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Formulation and Activity Testing of Sunscreen Nanoemulsion Cream Derived from 96% Ethanol Extract of Kersen Leaves (*Muntingia Calabura* L.)

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ABSTRACT

Kersen leaves contain secondary metabolite compounds, including flavonoids, phenolics, saponins, and tannins. Nanoemulsion has a colloidal dispersion that has a particle size between 10 and 200 nm. Nanoemulsion was incorporated into a cream base so that when used topically with a relatively small particle size, it can increase the penetration of active substances into the skin. This study aims to determine the physical characteristics and potential of 96% ethanol extract of kersenleaves as a sunscreen based on SPF value. This research is an experimental quantitative research which includes phytochemical screening, making nanoemulsion cream with concentrations of 2%, 4%, and 6%., testing the characteristics of the preparations, testing sunscreen activity in vitro using a UV-Vis spectrophotometer, and statistical data analysis with the SPSS program. The results of phytochemical screening of 96% ethanol extract of kersen leaves (Muntingia calabura L.) showed that the extract positively contained flavonoids, phenolics, tannins, and saponins. The results of the nanoemulsion research particle size test obtained a value of 17.65 nm, the zeta potential test (-18.30), and the transmittance percent test (94.224), which has a clear and transparent visual. Nanemulsion cream formulation with variations in the concentration of 96% ethanol extract nanoemulsion of kersen leaves at F0 (-), F1 (2%), F2 (4%), and F3 (6%) produced has good organoleptic properties and homogeneity, viscosity ranges from 2000 to 50,000 cPs, spreadability ranges from 5 to 7 cm, adhesive power is greater than 1 second, pH preparation is in the range of 4.5 to 6.5, and it is an oil-in-water emulsion type. In the results of the sunscreen activity test, the SPF value obtained at F0 (0.72) has no potential as a sunscreen, F1 (12.15) was in the maximum protection category, F2 (17.34) was in the ultra protection category, and F3 (30.56) was in the ultra protection category. Nanemulsion cream, a 96% ethanol extract of kersenleaves, has potential as a sunscreen. The most optimal nanemulsion cream formula was at an F3 concentration of 6%, with an SPF value of 30.53, entering the ultra protection category.

Keywords: *KersenLeaves, Cream, Nanoemulsion, Sunscreen*

INTRODUCTION

The skin is the largest organ in the body and serves as a protective barrier (Nurlaili, 2016). One of the skin's protective functions is to shield the body from exposure to UV radiation (Nafiah et al., 2024). Excessive exposure to UV radiation can cause harmful effects on the skin, specifically from ultraviolet (UV) radiation, which consists of approximately 95% UVA (320-400 nm) and 5% UVB (290-320 nm), while UVC radiation (<290 nm) does not reach the Earth's surface. Excessive UV exposure can cause harmful effects on the skin, namely from ultraviolet (UV) radiation, which consists of approximately 95% UVA (320-400 nm) and 5% UVB (290-320 nm), as well as UVC radiation (<290 nm) that does not reach the Earth's surface. Excessive exposure to UV radiation can cause sunburn, photoaging, and photocarcinogenesis. The stratum corneum absorbs approximately 70% of UVB radiation that reaches the skin, the epidermis absorbs 20%, and the upper dermis absorbs 10%. The epidermis partially absorbs UVA radiation, while the deeper layers of the dermis absorb 20%-30% of it.

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The energy from UVB radiation with shorter wavelengths is absorbed in greater quantities by the epidermis and keratinocyte DNA compared to UVA energy, which penetrates deeper into the dermal layers of the skin (Baran & Maibach, 2017).

Sunscreen is a cosmetic product containing sunscreen ingredients that protect the skin from the harmful effects of ultraviolet radiation by absorbing, reflecting, and scattering ultraviolet rays (BPOM RI, 2020). SPF (Sun Protection Factor) is the ratio of sunscreen protection against sunlight, serving as a measure of the effectiveness of sunscreen (Afivah et al., 2023). The effectiveness of sunscreen is determined by calculating the SPF (Sun Protection Factor) value through in-vitro testing using a UV-Vis spectrophotometer (Putriani et al., 2020).

Natural ingredients are being developed as alternative raw materials for sunscreen. Compared to chemical ingredients, natural ingredients have less potential for skin irritation, are more tolerable to human skin, and have less negative impact than chemical ingredients (Setiawan, 2010). Research on medicinal plants that have been passed down through generations by Indonesian communities is an initial step in exploring the potential of these plants (Edy and Parwanto, 2019).

