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Formulation and Evaluation of Nanoemulsions Combination of Black Cumin Seed Oil (*Nigella sativa* L.) and Moringa Seed Oil (*Moringa oleifera* L.) as Sunscreen

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ABSTRACT

Nanoemulsion preparations can improve quality and facilitate the penetration of active ingredients into the skin. Black cumin seed oil (*Nigella sativa* L.) and Moringa seed oil (*Moringa oleifera* L.) contain flavonoids that can absorb UV rays. This study aims to determine the physical properties and SPF value of nanoemulsion preparations as a sunscreen combination of black cumin seed oil and moringa seed oil. Nanoemulsion preparations with variations in the concentration of black cumin seed oil: moringa seed oil at F1 4:0, F2 0:4, F3 2:2, F4 3:1 and F5 1:3. Nanoemulsion preparations are evaluated for their physical and chemical properties such as organoleptis test, pH, viscosity, percent transmittance, particle determination test and SPF value. The results showed that variations in the concentration of black cumin seed oil and moringa seed oil affected to the test except in viscosity, statistical tests on pH tests and SPF tests have significant differences. The results of the transmittance percent test, perticle size test and polydispersity index of the preparation are in the ideal range. The potential zeta test results of the preparation do not fall within the ideal range. Black cumin seed oil and moringa seed oil in nanoemulsion preparations meet the parameters of good physical properties, organoleptis test, pH test, viscosity test, transmittance percent test, while in particle determination test it does not meet the requirements on potential zeta measurement. Formula 1 is the optimal formula of nanoemulsion preparations as sunscreens.

Keywords: *Black cumin seed oil, moringa seed oil, nanoemulsion, physical stability, SPF*

Introduction

Sunlight can exert detrimental effects on humans, particularly on the skin. These effects include erythema or skin redness, hyperpigmentation or dark spots, photoaging or premature skin aging, and skin cancer. (Hader & Jori, 2001). According to *International Agency for Research on Cancer* (IARC) in 2012 ultraviolet (UV) radiation consists of approximately 95% UVA and 5% UVB, with most UVB being absorbed by the ozone layer, while UVC is almost entirely blocked by the ozone layer.

The skin is the organ that covers the entire surface of the human body and functions as a protective barrier against external factors (Tortora & Derrickson, 2014). The skin needs to be protected from the harmful effects of UV radiation, and one of the protective measures is the application of topical preparations containing Sun Protection Factor (SPF). SPF is defined as the amount of UV radiation energy required to reach the Minimal Erythema Dose (MED) on the skin (Khan, 2018).

Protective cosmetics are cosmetic preparations that function to protect the skin from various environmental influences, such as the harmful effects of UV radiation, and are also commonly referred to as sunscreens (Tranggono & Latifah, 2007). Sunscreens work by absorbing radiation energy, scattering, and reflecting UV radiation from the exposed skin surface (Draelos & Thaman, 2006). Protective cosmetic preparations or sunscreens may be formulated in the form of creams, gels, lotions, and can also be developed as nanoemulsions.

Nanoparticle technology has recently become a new trend in the cosmetic industry because its nanoscale size can enhance product quality (Aziz *et al*., 2019). A nanoemulsion is a translucent emulsion system consisting of an oil and water mixture stabilized by surfactants and cosurfactants (Jusnita *et al*., 2019). The particle size of a nanoemulsion, ranging from 5–200 nm, can improve the penetration of active ingredients through the skin layers (Mardikasari *et al*., 2016).

Black cumin (*Nigella sativa* Linn.) is a plant known to contain antioxidants. According to Khoirunnisa Khoirunnisa (2019) black cumin seed oil exhibits antioxidant activity with an IC_{50} value of 18.126 μg/mL, where antioxidant compounds are considered very strong if the IC_{50} value is <50 (Molyneux, 2004). Antioxidants are essential in protective cosmetic products, as they function to safeguard the skin from free radical damage. The flavonoid with aromatic chromophores in black cumin also plays a role in neutralizing free radicals due to the presence of chromophore groups capable of absorbing UVA and UVB radiation *(*Wolf *et al*., 2001). Black cumin contains beneficial substances, including flavonol triglycosides, which are flavonoid compounds of the quercetin group (Merfort et al., 1997) and phenolic compounds, namely vanillic acid. The compound spectra were identified using RP-HPLC (Bourgaou et al., 2007). Flavonoids and phenolic compounds are responsible for the activity of plants. Flavonoids have several activities, including antiviral, antiplatelet, anti-allergic, anti-inflammatory, anti-tumor, and antioxidant properties (Buhler and Miranda, 2000). Furthermore, research by Rahmawaty *et al.* (2021) demonstrated that black cumin seed oil can help reduce creatinine levels in patients at risk of metabolic syndrome.

