JFSP Vol.9, No.2, May-August 2023, Page: 168-177 pISSN: 2549-9068, eISSN: 2579-4558

Jurnal Farmasi Sains dan Praktis

(JFSP)

http://journal.unimma.ac.id/index.php/pharmacy

PHYSICAL CHARACTERIZATION AND SUNSCREEN ACTIVITY OF NUTMEG OIL NANOEMULSION WITH ISOPROPYL MYRISTATE VARIATIONS

Siti Setianingsih[1], **Rizky Adityo Saputro**[2], **Vironica Rizky Fauziah**[2], **Wahyu Setyo Wibowo**[2], **Ayu Shabrina**[3]

[1]Departement of Pharmacology, Faculty of Pharmacy, Universitas Harapan Bangsa, Purwokerto 53182, Indonesia
[2]Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang 50273, Indonesia
[3]Departement of Pharmaceutical and Technology, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang 50273, Indonesia

shabrina@unwahas.ac.id

https://doi.org/10.31603/pharmacy.v9i2.8481

Article info:

Submitted : 06-01-2023

Revised : 06-07-2023

Accepted : 12-07-2023

Publisher:

Universitas Muhammadiyah Magelang

ABSTRACT

The nutmeg seed oil contains myristicin which can absorb UV rays. Nanoemulsion with isopropyl myristate can be used to increase the activity of sunscreen. This study aimed to determine the physical characteristics and effectivity of nutmeg oil (NMO) nanoemulsion with isopropyl myristate as an enhancer. The nanoemulsion was made with 6.4% NMO and variations of isopropyl myristate 1% (FI), 3% (FII), and 5% (FIII). The nanoemulsions were evaluated for physical characteristics such as appearance, pH, viscosity, transmittance percentage, particle size, and polydispersity index (PI). The in vitro SPF value was tested using a spectrophotometer, and sunscreen effectivity was determined by the Minimum Erythemal Dose (MED) value. Data obtained were analyzed using one-way ANOVA. This study showed that the colour of the NMO nanoemulsion was light yellow and clear, with a transmittance percentage of 97.5-97.9%. The pH FI, FII, and FIII results were 6.79±0.03, 6.84±0.04, and 7.02±0.03. The viscosity was 1.63 ± 0.81 to 1.82 ± 0.85 d.Pas with Newtonian rheology. The particle size was 14.3 ± 1.41 nm to 16.0 ± 2.13 nm, with PI less than 0.5. SPF value were 16.34 ± 5.50 (FI); 16.70 ± 5.20 (FII) and 17.80 ± 3.20 (FIII). MED values were 220.5 ± 6.34 (FI), 225.4 ±5.41 (FII), and 240.2 ±3.45 (FIII) minutes. The MED value showed that FIII was significantly different from FI and FII. Isopropyl myristate at 5% in nanoemulsion increases the effectivity of sunscreen.

Keywords: Enhancer; Nanoemulsion; Nutmeg oil; Sunscreen

1. INTRODUCTION

Chemical sunscreens work by absorbing and converting UV rays into heat and releasing them from the body. This type of sunscreen can absorb the skin and potentially irritate the skin layer (Minerva, 2019). Chemical sunscreens have side effects, such as irritation, pigmentation, and hormonal disorder in a woman that can cause cancer (Pirotta, 2020). Developing sunscreen from natural ingredients is necessary to prevent the side effect. One natural ingredient that has the potential as a sunscreen is nutmeg seed oil (Rahmadany et al., 2022). The myristicin content in nutmeg seed oil is the most significant compound in absorbing UV-B rays, with an SPF value of 19.44, categorized as extra protection (Ansory et al., 2020). Nutmeg oil in microemulsion contains α-pinene and β-phellandrene with an SPF value of 10.45 (Shabrina et al., 2022).

Sunscreen can be effective if it has a high SPF value which reflects the ability of the sunscreen to prevent erythema (Liony et al., 2014). Sunscreen works after absorbed into the skin and prevents UV radiation from sunlight (Panjaitan et al., 2017). Enhancing sunscreen penetration into the skin can increase its effectiveness.

Nanoemulsion systems can have good stability and penetration ability for topical use (Shaker et al., 2019). Isopropyl myristate (IPM) is one non-triglyceride oil with low molecular weight and high polarity characteristics, so it is suitable for the characteristics of nanoemulsions with a globule size of less than 1000 nm (Souto et al., 2022). Combining IPM and Tween, 80, as surfactants can increase viscosity and skin penetration (Abdullah et al., 2022). The combination of IPM and Tween, 80 in nanoemulsion of vitamin C, showed the highest skin penetration (Liston et al., 2022). Using IPM in the nanoemulsion formula can prevent intergranular agglomeration during storage so that the nanoemulsion becomes stable during the storage process (Hashim et al., 2019). IPM in nanoemulsion can also increase the permeation of active ingredients into the skin layer (Eichner et al., 2017).

