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Research Article

Formula Optimization of Sunscreen Nanoemulsion from *Centella asiatica* (L.) Extract with Combination Tween-80 and Propylene Glycol Surfactants

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ABSTRACT

Sunscreen protects against ultraviolet (UV) rays. It has a Sun Protection Factor (SPF) value. *Centella asiatica* (L.) is an herbal plant that contains flavonoid and triterpenoid compounds as antiphotoaging agents. Development of *Centella asiatica* (L.) extract in nanoemulsion preparation increases its solubility in water and absorption. This study aims to obtain the optimum formula of *Centella asiatica* (L.) nanoemulsion preparation from the combined use of Tween-80 and Propylene glycol (PG) surfactants and determine the effectiveness of *Centella asiatica* (L.) extract in vitro. The effectiveness of *Centella asiatica* (L.) extract was tested in vitro using ultraviolet-visible (UV-Vis) spectrophotometry expressed by SPF value. Development of *Centella asiatica* (L.) extract nanoemulsion formula using a combination of surfactants Tween-80 and Propylene glycol with factorial design 2 factors and 2 levels. P-value<0.05 indicated there is a statistically significant difference in the formula. *Centella asiatica* (L.) extract has protective power against UV rays at concentrations of 120, 240, and 360 ppm with SPF-values of 8.999, 19.219, and 29.569, respectively. Meanwhile, the nanoemulsion response analysis revealed the response of the nanoemulsion produced (p<0.05). The optimum formula obtained was found in the nanoemulsion formula with a combination of Tween-80 and PG at a low level.

INTRODUCTION

Ultraviolet (UV) radiation is categorized as a radiation that might have harmful effects on the skin, such as photoaging and photocarciogenesis (Puglia *et al.*, 2014). Photoaging causes skin aging, decreased collagen content, reduced skin elasticity, causes wrinkles, and rough skin surface (Pham *et al.*, 2014; Li *et al.*, 2021). Sunscreen is a topical preparation that can be applied to protect the skin from UV exposure. The level of protection against UV rays from sunscreen indicated by the presence of Sun Protection Factor (SPF) value (Kamel and Mostafa, 2015). Sunscreen is designed with some ingredients to protect against UV exposure. *Centella asiatica* (L.) is an herbal plant that can be used as an active ingredient in sunscreen formulations (Arina, Sarbani and Mohamed, 2023).

Gotu kola or *Centella asiatica* (L.) is an herbal plant that has potential as an antiphotoaging, antimelanogenesis, anti-inflammatory, and wound-healing agent (Prakash, Jaiswal and Srivastava, 2017; Arribas-López *et al.*, 2022). *Centella asiatica* (L) contains flavonoid and triterpenoid compounds that are responsible for the pharmacological activity in skin care. Triterpenoid compounds include asiaticoside, madecassoside, and asiatic acid (Sun *et al.*, 2020). Of these components, asiaticoside is one of the main triterpenoid compounds with the largest composition in *Centella asiatica* (L.) extract after madecassoside (Wang *et al*., 2020; Rizikiyan *et al*., 2022).

Asiaticoside is the main of triterpenoid compound in *Centella asiatica* (L.) extract which is efficacious as an antiphotoaging agent (Milani and Sparavigna, 2017). Its effectiveness as an antiphotoaging agent has been proven in a study by Huang *et al*. (2023) which showed that asiaticoside can prevent photoaging of the skin due to UV-A exposure through in vitro tests. Photoprotections of asiaticoside occurs through the mechanism of inhibiting HDF proliferation due to UV A exposure (Huang *et al*., 2023). In addition, asiaticoside also acts as an anti-melanogenesis agent through the mechanism of inhibiting excessive melanin production, which has the potential to cause melanogenesis and hyperpigmentation problems in the skin (Munirah, Faudzi and Mohamad, no date; Lu *et al*., 2019). Asiaticoside has a molecular weight of 959.12 g/mol, a water solubility of 307.347 μg/mL, and a partition coefficient of 2.24 (Zhang *et al*., 2016). The low solubility in water and the large molecular weight of asiaticoside have an inhibitory effect on the skin absorption process, causing limitations when formulated in topical preparations. To overcome these obstacles, asiaticoside can be developed and formulated in nanoemulsion preparations (Rigano and Lionetti, 2016). The small particle size of nanoemulsion facilitates the absorption of active substances to penetrate deeper layers of the skin (Rigano and Lionetti, 2016).