Kersen leaves (*Muntingia calabura* L.) contain secondary metabolites such as flavonoids, polyphenols, tannins, saponins, and alkaloids (Annisa & Najib, 2022). The secondary metabolites in the form of flavonoids and phenols found in kersen leaves, in addition to having antioxidant activity, are also utilized as sunscreen (Puspitasari & Wardhani, 2018). The 96% ethanol extract of kersenleaves shows antioxidant activity with an IC_{50} result of 43.29 ppm, classifying it as very strong because the IC_{50} value is <50 ppm (Putri & Najib, 2022). Kersenleaves have properties as a source of antioxidants, anti-inflammatory agents, antidiabetic agents, antibacterial agents, anthelmintics, antihyperlipidemic agents, and natural sunscreens (Sumarni et al., 2022).

The application of nanotechnology is one effective option because reducing the size of particles can improve effectiveness. Nanoparticle-based formulations can enhance the efficiency of absorption, distribution, and penetration of active ingredients into the skin (Yadwade et al., 2021). Nanotechnology can be applied in the form of nanoemulsions. Nanoemulsions are thermodynamically unstable colloidal dispersions of two immiscible liquids and are transparent dispersions of oil and water stabilized by interfacial films of surfactant and co-surfactant molecules. Nanoemulsions have particle sizes with diameters ranging from 10 to 200 nm (Tungadi, 2020). The use of nanoemulsions in the cosmetics industry is increasing due to their small particle size, which helps enhance the penetration of active ingredients into the skin, improve skin hydration, and increase the fluidity of the skin's outer layer, resulting in enhanced infiltration of active ingredients through the skin (Yadwade et al., 2021).

Nanemulsion cream is a delivery system in which the oil phase is emulsified into the aqueous phase in the form of droplets. This system can be either oil-in-water (O/W) or water-in-oil (W/O) (Hou & Sheng, 2023). Nanemulsion cream is a nanoemulsion-based formulation in the form of a semi-solid preparation applied topically (Zainol et al., 2015).

Previous research has been conducted on the formulation and stability of a vanishing cream formulation using 96% ethanol extract of kersen leaves (*Muntingia calabura* L.) as a skin-protecting sunscreen by Putri et al. (2022), the results of which indicated that the SPF value for F1 (2,145) falls under the minimal protection category, F2 with an SPF value of (7,793) under the extra protection category, F3 with an SPF value of (14,790) under the maximum protection category, and F4 with an SPF value of (21,767) falls into the ultra-protection category. Thus, the novelty of this study lies in the formulation and testing of the sunscreen activity of a 96% ethanol extract nanemulsion cream formulation of kersen leaves

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(*Muntingia calabura* L.). Based on the above, a study was conducted on the formulation and testing of the sunscreen activity of a nano-cream formulation of 96% ethanol extract of kersen leaves (*Muntingia calabura* L.). The nano-cream formulation was chosen because it is suitable for topical use, and it is expected that the nano-sized particles will be able to penetrate the skin more effectively.

MATERIALS AND METHODS

Equipment and Materials

Equipment

Analytical balance (Ohaus), porcelain dishes (Herma), mortar and pestle (Herma), thermometer, measuring cup (Herma), beaker (Herma), test tubes (Herma), test tube rack, tripod, wire mesh, Bunsen burner, wooden clamp, filter paper, dropper pipette, measuring flask (Herma), stopwatch, pH meter (Ohaus), water bath, watch glass, ruler, weighing pan (100 g), (200 g), and (500 g), glass plate, magnetic stirrer (B-One), sonicator (Jinyuanbao), homogenizer, particle size analyzer (Malvern Panalytical), UV-Vis spectrophotometer (Shimadzu), Brookfield viscometer (Digital viscometer NDJ-8S), moisture balance (Ohaus).

Materials

Kersen Leaves (PT. Lansida), 96% ethanol, Tween 80 (PT. Dian Fajar Medika), PEG 400 (PT. Dian Fajar Medika), VCO (Lansida), distilled water (PT. Nusa Kimia Indo), 96% ethanol (PT. Nusa Kimia Indo), stearic acid (Kimia Jaya Abadi), cetyl alcohol (Kimia Jaya Abadi), triethanolamine (Axxion), glycerin (Palapa Muda Perkasa), methyl paraben (PT. Dian Fajar Medika), propyl paraben (PT. Dian Fajar Medika), Methylene blue, Magnesium, HCl, concentrated H_2SO_4, NaOH, $FeCl_3$, Mayer's reagent.

Methods

This is a quantitative experimental study involving the preparation of a 96% ethanol extract Nanoemulsion cream from kersen leaves (*Muntingia calabura* L.) with varying extract concentrations, followed by physical property testing. The population and sample used were kersen leaves extracts produced by PT. Lansida. Non-probability sampling was used with a purposive sampling method. The research was conducted at the Pharmaceutical Technology Laboratory of the Cendekia Utama Kudus Health Technology Institute, and the nanoemulsion testing was carried out at the STIFAR “Yayasan Pharmacy” Laboratory in Semarang.

