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Research Article

Formulation of Nanoemulsion of *Centella asiatica* Leaves Extract as Active Ingredients to Produce Antioxidant Facial Serum

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Abstract: Free radicals can cause cell damage such as premature aging. To protect, ward off, and stabilize free radicals, antioxidant compounds can be used. Plants that can be used as a source of antioxidants are *Centella asiatica* (L.) Urban. This research aims to formulate a nanoemulsion of *Centella asiatica* leaf extract as an active ingredient of antioxidant serum. The steps of this research include (1) extraction by maceration and Microwave-Assisted Extraction (MAE); (2) extract characterization; (3) formulation of nanoemulsion using Self-Nanoemulsifying Drug Delivery System (SNEDDS) method; (4) nanoemulsion testing: stability test, antioxidant activity test, and irritation test, (5) nanoemulsion characterization which includes particle size, transmittance, pH, and viscosity. The results showed that: (1) yields of the maceration method and MAE were 14.60% and 17.54%; (2) antioxidant compounds in *Centella. asiatica* leaf extract are squalene, kaempferol, asiaticoside; The IC_{50} of the maceration and MAE extract were 93.152 ppm and 80.365 ppm; (3) nanoemulsions were made in 3 formulas (0.1; 0.3; and 0.5 g) with fixed variables of capryol 90, tween 20, and PEG 400 (1.5; 2.5; and 1); (4) stability test showed that only F1 was stable; the IC_{50} value of nanoemulsion is 2604.967 ppm; and the F1 irritation test showed no erythema and edema; (5) The particle size of F1 is 166.7 nm with a transmittance value of 97.4%, a pH of 5.33, and a viscosity of 75.35 cP.

Keywords: *Centella asiatica* leaves, Nanoemulsion, Antioxidant, Serum, SNEDDS

Introduction

The skin is the first part of the body that is badly affected by pollution and exposure to ultraviolet rays such as UV-A and UV-B can damage the skin by increasing the number of free radicals in the body [1]. An unhealthy lifestyle can also increase the number of free radicals in the body including the skin. About 80% of aging on the face is caused by free radicals from the effects of sun exposure. One of the right steps to counteract free radicals is by using cosmetics that contain antioxidant agents. Antioxidants are substances that can counteract the effects of free radicals [2]. Antioxidants can inactivate the development of oxidant reactions by binding to free radicals and reactive molecules so that they can inhibit cell damage. Another function of antioxidants is to neutralize free radicals and suppress the aging process [3].

Antioxidant products that can be employed to address this issue include antioxidant serums. Sources of antioxidants utilized in serums can be derived from natural or synthetic origins. However, the application of synthetic antioxidants is restricted due to potential side effects [4]. Currently, the utilization of natural ingredients has been explored as a reservoir of antioxidants in cosmetic formulations. One of the indigenous plants in Indonesia that can serve as a source of antioxidants is *Centella asiatica* [2].

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Centella asiatica is a wild plant that is well-known among Indonesians and is consumed as a traditional medicine [5]. *Centella asiatica* has compounds with antioxidant effects, namely phenols and triterpenes [3]. In addition, *Centella asiatica* also contains saponins, asiatic acid, and medexic. Asiaticoxide with asiatic acid and madexic acid is a strong antioxidant and can regenerate tissue levels by synthesizing collagen to remove dark spots and reduce wrinkles on facial skin. *Centella asiatica* can be used as an ingredient for facial skin care such as dull, wrinkled skin or skin that shows signs of aging [6]. *Centella asiatica* ethanol extract showed an antioxidant capacity of 43.198 ± 2.048 mg QE/g extract. In addition, *Centella asiatica* showed a very strong antioxidant potential with the IC_{50} value of the ethanol extract of 35.6±1.3 g/mL.

The goal of this study was to develop a stable nanoemulsion formula with potent antioxidant activity using *Centella asiatica* leaf extract. The hypothesis driving this research is that nanoemulsion formulations of *Centella asiatica* leaf extract exhibit greater antioxidant activity compared to non-nanoemulsified extracts. To enhance the quality and efficacy of the active components in this investigation, the *Centella asiatica* leaf extract will be transformed into a nanoemulsion. The integration of nanotechnology into cosmetics or skincare products enhances their effectiveness [7]. Nano-sized active ingredients possess the capability to penetrate deeper skin layers and interact more dynamically and efficiently as antioxidants.