Moringa (*Moringa oleifera* L.) can be utilized as an ingredient in cosmetics, with its seeds being one of the parts commonly used due to their antioxidant content. According to Utama (2022) moringa seed oil exhibits antioxidant activity with an IC_{50} value of 147.0277 μg/mL. One of the flavonoid groups present in moringa is quercetin (Krisnadi, 2015).

Methodology

Population and Sample

The population in this study consisted of black cumin seed oil produced in Yogyakarta and moringa seed oil produced in Blora Regency. The samples used in this research were black cumin seed oil branded Lansida and moringa seed oil branded Kelorina.

Instruments and Materials

Instruments

The instruments used in this study included a measuring cylinder (Herma), beaker glass (Herma), volumetric flask (Herma), analytical balance (Ohaus), homogenizer (DLAB), hot plate, pH meter (Ohaus), refrigerator, oven, Brookfield viscometer, centrifuge, UV-Vis spectrophotometer (Shimadzu), and particle size analyzer (Malvern).

Materials

The materials used in this study were black cumin seed oil (Lansida), moringa seed oil (Kelorina), Tween 80 (Brataco) as surfactan, propylene glycol (Brataco) as cosurfactan, methylparaben (Brataco) as preservative, propylparaben (Brataco) as preservative, and distilled water (Brataco) as a solvent.

Formulation

The nanoemulsion preparations were formulated using black cumin seed oil and moringa seed oil as active ingredients into five formulas with varying concentrations. The concentration of ingredients can be seen in the following table:

Table 1. Formula Design

Ingredients	**Formula (%b/v)**					**Function**
	1	**2**	**3**	**4**	**5**	
Black cumin seed oil	4	0	2	3	1	Active Ingredient (Oil Phase)
Moringa seed oil	0	4	2	1	3	Active Ingredient (Oil Phase)
Tween 80	36	36	36	36	36	Surfactant
Propylene glycol	10	10	10	10	10	Cosurfactant
Methylparaben	0,18	0,18	0,18	0,18	0,18	Preservative (Aqueous Phase)
Propylparaben	0,02	0,02	0,02	0,02	0,02	Preservative (Aqueous Phase)
Distilled water (Aquadest)	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Solvent (Aqueous Phase)

Formulation Procedure

Black cumin seed oil was mixed with moringa seed oil and propylene glycol as the oil phase, then homogenized using a homogenizer at speed 1 for 5 minutes.

Methylparaben and propylparaben were dissolved in preheated distilled water to form the aqueous phase. Tween 80, also preheated, was then added to the aqueous phase and homogenized for 5 minutes. Subsequently, the oil phase was gradually added into the aqueous phase, and both phases were homogenized together for 5 minutes at room temperature (Zulfa, 2020).

Physical Characterization Tests of Nanoemulsion Preparations

1) Organoleptic Test

   The organoleptic test was carried out by visually observing the color, odor, and physical form of the nanoemulsion preparation. The purpose of this test was to determine the appearance, aroma, and form of the nanoemulsion (Aisy *et al.*, 2021).

2) pH Test

   The pH test was performed by immersing the electrode into the sample. Prior to immersion, the electrode was rinsed with distilled water and dried with tissue, then inserted into the sample for measurement (Zulfa *et al.*, 2019).

3) Viscosity Test

   Viscosity was measured using a Brookfield viscometer. A 100 ml sample was placed in a beaker glass and fitted onto the solvent trap. Spindle no. 3 was selected at a rotation speed of 30 rpm. The measurement results were displayed on the digital screen (Az-Zahra *et al.*, 2022).

4) Centrifugation Test

   The centrifugation test was carried out by placing a centrifuge tube containing the sample into a centrifuge, operated at 3800 rpm for 30 minutes. Phase separation was observed after centrifugation (Panjaitan *et al.*, 2015).

5) Freeze–Thaw Stability Test

   The freeze–thaw stability test was conducted by storing the nanoemulsion preparation at 4°C for 24 hours, followed by transferring it to 40°C for another 24 hours (one cycle). The test was repeated for 6 cycles (12 days) (Fitriani *et al.*, 2016). Observations included organoleptic stability, pH value, and viscosity of the nanoemulsion (Budiarto *et al.*, 2020).