Nanoemulsion preparations were chosen because they have advantages of increasing the solubility of the active substance, provide good stability, producing transparent visual characteristics, small particle size, and having a high of surface area so that the absorption of the active ingredients is more maximized (Çinar, 2017). Nanoemulsion formula consists of an active substance, oil phase, water phase, surfactant, and co-surfactant. The selection of appropriate surfactant and co-surfactant components can produce a better nanoemulsion system. In this study, tween-80 and PG are selected as surfactant and co-surfactant in nanoemulsions. Tween-80 is a non-ionic surfactant that can form smaller emulsion droplets than other non-ionic surfactants (Sari *et al*., 2015). Meanwhile, propylene glycol (PG) is a co-surfactant that can be combined to support the solubilization of Tween-80 and increase their penetration of active substances in topical applications. In this study, formula optimization was carried out using a combination of Tween-80 and PG surfactants that will produce smaller nanoemulsion droplets and form a more stable oil-in-water emulsion (O/W) system (Rowe, 2017; Putri *et al*., 2021).

This study aimed to determine the optimum formula for *Centella asiatica* (L.) sunscreen nanoemulsion preparation optimized by using the combination of Tween-80 and PG, and to determine the effectiveness of *Centella asiatica* (L.) extract as a sunscreen preparation in vitro. The effectiveness test of *Centella asiatica* (L.) extract was conducted using an ultraviolet-visible (UV-Vis) spectrophotometry, which was expressed as the SPF value. The development of nanotechnology was done by manufacturing nanoemulsion preparations formulated using *Centella asiatica* (L.) extract containing asiaticoside as the active substance, olive oil as the oil phase, distilled water as the water phase, and a combination of surfactants such as Tween-80 and PG. The formula of *Centella asiatica* (L.) nanoemulsion was determined using a factorial design with two factors and two levels. The factors used in this study were Tween-80 and PG. The data for the optimum formula were measured and evaluated using Design Expert Version 13 software. Physical characteristic tests were conducted to determine the optimum nanoemulsion formula through several tests, such as the organoleptic test, droplet size test, zeta potential, transmission electron microscopy (TEM), pH test, percentage transmittance, and emulsion type test.

METHODS

Materials

The materials used in this study are *Centella asiatica* (L.) extract, ethanol p.a(Emsure®), n-hexane PA, ethyl acetate PA, diethyl-amine PA, asiaticoside comparator, phosphate buffer (Merck, Germany), virgin olive oil, Tween-80, PG, and 96% ethanol (Merck, Germany). The equipment used included Glassware (Pyrex), hotplate (Thermo), UV-Vis spectrophotometer (Shimadzu 1800 double beam), cuvette, analytical balance (Mettler Toledo), TLC plate silica gel GF254, oven (Memmert®), micropipette (Socorex®) magnetic stirrer, tweezers, sonicator, chamber, macerator, evaporator, sonicator bath (Elmason), homogenizer, 1.5 mL microtube, water purificator (Thermo), pH meter, particle size analyzer, stirrer, and Design Expert Version 13 software.

Centella asiatica (L.) Extraction

Centella asiatica (L.) extract is derived from dried *Centella asiatica* (L.) collected from the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Central Java, Indonesia. *Centella asiatica* (L.) has been determined at Sanata Dharma University's Faculty of Pharmacy, using the reference number 03/LKTO/Far-USD/05/2024. *Centella asiatica* (L.) was extracted by maceration method using 70% ethanol at a ratio (1:5 w/v). The powder was macerated for 24 h at 150 rpm. Filtration was performed using a Buchner funnel after 24 h of maceration. Then, re-maceration was carried out at 3x for 24 h with 250 mL of total solvent. Evaporation was performed and the extract was stored in a weighed porcelain cup.

Determination of Asiaticoside Compound Profile by Thin Layer Chromatography (TLC) Method

The 2000 ppm asiaticoside standard and 20,000 ppm *Centella asiatica* (L.) extract were prepared with ethanol. The stationary phase used Silica gel 60 F254 plates. A TLC plate was cut into 5 × 10 cm pieces with an elution length of 8 cm. A mixture of n-hexane, ethyl acetate, and diethylamine (80:20:2, v/v) was used as the mobile phase. Standard solutions and extracts were photographed on the TLC plate, then eluted in the mobile phase. After elution, the TLC plate was placed in an oven at 110 °C for 10 min. The stains were viewed using a 365 nm UV lamp (Rowe, 2017).