The research procedure involves the following steps: (1) extraction through maceration and Microwave-Assisted Extraction (MAE); (2) characterization of the crude Centella asiatica leaf extract; (3) formulation of the nanoemulsion using the Self-Nanoemulsifying Drug Delivery System (SNEDDS) approach [8]. Following BPOM regulations, nano preparations can serve as active cosmetic constituents if their size exceeds 100 nanometers. In this research, the nanoemulsion formula aligns with BPOM regulations; (4) nanoemulsion assessment: stability testing, antioxidant activity evaluation, and irritation testing; (5) characterization of the nanoemulsion encompassing particle size, transmittance, pH, and viscosity.

Materials and Methods

Materials

The instruments used in this research are Analytical Balance (Pioneer Ohaus PA 224), Microwave (Electrolux), Oven, Rotary Evaporator (Heidolph), UV-Vis double beam spectrophotometer (UH5300), Liquid Chromatography Mass Spectrometry (LC-MS/MS) (QTOF G2XS),, Particle Size Analyzer (PSA) (Horiba Scientific, Nanoparticle Analyzer SZ-100), pH meter, Viscometer (Brookfield), Sonicator (Model 3000 V/T Ultrasonic Homogenizer), and other research glassware according to work procedures.

The materials used in this research *Centella asiatica* leaves, distilled water, 70% technical ethanol, ethanol p.a, methanol, 2,2-diphenyl-1-picrihidazil (DPPH), filter paper, $CHCl_3$, $FeCl_3$, HCl, Magnesium powder, NaOH, CH_3COONa, $AlCl_3$, H_3BO_3, Wagner reagent, Capryol 90, Tween 20, Polyethylene Glycol (PEG) 400, and other chemicals that are by work procedures.

Sample Preparation

In this study, the collection and sampling of *Centella asiatica (L.) Urban* was carried out in the Banaran, Argomulyo, Cangkringan, Sleman Regency, Yogyakarta, Indonesia. Then, the *Centella asiatica* (L.) Urban leaves were washed with running water until clean, then dried in an oven at 50°C, then the dried leaves were ground into a fine powder with a blender [9].

Extraction of *Centella asiatica* Leaves Using Maceration

Centella asiatica leaves that had been washed and dried in the oven, were mashed with a blender so that they became powder. Then, 30 grams of *Centella asiatica* leaves powder was weighed and then soaked in 180 mL of 70% ethanol tightly closed for 48 hours (2×24 hours). Next, the extracted material is filtered through filter paper. Next, the extract was filtered through filter paper. Then, the filtrate was concentrated using a rotary evaporator at a temperature of 85°C. The yield of the extract was calculated [10].

Extraction of *Centella asiatica* Leaves Using Microwave Assisted Extraction (MAE)

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Centella asiatica leaves that have been washed and dried in an oven at a temperature of 50°C, and mashed with a blender so that they become powder. Then, 30 grams of *Centella asiatica* leaves powder was weighed and then soaked in 180 mL of 70% ethanol and tightly closed. The sample was then put in the microwave for 5 minutes at 100 watts of power stirring every 1 minute. Next, the extract was filtered with filter paper. Then, the filtrate was concentrated using a rotary evaporator at a temperature of 85°C. The yield of the extract was calculated [10].

Physical characterization of *Centella asiatica* leaves extract

The physical and chemical properties of Centella asiatica leaf extract were analyzed through assessments of color, scent, yield, and compound composition utilizing LC-MS/MS, along with conducting a DPPH test to ascertain the antioxidant potential of the extract.

Phytochemical Screening Test

Phytochemical screening tests on *Centella asiatica* leaf extract included tests for tannins, flavonoids, alkaloids, saponins, sterols, and triterpenoids. Identification of sterols and triterpenoids using the Salkowski test, Flavonoid test using Mg powder reagent and a few drops of concentrated HCl, Alkaloids test using 2% HCl and a few drops of Wagner's reagent, saponin test using distilled water.