6) Particle Size Analysis

   Particle size analysis was performed by diluting the sample in distilled water (1:100), mixing until homogeneous, and then analyzing particle size, polydispersity index (PDI), and zeta potential using a Particle Size Analyzer (PSA) (Wijiyanto *et al.*, 2016).

7) Percent Transmittance Test

   Percent transmittance was measured by diluting 1 ml of the sample in a 100 ml volumetric flask with distilled water. The solution was then analyzed at a wavelength of 650 nm using a UV-Vis spectrophotometer, with distilled water used as the blank *(Yuliani et al.*, 2016). Transmittance values of 90%–100% indicated that the sample had a transparent and clear visual appearance (Costa *et al.*, 2012).

Chemical Characterization of Nanoemulsion Preparations

SPF Test

The determination of the Sun Protection Factor (SPF) value in vitro was carried out by measuring the absorbance of the sample using a UV-Vis spectrophotometer at 5 nm intervals within the wavelength range of 290–320 nm, with each point measured three times (Adi & Zulkarnain, 2015). The SPF value was calculated using the Mansur equation.

$$\mathbf{SPF}_{spectrophotometric} : CF \times \Sigma EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Description:

CF : Correction factor (10)
Abs : Absorbance of the sample
EE(λ) : Erythemal effect spectrum caused by UV radiation at wavelength λ nm
I(λ) : Intensity of UV radiation at wavelength λ nm

Data Analysis

The analysis of pH, viscosity, particle size, and SPF values was carried out using SPSS version 20. The physical and chemical properties of the nanoemulsion combining black cumin seed oil and moringa seed oil were analyzed using hypothesis testing, namely normality and homogeneity tests. If the results of the normality and homogeneity tests show $p > 0.05$, indicating normally distributed and homogeneous data, statistical analysis was performed using One-Way ANOVA, followed by Games-Howell Post Hoc Test in case of significant differences. If the data were not normally distributed and homogeneous, non-parametric tests were applied using Kruskal-Wallis and Mann-Whitney statistical analyses.

Result and Discussion

The use of surfactants must be combined with co-surfactants to produce smaller and more stable particle sizes. In this study, the concentration ratio of surfactant to co-surfactant was 36:10. Tween 80 was selected as the surfactant because it is non-toxic and non-irritating. Propylene glycol was used as the co-surfactant to accelerate the formation of the nanoemulsion and to enhance the efficacy of parabens as preservatives, since the antimicrobial activity of methylparaben and other parabens can be reduced by non-ionic surfactants, such as polysorbate 80 (Rowe *et al*., 2009).

The nanoemulsion was prepared using a high-speed homogenizer. This method is influenced by homogenization speed and duration, which allows the formulation to require lower surfactant concentrations because the preparation undergoes particle-to-particle shear, thereby reducing particle size (Khoerunisa *et al*., 2020). Moreover, this method requires a shorter time for nanoemulsion formation. Higher homogenization speeds result in smaller particle sizes, and smaller particle sizes lead to better stability (Suprobo & Rahmi, 2015).

a. Organoleptic Test

The organoleptic test was conducted to observe the physical appearance of the nanoemulsion preparations. Organoleptic observations of each formula were carried out both before and after the freeze–thaw stability test. The results of the organoleptic evaluation of the nanoemulsion preparations are shown in Table 2.

Table 2. Organoleptic Test Results

Formula	Organoleptic (Color, Odor, Consistency)	
	Before	After
F1	Brownish clear, Characteristic odor, Semi-viscous	Brownish clear, Characteristic odor, Semi-viscous
F2	Yellowish clear, Characteristic odor, Semi-viscous	Yellowish clear, Characteristic odor, Semi-viscous
F3	Brownish clear, Characteristic odor, Semi-viscous	Brownish clear, Characteristic odor, Semi-viscous
F4	Brownish clear, Characteristic odor, Semi-viscous	Brownish clear, Characteristic odor, Semi-viscous
F5	Yellowish clear, Characteristic odor, Semi-viscous	Yellowish clear, Characteristic odor, Semi-viscous

Note:
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%

The organoleptic test results of the nanoemulsion preparations, which included color, odor, and consistency, showed that F1 and F4 had a clear light brown color due to the higher concentration of black seed oil, a characteristic odor, and a semi-viscous consistency. Formula F3 had a clear light brown color because the brown color from black seed oil was more dominant compared to the yellow color from moringa seed oil, also with a characteristic odor and semi-viscous consistency. Meanwhile, F2 and F5 had a clear light yellow color due to the higher concentration of moringa seed oil, with a characteristic odor and semi-viscous consistency.