Preparation of Kersen leaves Extract

Powder and 96% ethanol (1:10) were placed in a maserator for 3 x 24 hours, with the solvent replaced every 24 hours. Then,stirring was performed every 8 hours for 15 minutes, and followed by filtration. Next, The mash is concentrated using a rotary evaporator at a temperature of 50°C and a speed of 45 rpm. Repeat the evaporation process over a water bath until a concentrated extract is obtained, and calculate the extraction yield (Setyowati et al., 2019).

Moisture Content Test of Kersen leaves Extract

On the moisture balance device, 1 gram of concentrated kersen leaves extract was placed in aluminum foil, weighed, and the moisture content was measured by pressing the start button. The measurement was performed three times to obtain the percentage of moisture content in the kersen leaves extract (Pambudi et al., 2021).

Phytochemical Screening Test

Flavonoids

Wilstatter test

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A 0.5-gram sample of 96% ethanol extract of kersen leaves was placed in a test tube, dissolved in 10 mL of ethanol, then 0.1 grams of Mg powder and 5 drops of concentrated HCl were added and shaken vigorously (Pujiastuti & Zeba, 2021). A positive result is indicated by a red color (Ferdinan & Rizki, 2021).

Bate Smith-Matcalfe Test

A sample of 0.5 grams of 96% ethanol extract of kersen leaves was dissolved in 2.5 mL of distilled water and 2-4 drops of concentrated H_2SO_4 were added (Ferdinan & Rizki, 2021). It was then heated for 15 minutes in a water bath. A positive result is indicated by the formation of an orange color (Aribowo et al., 2021).

NaOH Test

A sample of 0.5 grams of 96% ethanol extract of kersenleaves is placed in a test tube, dissolved in 10 mL of ethanol, and then 5 drops of 10% NaOH are added (Pujiastuti & Zeba, 2021). A positive result for flavonoids is indicated by a light yellow color (Ferdinan & Rizki, 2021).

Phenolic

A 0.5-gram sample of 96% ethanol extract of kersenleaves was diluted with 3 mL of distilled water, then 2-3 drops of 5% $FeCl_3$ were added. The sample was considered to contain phenolic compounds if a blue-black color was observed (Mailuhu et al., 2017).

Tannin

A sample of 96% ethanol extract of kersenleaves was placed in a test tube (250 mg) and dissolved in 3 mL of warm water (Yuliana et al., 2023). Then, 1-2 drops of 1% $FeCl_3$ solution were added. A positive result indicated the formation of a dark green color (Indratmoko et al., 2020).

Saponin

A sample of 96% ethanol extract of kersenleaves was taken in an amount of 1 gram and dissolved with 5 mL of distilled water in a test tube, then 1 mL of distilled water was added and shaken vigorously for 1 minute (Pratiwi et al., 2023). Positive for saponins if a stable foam of 1–3 cm forms for 30 seconds (Ravelliani et al., 2021). The presence of saponins is indicated if, upon adding 1 drop of 2 N HCl, the foam does not disappear (Wibowo et al., 2024).

Alkaloids

A 0.5-gram sample of 96% ethanol extract of kersen leaves is dissolved in 2.5 mL of distilled water in a test tube, then 0.5 mL of 2% HCl and 2–3 drops of Mayer's reagent are added. A color change and white or yellowish precipitate indicate a positive alkaloid test (Pratiwi et al., 2023). The alkaloid test can be performed using the Mayer, Wagner, and Dragendorff methods (Wibowo et al., 2024).

Nanoemulsion Production

The nanoemulsion formula for 96% ethanol extract of kersen leaves (*Muntingia calabura* L.) (Annisa, 2021) is as shown in Table 1 below:

Table 1. Nanoemulsion Formulation

Material	Concentration (%)	Function
96% ethanol extract of kersen leaves	6	Active substance
VCO	5	Oil Phase
Tween 80	38	Surfactant
PEG 400	22	Ko- Surfactant
Aquadest ad	100	Water Phase

All ingredients were prepared, namely Tween 80, PEG 400, VCO, and 96% ethanol extract of kersen leaves. These were then placed in a beaker glass and mixed using a magnetic stirrer for 30 minutes, with aquadest added gradually. The addition of aquadest was stopped once the volume reached 100 mL. The preparation is transferred into a vial, sonicated for 15 minutes at 40°C using a bath-type sonicator (Rusminingsih et al., 2023).

Nanoemulsion Characteristic Test

Particle Size Test

The tool used for particle measurement is a particle size analyzer (PSA). Particle size was determined by diluting 1 ml of nanoemulsion into 50 ml of distilled water and homogenizing it. The measurement procedure involves taking 1 mL of the nanoemulsion sample and placing it into a cuvette. The cuvette containing the sample is then placed into the sample holder, the instrument is turned on, and the particle size menu is selected (Dienilah, 2022). The particle size requirements for nanoemulsions range from 10 to 200 nm (Tungadi, 2020).

