Extraction of Sungkai (*Peronema cannescens Jack)* leaves, Antioxidant Activity Test and Its Nanoemulsion Formulation

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Abstract. Sungkai (*Peronema canescens J.*) is an Indonesian native plant widely distributed on the islands of Kalimantan and Sumatra. Potential of these plants is related to the presence of secondary metabolites, which can be obtained through extraction. In its application, plant extract requires a delivery system that can penetrate well into the skin and maximise the efficacy of the active ingredients, such as nanoemulsion, which has high kinetic stability due to the smaller droplet size. The research was conducted to compare the yields from the two extraction methods, the presence of active compounds in the plants related to their properties as antioxidants and then formulated into nanoemulsion. The results showed that Sungkai leaves extract contains active metabolites such as alkaloids, flavonoids, tannins and saponins. The extraction of sungkai leaves yields 15.91% and 14.71% for CE and UAE methods. The total phenolic and flavonoid values were 27.74 and 41.88 mg GAE/g extract; 17.60 and 36.02 mg QE/g extract; and IC_{50} values of 50.78 and 53.50, included in the strong antioxidant category. Stable nanoemulsion formulation was obtained by adding 1 gram of olive oil with a homogenisation speed of 15000 rpm, with particle size of 83.4 nm and a polydispersity index of 0.455.

1 INTRODUCTION

Sungkai (*Peronema cannescens jack*) or known as jati sabrang, ki sabrang, sungkai kurus, or sekai is a Indonesian native plant which is widely distributed on Kalimantan and Sumatra Island. The leaves and roots of sungkai have also long been used as traditional medicine in the Dayak Tunjung tribe as a medicine for fever, diuretic and rheumatic pain, and the Lembak Empat tribe in Bengkulu still uses the young leaves of sungkai to maintain health, treat high fever and malaria [1]. Currently, sungkai research focuses on leaves as an extract which has potential as an antipyretic [2] anti-inflammatory [3,4], antioxidant [5–8], antibacterial [9–11], Antihyperuricemia [12], anticancer [13,14], and antidiabetic [15,16].

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The exciting potential of the sungkai leaf is related to the presence of secondary metabolites/phytochemicals. Sungkai leaf extract contains phytochemicals such as alkaloids, phenolics, flavonoids, tannins, and saponins. The literature shows that phenolics are the most abundant and structurally diverse plant phytoconstituents [17]. The high phenolic content in the plant has been related to its antioxidant activity, which plays a role in preventing the development of age-related diseases, especially those caused by oxidative stress [18]. Oxidative stress is associated with various conditions, including cardiovascular disease, cancer, hypertension, diabetes, neurogenerative diseases, aging, etc. Therefore, a balance needs to be a concern, and antioxidants play an essential role in achieving this balance because antioxidants neutralize or destroy *reactive oxygen species* (ROS) or *free radicals* before they damage cells [19].

Secondary metabolites in plants can be obtained through extraction methods, while the critical purpose of extraction is represented by high efficiency and efficacy. The extraction result can express efficiency, while efficacy refers to the potential bioactivity and efficacy. So, the proper extraction method is essential in every extraction case.

Conventional extraction methods such as maceration are still popular for extracting natural materials; even though the process is very easy and simple, this method has several disadvantages in terms of time and the amount of solvent used. The alternative developed in this research is the use of the ultrasonic-assisted extraction (UAE) method. This method utilizes ultrasonic waves, which can destroy leaf cells, thereby speeding up the mass transfer of bioactive compounds from inside the cells to the solvent. This can increase the effectiveness and efficiency of extraction, especially the amount of solvent and the time required during the process [20].

Several studies have proven that using UAE provides better results regarding phenolic and flavonoid values and can function as an antioxidant [19,20]. Kautsari, et al. 2019 [21] extracted turmeric (*Curcuma longa Linn*) using various maceration, sonication, and microwave methods. The highest quality of curcuminoids is found in simplicia extract by sonication, followed by simplicia extract by maceration. Adiwibowo, et al. 2020 [22] extracted the starfruit's (*Avverhoa bilimbi L.*) fruit, leaves, and petioles. The results showed that the sonication extraction method gave comparable results to maceration extraction but with a relatively shorter extraction time.