After the freeze–thaw test, the organoleptic characteristics of the nanoemulsion preparations in each formula did not show any changes in color, odor, or consistency. This indicates that the preparations were stable in terms of organoleptic properties. Nanoemulsions are characterized by a clear and transparent physical appearance, which is due to their small particle size. The smaller the particle size, the more transparent the resulting emulsion. Conversely, the larger the particle size, the greater the scattering of visible light, which causes the emulsion to appear more turbid (Aisy *et al.*, 2021).

b. pH Test

The pH test was conducted to determine the acidity level of the nanoemulsion preparations. The pH values were measured using a pH meter. The pH observations were carried out for each formula before and after the freeze–thaw stability test. The results of the pH test of the nanoemulsion preparations are shown in Table 3.

Table 3. pH Test Results

Formula	pH (Mean ± SD)	
	Before	After
F1	5,63 ± 0,01	5,15 ± 0,01
F2	5,74 ± 0,03	5,25 ± 0,01
F3	5,74 ± 0,01	5,25 ± 0,03
F4	5,64 ± 0,01	5,16 ± 0,01
F5	5,76 ± 0,02	5,26 ± 0,01

Note:
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%

The pH values that comply with the Indonesian National Standard (SNI) 16-4399-1996 for sunscreen preparations range from 4.5 to 8. The results of pH measurements for each formula showed that all formulations met the SNI requirements. Based on the pH test results, F1 and F4 exhibited lower (more acidic) pH values due to the higher concentration of black seed oil. Formula F3 showed a higher (more basic) pH value, as the effect of moringa seed oil was more dominant. F2 and F5 also demonstrated more basic pH values because they contained higher concentrations of moringa seed oil. It can be concluded that formulas containing higher concentrations of black seed oil tend to have a more acidic pH, as black seed oil itself has a lower pH.

After undergoing the freeze–thaw stability test, the pH values of the nanoemulsion preparations decreased. This pH reduction is suspected to be caused by the influence of CO_2 in the nanoemulsions, where CO_2 from the air reacts with the aqueous phase of the nanoemulsion, forming an acid. Additionally, the decrease in pH may also result from the hydrolysis of Tween 80, releasing sorbitan monooleate fatty acids, and could also be affected by environmental factors such as light exposure and humidity (Iskandar *et al.*, 2021).

Based on statistical analysis, the normality test results showed that F1, F3, and F4 were not normally distributed with a significance value of 0.000 (p-value < 0.05), while the homogeneity test results indicated that the data were homogeneous with a significance value of 0.341 (p-value > 0.05). The Kruskal–Wallis test was then conducted to determine whether there were significant differences, and the results showed a significant difference with a significance value of 0.023 (p-value < 0.05).

Further analysis using the Mann–Whitney test revealed that no significant differences were found between F1 & F4, F2 & F3, F2 & F5, and F3 & F5 (p-value > 0.05). However, significant differences were observed in F1 & F2, F1 & F3, F1 & F5, F2 & F4, F3 & F4, and F4 & F5 (p-value < 0.05). This indicates that variations in the concentrations of black seed oil and moringa seed oil significantly influenced the pH values. Specifically, black seed oil, which has a more acidic pH, caused formulas with higher concentrations of this oil to exhibit lower (more acidic) pH values.

c. Viscosity Test

The viscosity test was conducted to determine the thickness of the nanoemulsion preparations. The viscosity of each formula was observed before and after the freeze–thaw stability test. The results of the viscosity test of the nanoemulsion preparations are shown in Table 4.

Table 4. Viscosity Test Results

Formula	Viscosity (cPs) (Mean ± SD)	
	Before	Before
F1	488,30 ± 0,44	499,30 ± 0,23
F2	488,50 ± 0,26	499,50 ± 0,44
F3	488,50 ± 0,17	499,60 ± 0,35
F4	488,30 ± 0,32	499,60 ± 0,21
F5	488,50 ± 0,31	499,40 ± 0,30

Note:
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%

The results of viscosity measurements for each formula showed that the preparations had ideal viscosity values. Based on the viscosity test, the nanoemulsion formulations F1–F5 demonstrated non-significant differences in viscosity, which was attributed to the fact that the total concentration of oil used in all formulas was the same, namely 4%.