Identification of Flavonoid Compounds with UV-Vis Spectrophotometer and Shift Reagent

A total of 25 mg of extract was dissolved in 10 mL of methanol in a beaker and stirred until homogeneous, then transferred to a tube and centrifuged for ± 10 minutes. After the filtrate was separated from the precipitate, each filtrate was then transferred into 5 test tubes. In test tube I, 3 mL of pure filtrate was pipetted. In test tube II, 3 mL of the filtrate was pipetted and 3 drops of NaOH solution were added and then shaken until homogeneous. In test tube III, 3 mL of the filtrate was pipetted and 6 drops of 1% $AlCl_3$ solution was added and then shaken until homogeneous. In test tube IV, 3 mL of the filtrate was pipetted, and 250 mg of sodium acetate (CH_3COONa) was added and then shaken until homogeneous. Test tube V pipetted 3 mL of filtrate and added 250 mg of Sodium Acetate (CH_3COONa), stirred until homogeneous, and added 150 mg of Boric Acid (H_3BO_3), the mixture was shaken again until homogeneous. Then put each sample into a cuvette for analysis using a UV-Vis Spectrophotometer and observe the spectrum at a wave number of 200-800 nm [11].

Analysis LC-MS/MS of *Centella asiatica* Leaves Extract

The *Centella asiatica* leaves extract was characterized to determine the content of secondary metabolites contained in the *Centella asiatica* leaves extract using LC-MS/MS. This is done by making a 1000 ppm *Centella asiatica* leaves extract solution [12]. The optimization of the LC-MS/MS instrument is in Table 1.

Table 1. LC-MS/MS Operating Conditions

LC-MS/MS Operating Conditions	**Information**
Coloumn and mobile phase	Agilent C18 with dimensions of 4.0 mm (id) x 250 mm (length) and a particle size of 1.8 m
Mobile phase A	Water containing 0.1% acetic acid (solvent A)
Mobile phase B	Acetonitrile containing 0.1% acetic acid (solvent B)
Temperature	50˚ C
Flow Rate	1,00 mL/min
Injection volume	20 µL
Run Time	30 min

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The Formulation of *Centella asiatica* Leaves Extract Nanoemulsion

Before the formulation of *Centella asiatica* leaves extract was tested by the DPPH method, the best antioxidant was selected using a UV-Vis spectrophotometer, and the IC_{50} value was calculated from the absorbance value obtained. The manufacture of a nanoemulsion formula for *Centella asiatica* leaves extract uses the main ingredients, namely *Centella asiatica* leaves extract, capryol 90 as the oil phase, tween 20 as a surfactant, and PEG 400 as a co-surfactant [13].

The compositions were prepared based on the specifications in Table 2, and subsequently, the SNEDDS technique was employed. The extract from *Centella asiatica* leaves was combined with Tween 20 as a surfactant and then subjected to sonication for two rounds of four minutes each. Following this, PEG 400 was introduced as a co-surfactant, and the mixture was sonicated for two rounds of four minutes again. Afterward, capryol 90 was incorporated as the oil component, followed by another round of sonication for two sets of four minutes. The ensuing nanoemulsion formulation was then progressed to the subsequent phase to identify the most effective preparation [14].

Table 2. Nanoemulsion Formula Ratio

Formula	Extract	:	Tween 20	:	PEG 400	:	Capryol 90
F1	0.2		2.5		1		1.5
F2	0.6		2.5		1		1.5
F3	1		2.5		1		1.5

Stability Test

The nanoemulsion was continuously kept warm at a temperature of 37°C and subjected to homogenization using a vortex machine for 30 seconds. It was monitored every hour over 4 hours to assess its stability. The criteria for deeming the nanoemulsion stable involved the absence of layer formation, clumping, or sedimentation.

pH Test

The pH meter was calibrated in buffer then the electrode was rinsed with distilled water, then dried with a soft tissue. The electrode was dipped into the nanoemulsion preparation until the pH meter showed a stable reading.