High-quality natural ingredient extracts have the potential to be applied in various fields, such as cosmetics. Cosmetic preparations derived from natural "*back to nature*" ingredients have become a widely discussed campaign because they reduce synthetic chemicals' risks and long-term effects. In practice, using plants as cosmetics requires a delivery system that can penetrate well into the skin and maximize the efficacy of the active ingredients. One popular and widely used cosmetic delivery system, which is applied topically, is nanoemulsion. Nanoemulsion is a heterogeneous mixture consisting of one or more liquids that do not mix and are dispersed in another liquid in droplets with sizes ranging from 20–200 nm. Nanoemulsions are popular because of their stable advantages due to their small size and high surface area, making them ideal for use as carriers. Nanoemulsion formulation can also minimize the occurrence of creaming, coalescence, flocculation, sedimentation, and Ostwald ripening [23].

This study compared conventional extraction methods with maceration and ultrasonic-assisted extraction (UAE). The extracts obtained were then tested for phytochemicals qualitatively. Total phenolic, total flavonoid and DPPH antioxidant tests were also carried out to determine the antioxidant quantitatively. The following nanoemulsion formulation used the sungkai leaf extract from the UAE method.

2 MATERIALS AND METHODS

2.1 Material

Fresh sungkai leaves were collected from Bengkulu. The leaves of the plant were dried at room temperature, cut, and ground into powder by a mechanical grinder, filtered with a 32 mesh sieve, and then stored at -6 ºC until use. The reagent used included ethanol 70% was purchased in the local market, methanol (Merck), NaOH (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich), Quercetin (Sigma Aldrich, USA), Gallic Acid, Folin-Ciocalteu, aluminium chloride ($AlCl_3$), $NaNO_2$, Na_2CO_3, ferric chloride ($FeCl_3$), HCl, H_2SO_4, Alkaloid reagent (dragendorff, mayer, and bouchardart), aquadest, olive oil and virgin coconut oil (VCO), tween 80, and span 80. The equipment used in this study are digital analytical scales, hotplate magnetic stirrer, Ultrasonicator (Sonics Vibra-cell), Ultra turrax IKA T25, Rotary Evaporator Eyela N-1300, particle size analyser (PSA) Horiba SZ-100, spectrophotometer UV-VIS Agilent, and spectrophotometer IR Shimadzu FTIR Prestige 21.

2.2 Methods

2.2.1 Extraction of Sungkai Leaves

Conventional extraction (CE): Sungkai leaves were extracted with 70 % ethanol at solid: solvent ratio of 1:7 for three days at room temperature with three repetitions. The crude extracts were filtered and concentrated by a rotary evaporator at 50∘ C, and then the extract obtained was kept at 4∘ C until further use.

Ultrasonic-Assisted Extraction (UAE): Sungkai leaves were extracted with 70% ethanol, the ratio solid: solvent 1:7, and the amplitude 40 kHz, with various extraction times (10, 30, and 60 minutes) with three repetitions. The crude extracts were filtered and concentrated by a rotary evaporator at 50∘ C, and then the extract obtained was kept at 4 ∘ C until further use.

2.2.2 Phytochemical Analysis

Phytochemical tests were carried out on crude extracts of *P. cannescens jack* based on references from Harborne. The compounds identified were alkaloids, flavonoids, tannins, and saponins.

2.2.3 Determination of Total Phenolic Content (TPC)

The gallic acid standard was prepared with 1 mg gallic acid and dissolved in 1 ml MeOH (1000 µg/mL). The primary solution was then made into serial concentrations of 5, 10, 20, 30, and 40 µg/ml. For testing, 1 mg (both CE and UAE extracts) was weighed in 1 ml MeOH (1000 µg/mL), then 250 µl was taken for testing. The sample solution (500 µl) and standard gallic acid solution (25, 50, 100, 150, and 200 µL) were pipetted into a test tube, made up to 4 mL by adding distilled water and 250 µl of Folin-Ciocalteau was added, and then shaken. The solution was left for 8 minutes, then 750 µl of 20% Na_2CO_3 was added and shaken until homogeneous and left for 2 hours at room temperature. Absorbance was measured at a wavelength of 765 nm using a UV-visible spectrophotometer (Agilent Technologies Cary 60). Measurements were carried out in three repetitions. TPC was reported as milligram gallic acid equivalent per gram of MAP extract (mg GAE/g extract) [24].