Furthermore, IPM is known as the best enhancer for cosmetics due to its ability to disrupt the skin barrier by physical methods (Iliopoulos et al., 2022). The previous research showed that the microemulsion of nutmeg seed oil has antioxidant activity (Shabrina et al., 2021). Furthermore, the microemulsion of nutmeg seed oil showed good physical stability and was proven to have sunscreen activity with an SPF value of 12 (Rahmadany et al., 2022). In order to increase the absorption of sunscreen into the skin, it is necessary to produce nanoemulsions with enhancers, such as isopropyl myristate (IPM). This study aimed to determine the physical characteristics and sunscreen effectivity of nutmeg seed oil nanoemulsion with isopropyl myristate variations.

2. METHODS

2.1. Tools

The tools used in this study were a magnetic stirrer (Scilogex®), UV-Vis spectrophotometer (Shimadzu®), particle size analyzer (SZ-100®), viscometer (Rheosys Merlin VR II®), micropipette (Scilogex®), pH meter (Electro Lab®), vortex, nonirritant bandage (Hypafic®) and sterile gauze (Onemed®).

2.2. Materials

The active ingredient used in this study was nutmeg seed oil obtained from PT. Nusaroma Essential Indonesia, completed with a certificate of analysis (CoA). The materials used were technical grade of tween 80, propylene glycol, isopropyl myristate, and benzyl alcohol. Methanol was used for in vitro analysis with analytical grade. All materials were obtained from CV. Multi Kimia Raya Semarang. The positive control used was Parasol SPF 25++ Spray Sunscreen.

2.3. Preparation of Nutmeg Seed Oil Nanoemulsion

Nutmeg seed oil nanoemulsion was prepared by dividing the materials into the water and oil phases (**Table 1**). The water phase was prepared by heating the purified water to a temperature of 30 °C then tween 80; propylene glycol and benzyl alcohol were dissolved in the heated purified water while stirring using a magnetic stirrer at 30 °C at 700 rpm until the phase was clear. The oil phase consisted of nutmeg seed oil and isopropyl myristate and was heated at 30 °C. The oil phase was then added dropwise into the water phase while stirring with a magnetic stirrer at 30 °C at 1200 rpm for 30 minutes (Shabrina & Khansa, 2022). The nutmeg seed oil nanoemulsion was examined for its physical characteristics and effectivity.

Table 1. Formulation of nutmeg seed oil nanoemulsion

Materials	Concentration (%)		
	FI	FII	FIII
Nutmeg Oil	6.4	6.4	6.4
Isopropyl Myristate	1	3	5
Tween 80	40	40	40
Propylene glycol	20	20	20
Benzyl Alcohol	1	1	1
Purified water up to	100	100	100

2.4. Evaluation of The Physical Characteristics of Nutmeg Seed Oil Nanoemulsion

The nutmeg seed oil nanoemulsion was carried out by visually observing the colour, odour, and phase separation. The nanoemulsion pH was tested using 10 mL of the sample. Viscosity and rheology tests were carried out with 15 mL of the sample at 12 rpm with the number of rounds six times (the length of each round was 30 seconds) using a cone and plate viscometer. The clarity test was done using a 3 mL sample and then analyzed using a spectrophotometer. Particle size and polydispersity index tests were conducted using a Particle Size Analyzer (PSA) with 5 mL of sample. Each formula was analyzed three times.

2.5. In Vitro Tests and Effectivity of Nutmeg Seed Oil Nanoemulsions

The determination of SPF value begins by determining the Correction Factor (CF) value with a Parasol SPF 25++ as a comparison and positive control. The product was chosen for the comparison due to the similarity of the ingredients. Parasol SPF 25++ was weighed for 1 gram and dissolved up to 10 mL using methanol. The absorbance of the Parasol SPF 25++ was measured with a UV spectrophotometer at a wavelength of 290-320 nm with 5 nm intervals. The results were calculated using the Mansur formula to determine the CF value as follow Eq. 1.

$$SPF = CF\ x \sum_{290}^{320} EE\ (\lambda) x\ I\ (\lambda)\ x\ A\ (\lambda) \tag{1}$$

Where:

CF: Correction Factor

EE x I: Total erythema oedema and intensity of UV rays based on a spectrophotometer

A: Absorbance of the sample

Determination of the in vitro SPF value of nutmeg seed oil nanoemulsion was carried out according to the CF. The Nanoemulsion sample of FI, FII, and FIII was weighed for 1 gram, then dissolved with methanol up to 10 mL. The results obtained were calculated using the Mansur formula above.