Zeta Potential Test

Zeta potential is analyzed using a particle size analyzer (PSA). Zeta potential was determined by diluting 1 ml of nanoemulsion into 50 ml of distilled water and homogenizing it. A 2 mL sample of nanoemulsion is taken and placed into a cuvette, which is then inserted into the holder, and the zeta potential mV menu is selected (Mannuela, 2016). The ideal zeta potential value for a nanoemulsion formulation is less than -30 mV and greater than +30 mV, indicating a more stable dispersion (Indratmoko et al., 2020).

Transmittance Percentage Test

Transmittance percentage is measured at 650 nm using a UV-Vis spectrophotometer at the beginning of the observation after the formulation is prepared by dissolving 1 mL of the sample into a 100 mL volumetric flask using distilled water, homogenized with a homogenizer for 5 minutes (Siqhny et al., 2020). Nanoemulsions have a clear and transparent appearance, as evidenced by a transmittance percentage of 90–100% (Nasiro et al., 2023).

Nanemulsion cream Production

This study used variations in the concentration of 96% ethanol extract nanoemulsion from kersenleaves, referring to previous research based on the best SPF value at a concentration of 4% F3 (Putri et al., 2022).

Table 2. Nanemulsion cream Formulation

Materials	Concentration (%)				Function
	F0	F1	F2	F3	
Nanoemulsion	-	2	4	6	active substance
Stearic acid	10	10	10	10	emulsifying agent
Cetyl Alcohol	2	2	2	2	tickening agent
Trietanolamin	0,2	0,2	0,2	0,2	emulsifying agent
Gliserin	5	5	5	5	emolien
Metil paraben	0,18	0,18	0,18	0.18	preservative
Propil paraben	0,02	0,02	0,02	0,02	preservative
Aquadest ad	100	100	100	100	solven

Description :
F0 = Without 96% ethanol extract of kersenleaves
F1 = Formulation of 96% ethanol extract of kersenleaves with a concentration variation of 2%

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F2 = Formulation of 96% ethanol extract of kersenleaves with a concentration variation of 4%
F3 = Formulation of 96% ethanol extract of kersenleaves with a concentration variation of 6%

The production of 96% ethanol extract nanemulsion cream from kersen leaves is carried out by first producing the cream base. The cream base is produced by melting the oil phase (stearic acid, cetyl alcohol, and propyl paraben) in a porcelain dish over a water bath until all ingredients are melted (temperature maintained at 70°C). The aqueous phase (triethanolamine, glycerin, methyl paraben, and distilled water) is placed in a porcelain dish over a water bath until it melts (temperature maintained at 70°C). The oil phase is poured into a warm mortar, and the water phase is added gradually while stirring until a creamy mass forms (Putri et al., 2022). The nanoemulsion of 96% ethanol extract of kersenleaves is added to the cream base gradually and stirred until homogeneous (Ningsih & Atiqah, 2020).

Nanoemulsion Cream Characteristic Test

Organoleptic Test

The nanoemulsion cream preparation is organoleptically observed using the five senses to assess its shape, color, and smell (Salsabila et al., 2024). The criteria for the cream formulation include a creamy consistency that spreads easily, a soft texture, and a non-irritating odor, in accordance with SNI 16-4399-1996 (National Standards Agency, 1996).

Homogeneity Test

The nanoemulsion cream is weighed at 0.5 grams and applied thinly and evenly on a watch glass, then observed for the presence of coarse particles on the watch glass. The cream composition must be homogeneous without any unmixed particles or lumps (Sari et al., 2021).

Viscosity Test

This test uses a Brookfield viscometer. The viscosity test is conducted to determine the thickness of a formulation. 100 grams of nanoemulsion cream preparation is put into a beaker glass.beaker glass, then spindle no. 3 is attached and inserted into the cream formulation, and the rotor is run at a speed of 30 rpm. Once the Brookfield viscometer displays a stable reading, the results are recorded (Tahir et al., 2024). The viscosity requirements for nanoemulsion cream formulations are within the range of 2000–50,000 cPs according to SNI 16-4399-1996 (National Standards Agency, 1996).

Spreadability Test

A 0.5-gram sample of nanoemulsion cream was placed on a spreadability testing device/watch glass and covered with a watch glass on top. A load of 200 grams is applied on top of the watch glass (Clarista et al., 2024). The load is left for 1 minute, and the diameter of the cream spread after the load is applied is recorded. Good spreadability requirements are within the range of 5–7 cm (Salsabila et al., 2024).

Adhesion Test

The test was conducted by placing 0.5 grams of nanoemulsion cream on a glass plate and covering it with another glass plate on top, then pressing it with a 500-gram weight for 5 minutes. The weight was then replaced with a 100-gram weight, and the two glass plates that were adhered together were separated, and the time until the two glass plates separated was recorded (Sari et al., 2021). The acceptable time requirement for the adhesion strength test on the nanoemulsion cream formulation is >1 second (Murdiana et al., 2022).