2.2.4 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was estimated by the aluminium chloride colouring method [25]. Quercetin and samples were prepared at 4 mg, each dissolved in 4 ml methanol (1000 µg/ml stock solution). A standard curve was prepared with 5, 10, 20, 30, and 40 µg/ml standard solutions by pipetting quercetin standard solutions (25, 50, 100, 150, and 200 µl) into a test tube. Sample measurements were carried out by pipetting 250 µL into a reaction tube. The solution was added with 2 ml of distilled water and 150 µl of 5% $NaNO_2$. After 5 minutes, 150 µl of 10% $AlCl_3$ was added. Six minutes later, 2 ml of 1M NaOH was added, and the volume was increased to 5 ml with distilled water. The mixture was homogenised, and the absorbance λ 510 nm was measured using a UV-Vis spectrophotometer. TFC was reported as a milligram of quercetin equivalent per gram of extract (mg QE/g extract). Quercetin and samples were weighed at 4 mg, each dissolved in 4 ml methanol (1000 µg/ml stock solution). A standard curve was prepared with 5, 10, 20, 30, and 40 µg/ml standard solutions by pipetting quercetin standard solutions (25, 50, 100, 150, and 200 µl) into a test tube. Sample measurements were carried out by pipetting 250 µL into a reaction tube. The solution was added with 2 ml of distilled water and 150 µl of 5% $NaNO_2$; after 5 minutes, 150 µl of 10% $AlCl_3$ was added. Six minutes later, 2 ml of 1M NaOH was added, and the volume was increased to 5 ml with distilled water. The mixture was homogenised, and the absorbance λ 510 nm was measured using a UV-Vis spectrophotometer. TFC was reported as a milligram of quercetin equivalent per gram of extract (mg QE/g extract).

2.2.5 Antioxidant Activity in Sungkai Leaves Extract

The antioxidant activity of the extract against DPPH free radicals was measured according to the method of Dewi et al., 2014 [26]. Amount of 4 mg of extract was weighed and dissolved in 4 mL of methanol to obtain a sample stock solution of 1000 ppm. The test was carried out by varying the sample concentration until the concentration in the test solution was 10, 50, 100, and 200 µg/mL. Standard solution (1-20 µg/ µL) in 2 ml methanol was added to 0.5 ml DPPH solution (1 mM in methanol). The mixture was shaken and left at room temperature for 30 minutes. The resulting absorption was measured at a wavelength of 515 nm. The percent inhibition of the sample is calculated based on the difference in absorption between the blank and the sample. The test was carried out three times. Quercetin was used as a comparison.

2.2.6 Formulation of Sungkai Nanoemulsion

The method of making sungkai nanoemulsion (SNE) was based on a study by Atun et. al [27] with some modifications. Variations of SNE formulation can be seen in Table 1. SNE was made by mixing 250 mg of sungkai extract with the aqueous phase (tween 80 and distilled water) and stirring until homogeneous. Then, different amounts of olive oil (0.5 grams, 1 gram, and 2 grams) were added drop by drop while homogenized using an ultra turra by varying the speed in the range of 10,000-20,000 rpm for 30 minutes. The formulated Sungkai nanoemulsion was then tested for physical properties such as stability, particle size, and polydispersity index.

Table 1. Formulation of Sungkai Nanoemulsion (SNE) and its variations.

No	Sample Name	Sungkai Extract (mg)	Olive Oil (gr)	Tween 80 (gr)	Aquadest (gr)	Homogenizing Speed (rpm)
1	SNE A	250	0.5	2	47.5	10.000
		250	1.0	2	47.0	10.000
		250	2.0	2	46.0	10.000
2	SNE B	250	0.5	2	47.5	15.000
		250	1.0	2	47.0	15.000
		250	2.0	2	46.0	15.000
3	SNE C	250	0.5	2	47.5	20.000
		250	1.0	2	47.0	20.000
		250	2.0	2	46.0	20.000

3 RESULTS AND DISCUSSION

3.1 Extraction of Sungkai Leaves

Extraction is the initial stage in utilizing natural materials for the food, medicine, cosmetics, etc. industries. This stage is an essential process as the extraction results reflect the efficiency and efficacy of the resulting extract. In this study, the authors compared the conventional extraction method (CE) with the ultrasonication-assisted extraction method (UAE). Both extraction methods were performed with the same solvent (70% ethanol) and solid: solvent ratio (1:7). CE was performed without variation, while UAE was performed with variation in extraction time (10, 30, and 60 min).