Health Research Ethics Committee of Fakultas Kedokteran Universitas Muhammadyah Yogyakarta approved the sunscreen effectivity test with number 034/ EC-KEPK-FKIK UMY/V/2021. The sunscreen effectivity was determined using rabbit skin. The total animal used in this research were five rabbits, including unprotected skin and protected skin with Parasol, FI, FII and FIII. The back of the rabbits was depilated 24 hours before treatment using a shaver. The back skin of animals that had been shaved was divided into five sections for treatment: FI, FII, FIII, positive control (Parasol SPF 25++), and negative control. Each section was marked with a size of 4x4 cm. The animals were exposed to 311 nm UV light. Observations were made by counting the number of red spots (erythema) appearing in each test animal group. The time was measured from the beginning of UV light exposure until the erythema appeared. This measurement was calculated as Minimum Erythemal Dose (MED). The MED was calculated by comparing the exposure time of unprotected skin (negative control) with protected skin in every section (FI, FII, FIII, and positive control) (Heckman et al., 2013).

2.6. Data Analysis

The organoleptic, polydispersity index (PI), and rheology data were described descriptively. The pH, viscosity, transmittance percentage, particle size, and effectivity of sunscreens for each formula were statistically analyzed using one-way ANOVA. The in vitro and MED sunscreens data were also analyzed using an independent T-test to compare the level of effectivity.

3. RESULTS AND DISCUSSION

3.1. Physical Characteristics Result of Nutmeg Oil Nanoemulsion

The physical appearance of the nutmeg oil nanoemulsion can be seen in **Figure 1**. The nanoemulsion of nutmeg oil in FI, FII, and FIII was yellow and clear, with a distinctive odour of nutmeg oil. All formulas are homogeneous, no separation occurs, and consist of one phase.

The physical characteristics of the nutmeg oil nanoemulsion can be seen in **Table 2**. The results showed that all nutmeg seed oil nanoemulsion formulas had a suitable pH value for topical use at a pH of 4.5-8.0 (Ahmady et al., 2020). The pH of nutmeg seed oil before being incorporated into the nanoemulsion system was 6.70 ± 1.50. The excipients from the nanoemulsion system, such as surfactants, cosurfactants, and enhancers, showed a pH of 6.0-7.5. The nanoemulsion system of nutmeg oil experienced an increase in pH but did not show any significant difference compared to nutmeg seed oil and the excipients. The pH of FIII showed the highest value and was significantly different ($p < 0.05$) compared to FI and FII. This result indicates that the concentration of IPM affected the pH. According to the previous study, a higher concentration of IPM would increase the system's pH (Pakki et al., 2019).

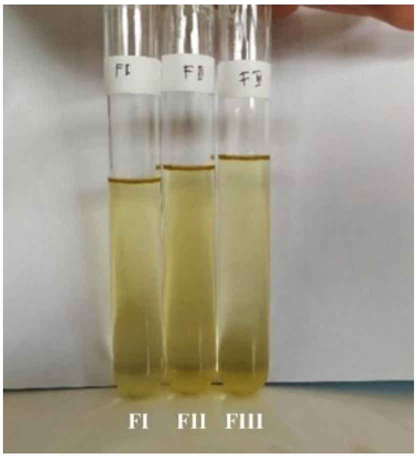

Figure 1. Nutmeg Oil Nanoemulsion with Isopropyl Myristate Variations

The high viscosity value shows that the system could spread and penetrate the skin barrier (Nastiti et al., 2017). The combination of IPM and Tween 80 will increase the viscosity of the nanoemulsion system (Bardhan et al., 2013). This situation can increase the stability of the nanoemulsion due to the stable globule formation so that the nutmeg seed oil content can be maintained in the system (Thomas et al., 2014). IPM has low interfacial tension and viscosity, which is advantageous in manufacturing nanoemulsions because it produces smaller globule sizes than other mineral oil with large molecular sizes with long hydrocarbon fatty acids acid chains (Muthi, 2016). IPM can integrate with the lipid bilayer and bind to the ester group on the hydrophilic compound so that the structure of the stratum corneum bilayer membrane becomes more tenuous (Jiang et al., 2017).

Table 2. Physical Characteristics of Nutmeg Oil Nanoemulsion with Isopropyl Myristate Variations

Formula	Physical Characteristics*				
	pH	**Viscosity (d.Pas)**	**Transmittance Percentage (%)**	**Particle Size (nm)**	**Polydispersity Index**
FI	6.79±0.03	1.78 ± 0.90	97.5 ± 1.41	16.0 ± 2.13	0.436 ± 0.210
FII	6.84±0.04	1.82 ± 0.85	97.7 ± 1.55	14.3 ± 1.41	0.331 ± 0.224
FIII	7.02±0.03[a]	1.63 ± 0.81	97.9 ± 1.18	15.0 ± 1.35	0.203 ± 0.231

*Data displayed n = 3 ± standard deviation

a = significantly different from FI and FII

IPM is a class of fatty acid esters that meet the safety requirements of cosmetics and are widely used due to their effectiveness (Mawazi et al., 2022). The rheology result of the nutmeg oil nanoemulsion can be seen in **Figure 2** and **Figure 3**. All of the nutmeg oil nanoemulsion formulas had a Newtonian flow, defined as a fluid with a linearly proportional shear stress with a velocity gradient in a direction perpendicular to the shear plane (Marques et al., 2018). These results indicate that the droplets formed were tiny, so the preparations resemble solutions or liquids and can flow easily (Hasrawati et al., 2016; Zhao et al., 2016). Medium-chain fatty acids such as IPM have been used in mixed micelle nanoemulsion formulations as a good absorption of active substances and as permeation enhancers. This research was in line with Zhao et al (2016) that combining IPM and Tween 80 will increase the system's viscosity. The statistical result showed that there was no significance difference between all formulas.