Determination of SPF-value of Gotu Kola Extract

Gotu kola extract was taken as 1.2 mg, 2.4 mg, and 3.6 mg then diluted with ethanol p.a to 10 mL (120 *xg*, 240 *xg,* and 360 *xg*). A test uptake curve was constructed in a cuvette with a wavelength between 290−320 nm. The absorbance at each concentration was recorded and the SPF value was calculated using the Mansur method (Yang *et al.*, 2018). The SPF value was determined using the Mansur equation as follows:

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times \mathrm{abs}(\lambda)$$

where CF is the Correction Factor, EE is the Erythema Effect, I is Intensity Spectrum of the Sun, and Abs is Absorbance of the sample (Arina, Sarbani and Mohamed, 2023).

Preparation of Nanoemulsion *Centella asiatica* (L.)

Nanoemulsions were prepared using four formulas that had been determined using a factorial design with two factors and two levels (Table 2). The nanoemulsion preparation was made by mixing the active substance in the form of *Centella asiatica* (L.) powder containing asiaticoside, tween-80, PG, and olive oil into a glass beaker. Subsequently, the mixture was stirred in a glass beaker using a magnetic stirrer at 1000 rpm for 10 min. Next, the water phase was added to the mixture by adding distilled water that had been heated at a temperature of 70 °C. Distilled water was added slowly into a glass beaker. The stirring speed was increased to 1250 rpm for 10 min. After that, the mixture was homogenized for 2 min and sonicated for 40 min (Yuliani *et al.*, 2016; Arianto and Cindy, 2019).

Table 1. Formula of *Centella asiatica* (L.) Nanoemulsion

Materials	Use
Centella asiatica (Asiaticoside)	Active substance
Tween-80	Surfactant
Propylene Glycol	Co-surfactant
Olive Oil	Oil Phase
Phosphate Buffer	Water Phase

Table 2. Design Factorial Formulations

	Formula (g)			
Materials	F1	Fa	Fb	Fab
Asiaticoside	0.5	0.5	0.5	0.5
Tween 80	10	12	10	12
Propylene Glycol	3	3	4	4
Olive Oil	0.5	0.5	0.5	0.5
Phosphate Buffer	100	100	100	100

Organoleptical Test

Organoleptic testing of *Centella asiatica* (L.) nanoemulsion preparation was carried out by observing the color, odor, homogeneity, and phase separation of the formed nanoemulsion (Hanifah and Jufri, 2018; Arianto and Cindy, 2019).

Emulsion Type Test

The emulsion-type-test was conducted using the dilution method by dissolving the sample in water (1:100) and oil (1:100). Nanoemulsion is classified as oil-in-water (O/W)-type if the sample is soluble in distilled water. If the nanoemulsion is classified as a water-in-oil (A/M)-type, the sample is completely dissolved in the oil phase (Yuliani *et al*., 2016).

pH Test of Preparations

A pH test was carried out using a pH meter. As much as 1 g nanoemulsion sample was dissolved in 10 mL of distilled water. The pH meter was dipped into the sample and the pH value listed on the display showed the pH results of the nanoemulsion (Yuliani *et al*., 2016; Hanifah and Jufri, 2018).

Droplet Size Test

Droplet-size tests were conducted using the optimum nanoemulsion formula. Droplet measurements with Dynamic Light Scattering (DLS) were performed using a Particle Size Analyzer (PSA). A total of 10 mL of sample was taken and then inserted into the cuvette. The samples were analyzed using a PSA instrument to determine the size distribution of nano particles. The polydispersity index and zeta potential values were obtained in the same manner using a PSA instrument (Masakke *et al*., 2015; Yuliani *et al*., 2016; Jiang, Zhou and Chen, 2022).

Zeta Potential Test

A Particle Size Analyzer instrument was used to measure the zeta potential of nanoemulsion samples. At total of 10 mL sample was dissolved in distilled water with a ratio of 1:1 and analyzed using a PSA instrument.