Based on Table 2, which refers to yield quantity, conventional extraction produces more yield than the average yield of the UAE method. However, based on extraction time, the UAE method is superior to the conventional method. In the time variation performed with the UAE method, the resulting yield is proportional to the increase in extraction time.

Table 2. Comparation yield from two extraction methods of Sungkai leaves.

Extraction method	Dry weight (gram)	Cycle & time	Ratio solid: solvent (ml)	Crude extract weight	% yield
CE	100	3 x 24 hours	1:7	15.9156	15.91 %
UAE	20.1248	3 x 10 minute	1:7	2.5252	12.54 %
UAE	20.0941	3 x 30 minute	1:7	2.9134	14.49 %
UAE	20.0877	3 x 60 minute	1:7	3.4357	17.10 %

Note:
CE: Conventional extraction
UAE: Ultrasound-Assisted Extraction

The longer contact time between the sample and the solvent causes an increase in the amount of active substance extracted. This has similar results in research by Kristina et al. 2022 who showed time variations (5-30 minutes). The longer extraction time increased extraction yield at a certain point, 25 minutes, and decreased at 30 minutes [28].

Likewise, Xu et al. 2013 Compared the extraction time (15-90 minutes) to the yield in the CE and UAE methods. The CE method produces more yield as the extraction time increases at 15-60 minutes and decreases at 75-90 minutes, while in the UAE method, the maximum yield is obtained at 15-30 minutes and decreases as the extraction time increases. Conventional extraction takes longer, and the mass transfer process tends to slow down compared to the UAE method. However, in the UAE extraction, the maximum yield was obtained at 30 minutes and decreased in the next time extraction, which is probably due to the chemical decomposition of the active content of the extract as a result of the ultrasonic cavitation force for too long [29]. Based on some of the studies above, the UAE extraction method for extracting natural materials is optimal at an extraction time of around 30 minutes. [28–30].

UAE extraction is more effective and efficient based on extraction time. The yield obtained by the UAE method is also similar to the yield produced by the conventional method. Therefore, sungkai leaf extraction can be carried out using the UAE method.

3.2 Phytochemical Study

Phytochemical testing is performed as an initial screening to determine the extract's constituents or class of compounds. Generally, phytochemicals/metabolites in plants are divided into primary and secondary. Primary metabolites have functions directly related to plant survival, while secondary metabolites are compounds obtained through biosynthetic mechanisms with various pathways from primary metabolism. Secondary metabolites in plants have unique characteristics and vary from one plant to another, which is a potential of interest to the pharmaceutical, cosmetic, and other chemical industries.

In this study, phytochemical testing was conducted on extracts from the UAE method. Table 2 shows that sungkai leaf extract contains alkaloid, flavonoid, tannin and saponin phytochemicals. The same phytochemical test results were also shown by the study of Latief et al., 2021 which uses maceration extraction method with 70% ethanol solvent. So, it can be said that qualitatively, the group of compounds produced from the UAE extraction method has similarities with conventional extraction results.

Table 3. Phytochemical analysis of sungkai leaves extract.

No	Phytochemical analysis	Reagent	Results	Color
1	Alkaloid	Bouchardart Meyer Dragendroff	+ - +	Precipitate of brown-orange - Precipitate of black-brown
2	Flavonoid	Mg powder, HCl	+	Brownish-yellow
3	Tanin	$FeCl_3$ 1%	+	Dark green-brown
4	Saponin	Hot water	+	Stabilised foam after 5 minutes
(+) Positive (-) Negative				

3.3 Total Phenolic Content and Total Flavonoid Content

In Table 3, the results of phytochemical testing are presented qualitatively. Sungkai leaves extract contains various secondary metabolites, including phenolic and flavonoid. Phenolic and flavonoid compounds are the dominant secondary metabolites in plants. Both groups of compounds are widely developed because of their potential as antioxidants/free radical fighters.