Transmittance Test

A total of 1 mL of nanoemulsion was dissolved in a 100 mL volumetric flask using distilled water. The solution was measured percent transmittance at a wavelength of 650 nm using a UV-Vis spectrophotometer with distilled water as a blank [15].

Viscosity Test

Measurement of the viscosity of the preparation using a viscometer [16]. The viscometer used is the BROOKFIELD DV2T brand which operates at a certain shear rate. The preparation is poured sufficiently on the sample cup and its viscosity is measured.

Antioxidant Test with DPPH

DPPH was dissolved according to the sample solvent (ethanol) and the absorbance was measured at a wavelength of 515-520 nm. The DPPH solution was dripped with a sample solution and then the

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absorbance was measured again. After obtaining the absorbance value, the percent inhibition (%) of the sample against DPPH was calculated. Antioxidant activity is calculated as the percentage of inhibition of DPPH (percentage of scavenging effect). Antioxidant activity was measured by the IC_{50} value which is the sample concentration required to provide 50% inhibition.

Particle Size Characterization

Nanoemulsion was characterized by particle size and polydispersity index by dissolving in distilled water, then the clear solution was determined by particle size and polydispersity index using PSA [17].

Irritation test

The test animals used were male New Zealand rabbits with an experimental time of 72 hours. Rabbits that have their backs shaved are applied with nanoemulsion formula, then covered with gauze. Erythema and edema were observed at 24, 48, and 72 hours after exposure [18].

Results and Discussions

Physical characterization of *Centella asiatica* leaves extract

The *Centella asiatica* leaves extract obtained is a dark green viscous extract with a distinctive leaf aroma. The yield of the extraction results can be seen in Table 3. From the results obtained from the maceration extraction technique and MAE in Table 3, it can be concluded that the MAE extraction technique produces higher yields, so it is recommended that further tests be carried out using MAE extract. The compounds contained in *Centella asiatica* leaf extract have high thermal stability, so they are not degraded when extracted using the MAE extraction technique. Other research shows that the content of Phenolic, Flavonoids, Triterpenoids, and Ascorbic acid compounds in the MAE extract is higher compared to the results of the Maceration extract [19].

Antioxidant Activity of *Centella asiatica* Leaves Extract

The results of the antioxidant activity test are expressed by the IC_{50} value, which is the concentration of an inhibitor that reduces the response of binding by half. The smaller the IC_{50} value, the stronger the antioxidant activity. Specifically, a compound is said to have very strong antioxidant activity if the IC_{50} value is less than 50 ppm; strong for IC_{50} is 50-100 ppm; moderate if IC_{50} is 100-150 ppm; and weak if IC_{50} is 151-200 ppm [20]. Based on Table 3, the results of *Centella asiatica* leaf extract samples from maceration and MAE methods have strong antioxidants because they have IC_{50} values of 93.152 ppm and 80.365 ppm, respectively. According to the data in Table 3, it is evident that utilizing MAE for extraction results in a greater yield and a more potent IC_{50} value compared to the maceration technique. Therefore, the MAE extraction technique is recommended as the most efficient approach for extracting beneficial antioxidant compounds from *Centella asiatica (*L) Urban.

Table 3. Yield and IC_{50} of *Centella asiatica* Leaves Extract

Extraction method	Yield	IC_{50}
Maceration	14.60±0.74	93.152±5.92
MAE	17.54±0.54	80,365±4.70

Phytochemical Screening Result

This phytochemical test is a preliminary qualitative test regarding the content of secondary metabolite compounds before quantitative antioxidant activity testing. The phytochemical results showed that *Centella*

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asiatica leaf extract contained alkaloids, saponins, flavonoids, polyphenols, and triterpenoids. The results of other studies also show that *Centella asiatica* leaf extract contains these compounds [21].

Identification of Flavonoid with Shift Reagent

Based on Table 5 the spectra of extract + methanol solution have two maximum absorptions at 322 nm (band I) and 248 nm (band II). Compounds that have maximum absorption in the wavelength range of 310-330 nm (band I) and 245-275 nm (band II) are included in the Isoflavone group [12].