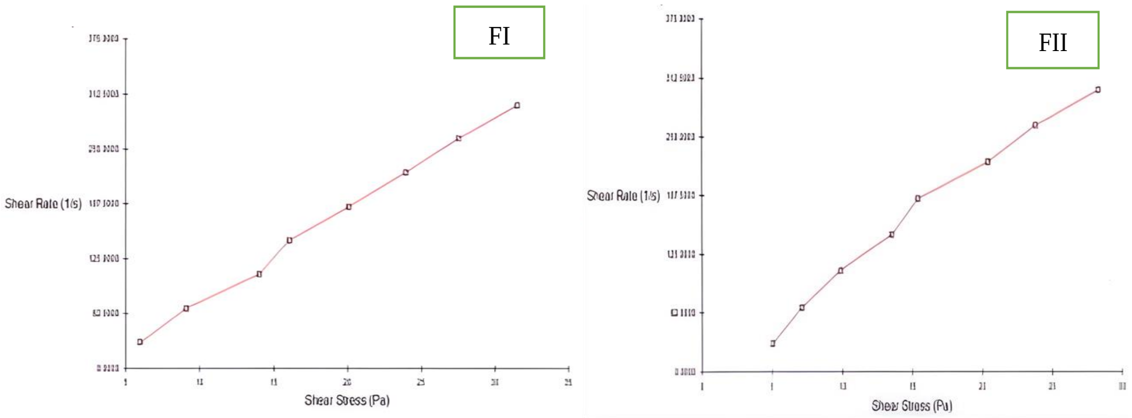

Figure 2. Rheogram of FI and FII Nutmeg Oil Nanoemulsion with Isopropyl Myristate Variations

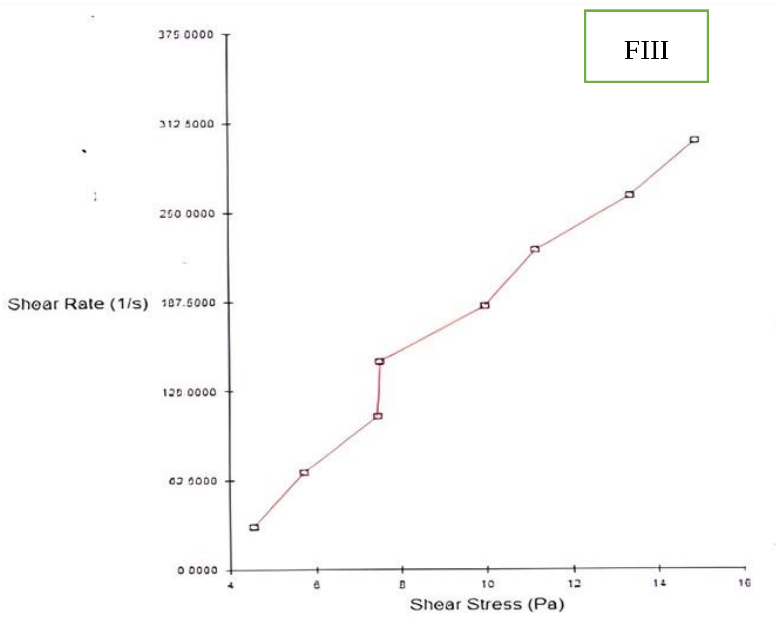

Figure 3. Rheogram of FIII Nutmeg Oil Nanoemulsion with Isopropyl Myristate Variations

The transmittance percentage shows the clarity of the nanoemulsion system. Based on **Table 2**, the transmittance percentage meets the nanoemulsion requirements. The ideal transmittance requirement for nanoemulsion is 90-100% (Gurpreet & Singh, 2018). The transmittance percentage in each formula did not show any significant difference. It showed that IPM did not affect the clarity of the nanoemulsion system (Dalibera et al., 2021).