The results of measurements of TPC and TFC are presented in Table 4. The total phenolic value of sungkai leaf extracts using CE and UAE methods was 27.74 ± 0.19 mg GaE/g and 41.88 ± 0.15 mg GaE/g, respectively, and the total flavonoid value of CE and UAE methods was 17.60 ± 0.50 mg QE/g and 36.02 ± 0.02 mg QE/g, respectively. Based on the results, the UAE method provides a better TPC and TFC content value than the conventional method.

Table 4. Total phenolic content, total flavonoid content, and antioxidant for CE and UAE of sungkai leaves extract.

Sample Name	Extraction Methods	Total Phenolic content (mg GaE/g extract)	Total Flavonoid content (mg QE/g extract)
Sungkai leaves extract	Maceration	27,74 ±0,19	17,60 ±0,50
	UAE	41,88 ±0,15	36,02 ±0,02

The UAE method has the advantage of producing quality extracts compared to other conventional methods. This is related to the cavitation force generated from the process. In principle, the enhanced extraction of solvents from materials by UAE is due to the mechanical effects of acoustic cavitation. Applying ultrasonic waves to a solution will cause the molecules to oscillate about their equilibrium position. When sufficiently large ultrasonic energy is applied, stretching and breaking of molecular bonds between solutions will occur. When the bubbles burst and come into contact with the cell wall, shock waves are formed with liquid jets that rupture the cell wall and increase mass transfer [31,32].

The results of this research are also supported by a study conducted by Um et al., 2018 and El Maaiden et al., 2022 which states that the TPC and TFC values with the UAE method are better than the conventional method. Therefore, UAE extraction is a suitable method to extract phenolic compounds and flavonoids [33,34].

3.4 Antioxidant Activity of Sungkai Leaves using DPPH Methods

Antioxidant testing was carried out using the DPPH method. This method is the most common and widely used because the process is easy and simple. The basic principle of measuring antioxidants with the DPPH method is the interaction between free radicals with hydrogen or electrons released by antioxidant compounds so that dpph radicals become stable, which is then marked by a reduction in the intensity of purple to yellow color in the test solution.

In this test, quercetin standard is a strong antioxidant with an IC_{50} value of 11.15 ± 0.02. The antioxidant test results of sungkai leaf extracts are presented in Table 5. The antioxidant values with conventional and UAE extraction methods were 50.78 ± 0.17 µg/mL and 53.50 ± 0.11, respectively. Based on the IC_{50} values obtained from both extraction methods, the antioxidants in the sungkai leaf samples were strong antioxidant categorized as 50-100 µg/mL. The IC_{50} value of conventional extraction is slightly higher than that of UAE extraction.

Several other studies conducted antioxidant testing on sungkai leaf extract. Okfrianti et al. 2022 compared the antioxidant test of young and old sungkai leaves, the IC_{50} values obtained were similar to the results in this study, which were 50.8 and 52.8 ppm [6]. Pindan et al. 2021 conducted antioxidant tests on sungkai leaves fractionated with various solvents, the IC_{50} results for crude extract, hexane fraction, ethyl acetate fraction, and residual ethanol fraction were 25,549; 607,475; 12,986; and 15,766 ppm, respectively. The antioxidant value of the crude extract is already classified as a very strong antioxidant, but the fractionation results with semi-polar solvents actually improve the quality of these antioxidants [35]. Antioxidant compounds are generally derived from polar compounds such as phenolics and flavonoid derivatives, so extraction and fractionation using polar solvents can maximize the withdrawal of antioxidant active substances in plants.

Although in Table 3, it is mentioned that the TPC and TFC values of extracts with the UAE method are better than the conventional method, in the quantitative examination of the antioxidant test with the DPPH method, the results are not directly proportiona1. This can occur because the antioxidants in the extracts of conventional extraction results can come from other groups of compounds besides phenolic and flavonoid groups, which may be less available in extracts with the UAE method.