***Transmission Electron Microscopy* (TEM)**

Transmission Electron Microscopy (TEM JEOL JEM 1400) was used to visualize the shape and morphology of nanoemulsions. One drop of the sample was dripped onto a copper grid, allowed to dry and kept for 24 h at room temperature. The sample was analyzed using a TEM instrument under a 100-200 kV microscope (Hanifah and Jufri, 2018; Kristiani *et al*., 2019).

Data Analysis and Formula Optimization of *Centella asiatica* (L.) Nanoemulsion

The factorial design was used as a statistical method to optimized the nanoemulsion formula. Factorial design was done with two factors and two levels using Design expert Version 13. The optimized formulation parameters included the Tween-80 concentration (X1) and PG concentration (X2). Factorial design optimization was conducted by presenting the results of factors against the responses in the form of particle size, zeta potential, and pH of the nanoemulsion produced. The normal distribution of data was analyzed using Two-Way Analysis of Variance (ANOVA) with 95% confidence level. The p-value<0.05 indicated significant results for the nanoemulsion characteristics. The optimum nanoemulsion formula was determined through data analysis by finding the Contour Plot of each factor of the nanoemulsion using Design of Experiment (DOE). A desirability analysis approach was used to determine the optimal response conditions.

RESULTS AND DISCUSSION

Compound Profile of Asiaticoside in *Centella asiatica* (L.) Extracts

Analysis of asiaticoside compound profile in *Centella asiatica* (L.) extract was performed using thin-layer chromatography (TLC). Tests were carried out using asiaticoside 2000 xg and *Centella asiatica* (L.) extract 20,000 xg in 70% ethanol p.a. The mobile phase was n-hexane: ethyl acetate: diethylamine (80:20:2), and the stationary phase was silica gel 60 F254, with an elution distance of 8. Theoretically, according to the Indonesian Herbal Pharmacopoeia, asiaticoside has an Rf-value of approximately 0.33.

Qualitative test results with TLC showed at Figure 1 that the asiaticoside compound was on spot 1, with an Rf-value of 0.344. This is also in line with research conducted by Wagner *et al*. (1996), which showed that the Rf-value of asiaticoside ranged from 0.35. Meanwhile, spot (2) has a Rf value of 0.21, and is assumed to be madecassoside, whereas spot (3), with a Rf of 0.18 is assumed to be asiatic acid.

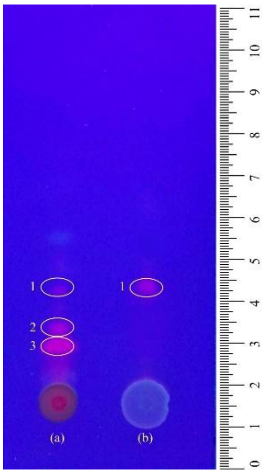


Figure 1. Thin Layer Chromatography (TLC) Test Results. (a) Sample; (b) Asiaticoside standard; (1) Spot Asiaticoside; (2) Spot Madecassoside; (3) Spot Asiatic Acid

Table 3. *In-vitro* determination of SPF-value

No	Wavelength (nm)	EE (λ) × I	*Centella asiatica* 120 *xg* (absorbance)	*Centella asiatica 240 xg* (absorbance)	*Centella asiatica* 360 *xg* (absorbance)
1	290	0.015	0.772±0.001	1.650±0.016	2.395±0.022
2	295	0.0817	0.817±0.007	1.784±0.041	2.588±0.009
3	300	0.2874	0.850±0.009	1.877±0.027	2.683±0.021
4	305	0.3278	0.888±0.015	1.923±0.020	3.278±0.525
5	310	0.1864	0.937±0.013	1.948±0.014	3.011±0.300
6	315	0.0837	1.102±0.006	2.129±0.032	2.944±0.022
7	320	0.0180	1.067±0.015	2.220±0.016	3.213±0.315
Protection Type SPF Value			8.999 Low	19.219 Medium	29.569 High

In Vitro SPF Test of *Centella asiatica* (L.) Extracts

SPF is a value that indicates the level of photoprotective effectiveness of sunscreen preparations in protecting the skin against of UVA rays (PA). The SPF indicates the amount of UV energy needed to cause a minimal erythema dose (MED) on the skin protected by sunscreen products (Arina, Sarbani and Mohamed, 2023). The SPF-value consisted of three categories, namely the range of 6-10 (low category), 15-25 (medium category), 30-50 (high category), and 50+ (very high category) (Ngoc *et al*., 2019). The higher the SPF-value, the product more effective in protecting the skin from the adverse effects of UV rays. In this study, the SPF-value was determined using UV-Vis spectrophotometry in-vitro (Yang *et al*., 2018).