A good nanoemulsion has a particle size of 10-100 nm (Abdullah et al., 2022). **Table 2** showed that all of the nutmeg oil nanoemulsion systems had a particle size of less than 100 nm and did not show any significant difference in each formula. The surfactants, cosurfactants, and enhancers such as IPM could affect the size of nanoemulsion globules; increasing the adsorption of surfactants at the oil and water interface can support the formation of small globules size (Cho, 2016; Janakiraman et al., 2022). Particle measurements are essential to determine the stability of nanoemulsion systems. If the particle size is less than 100 nm, the aggregation rate will be lower, and the phase separation of the nanoemulsion can be prevented. The decreasing globule size can extend the shelf life of the nanoemulsion system; besides, the system is not easily degraded and absorbed into the skin barrier (Elsabahy, 2017). A lower PI value of less than 1 indicates that the nanoemulsion is monodisperse (Yakoubi et al., 2021). The PI of this research showed that all formulas had a PI below 0.5 and did not show any significant difference. This result indicates that IPM combined with surfactant and co-surfactant will reduce the particle size and PI (Argenta et al., 2014).

The role of Tween 80 as a surfactant and PEG 400 as a cosurfactant can affect the final result of the particle size value of the nanoemulsion. The surfactant will be adsorbed on the surface of the oil phase droplets and form micelles which can lower the interfacial tension resulting in a good nanoemulsion with small particle size. Then cosurfactants will help prevent phase separation from recombining (Caya et al., 2020). IPM can affect particle size because IPM is a non-triglyceride oil which can be advantageous in making nanoemulsions compared to mineral oil. After all, it is easier to produce small globule sizes to obtain stable nanoemulsions (Seo et al., 2020). This is in line with the research of Shabrina et al. (2020) that nanoemulsions are stable by reducing the size of the globules using surfactants and IPM.

3.2. In Vitro SPF Result and Effectivity of Nutmeg Seed Oil Nanoemulsions

The correction factor is determined from a sunscreen product with a known SPF value. This correction factor is a tolerance limit for using a spectrophotometer and solvents to obtain accurate results. The absorbance value is measured using a spectrophotometer with a wavelength of 290-320 nm and then calculated using the Mansur formula (Zarkogianni & Nikolaidis, 2016). The correction factor was determined from the Parasol SPF 25+ PA++ resulting in a value of 6.68. Factors that can affect the determination of the SPF value include the absence of an appropriate method to evaluate sunscreen products, the combination of ingredients, and concentration of sunscreen, the use of different solvents, and the emulsion type. Those parameters will affect and interact with the product component. Others include the emulsifier used in a formulation, the addition of other active substances, the pH system, the viscosity, and the emulsion (Putu & Artini, 2020). The sunscreen effectivity of nutmeg oil nanoemulsion can be seen in **Table 3** and **Figure 4**.

Table 3. In Vitro SPF Result and MED of Nutmeg Oil Nanoemulsion with Isopropyl Myristate Variations

Formula	MED (minutes)	SPF Value from MED	SPF Value in Vitro
Negative control (Unprotected skin)	13.5 ± 7.41	-	-
FI	220.5 ± 6.34[c,d]	16.34 ± 5.50 [c,d]	15.35 ± 1.16
FII	225.4 ±5.41 [c,d]	16.70 ± 5.20 [c,d]	14.80 ± 2.40
FIII	240.2 ±3.45[a,b,d]	17.80 ± 3.20 [a,b,d]	15.72 ± 1.33
Positive control (Parasol SPF 25++)	328.2 ± 4.50[a,b,c]	24.31 ± 4.30 [a,b,c]	6.68 ± 1.12 [a,b,c]

Note:

n = Data displayed n = 3 ± standard deviation

a: Significantly different from FI

b: Significantly different from FII

c: Significantly different from FIII

d: Significantly different from positive control

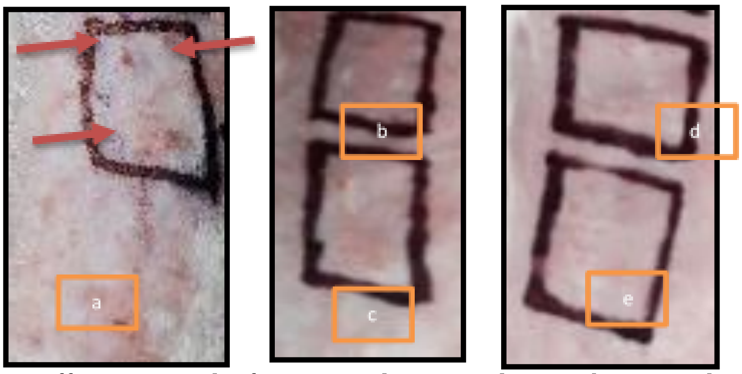

Figure 4. Sunscreen Effectivity Result of Nutmeg Oil Nanoemulsion with Isopropyl Myristate Variation: a = Negative control with erythema; b = FI; c = FII; d = FIII; e = Positive control

The results showed significant differences in the SPF and MED values for FI and FII compared to FIII. The differences were caused by the concentration of isopropyl myristate (IPM). IPM plays an essential role in the absorption of the nanoemulsion system into the skin. IPM can increase the absorption of products into the skin, whereas sunscreen from natural ingredients can provide effectiveness when the preparation can be absorbed into the skin (Bhalke et al., 2020). A high concentration of IPM can change the rigid structure of lipid structures and reduce the tension of the stratum corneum, thereby increasing penetration. The active substances are absorbed into the skin layers (Eichner et al., 2017). In addition, isopropyl myristate in the nanoemulsion system also acts as a co-surfactant and provides good stability for nanoemulsion (Dalibera et al., 2021). IPM and tween 80 in nanoemulsion systems are known to increase sunscreen effectivity (Hashim et al., 2019). This indicates that the higher concentration of IPM as an enhancer, the effectivity of the sunscreen could be increased.