Table 5. The IC_{50} value for DPPH free radical scavenging of *P. cannescens*

No	Sample	IC_{50} (µg/mL)	Activity
1	Quercetin (standart)	11,15± 0,02	Very active
2	Maceration P. cannescens jack	50,78± 0,17	Active
3	UAE P. cannescens jack	53,50± 0,11	Active

Extracts were considered active as below(Molyneux, 2004) IC_{50}= <50 (µg/mL) very active, IC_{50}= 50-100 (µg/mL) active, IC_{50} = 100-500 (µg/mL) less active , IC_{50}= >500 (µg/mL) not active

3.5 Formulation of Sungkai Nanoemulsion (SNE)

In general, nanoemulsions are classified into two types: water-in-oil (w/o) nanoemulsions and oil-in-water (o/w) nanoemulsions, but oil-in-water (o/w) emulsions are more widely used in cosmetic formulations. In this study, the nanoemulsion formulation made was oil in water (O/W), and the oil phase dispersed into the water phase.

Nanoemulsions are kinetically stable and thermodynamically unstable, so their formulation is highly dependent on the formation process of nano-scale droplets. The use of low surfactants makes nanoemulsions require a high-energy input methodology to achieve a stable formulation. Therefore, this study used a high-speed homogenisation method using an ultra-turrax device. High-speed homogenisation is the most commonly used method for nanoemulsion preparation. During this process, several forces, such as hydraulic force, turbulence, and cavitation, work together to produce nanoemulsions with very small droplet sizes [36]. Formulations were made with varying oil concentrations and varying homogenisation speeds. The data results are presented in Table 6 below.

Table 6. The Data particle size and polydispersity index (PI) of sungkai nanoemulsion (SNE).

Sample Name	Olive oil (g)	Homogenation speed (rpm)	Particle Size	Polydispersity Index (PI)	Picture

SNE A1	0.5	10000	NA*	N/a	
SNE A2	1.0	10000	174.6	0.451	
SNE A3	2.0	10000	140.6	0.405	
SNE B1	0.5	15000	NA*	N/a	
SNE B2	1.0	15000	83.4	0.455	
SNE B3	2.0	15000	88.5	0.410	
SNE C1	0.5	20000	242.6	0.645	
SNE C2	1.0	20000	97.5	0.410	
SNE C3	2.0	20000	101.9	0.452	

*NA: this data is not taken because the particles are not nano-sized and the PI value exceeds 1

Physically, the nanoemulsion of sungkai is green-brown in color, the variation of oil addition makes the color of the nanoemulsion more turbid. Particle size and Polydispersity Index (PI) are important parameters in testing the physical stability of nanoemulsions. Polydispersity Index (PI) refers to the value of homogeneous particle distribution in a solution. The PI ranges from 0-1, where if the value is close to 0 then the particle distribution is said to be homogeneous, but if the value is close to 1 then the distribution is heterogeneous.

A larger PI value indicates that the surface is uneven, and the more diverse the surface, the easier it is to agglomerate or precipitate.

The test results of sungkai nanoemulsion (Table 6) in this study ranged from 83.4 nm-242.6 nm with PI 0.405-0.645. The particle size range can still be categorized as a nanoemulsion, although cosmetics categorize nanoemulsions with a particle size of 1-100 nm. The average PI value is 4.6, it can be concluded that the nanoemulsion formed is distributed homogeneously.

In this study, the best nanoemulsion formulation was obtained by adding 1 gram of oil and a homogenizer speed of 15000 rpm, which produced a clear brownish nanoemulsion with a particle size of 83.4 nm and PI 0.455. Particle size values below 100 with PI less than 0.5 can indicate a stable preparation..

3.5.1 Effect of Oil Addition on SNE Particle Size

The O/W nanoemulsion formulation of sungkai uses olive oil as the oil phase. Olive oil has a low viscosity. Olive oil was chosen because this oil is widely used in the food industry and cosmetics. Olive oil contains minerals and vitamins that are good for maintaining skin health.

In this study, the weight variation of olive oil was carried out starting from 0.5 grams, 1 gram, and 2 grams. In the table above, the physical appearance of nanoemulsions with less oil tends to be clear, and nanoemulsions with more oil tend to be cloudy. The relationship between the addition of oil in nanoemulsion formulations and particle size can be seen in Figure 1.