The SPF-value of the extract was tested at three different concentrations, namely 120 ppm, 240 ppm and 360 ppm in 70% ethanol p.a. UV-Vis spectrophotometry was performed using the Mansur equation. The SPF test results (Table 3) showed that the higher the concentration of Gotu kola-extract solution, the higher of SPF-value produced. The presence of chromophore group in *Centella asiatica* (L.) extract supports its ability and effectiveness as an antiphotoaging agent in providing effective protection against UV rays. These results indicate that based on the results of in-vitro tests, *Centella asiatica* (L.) extract is proven to have activity as a UV protector.

Preparation of *Centella asiatica* (L.) Nanoemulsion

In this study, the maceration extraction method for Gotu kola aims to separate the active compounds of the plant and maintain the content of compounds that are not heat-resistant. Gotu kola maceration can be used to extract several compounds, such as triterpenoids, flavonoids, saponins, alkaloids, phenolics, tannins, and carotenoids, depending on the type of solvent and the extraction time (Chowdhury *et al.*, 2016). Research conducted by Monton *et al.* (2019) showed that the results of maceration extraction of Gotu kola herb for madecassoside, asiaticoside, madecasid acid, and asiatic acid were 0.855 %, 0.174 %, 0.053 %, and 0.025 % respectively at 60 °C for 120 min. In this study, the yield of Gotu kola herb extract reached 30.6 %, which met the requirement of not less than 7.3 %.

Nanoemulsion preparations were chosen to increase the low solubility of asiaticoside and its absorption for topical use (Arianto and Cindy, 2019). The selection of oil component in nanoemulsion formulations is an important factor in the ability of the oil phase to dissolve active substances into the oil phase dispersed in the aqueous phase. The oil phase used is olive oil which acts as an emollient and is beneficial for maintaining moisture, softness, and antiaging of the skin (Hakim, Arianto and Bangun, 2018; Chrismaurin *et al.*, 2023). The use of surfactants and co-surfactants in the formulation supports the formation of nanoemulsion systems. Nanoemulsions were formulated with the combined use of Tween-80 surfactant and Propylene glycol cosurfactant. Tween 80 is hydrophilic and can be used to produce O/W type nanoemulsions (Sari *et al.*, 2015). Tween-80 is non-ionic surfactants used to reduce interfacial tension and minimize skin irritation (Faria-Silva *et al.*, 2020). PG is a co-surfactant that can facilitate the solubilization of Tween-80 surfactant in forming a more stable oil-in-water nanoemulsion system and increase their penetration of active substances in topical applications. The combination of Tween 80 and PG maintains the balance between hydrophobic and hydrophilic groups, which is expected to produce smaller nanoemulsion droplets and form a more stable oil-in-water (O/W) emulsion system (Rowe, 2017; Putri *et al.*, 2021).

Organoleptic Test Evaluation of Nanoemulsion

The resulting of *Centella asiatica* (L.) nanoemulsion organoleptic test showed a transparent light yellow color consistency, a distinctive aroma like olive oil, homogeneous, and no phase separation (Figure 2).

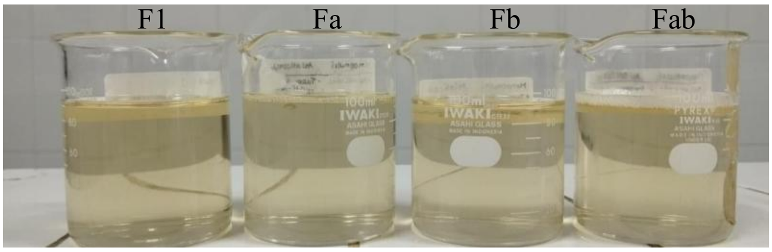


Figure 2. Preparation of *Centella asiatica* (L.) nanoemulsion

Table 4. Results of Physical Characteristics of *Centella asiatica* (L.) Nanoemulsion