Emulsion Type Test

Performed using the staining method. The emulsion type is determined by taking a sample of the nanoemulsion cream and placing it on a watch glass, then adding 1 drop of methylene blue and stirring until homogeneous. An oil-in-water (O/W) emulsion is indicated by a uniform blue color distribution in the sample (Clarista et al., 2024).

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SPF Test

The determination of sunscreen effectiveness based on SPF values is conducted in vitro using a UV-Vis spectrophotometer with a wavelength range of 290–320 nm at intervals of 5 nm. The blank used is a 96% ethanol solution, and its absorbance can be calculated (Higyeungsi et al., 2023). The SPF level test was performed using a UV-Vis spectrophotometer. A 0.1-gram sample of each nanoemulsion cream formulation was weighed, placed in a 10-mL volumetric flask, and filled with 96% ethanol to the mark. The resulting solution was then measured for absorbance using a UV-Vis spectrophotometer at a wavelength of 290-320 nm. The absorbance was recorded at intervals of 5 nm. The SPF value was calculated using Mansur's formula (Erwiyani et al., 2021).

$$\boxed{\text{SPF} = \text{CF} \times \sum \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda)}$$

Explanation:

CF	: Correction factor (10)
Abs	: Absorbance of sunscreen product
EE	: Effectiveness of erythema caused by UV rays at wavelength λnm
I	: Intensity of UV rays at wavelength λ nm

RESULTS AND DISCUSSION

The evaluation of water content in 96% ethanol extract of kersen leaves (*Muntingia calabura* L.) aims to determine the water content in the extract. The water content results are shown in Table 3 as follows:

Table 3. Water Content Results of Kersen leaves Extract

Sample of 96% Ethanol Extract of Kersen Leaves	
Replication 1	10,02%
Replication 2	10,09%
Replication 3	10,19%

Based on the data in Table 3 above, the average water content of 96% ethanol extract of kersen leaves (*Muntingia calabura* L.) was 10.1%. This water content of 96% ethanol extract meets the requirements according to Voight (1994), who states that the water content in thick extracts is in the range of 5-30% extract.

Phytochemical Screening of 96% Ethanol Extract of Kersen Leaves (*Muntingia calabura* L.)

Phytochemical screening was conducted to determine the content of secondary metabolites in 96% ethanol extract of Kersen leaves (*Muntingia calabura* L.), including flavonoids, phenolics, tannins, saponins, and alkaloids. The results of the phytochemical screening, as shown in Table 4, are as follows:

Table 4. Phytochemical Screening Results

No	Compund	Reagent	Result	Description
1.	Flavonoids (*Wilstatter*)	HCl concentrated + Mg powder	(+)	Formation of red color
	(*Bate Smith-Matcalfe*)	H_2SO_4 concentrated	(+)	Formation of orange color

No	Compund	Reagent	Result	Description
	(NaOH)	NaOH 10%	(+)	Formation of yellow color
2.	Phenolic	$FeCl_3$ 5%	(+)	Formation of a dark blue color
3.	Tannin	$FeCl_3$ 1%	(+)	A blackish-green color has formed.
4.	Saponin	HCl 2N	(+)	Stable foam formation
5.	Alkaloids	HCl 2 N + Mayer's reagent	(-)	No white deposits formed

Description:
(+) = contains secondary metabolite compounds
(-) = does not contain secondary metabolite compounds

The phytochemical screening results in Table 4 above show that the 96% ethanol extract of kersen leaves (*Muntingia calabura* L.)e for flavonoids, phenolics, tannins, and saponins. In the flavonoid test, there were three tests, namely the Wilstatter test, the Bate Smith-Matcalfe test, and the NaOH test.

The Willstatter test showed a positive result, indicated by the formation of a brick-red color. Consistent with the research by Vonna et al. (2021), the ethanol extract of kersen leaves contains flavonoid compounds, as evidenced by the formation of a brick-red solution after reacting with Mg powder and HCl. According to the research by Aribowo et al. (2021), the extract tests positive for flavonoids belonging to the flavonol and flavanone groups if a color change to red occurs, which is accompanied by the formation of red benzopyridinium salts resulting from the reduction of a polyhydroxy group in flavonol by magnesium in hydrochloric acid. According to Arum (2012), cited in Aditiya (2022), kersen leaves contain flavonoid components such as flavon and flavonol.

The Bate-Smith-Metcalfe test showed positive results, indicated by the formation of an orange color. According to the research by Aribowo et al. (2021), the extract was positive for anthocyanidin flavonoids if there was a color change to orange. In line with the research by Anisa and Najib (2022), kersenleaves contain phenolic and flavonoid compounds.