4. CONCLUSION

Nutmeg seed oil nanoemulsion with 1% to 5% isopropyl myristate showed physical characteristics that fulfil the requirements of the nanoemulsion system. Isopropyl myristate at 5% in the nutmeg seed oil nanoemulsion system showed the highest sunscreen effectivity on rabbits skin and was categorized as ultra-protection. For future research, clinical trials can be carried out to minimize animal harm, and cosmetics can be claimed as dermatologically tested.

5. ACKNOWLEDGMENT

The author would like to thank the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for providing grants through Penelitian Dosen Pemula (PDP) scheme with contract number 018/LL6/PB/AK.04/2022.04/LPPM.

6. REFERENCES

Abdullah, N. A., Jufri, M., Mun'im, A., & Saputri, F. C. (2022). Formulation and Evaluation of Two Celastrol Nanoemulsions Prepared From Two Oils: Isopropyl Myristate and Virgin Coconut Oil. *International Journal of Applied Pharmaceutics*, *14*(2), 267–275. https://doi.org/10.22159/ijap.2022v14i2.43509

Ahmady, A., Amini, M. H., Zhakfar, A. M., Babak, G., & Sediqi, M. N. (2020). Sun protective potential and physical stability of herbal sunscreen developed from afghan medicinal plants. *Turkish Journal of Pharmaceutical Sciences*, *17*(3), 285–292. https://doi.org/10.4274/tjps.galenos.2019.15428

Ansory, H. M., Sari, E. N., Nilawati, A., Handayani, S., & Aznam, N. (2020). Sunscreen and Antioxidant Potential of Myristicin in Nutmeg Essential Oils (*Myristica fragrans*). *2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019)*, *26*(1), 138–142. https://doi.org/10.2991/ahsr.k.200523.034

Argenta, D. F., de Mattos, C. B., Misturini, F. D., Koester, L. S., Bassani, V. L., Maria Oliveira SimõEs, C., & Teixeira, H. F. (2014). Factorial design applied to the optimization of lipid composition of topical antiherpetic nanoemulsions containing isoflavone genistein. *International Journal of Nanomedicine*, *9*(1), 4737–4747. https://doi.org/10.2147/IJN.S67732

Bardhan, S., Kundu, K., Saha, S. K., & Paul, B. K. (2013). Physicochemical studies of mixed surfactant microemulsions with isopropyl myristate as oil. *Journal of Colloid and Interface Science*, *402*, 180–189. https://doi.org/10.1016/j.jcis.2013.04.008

Bhalke, R. D., Kulkarni, S. S., Kendre, P. N., Pande, V. V, & Giri, M. A. (2020). A facile approach to fabrication and characterization of novel herbal microemulsion-based UV shielding cream. *Future Journal of Pharmaceutical Sciences*, *6*(1), 1–10.

Caya, D. N., Aryani, R., & Priani, S. E. (2020). Kajian Pustaka Pengaruh Penambahan Surfaktan dan Kosurfaktan Terhadap Karakteristik Sediaan Mikroemulsi. *Prosiding Farmasi*, *6*(2). https://karyailmiah.unisba.ac.id/index.php/farmasi/article/view/24682

Cho, W. G. (2016). Stability of W/O Nanoemulsions with Low Viscosity Prepared by PIC Method. *Journal of the Society of Cosmetic Scientists of Korea*, *42*(2), 127–133. https://doi.org/10.15230/scsk.2016.42.2.129

Dalibera, N. C., Zanin, M. H. A., Guimaraes, K. L., de Oliveira, L. A., & de Oliveira, A. M. (2021). Optimized formulation of thermoresponsive nanoemulsion-based gel for enhanced oil recovery (EOR) application. *Applied Petrochemical Research*, *11*(2), 155–163. https://doi.org/10.1007/s13203-021-00269-9

Eichner, A., Stahlberg, S., Sonnenberger, S., Lange, S., Dobner, B., Ostermann, A., Schrader, T. E., Hauß, T., Schroeter, A., Huster, D., & Neubert, R. H. H. (2017). Influence of the penetration enhancer isopropyl myristate on stratum corneum lipid model membranes revealed by neutron diffraction and 2H NMR experiments. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1859*(5), 745–755. https://doi.org/https://doi.org/10.1016/j.bbamem.2017.01.029

Gurpreet, K., & Singh, S. K. (2018). Review of nanoemulsion formulation and characterization techniques. *Indian Journal of Pharmaceutical Sciences*, *80*(5), 781–789.