Formula	pH	Particle size (nm)	Zeta Potential	Polydispersity Index	Transmittance (%)
1	6.34±0.028	13.36 ± 0.252	−4.16 ± 0.208	0.359 ± 0.047	95.4 ± 0.1
A	6.36±0.021	11.7 ± 0.264	−3.1 ± 0.1	0.301 ± 0.038	96.16 ± 0.23
B	6.36±0.02	13.2 ± 0.519	−2.23 ± 0.153	0.441 ± 0.046	93.73 ± 0.38
AB	6.38±0.112	11.8 ± 0.624	−3.13 ± 0.473	0.127 ± 0.009	96.9 ± 0.3

Table 5. Statistical Analysis of Physical Properties

Source	Particle Size		Zeta Potential		pH	
	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value
Model	0.0025	11.95	0.0002	25.02	2.2257	1.80
A-Tween 80	0.0003	35.56	0.6125	0.2778	0.1148	3.13
B-PG	0.9001	0.0168	0.0003	36.10	0.1728	2.24
AB	0.6181	0.2689	0.0003	38.68	0.0185	0.0185

Emulsion Type Test

The nanoemulsion-type test aimed to determine the type of nanoemulsion formed. *Centella asiatica* (L.) nanoemulsion categorized as an oil-in-water (O/W) nanoemulsion type because the nanoemulsion sample can dissolve in the water phase used. These results indicate that the nanoemulsion system was oil-dispersed in the aqueous phase (Kumar, Soni and Prajapati, 2017). The O/W type nanoemulsions are preferred because they accelerate the penetration of lipophilic drugs into the deeper layers of the skin (Hanifah and Jufri, 2018).

Transmittance Percentage Value Measurement

The percentage transmittance value is used to measure the clarity of a particle. The percentage transmittance value meets the requirements and reaches the nanometer scale when the absorbance value obtained is close to 100% (Huda and Wahyuningsih, 2018). Based on the results of the measurement carried out, the nanoemulsion preparations in the four formulas produced a transmittance percentage value that was in the range of 90-100%. This indicates that the resulting preparation has reached the nanometer scale. Of the four nanoemulsion formulas, the percentage transmittance value produced was within the required range, and the percentage transmittance from the highest to the lowest value was in the order AB, A, 1, and B (Table 4). However, the measurement of the percentage transmittance value that is close to 100% does not guarantee that the resulting preparation will form a smaller particle size, so it is necessary to test the particle size to strengthen the test results carried out.

***Particle Size* Test**

Particle size measurement or particle size analysis (PSA) was performed using dynamic light scattering (DLS). In this study, the expected particle size resulted in nanoemulsion preparations in the range 10-200 nm (Redhita *et al*., 2022). Based on the particle measurement results of the four nanoemulsion formulas, it was found that the particle sizes from smallest to largest were respectively: 11.7 ± 0.264 nm in formula A, 11.8 ± 0.624 nm in formula AB, 13.2 ± 0.519 *nm* in formula B, and 13.36 ±0.252 nm in formula 1 (Table 4). These results indicate that nanoemulsion preparations are included in the nanoparticle preparation category because they have particle sizes in the range of 10-200 nm.

The measurement results for *Centella asiatica* (L.) nanoemulsion particles were also reinforced by the measurement results of the polydispersity index value to measure the particle size distribution in the system (Table 4). If the polydispersity index value is close to zero, the particle distribution indicates a monodisperse system. A polydispersity index value in the range of 0.01-0.7 indicates a good monodisperse system with a narrow range of distribution and a uniform formula. Conversely, if the polydispersity index value is close to one, it indicates a polydisperse system with a wide distribution system (Dewandari, Sofwan and T, 2019; Aini, Wijayatri and Pribadi, 2022). From the polydispersity index measurement results, all nanoemulsion formulas have a polydispersity index value <0.7 with the smallest value in formula AB of 0.127±0.009 and the largest value in formula B amounting to 0.441±0.046. This indicated that the four nanoemulsion formulations were monodisperse systems.

Zeta Potential

Zeta potential is used to measure the electrokinetic potential of a particle and test the stability of a nanoemulsion system. Nanoemulsions indicated to have a good stability if the zeta potential value in the range of +30 mV to - 30 mV (Munirah, Faudzi and Mohamad, 2020; Smith *et al*., 2017). The results of the zeta potential measurements showed that the four nanoemulsion formulas were in the range of −2.23 ± 0.153 to −4.16±0.208 (Table 4). These results indicate that the four nanoemulsion formulas meet the requirements of good zeta potential, with the highest potential zeta value in Formula B and the lowest potential zeta value in formula 1.

pH Test of Preparation

Preparation pH testing is conducted to determine the acidity level of a preparation. Nanoemulsions for topical preparation have a pH-value requirements in the range of 4.5-6.5. The pH range accordance with the skin pH to prevent skin irritation (Arianto and Cindy, 2019; Redhita *et al*., 2022). The use of phosphate buffer as the aqueous phase contributed to the pH stability of the produced nanoemulsion.