The NaOH test showed positive results, indicated by the formation of a yellow color. This is in line with Hadi's (2019) research, which stated that the flavonoid test with the addition of 10% NaOH to kersen leaves showed positive results, indicated by a bright yellow color. According to the research by Kusnadi and Devi (2017), the compound kristin, which is a derivative of the flavon compound, acts as a base catalyst when 10% NaOH is added, causing the breakdown of flavonoid compounds into acetophenone molecules due to the breaking of bonds in the isoprene structure. In line with the research by Anisa and Najib (2022), the color of the kersen leaves extract in the flavonoid test after the addition of 10% NaOH indicates the presence of flavonoids classified as phenolic compounds.

In the phenolic test of 96% ethanol extract of kersen leaves, a positive result was obtained, indicated by the formation of a dark blue color. In line with the research by Rusita et al. (2021), the ethanol extract of kersenleaves was positive for phenolic compounds, indicated by a color change from yellow to dark blue. According to the research by Suhaenah et al. (2023), the $FeCl_3$ reagent will react with a color change due to the formation of a covalent coordination bond between iron (III) ionAnisa and Najib's (2022) research confirms the presence of phenolic compounds in kersenleaves.eaves.

The tannin compounds in kersen leaves extract are characterized by a color change to blackish green. In line with the research by Indratmoko et al. (2020), ethanol extract of kersen leaves positively contains tannin compounds, characterized by a blackish green color. According to the research by Putri and Lubis (2020), this color change occurs due to a reaction

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between the hydroxyl groups present in the tannin compounds and the 1% $FeCl_3$ reagent. Anisa and Najib's (2022) research confirms the presence of tannin compounds in kersen leaves.

Ethanol extract of kersen leaves was found to contain saponins, as shown by the formation of stable foam. In line with the research by Vonna et al. (2021), the ethanol extract of kersen leaves contains saponin compounds, indicated by the formation of foam, and when 2N HCl is added, the foam does not disappear. Anisa and Najib's (2022) research confirms the presence of saponin compounds in kersen leaves est, no positive results were observed in the form of white precipitation. In line with the research by Vonna et al. (2021), ethanol extract of kersen leaves does not contain alkaloid compounds. According to Ferdinan and Rizki (2021), the absence of white precipitation in the alkaloid test is due to the absence of potassium-alkaloid complexes.

Nanoemulsion Characteristic Test

The results of the characteristic test of the 96% ethanol extract nanoemulsion of kersen leaves can be seen in Table 5.

Table 5. Nanoemulsion Characteristics Test

Test	Result
Particle Size	17,65 nm
Polydispersity Index	0,3
Potential Zeta	-18,30 mV
Transmittance Percentage	94,224%

Source: Processed primary data (2025)

The results obtained in Table 5 of the nanoemulsion particle size test are (17.65 nm), which meet the requirements for good particle size. In line with Tungadi's (2020) research, the ideal particle size for nanoemulsions ranges from 10 to 200 nm. According to Pratiwi et al.'s (2018) research, nanoemulsion particle sizes <90 nm can be more stable during sedimentation. In the study by Indratmoko et al. (2020), it was shown that a higher concentration of Tween 80 compared to PEG 400 can affect particle size and clarity, where an increase in Tween 80 concentration can reduce particle size by 12.4 nm and improve clarity by 98.5%. According to the study by Yadwade et al. (2021), smaller particle sizes can help increase the penetration of active substances into the skin.

The polydispersity index value for the nanoemulsion formula is (0.3), indicating that the nanoemulsion has good uniformity in droplet size distribution, as evidenced by the range of values meeting the polydispersity index requirements. In line with the study by Adi et al. (2019), it was stated that a polydispersity index value <0.5 falls under monodisperse, indicating uniform droplet size. According to the research by Wahyuningsih and Putranti (2015), the polydispersity index value is used to estimate the distribution range of particle sizes in a formulation and to determine the presence or absence of aggregation or particle clustering.

The results of the nanoemulsion zeta potential test are (-18.30), which meet the zeta potential test requirements. This indicates that the resulting nanoemulsion has good stability. In line with the research by Khoshnevisan and Barkhi (2015), which states that the ideal zeta potential value is in the range of <-30 mV or >+30 mV, indicating that if the zeta potential has a large positive or negative value on the globule surface, the dispersion formed will be more stable. In the study by Jafari and McClements (2018), small particle size combined with a large zeta potential value can enhance the stability of nanoemulsions. The zeta potential value in maintaining stability generally falls within the range of <-30 mV or >+30 mV, which can significantly impart surface charge to the globules. A large negative zeta potential causes greater repulsive forces between globules, thereby preventing aggregation and the formation of a separation phase. According to Handayani et al. (2018), a low pH produces a positive zeta potential, while a high pH produces a negative zeta potential. Tween 80, as a nonionic surfactant, reduces the zeta potential.