SNE A1 and B1 show that the Particle size analyzer cannot display the particle size when adding a variation of 0.5 grams of oil. This can happen because the amount of oil is not enough to cover the extract/active ingredient. So the formulation's extract/active ingredient particles still move around freely or are only dissolved in the water phase so that the nanoemulsion is not formed. As for SNE formulas A2 and A3, adding oil causes a decrease in particle size. This is different from the SNE B2-B3 and SNE C2-C3 variations, which show that adding olive oil to the formulation causes an increase in particle size. A similar situation was described in studies [37,38]. Formulation with more oil causes the amount of surfactant to decrease so that the surface tension decreases and can result in instability or separation of the nanoemulsion.

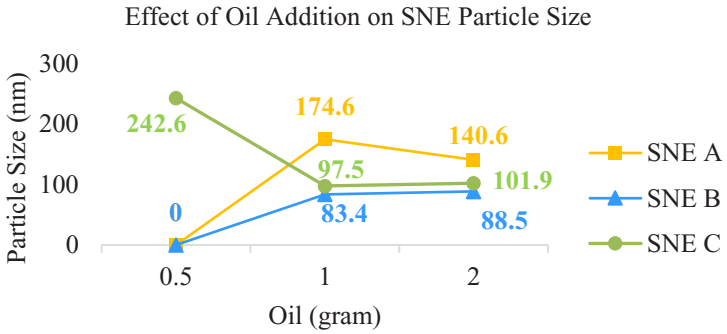

Fig 1. Effect of Oil Addition on SNE Particle Size

3.5.2 Effect of Homogenizer Speed on SNE Particle Size

Homogeniser speed is one of the essential factors in nanoemulsion formation. The effect of homogenizer speed on particle size can be seen in Figure 2. In SNE 1 with speeds of 10000 and 15000 rpm, particle size values could not be shown on the particle size analyzer. In addition to the small amount of oil, this could also be due to the homogenization process, which has a speed of 10000-15000 rpm and is not strong enough to produce a nanoemulsion. However, the SNE 1 sample with a speed of 20000 rpm showed a particle size value of 242.6. This speed can form nanoemulsions even with a large size >200 nm, and the PI value of 0.645 indicates a heterogeneous distribution.

In the other two variations, SNE 2 and 3, increasing the homogenizer speed from 10000 to 15000 rpm caused a decrease in particle size. Still, the particle size increased again when the homogenizer speed became 20000 rpm. This can be due to the energy generated when the 20.000 rpm speed is used, so the particles in the sample will tend to agglomerate and form a larger size. Other studies also explain that high-pressure homogenization in the maximum emulsification process does not significantly reduce particle size because the interface layer reaches a saturation state [39].

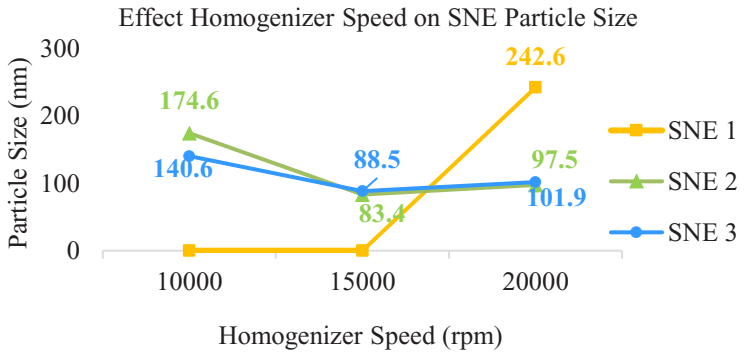

Fig 2. Effect of Homogenizer Speed on SNE Particle Size

4 Conclusion

In this study, sungkai leaves extract was shown to have secondary metabolite compounds from the alkaloid, flavonoid, tannin and saponin groups. The extraction of sungkai leaves with conventional extraction (CE) and ultrasound-assisted extraction (UAE) gave 15.91% and 14.71%, respectively. Each extraction method's total phenolics and flavonoids were 27.74 and 41.88 mg GAE/g extract and 17.60 and 36.02 mg QE/g extract, respectively. Antioxidant tests with the DPPH method gave IC_{50} results of 50.78 and 53.50, which are included in the strong antioxidant category. The best nanoemulsion formulation was obtained in the SNE B2 formulation with a variation of olive oil addition of 1 gram and a homogenization speed of 15000, which produced a stable nanoemulsion with a particle size of 83.4 nm and a polydispersity index of 0.455.

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