Data Analysis

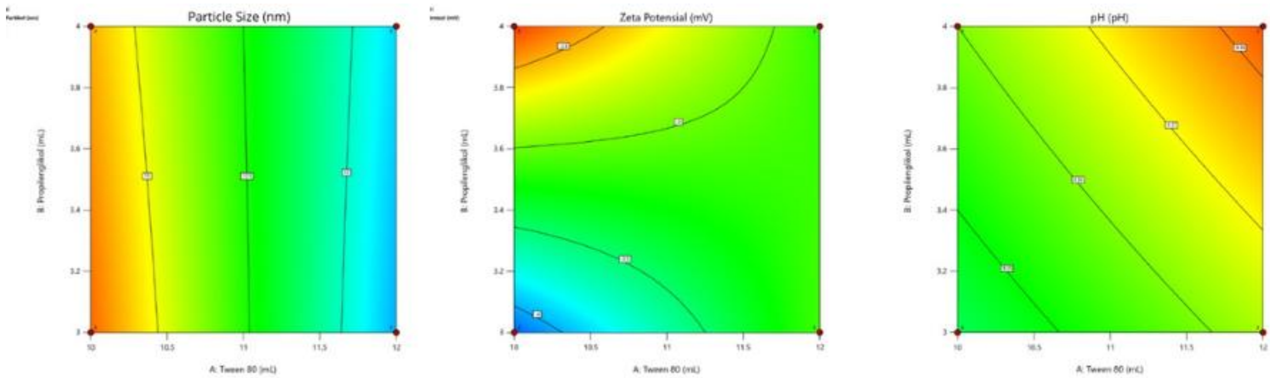
Data analysis was conducted using Design Expert Version 13 to verify the impact of Tween-80 and PG on several factors as particle size, zeta potential, and pH measurement. P-value < 5% or p-value < 0.05 indicates a significant response (Hendrawan *et al*., 2016) . The particle size response shows significant because the p-value was<0.05. The F-value of 11.95 indicates that Tween-80 and PG have a significant effect on the particle size response of the nanoemulsion. The addition of Tween-80 to the nanoemulsion formula has the greatest effect of 81.1039 % on the particle size response of the nanoemulsion produced. The factors of tween-80 and PG affected the response of the nanoemulsion particle size, which is in the range of 12-13 nm.

Zeta responses also showed significant result because the p-value was<0.05. This is supported by the F-value obtained at 25.02 which indicates that the model used was significant. Similar to the particle size response, the results of this analysis showed that the Tween-80 and PG factors were able to have a significant effect on the nanoemulsion potential zeta response with the contribution in the interaction which is 45.567% on the potential zeta response of the nanoemulsion produced. This shows that the combination of the surfactant Tween-80 and the co-surfactant PG can increase the zeta potential response of nanoemulsion and stability of the nanoemulsion produced. The Figure 3b shows the optimum area of Tween-80 and PG with zeta potential response. This area meets the desired of zeta potential criteria in the range of -30 mV to +30 mV. In this study, zeta potential results in the range -4 mV to -2.5 mV that indicated the stability of the produced nanoemulsions.

Meanwhile, the results of the statistical analysis of the pH response showed that the resulting model was not significant because the p-value was >0.05. The smallest F-value produced was 1.8, which can affect the results of the factor analysis on the pH response which is not significant. This result indicates that the addition of Tween-80 and PG did not significantly affect the pH response of the nanoemulsion. Their contributions to the formation of the nanoemulsion system were almost the same. However, the results of the nanoemulsion pH test are in the required pH range of 4.5-6.5.