The results of the nanoemulsion transmittance test are (94.224%), indicating that the nanoemulsion produced has a clear and transparent appearance, thus meeting the transmittance requirements. This aligns with the research by Nasiro et al. (2023), who stated that a transmittance percentage of 90%-100% indicates that the formulation is transparent and clear, and a transmittance percentage approaching 100% means that the formed globules have reached nanometer-sized dimensions. According to Widyastuti and Saryanti (2023), a nanoemulsion can be considered good if it has a transmittance percentage of 90%-100%, which indicates that the nanoemulsion has small particle sizes. According to Kusumawardani's research (2019), a transmittance percentage close to 100% indicates that the oil content in the formulation is smaller than the surfactant and co-surfactant, thereby providing good clarity. According to Huda and Wahyuningsih (2016), a higher surfactant-to-cosurfactant ratio in the formulation can influence emulsion droplet size, such that smaller droplets result in clearer formulations, thereby increasing the percentage transmittance.

Nanoemulsion Cream Characteristics Test

Table 6. Test results of physical characteristics of nanoemulsion cream 96% ethanol extract of kersen leaves

Test	F0	F1	F2	F3
Organoleptic	white, characteristic odor, and semi solid	light brown, characteristic odor, and semi solid	light brown, characteristic odor, and semi solid	dark brown, characteristic odor, and semi solid
Homogeneity	homogeneous	homogeneous	homogeneous	homogeneous
pH	6,49 ± 0,01	6,45 ± 0,02	6,41 ± 0,03	6,30 ± 0,02
Spreadability (cm)	5,1 ± 0,1	5,3 ± 0,1	5,6 ± 0,1	5,9 ± 0,1
Adhesion test (second)	2,52 ± 0,03	2,29 ± 0,04	2,12 ± 0,03	1,44 ± 0,05
Viscocity (cps)	3931,433 ± 0,35	3930,600 ± 0,36	3929,467 ± 0,35	3928,633 ± 0,25
Cream Type	M/A	M/A	M/A	M/A

Based on the results of the organoleptic test observations in Table 6 above, the color of the nanoemulsion cream preparation was found to be white in F0, while the formulations with the addition of 96% ethanol extract of kersen leaves in F1, F2, and F3 ranged from light brown to dark brown. After observation, it can be concluded that the higher the concentration of extract used, the darker the color of the formulation. In the odor observation of the nanemulsion cream formulations in F1, F2, and F3, the formulations had the characteristic odor of 96% ethanol extract of kersen leaves. In the texture observation of the nanemulsion cream formulations in F0, F1, F2, and F3, the formulations had a semi-solid texture. This aligns with the research by Wijayanti and Maulana (2023), where the addition of extract to the cream formulation affects its odor and color. The brown color and characteristic odor originate from the extract used. The higher the concentration of ethanol extract from kersen leaves used, the darker the brown color becomes.

Result of the homogeneity test, the nanemulsion cream preparations in F0, F1, F2, and F3 showed homogeneous results, indicated by the absence of coarse particles when applied to a watch glass. In line with the research by Pratasik et al. (2019), a cream is considered homogeneous if the cream formulation is free from clumped particles and no coarse particles are visible when applied to transparent glass. According to the research by Murdiana et al. (2022), the homogeneity test showed that the preparation did not contain coarse particles due to the melting process and the perfect mixing of the oil and water phases.

Results of the viscosity test, it can be concluded that the nanemulsion cream preparations in F0, F1, F2, and F3 meet the ideal viscosity parameters for preparations. According to the National Standards Agency (1996), the viscosity value for ideal topical formulations is within the range of 2,000–50,000 cPs. Increasing the extract concentration was found to reduce the viscosity value. This aligns with the research by Putri et al. (2022), which stated that adding kersen leaf extract reduces the viscosity of the cream. According to the research by Erwiyani et al. (2021), creams containing the highest extract concentration will result in lower viscosity values, influenced by the consistency of the extract as a thick liquid, thereby producing a nanoemulsion cream formulation that is increasingly thinner.

The spreadability test aims to determine the spreadability of the nanemulsion cream formulation when applied to the skin surface. Based on the results of the spreadability test, all formulations in the nanemulsion cream preparation have met the spreadability test requirements. According to research by Salsabila et al. (2024), good spreadability requirements are in the range of 5-7 cm. Based on the results obtained, it can be concluded that the higher the concentration of the 96% ethanol extract nanoemulsion from kersen leaves, the greater the spreadability. This aligns with the research by Erwiyani et al. (2021), which states that increasing the concentration of the extract with a more dilute extract consistency results in a cream with greater spreadability.