Several factors can influence viscosity, including the viscosity of the dispersed phase, the concentration of the dispersed phase, as well as the type and concentration of emulsifier used (Jusnita & Syurya, 2019). After the freeze–thaw stability test, the viscosity of the nanoemulsion preparations showed an increase compared to before the test. This increase occurred due to the effect of alternating low and high temperatures during the freeze–thaw cycles (Az-Zahra *et al.*, 2022).

Based on statistical analysis, the normality test indicated that the data were normally distributed (p-value >0.05), and the homogeneity test confirmed that the data were homogeneous with a significance value of 0.393 (p-value >0.05). The One-Way ANOVA test was then performed to determine whether there were differences between the groups, and the results showed no significant differences, with a significance value of 0.832 (p-value >0.05). This means that there were no significant differences among the formulas, leading to the conclusion that variations in the concentrations of black seed oil and moringa seed oil did not affect viscosity values, since the total oil concentration used in each formula was the same, namely 4%.

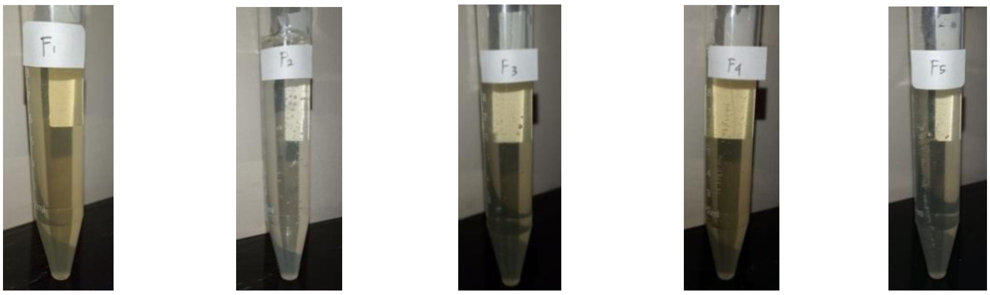
d. Centrifugation Test

The centrifugation test was performed immediately after the nanoemulsion preparations were made, with measurements taken once for each formula. The results of the centrifugation test of the nanoemulsion preparations are shown in Table 5.

Table 5. Centrifugation Test Results

Formula	Sentrifugasi		
	Sedimentation	Phase Separation	Turbidity
F1	-	-	-
F2	-	-	-
F3	-	-	-
F4	-	-	-
F5	-	-	-

Note:
- : not present;
+ : present
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%



Formula 1 Formula 2 Formula 3 Formula 4 Formula 5

Figure 1. Centrifugation Test Results for Each Formula

After centrifugation testing on each formula, the results showed no sedimentation, phase separation, or turbidity in any of the formulas. These findings indicate that the nanoemulsion preparations exhibited good physical stability. The use of Tween 80 as a surfactant can form a film layer on the droplet surface, as Tween 80 is a non-ionic surfactant that is not affected by acidic conditions, thereby remaining active in protecting the oil–water interface. This mechanism prevents globule coalescence within the dispersion medium and inhibits phase separation. Phase separation in the formulation typically indicates the breakdown of the nanoemulsion caused by oil droplets no longer being protected by the surfactant and co-surfactant (Pratiwi *et al.*, 2018).

e. SPF Test

The SPF test was conducted to determine the Sun Protection Factor (SPF) value of the nanoemulsion preparation combining black cumin seed oil and moringa seed oil. The SPF value was measured using a UV-Vis spectrophotometer by calculating the absorbance values based on the Mansur equation. The SPF test results of the nanoemulsion preparations are presented in Table 6.

Table 6. SPF Test Results

Formula	SPF (Mean ± SD)	Category
F1	38,23 ± 0,69	Ultra
F2	30,89 ± 0,76	Ultra
F3	36,76 ± 1,98	Ultra
F4	37,72 ± 1,35	Ultra
F5	35,60 ± 0,46	Ultra

Note:
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%

Based on the SPF values obtained, formulas F1–F5 fall into the ultra protection category because their SPF values were greater than 15. Significant differences were observed between F1&F2, F1&F5, F2&F4, F2&F3, and F2&F5. This finding indicates that formulations containing black cumin seed oil exhibited higher SPF values. This is consistent with Alhabsyi *et al*. (2014), who reported that the higher the antioxidant activity, the greater the SPF value obtained. According to Khoirunnisa (2019) black cumin seed oil has higher antioxidant activity than moringa seed oil, with an IC_{50} value of 18.126 µg/mL, while moringa seed oil shows an IC_{50} value of 147.0277 µg/mL (Utama, 2022) Therefore, formulations containing black cumin seed oil resulted in higher SPF values.