Particle Morphology

Morphological observations using TEM (Figure 4) were used to determine the size and morphology of the droplet surface through bright-field enhancement combined with a diffraction model to produce a higher-resolution nanoemulsion structure (Faria-Silva *et al.*, 2020). Other results of the nanoemulsion morphology with TEM showed that the shape or morphology of the nanoemulsion particles containing the active substance asiaticoside was a monodisperse system with particle sizes in the range of 11.7-13.6 nm. The particles tended to be spherical with a smooth surface and no aggregation or separation was observed in the produced nanoemulsion system. The spherical morphology of the nanoemulsion shows that the inner part is the oil phase in the form of olive oil, whereas the thin line on the outside shows that surfactants in the form of Tween-80 and PG appear to envelop the nanoemulsion droplets. This is in line with the results of the nanoemulsion emulsion-type test, which showed that this nanoemulsion was of the oil-in-water (O/W) type (Munirah, Faudzi and Mohamad, 2020; Hanifah and Jufri, 2018; Kristiani *et al.*, 2019).



(a) (b) (c)

Figure 3. *Contour Plot* of Tween-80 and PG on Particle Size (a), *Contour Plot* of Tween-80 and PG on Zeta Potential (b), *Contour Plot* of Tween-80 and PG on pH

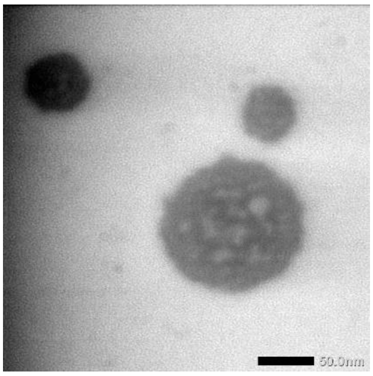


Figure 4. TEM Morphology of Nanoemulsion

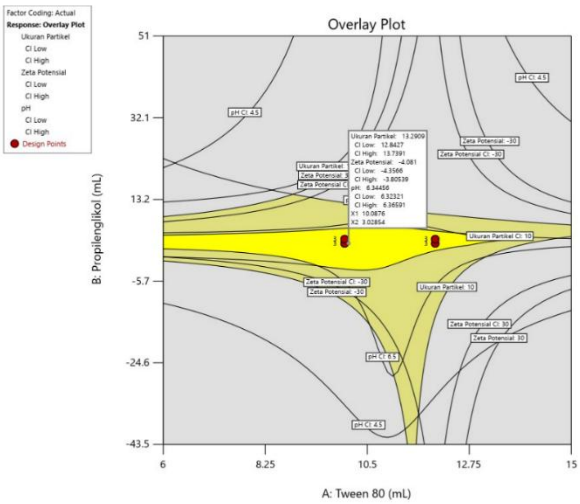


Figure 5. Optimization Plot of Particle Size, Zeta Potential, and pH Respons

Optimization of *Centella asiatica* (L.) Nanoemulsion Formula

Optimization of the nanoemulsion formula was performed using Design Expert Version 13. Formula optimization was carried out on the response of particle size, zeta potential, and pH (Figure 5). Based on the results of the analysis, the optimum nanoemulsion formula using a combination of Tween-80 and PG surfactants was found in a low-level formula, with a desirability value of 1. The desirability value is said to be good if the value in the range of 0-1. A value of zero indicates that one or more results are outside the undesirable limits, whereas a value of one indicates an ideal result (Mang *et al*., 2015; Riswanto *et al*., 2019). The desirability value at the optimum formula shows the expected results. This optimization result showed that the addition of Tween-80 and PG has significantly impacted the physical properties of sunscreen nanoemulsion (particle size, zeta potential, and pH).

CONCLUSIONS

Centella asiatica (L.) extract was proven to have activity as a UV protector as shown through the results of the in vitro Sun Protection Factor (SPF). The effectiveness of *Centella asiatica* (L.) extract was demonstrated in a sunscreen preparation formula using a combination of Tween-80 and PG surfactants. The *Centella asiatica* (L.) nanoemulsion preparation formula is an oil-in-water-type nanoemulsion that has good physical properties, such as the color of the preparation in the form of light yellow, consistency in the form of a solution and transparency, particle size that shows nanosized particles, and the zeta potential and pH values produced are in the required range. Based on the analysis of Design of Experiment (DOE) data, the optimization result that was the optimum formula was obtained with a concentration of Tween-80 and PG concentration at low level. From this study, the combination of Tween-80 and PG has significantly impacted responses on particle size, zeta potential, and pH of the *Centella asiatica* (L.) nanoemulsion.

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CONFLICT OF INTEREST

The authors declared that no conflict of interest should arise on this research article.

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