Hashim, D. M., Sheta, N. M., Elwazzan, V. S., & Sakran, W. S. (2019). Enhancing the Sunscreen Efficacy of Bemotrizinol Micropigment By Using O/W Nanoemulsion Topical Preparations. *International Journal of Pharmacy and Pharmaceutical Sciences*, *11*(7), 47–56. https://doi.org/10.22159/ijpps.2019v11i7.32652

Hasrawati, A., Hasyim, N., & Irsyad, N. A. (2016). Pengembangan Formulasi Mikroemulsi Minyak Sereh (*Cymbopogon nardus*) Menggunakan Emulgator Surfaktan Nonionik. *Jurnal Fitofarmaka Indonesia*, *3*(1), 151–154. https://doi.org/10.33096/jffi.v3i1.176

Heckman, C. J., Chandler, R., Kloss, J. D., Benson, A., Rooney, D., Munshi, T., Darlow, S. D., Perlis, C., Manne, S. L., & Oslin, D. W. (2013). Minimal Erythema Dose (MED) testing. *Journal of visualized experiments : JoVE*, *75*, 1–5. https://doi.org/10.3791/50175

Iliopoulos, F., Sil, B. C., & Evans, C. L. (2022). *The role of excipients in promoting topical and transdermal delivery : Current limitations and future perspectives*. *December*, 1–10. https://doi.org/10.3389/fddev.2022.1049848

Janakiraman, A. K., Islam, T., Liew, K. Bin, Elumalai, M., & Hanish Singh, J. C. (2022). Improved oral bioavailability of poorly water-soluble vorinostat by self-microemulsifying drug delivery system. *Beni-Suef University Journal of Basic and Applied Sciences*, *11*(1), 1115–1125. https://doi.org/10.1186/s43088-022-00279-z

Jiang, Q., Wu, Y., Zhang, H., Liu, P., Yao, J., Yao, P., Chen, J., & Duan, J. (2017). Development of essential oils as skin permeation enhancers: Penetration enhancement effect and mechanism of action. *Pharmaceutical Biology*, *55*(1), 1592–1600. https://doi.org/10.1080/13880209.2017.1312464

Liony, B., Suhartiningsih, D. H., & Pd, M. (2014). *Protection Factor (SPF) pada Hasil Jadi Krim Tabir Surya*. *03*, 209–216.

Liston, L. S., Rivas, P. L., Sakdiset, P., See, G. L., & Arce, F. (2022). Chemical Permeation Enhancers for Topically-Applied Vitamin C and Its Derivatives: A Systematic Review. *Cosmetics*, *9*(4). https://doi.org/10.3390/cosmetics9040085

Marques, T. Z. da S., Santos-Oliveira, R., De Siqueira, L. B. de O., Da Silva Cardoso, V., De Freitas, Z. M. F., Da Silva Ascenção Barros, R. D. C., Villa, A. L. V., de Bustamante Monteiro, M. S. de S., Dos Santos, E. P., & Ricci-Junior, E. (2018). Development and characterization of a nanoemulsion containing propranolol for topical delivery.

International Journal of Nanomedicine, *13*, 2827–2837. https://doi.org/10.2147/IJN.S164404

Mawazi, S. M., Ann, J., Othman, N., Khan, J., Alolayan, S. O., Al Thagfan, S. S., & Kaleemullah, M. (2022). A Review of Moisturizers; History, Preparation, Characterization and Applications. *Cosmetics*, *9*(3), 1–19. https://doi.org/10.3390/cosmetics9030061

Minerva, P. (2019). Penggunaan Tabir Surya Bagi Kesehatan Kulit. *Jurnal Pendidikan Dan Keluarga*, *11*(1), 87. https://doi.org/10.24036/jpk/vol11-iss1/619

Muthi, B. (2016). Formulation And Optimization Of Caffeine Nanoemulsion Using Factorial Design Study. *Icmhs*, 6–9.

Nastiti, C. M. R. R., Ponto, T., Abd, E., Grice, J. E., Benson, H. A. E., & Roberts, M. S. (2017). Topical nano and microemulsions for skin delivery. *Pharmaceutics*, *9*(4), 1–25. https://doi.org/10.3390/pharmaceutics9040037

Pakki, E., Rewa, M., & Irma, N. (2019). The Effectiveness of Isopropyl Myristate as Enhancing Agent in the Antioxidant Cream of Kasumba Turate Seed (Carthamus tinctorius L.) Efektivitas Bahan Peningkat Penetrasi, Isopropil Miristat Dalam Krim Antioksidan Ekstrak Biji Kasumba Turate (Carthamus t. *Journal of Pharmaceutical and Medicinal Sciences*, *4*(2), 44–50.