The adhesion test aims to determine the ability of the nanemulsion cream formulation to adhere when applied to the skin. Based on the results of the adhesion test in Table 13 above, all formulas, namely F0, F1, F2, and F3, meet the requirements for cream preparations. This is in line with the research by Murdiana et al. (2022), which states that the adhesion test requirement for cream preparations is >1 second. The highest adhesion test results were found in F0, which did not contain ethanol extract of kersen leaves, while the lowest adhesion was found in formula 3, which contained a 6% concentration of nanoemulsion ethanol extract of kersen leaves. Based on these results, the formula without extract is inversely proportional to the formula with the highest extract addition. This aligns with the research by Putri et al. (2022), which states that increasing the concentration of ethanol extract of kersen leaves reduces the adhesion value.

The pH test aims to ensure the safety of the nanemulsion cream formulation's pH to match the skin's pH. The optimal pH range is between 4.5 and 6.5 (Tranggono & Latifah, 2007). Based on the results of the pH test, the nanemulsion cream preparations F0, F1, F2, and F3 fall within the safe pH range for skin because they are still within the normal pH range. The pH value of the base (F0) is higher than that of the cream containing kersen leaves extract. The higher the concentration of extract used, the more acidic the cream's pH becomes, as indicated by the lower pH values shown in Table 14. The decrease in pH is due to the kersen leaves extract containing acidic compounds such as vitamin C. This aligns with Lirang's (2021) research, which states that every 100 grams of kersenleaves contain 80.5 mg of vitamin C. In this study, a pH test was also conducted on kersen leaves extract, and the results showed a pH value of 3.92, which falls within the acidic pH range.

The emulsion type test aims to determine whether the nanemulsion cream formulation belongs to the A/M or M/A type using a staining method. Based on the data from the emulsion type test using methylene blue solution. F0, F1, F2, and F3 are classified as oil-in-water (O/W) emulsions in all formulas. In line with the research by Clarista et al. (2024), O/W emulsion creams are characterized by a uniform distribution of blue color in the formulation. According to the research by Pratasik et al. (2019), O/W emulsions form because the volume of the oil phase (dispersed phase) used is smaller than the water phase (dispersing phase), causing oil globules to disperse into the water phase.

SPF Value Test

The SPF value test aims to determine the SPF value obtained from the 96% ethanol extract nanemulsion cream formulation of kersen leaves (*Muntingia calabura* L.), conducted in vitro using a UV-Vis spectrophotometer. As shown in Table 7 below:

Table 7. Test SPF

Formula	Value SPF ± SD	Wavelength	Sunscreen Protection Category
F0	0,72 ± 0,02	290-320	-
F1	12,15 ± 0,47	290-320	Maximum Protection
F2	17,34 ± 0,73	290-320	Ultra Protection
F3	30,56 ± 0,99	290-320	Ultra Protection

Based on the SPF test results in Table 7 above, the SPF value obtained for F0 is (0,72), meaning it does not have the potential to be used as sunscreen. F1, with an SPF value of (12.15), falls into the maximum protection category. F2, with a value of (17,34), falls into the ultra protection category, and F3, with a value of (30.56), also falls into the ultra protection category. In this study, the highest SPF value for the 96% ethanol extract nanemulsion cream formulation of kersen leaves was obtained at a concentration of 6%, with an SPF value of (30.56). This is because the extract concentration in F3 is the highest compared to other formulations. This finding aligns with the research by Putri et al. (2022), which showed that the higher the concentration of kersen leaf extract, the higher the SPF value produced.

According to research by Puspitasari and Wardhani (2018), kersen leaves contain flavonoids and phenolics, which not only have antioxidant activity but can also function as sunscreen. This aligns with the research by Putri and Najib (2022), which states that 96% ethanol extract of kersen leaves contains antioxidant activity with a value (IC_{50} = 43.29 ppm), classified as a very strong antioxidant. In the study by Prasiddha et al. (2016), it was stated that flavonoid compounds have the potential to act as sunscreen because they contain chromophore groups. Chromophore groups are conjugated aromatic systems that can absorb UV light at both UVA and UVB wavelengths. Phenolic compounds have conjugated double bonds that undergo resonance when exposed to UV (ultraviolet) light. The conjugated system of a phenolic compound with the chemical compounds present in sunscreen is what gives it photoprotective potential. According to Pangemanan et al. (2020), the higher the content of phenolic compounds in the extract, the greater the ability of the sunscreen to protect the skin from damage caused by UV radiation exposure.

CONCLUSION

Physical property tests on nanoemulsions and nanemulsion creams meet the requirements for good physical characteristics. Tween 80, PEG 400, and VCO can influence the physical characteristics of nanoemulsions. Variations in the concentration of 96% ethanol extract of kersen leaves affect the physical characteristics of nanemulsion creams. The most optimal SPF test results were obtained in formula 3 at a 6% concentration, yielding an SPF value of (30,56) in the ultra-protection category.

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