen products, as well as differences in the composition of emollients and emulsifiers in the formula although the products were liquid (Putu & Artini, 2020). Determination of the SPF value in vitro was carried out using 5 nm intervals at a wavelength of 290-320 nm (Mansur et al., 1986). The wavelength of 290 nm-320 nm is included in the UV-B category which has energy that can penetrate the epidermis layer of the skin (the outermost layer of skin) so that its effects can be seen directly on the skin (Rantika et al., 2020). The data on the SPF value stability of FI, FII and FIII of NM are shown in the Table 3.

Table 3. Stability of NSM SPF Value after 4 weeks of storage

Formula	SPF Value*				
	Before Storage	Week 1	Week 2	Week 3	Week 4
F1	10.31 ± 0.95	10.38 ± 1.04	10.37 ± 1.03	10.37 ± 1.05	10.38 ± 0.96
F2	10.47 ± 0.87	10.42 ± 0.78	10.41 ± 0.88	10.46 ± 0.84	10.45 ± 0.83
F3	10.45 ± 0.85	10.47 ± 0.95	10.45 ± 0.91	10.43 ± 0.77	10.41 ± 0.87

*Mean±SD, n=3. SD: Standard deviation

The table showed that the SPF of all formula were included in the category of maximum protection in the range of 8-15 (BPOM, 2014). The data showed that there were no significant difference of the SPF value after 4 weeks of storage ($p > 0.05$). The data also showed that each formula also did

not show any significant different of the SPF value ($p > 0.05$) The combination of tween 80 and PEG 400 can provide thermodynamically stable dosage result and is compatible with the active substances used. The previous study of the Tween 80 and PEG 400 combination in nutmeg oil showed stable microemulsion especially on physical and antioxidant activity within 6 cycles (Shabrina et al., 2021). The Tween 80 has long-chain fatty acid and are available to decrease the interfacial tension between the nutmeg oil and cosurfactants, which provides clear Microemulsion (Handa et al., 2021). The PEG 400 molecule has two parallel helical bonds that form molecular complexes with active substances and cosurfactants in a circular chain formation to form intramolecular dispersions that can maintain stability of drug molecules (Seo et al., 2020). Both Tween 80 and PEG 400 could protect active ingredients from thermal and light exposure so that the microemulsion activity would be as stable as the physical appearance (Handa et al., 2021; Lv et al., 2018).

The sunscreen ability from NSM was also influenced by the compounds of nutmeg seed oil (Ansory et al., 2019). The SPF value were in line with major compounds detected in NSM, namely α-pinene and β-phellandrene. α-pinene could increase cells viability in mice skin after UV-A exposure for 10 days (Karthikeyan et al., 2019). Pretreatment of α -pinene and β-phellandrene before UV-A exposure in rats also increased significantly SOD, CAT and GPX in mice skin (Karthikeyan et al., 2018). The sunscreen activity of all NSM formula were categorized as maximum protection.

CONCLUSION

Major compounds in nutmeg seed oil such as α -pinene and β-phellandrene were detected after being formulated in microemulsion. Sunscreen activity from nutmeg oil microemulsion using various concentration tween 80 and PEG 400 were as stable as the physical appearance which SPF value were categorized as maximum protection.

ACKNOWLEDGEMENT

The author would like to thank the Ministry of Research and Technology/National Research and Innovation Agency (Ristekdikti) for funding this research through the Penelitian Dosen Pemula (PDP) program in 2021 according to SK No. 1867/E4/AK.04/2021 with contract number 067/E4.1/AK.04.PT/2021.

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