Panjaitan, M., Ramadhan, A. M., & Rahmawati, D. (2017). Aktivitas Tabir Surya Ekstrak Etanol Herba Kerokot (*Lygodium microphyllum*). *Proceeding of the 5th Mulawarman Pharmaceutical Conferences*, *April*, 23–24. https://doi.org/10.25026/mpc.v5i1.234

Pirotta, G. (2020). Sunscreen Regulation. In *Sunscreen Regulation in The World* (Nomor November, hal. 1–12). Springer Nature Switzerland AG. https://doi.org/10.1007/698

Putu, N., & Artini, R. (2020). Validasi Dan Verifikasi Hasil Uji Sun Protection Factor (SPF) Pada Sediaan Sunblock Dan Sunscreen Bermerk Dengan Metode Spektrofotometri. *The Journal Of Muhammadiyah Medical Laboratory Technologist*, *3*(1), 29–38.

Rahmadany, S. E., Nida, A. Z., Fithria, R. F., & Shabrina, A. (2022). Uji Iritasi dan Aktivitas Tabir Surya Secara in Vitro Minyak Biji Pala Dalam Sistem Mikroemulsi Dengan Variasi Tween 80-Etanol. *Jurnal Ilmu Farmasi dan Farmasi Klinik*, *18*(2), 47. https://doi.org/10.31942/jiffk.v18i2.5957

Seo, S. H., Kim, E., Joo, Y., Lee, J., Oh, K. T., Hwang, S. J., & Choi, K. Y. (2020). A mixed micellar formulation for the transdermal delivery of an Indirubin analog. *Pharmaceutics*, *12*(2). https://doi.org/10.3390/pharmaceutics12020175

Shabrina, A., & Khansa, I. S. M. (2022). Physical Stability of Sea Buckthorn Oil Nanoemulsion with Tween 80 Variations. *Indonesian Journal of Pharmaceutical Science and Technology*, *Supplement*(1), 14–21.

Shabrina, A., Safitri, E. I., Fithria, R. F., Munir, M., & Sumantri, S. (2022). Chemical qualitative analysis and spf value stability of nutmeg seed oil in microemulsions with tween 80 and PEG 400 as surfactants and cosurfactants. *Pharmaciana*, *12*(1), 106. https://doi.org/10.12928/pharmaciana.v12i1.21997

Shabrina, A., Safitri, E. I., & Pratiwi, I. (2021). Uji Stabilitas Fisik Dan Antioksidan Mikroemulsi Minyak Biji Pala Dengan Variasi Tween 80 – Peg 400. *Media Farmasi*, *17*(1), 25–31.

Shaker, D. S., Ishak, R. A. H., Ghoneim, A., & Elhuoni, M. A. (2019). Nanoemulsion: A review on mechanisms for the transdermal delivery of hydrophobic and hydrophilic drugs. *Scientia Pharmaceutica*, *87*(3). https://doi.org/10.3390/scipharm87030017

Souto, E. B., Cano, A., Martins-Gomes, C., Coutinho, T. E., Zielińska, A., & Silva, A. M. (2022). Microemulsions and Nanoemulsions in Skin Drug Delivery. *Bioengineering*, *9*(4), 1–22. https://doi.org/10.3390/bioengineering9040158

Thomas, S., Vieira, C. S., Hass, M. A., & Lopes, L. B. (2014). Stability, cutaneous delivery, and antioxidant potential of a lipoic acid and α-tocopherol codrug incorporated in microemulsions. *Journal of Pharmaceutical Sciences*, *103*(8), 2530–2538. https://doi.org/10.1002/jps.24053

Yakoubi, S., Kobayashi, I., Uemura, K., Nakajima, M., & Isoda, H. (2021). Optimization and Modeling by Response Surface Methodology (RSM): Enhancement of Their Antimicrobial Potential and Bioavailability in Nanoscale Food Delivery System. *Foods*, *10*(3149), 1-28.

Zarkogianni, M., & Nikolaidis, N. (2016). Determination of Sun Protection Factor (SPF) and Stability of Oil-in-Water Emulsions Containing Greek Red Saffron (Crocus Sativus L.) as a Main Antisolar Agent. *International Journal of Advanced Research in Chemical Science*, *3*(7), 1–7. https://doi.org/10.20431/2349-0403.0307001

Zhao, C., Quan, P., Liu, C., Li, Q., & Fang, L. (2016). Effect of isopropyl myristate on the viscoelasticity and drug release of a drug-in-adhesive transdermal patch containing blonanserinEffect of isopropyl myristate on the viscoelasticity and drug release of a drug-in-adhesive transdermal patch containing bl. *Acta Pharmaceutica Sinica B*, *6*(6), 623–628. https://doi.org/10.1016/j.apsb.2016.05.012