The addition of $AlCl_3$ reagent experienced a shift of band I by 40 nm, and band II by 8 nm, indicating the presence of a 5-OH group. The addition of CH_3COONa and H_3BO_4 reagents shifted the I band to 42 nm and band II to 4 nm, indicating the presence of an o-group in the OH ring A (6, 7 or 7, 8). Based on the results of the shifting wavelength with the shift reagent, the possible compounds contained in the *Centella asiatica* leaves extract are 5,6,7-trihydroxy isoflavones.

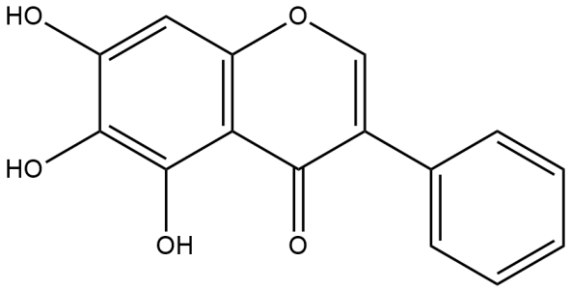

Figure 1. 5,6,7-trihydroxy isoflavones

Phytochemical Contents of *Centella asiatica* Leaves Extract Using LC-MS/MS

Analysis of secondary metabolites of *Centella asiatica* leaves extract using LCMS/MS. Characterization using LCMS/MS is one of the modern chromatographic analyses that can be used in quantitative and qualitative tests to determine the profile of a metabolite [22]. The results of the analysis using LCMS/MS obtained several chromatogram peaks with retention times shown in Figure 2.

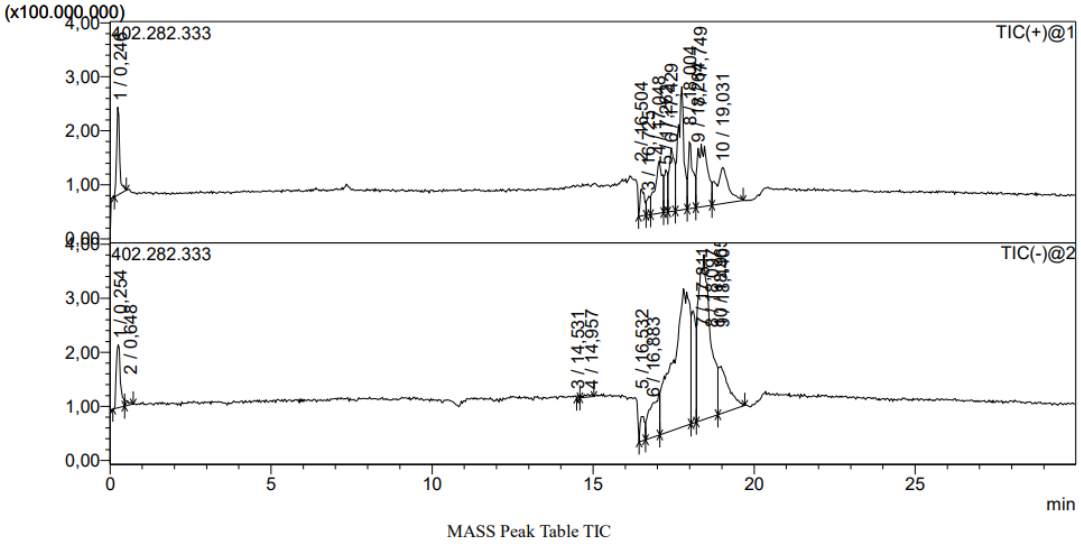

Figure 2. LC-MS chromatogram of ethanol extract of *Centella asiatica* leaves

Table 4. Antioxidant compounds of ethanol extract of *Centella asiatica* leaves

Compound	Formula	m/z	Area
Squalene	$C_{30}H_{50}$	409.35	1335361522

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Kaempferol 3- [6”-(3- hydroxy-3-methylglutaryl) glucoside]- 7-glucoside	$C_{33}H_{38}O_{20}$	753.65	1509283683
Asiaticoside	$C_{48}H_{78}O_{19}$	960.7	2478796209