According to Sari & Islamiyati (2023) , the SPF value of moringa seed oil (*Moringa oleifera* L.) in cream formulations ranged between 12–19. Meanwhile, Kale *et al*. (2010) reported that black cumin seed oil (*Nigella sativa* L.) in cream formulations had an SPF value of 1.05. In contrast, in this study, the SPF value of moringa seed oil in nanoemulsion formulations was 30.89, and that of black cumin seed oil was 38.23. This demonstrates that nanoemulsion-based sunscreen formulations can produce higher SPF values compared to cream-based formulations. Differences in SPF values may be influenced by variations in the extraction process to obtain the oil as an active ingredient, as well as differences in formulation dosage forms.

Nanoemulsions are drug delivery systems that can enhance drug bioavailability by increasing the solubility of active ingredients, accelerating drug release, improving skin penetration, and boosting overall effectiveness (Karthik *et al*., 2017). In line with Rehman *et al*., (2017) nanoemulsion systems can be used for transmucosal and transdermal delivery routes, thereby effectively improving bioavailability.

Based on statistical analysis, the normality test showed that the data were normally distributed (p-value >0.05), while the homogeneity test indicated non-homogeneity with a significance value of 0.043 (p-value <0.05). The One-Way ANOVA test revealed significant differences among groups (p-value <0.05). Further Post Hoc Games-Howell analysis showed no significant differences between F1&F3, F1&F4, F3&F4, F3&F5, and F4&F5 (p-value >0.05). However, significant differences were observed between F1&F2, F1&F5, F2&F3, F2&F4, and F2&F5 (p-value <0.05).

These results indicate that variations in the concentration of black cumin seed oil and moringa seed oil significantly influence SPF values. This is because black cumin seed oil possesses stronger antioxidant activity, which contributes to higher SPF values. According to Alhabsyi *et al*., (2014), increased antioxidant activity correlates with higher flavonoid content, thereby enhancing sunscreen activity, since the chromophore groups in flavonoids are capable of absorbing UV radiation.

f. Transmittance Test

The percentage of transmittance was measured using a UV-Vis spectrophotometer. The results of the transmittance test of the formulations are presented in Table 7.

Table 7. Transmittance Test Results

Formula	Transmittance (%) (Mean ± SD)
F1	100,62 ± 0,12
F2	100,67 ± 0,08
F3	100,54 ± 0,05
F4	100,51 ± 0,06
F5	100,45 ± 0,10

Note:
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%

A transmittance percentage of 90–100% indicates that the sample exhibits a clear and transparent visual appearance (Costa *et al*., 2012). The results of the transmittance test for each formulation showed that all formulations had transmittance values greater than 90%. This indicates that the nanoemulsion preparations were clear, had particle sizes approaching the nanometer range, and met the transmittance requirements for nanoemulsion systems. The presence of surfactants can increase the percentage of transmittance, as their primary function is to reduce the interfacial tension between the oil and water phases, thereby producing smaller nanoemulsion particle sizes (Beandrade, 2018).

g. Particle Size Analysis

Particle size analysis was conducted to determine the particle size, polydispersity index (PI), and zeta potential of the nanoemulsion formulations. The results are presented in Table 8.

Table 8. Particle Size Analysis Results

Formula	PSA Test (Mean ± SD)		
	Particle Size (nm)	PI	Zeta potential (mV)
F1	10,65 ± 0,36	0,21 ± 0,04	-9,54 ± 0,20
F2	10,34 ± 0,22	0,12 ± 0,04	-7,85 ± 1,04
F3	10,39 ± 0,17	0,11 ± 0,00	-8,47 ± 0,37
F4	10,55 ± 0,05	0,13 ± 0,01	-7,17 ± 2,61
F5	10,54 ± 0,05	0,09 ± 0,02	-6,46 ± 1,57

Note:
F1 = Black seed oil 4%
F2 = Moringa seed oil 4%
F3 = Black seed oil 2% and moringa seed oil 2%
F4 = Black seed oil 3% and moringa seed oil 1%
F5 = Black seed oil 1% and moringa seed oil 3%

The particle size of nanoemulsion preparations generally ranges between 5–200 nm (Mardikasari *et al.*, 2016). According to Jusnita (2014) particle size is influenced by homogenization speed and duration—higher speeds and longer homogenization times yield smaller particle sizes. Additionally, Khoirunnisa (2019) reported that increasing concentrations of surfactants and co-surfactants further reduce particle size. This effect is attributed to Tween 80’s ability to reduce surface tension, thereby facilitating droplet formation and stabilization.