Three compounds (Table 6) were confirmed to have antioxidant activity based on references and previous studies. The first compound that has antioxidant activity is squalene. Squalene is a compound belonging to the triterpenoid group and an intermediate compound in the biosynthesis of sterol compounds both in plants and in animals. Squalene compounds are natural antioxidants that have anti-radical and antioxidant properties depending on how they are stored and treated during the production process [23]. Squalene dissolves well in ethanol solvents, although it is nonpolar, it is based on squalene MSDS. Squalene functions as an antioxidant in the skin that is experiencing oxidative stress due to exposure to ultraviolet light. Squalene can also reduce the toxic effects of drugs consumed and has anti-tumor activity [23]. Squalene functions as an antioxidant, and can maintain moisture and skin softness. Squalene has great opportunities in the cosmetic industry, squalene can be used as cosmetic preparations in the form of emulsion as a technology for squalene preparations [24]. The structure of squalene and the reaction of squalene with free radicals can be seen in Figure 4

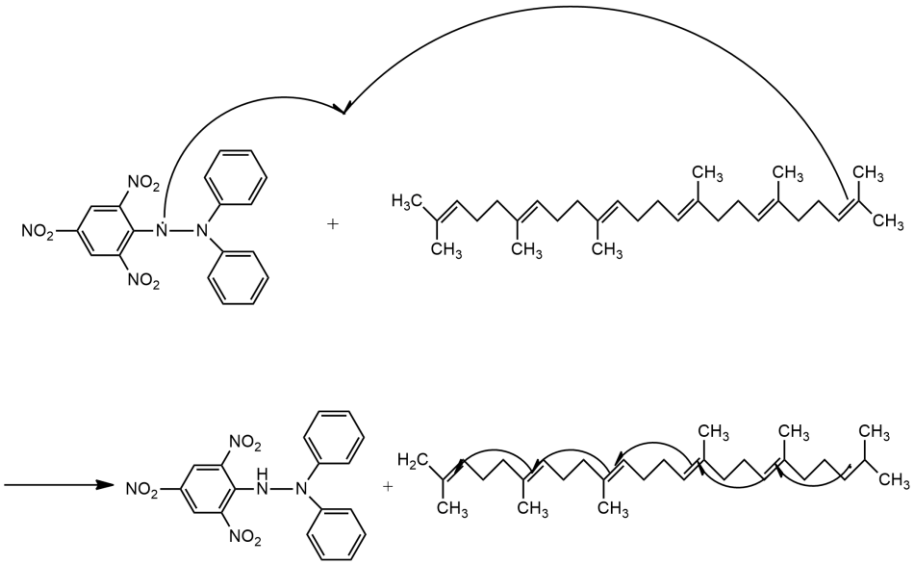

Figure 4. Reaction Squalene and DPPH

The second compound that has antioxidant activity is kaempferol, a flavonoid bioactive compound. Flavonoid compounds have antioxidant effects caused by free radical scavenging through hydrogen proton donors from flavonoid hydroxyl groups [25]. Kaempferol is a flavonoid aglycone that is mostly found in the form of glycosides. Kaempferol 3-[6”-(3-hydroxy-3-methylglutaryl) glucoside]-7-glucoside is a derivative of the glycosylated kaempferol compound [17]. The structure of kaempferol and the reaction of kaempferol with free radicals can be seen in Figure 6.

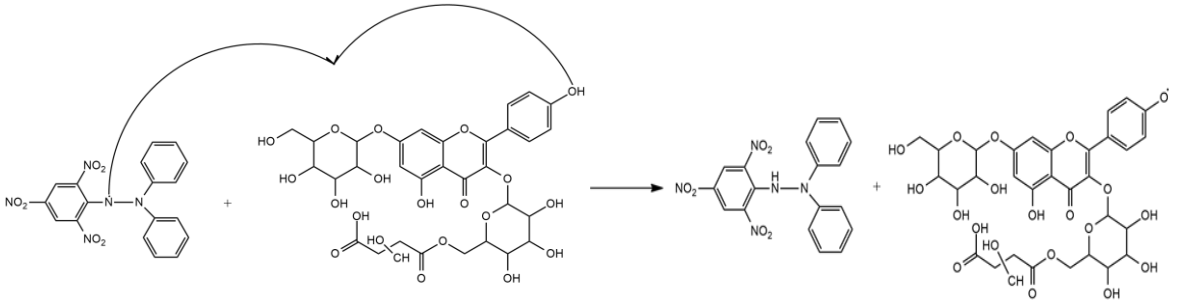

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Figure 6. Reaction Kaempferol and DPPH

The primary antioxidant in *Centella asiatica* is asiaticoside, identified as the third antioxidant compound. Asiaticoside exhibits potent antioxidant properties. The concentration of asiaticoside varies across different parts of the plant, with the leaves containing the highest amount. Figure 8 illustrates the structure of asiaticoside and its interaction with free radicals.

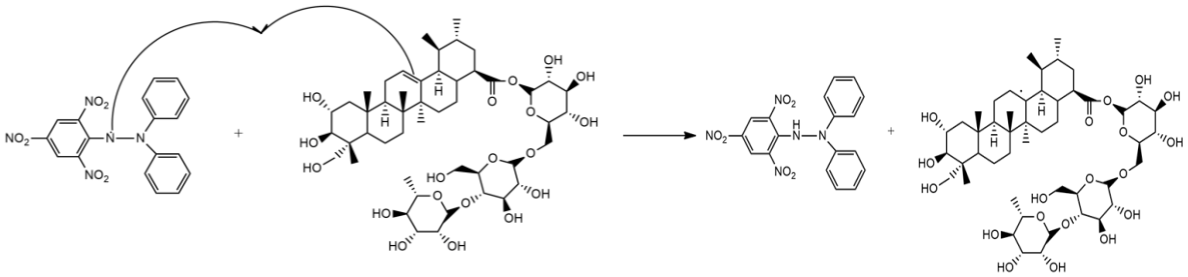

Figure 8. Reaction Asiaticoside and DPPH

Characterization and activity test of nanoemulsion

Stability of Nanoemulsion

Stability refers to a cosmetic item's capacity to uphold its quality, encompassing factors such as its effectiveness, potency, and purity. Evaluations of stability can be observed in Table 7, demonstrating the product's quality assurance.

Table 5. Stability of Nanoemulsion

Formulas	Stability	Indicator
F1	Stable	Homogenous emulsion, no precipitate, no layer
F2	Unstable	Precipitation
F3	Unstable	Precipitation

Analyzing Table 6, it is evident that F1 exhibits superior stability compared to F2 and F3, evident from the absence of precipitate formation. This might be attributed to the heightened attractive forces between larger droplets, coupled with decreased repulsion among them. The diminished electrostatic repulsion energy facilitates faster aggregation and sedimentation. Given its stability, F1 stands out as the preferred formula.

pH of Nanoemulsion

The purpose of the pH test is to gauge the acidity level of the formulation, which is linked to potential skin irritation. Deviation from the skin's pH can heighten the likelihood of skin irritation and discomfort. Table 8 displays the outcomes of the pH examination.

According to the data in Table 7, the pH levels of the three nanoemulsion formulations fall within a safe range for skin application. Facial skin typically maintains a pH range of 4 to 6, ensuring the nanoemulsions' skin-friendly usage without irritating [26]. If the pH temporarily exceeds 6.5, it may cause skin dryness, while a pH below 4.5 can lead to skin irritation [27].

Transmittance of Nanoemulsion

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The percent transmittance test was carried out to measure the clarity of the emulsion formed. The measurement of percent transmittance is one of the important factors in observing the physical properties of the formed nanoemulsion [28]. The outcomes of this transmittance test are available in Table 8.

Transmittance test results show that the salam leaves nanoemulsion formulas produced a clear dispersion with a percent transmittance value ranging from 92.2 to 96.6%. The test results for the clarity of the *Centella asiatica* leaves nanoemulsion produced a clear dispersion with a transmittance value of more than 90% which indicates that the droplet size is small. This is due to the use of Tween as a surfactant that can form a nanoemulsion system spontaneously when dispersed [14].