The particle size results showed mean values of 10.65 nm (F1), 10.34 nm (F2), 10.39 nm (F3), 10.55 nm (F4), and 10.54 nm (F5). All formulations met the nanoemulsion particle size requirements. Particles smaller than 90 nm are known to prevent creaming in nanoemulsion systems (McClements, 2012).

In cosmetic preparations formulated as nanoemulsions, it is expected that the system can penetrate the skin layers down to the dermis, since this layer contains numerous blood vessels that provide nutrients and maintain the balance of skin regeneration processes (Rismana *et al.*, 2014). The particle size of lipid vesicles has a significant impact on the delivery of bioactive compounds into the skin. Vesicles with a diameter of ≥600 nm are unable to deliver active substances beyond the stratum corneum; vesicles with a diameter of ≤300 nm can transport actives into deeper skin layers; while vesicles with a diameter of ≤70 nm are capable of delivering active substances into both the dermis and epidermis (Verma *et al.*, 2003;

Hua, 2015). Particles ranging from 10–210 nm can penetrate the skin via the transfollicular route (Geusens *et al.*, 2011; Zeb *et al.*, 2016). Based on the particle size obtained, the nanoemulsion formulation combining black cumin seed oil and moringa seed oil is expected to penetrate the skin layers as deep as the dermis.

The polydispersity index (PDI) is used to determine particle uniformity within the nanoemulsion system. A good PDI value ranges from 0 (monodisperse particles) to 0.5 (broad particle size distribution) (Adi *et al.*, 2019). The PDI values of all formulations were <0.5, indicating that the droplets formed were sufficiently uniform and met the criteria for an acceptable PDI. PDI is an important parameter of formulation stability, since lower values are associated with greater stability over long-term storage (Gao *et al.*, 2008).

Zeta potential reflects the surface charge of colloidal particles and is a critical indicator of nanoemulsion stability, as similar charges generate electrostatic repulsion that prevents particle aggregation. For a colloid to be considered electrostatically stable, the zeta potential should be greater than ±30 mV (Akhtar *et al.*, 2012). The zeta potential values obtained in all formulations were less than –30 mV, indicating relatively weak inter-particle repulsion and limited ability to prevent coalescence, suggesting that the nanoemulsion stability was not predominantly maintained by electrostatic repulsion. However, zeta potential is not the sole parameter determining nanoemulsion stability (Shah *et al.*, 2014).

The negative zeta values observed were attributed to free fatty acids present in the formulation (Beandrade, 2018). Additionally, most dispersed particles in aqueous systems tend to exhibit negative charges due to preferential adsorption of hydroxyl ions (Handayani *et al.*, 2018). Several factors influence zeta potential, including pH. At higher pH, zeta potential values tend to decrease (more negative), while at lower pH, they may become more positive. Other influencing factors include conductivity and concentration changes caused by the addition of ionic surfactants (Amyliana & Agustini, 2021). In this study, Tween 80, a non-ionic surfactant, was employed; such surfactants typically lower the absolute value of zeta potential(Handayani *et al.*, 2018).

Conclusion

Based on the results of this study, it can be concluded that, black cumin seed oil (*Nigella sativa* L.) and moringa seed oil (*Moringa oleifera* L.) can be formulated into nanoemulsion preparations. The nanoemulsion preparations of black cumin seed oil (*Nigella sativa* L.) and moringa seed oil (*Moringa oleifera* L.) met the parameters of good physical properties in organoleptic tests, pH tests, viscosity tests, percent transmittance tests, centrifugation tests, and freeze-thaw stability tests. However, in particle characterization, the zeta potential values did not meet the required criteria for nanoemulsion stability. The optimal nanoemulsion formula as a sunscreen was obtained in Formula 1 with a concentration of 4% black cumin seed oil.

Conflict of interest

The authors declare that they have no competing interests

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