Viscosity of Nanoemulsion

The viscosity test aims to determine the thickness of the preparation related to the ease when applied to the skin. The dispersion of the preparation is influenced by the viscosity of the preparation, the viscosity of the gel is inversely proportional to the dispersion produced. The higher the concentration of the gelling agent to be used, it will increase the resistance of the gel to flow and spread [29]. The results of the viscosity test can be seen in the table 8.

The findings indicate that increased extract content leads to higher viscosity. Within the trio of formulas, solely F1 exhibits a viscosity below 100 cP, classifying it as having an optimal viscosity level. For topical applications, an ideal nanoemulsion viscosity typically falls within the range of 1-100 cP [19].

Table 8. pH, transmittance, and viscosity of nanoemulsion

Formulas	pH	% Transmittance	Viscosity (cP)
F1	5.33	97.4	75.35
F2	5.07	96.8	100.5
F3	5.04	95.1	179.1

Antioxidant Activity of Nanoemulsion

An evaluation of the antioxidant properties of a nanoemulsion prepared from *Centella asiatica* leaf extract was performed to determine the IC50 concentration. This IC50 value signifies the potential usage of the nanoemulsion as an active ingredient in antioxidant serum production. The results of the antioxidant test for the nanoemulsion derived from *Centella asiatica* leaf extract indicated an IC50 value of 2604.967 ppm. However, this value categorizes the antioxidant capacity of the nanoemulsion preparation as weak, given that the IC50 exceeds 200 ppm. This outcome can be attributed to the presence of surfactants, cosurfactants, and oil that encase the extract, necessitating additional time for DPPH to interact with the extract [3].

The reason for this is that merely 3.84% of the entire preparation constitutes the amount of extract added. The IC50 value for the *Centella asiatica* leaves extract in the nanoemulsion stands at 100.0307 ppm. Although there is a minor decline in antioxidant efficacy, it is not substantial and still falls within the classification of robust antioxidants.

Particle Size of Nanoemulsion

The result of particle size identification using PSA showed that the nanoemulsion of *Centella asiatica* leaves extract (F1) had a particle size of 166.7 nm. The particle size of nanoemulsion is included in the category of nanoparticles with a diameter range of less than 500 nm. The polydispersity index value of the nanoemulsion which is less than 0.5 is 0.413, describing the particle size distribution of the nanoemulsion preparation having a good level of homogeneity.

Nanoemulsions had a good particle size as an ingredient for cosmetics because they were not too small (< 100 nm). Nanomaterials with a diameter that is too small in the range of 10-100 nm can enter the

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body [30]. One of the ways to reduce the possibility of nanomaterials entering the body is to increase the particle size (>100 nm).

Irritation Test Result

Conducting an irritation test is a prerequisite before introducing cosmetic items to the market. This procedure aims to mitigate potential adverse reactions resulting from product usage [31]. Table 11 illustrates the outcomes of the irritation test.

Table 6. Irritation Test Result

Sample	24 Hours		48 Hours		72 Hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
F1	-	-	-	-	-	-

Description: (-): Has not caused erythema/edema

(+): Has caused erythema/edema

According to the findings from the irritation test outlined in Table 11, rabbits exhibited no signs of irritation (such as redness or swelling) at 24, 48, and 72 hours following the application of the nanoemulsion. This absence of irritation can be attributed to the fact that nanoemulsions are composed of non-irritating substances.

Conclusion

Centella asiatica leaves extract nanoemulsion (F1) has a particle size of 166,7 nm with good particle size homogeneity. The antioxidant activity of nanoemulsion (F1) has an IC50 value of 100.0307 ppm which is included in the category of weak antioxidants. The results showed that the nanoemulsion preparation decreased antioxidant activity compared to the extract, this was due to the nanoemulsion formula affecting antioxidant activity. According to the study findings, the antioxidant activity of the F1 nanoemulsion was found to be less potent compared to the raw *Centella asiatica* leaf extract. Hence, there's a need to create novel formulations to achieve nanoemulsions with enhanced antioxidant properties.

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