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Formulation and Characterization of Sungkai Leaf Extract Nanoemulsion (*Peronema canscens* Jack)

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Abstract: *Sungkai* (*Peronema canescens* Jack) *leaves are empirically used for various treatments, and their efficacy can be enhanced using nanoparticle technology, especially nanoemulsions. The purpose was to find the best nanoemulsion formula (NES) containing sungkai leaf extract (EDS). Sungkai leaves were extracted with 70% ethanol, and the compounds were determined by liquid chromatography-mass spectrometry. NES was formed by mixing EDS (260, 400, and 530 mg), VCO, tween80, PEG400, soy lecithin, sodium alginate, and deionized water. The NES formed were characterized by particle size, polydispersity index (PDI), zeta potential, pH, viscosity, percent transmittance, and physical stability test. The results showed that EDS contains at least seven compounds, with eupatilin as a dominant compound. The characterization results obtained NES with a particle size range of 270–520 nm with a PDI value of 0.413–0.608. NES was categorized as stable with a zeta potential value of -45.9 to -48.7 mV and no phase separation based on the centrifugation test. NES has a viscosity of about 1.90–2.03 cP, pH 7, and a percent transmittance of 93–98%. The best formula is the formula with EDS F1 (260 mg). Thus, NES has the potential for the development of multiple treatment targets.*

Keywords*: eupatilin, LC-MS, percent transmittance, PDI, zeta potential*

INTRODUCTION

Sungkai (*Peronema canescens* Jack), also known as Ki Sabrang or Jati Sabrang is a plant whose wood is used by Indonesian. This plant can be found in West Sumatra, Bengkulu, Jambi, South Sumatra, West Java, and almost all parts of Kalimantan. Apart from the wood, local people have used sungkai leaves empirically for various treatments, such as hypertension, fever, malaria, toothache, ringworm, intestinal worms, diarrhea, and bloating (Ibrahim and Kuncoro 2012; Latief et al. 2021).

The various benefits of sungkai leaves are related to the secondary metabolites they contain. Sitepu's research (2020) reported that the phytochemical test of the methanol extract of sungkai leaves positively contained alkaloids, flavonoids, anthraquinones, glycosides, tannins, and steroids. Previous studies have also explored the benefits of sungkai leaves scientifically, such as antibacterial (Ibrahim and Kuncoro 2012), anti-hyperuricemia (Latief et al. 2021), antioxidants, antityrosinase (Fadlilaturrahmah et al. 2021), anticholesterol (Annisa 2021), anti-inflammatory (Latief et al. 2021), antipyretic

(Brata and Wasih 2021), antidiabetic (Aini 2022), and immunostimulatory (Sistiany 2022). However, these studies only focus on utilizing sungkai leaves as extract preparations. The efficacy of the sungkai leaf extract can be further enhanced by nanoparticle technology. Nanoparticle technology can increase the effectiveness of drugs due to the small particle size, which increases drug distribution to specific areas of the drug, protects drug entities in the systemic circulation, and allows for sustainable drug release (Deng et al. 2020). Nanoemulsion is one form of nanoparticle used in drug delivery systems (Sutradhar and Amin 2013). Nanoemulsion is an emulsion that has a size on the nanometer scale. The nanoemulsion consists of a thermodynamically stable mixture of oil and aqueous phases. Drug delivery of nanoemulsions can increase the bioavailability of drugs or bioactive compounds (Kumar et al. 2019). Therefore, this study aimed to determine the optimum sungkai leaf extract nanoemulsion formula.

RESEARCH METHODS

Materials and Tools

The material used in this research is sungkai leaves which come from the Sukabumi area, West Java, and have been identified by Dr. Ir. Iwan Hilwan, MS. as a dendrology and forest botanist expert, 70% ethanol (food grade), virgin coconut oil (VCO) (GONAVCO-food grade), tween 80 (Sigma-Aldrich), polyethylene glycol (PEG) 400 (AlphaChem-USP grade), soy lecithin (PT. Multi Kimia Raya Nusantara), sodium alginate (Sigma-Aldrich), and deionized water. The tools used are a magnetic stirrer, water bath sonicator, glassware, liquid chromatography-mass spectrometry/LC-MS (Waters, USA), particle size analyzer/PSA (Horiba SZ 100z), pH meter, TV-10 viscometer (Toki Sanyo), UV-Vis spectrophotometer and centrifugation.

Methods

Extraction of Sungkai Leaves

The leaves of sungkai are dried to become simplicia. The simplicia was then macerated with 70% ethanol (1:10) in a closed vessel for 3×24 hours. After that, the macerate was concentrated using a rotary evaporator to obtain a thick extract. Sungkai leaf extract, referred to as EDS, is stored at ±4°C until reused. The content of chemical compounds in the extract was determined using LC-MS/MS

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

EDS composition analysis was carried out using Ultra Performance Liquid Chromatography (UPLC) equipped with a Xevo G2-S Q-TOF detector (Waters, USA) operating in positive ion electrospray mode. EDS (5 µL) was injected into the C18 column (1.8 µm 2.1×100 mm) HSS. The flow rate was set at 0.2 mL/min and the column temperature at 50°C. The mobile phase consisted of solvent A (water + 5 mM ammonium formate) and solvent B (acetonitrile + 0.05% formic acid).

Preparation of Sungkai Leaf Extract Nanoemulsion (NES)

Sungkai leaf extract nanoemulsion formula, from now on referred to as NES, was prepared using low-energy and high-energy methods referring to the research of Son et al. (2019) with a modification of the oil phase type. Comparison of VCO as the oil phase with surfactants (tween 80) refers to Listyorini et al. (2018) with a ratio of 1:8. The preparation of NES begins with the preparation of a primary emulsion by mixing EDS with VCO, then dispersing it with tween 80 (surfactant) and PEG 400 (cosurfactant) with light stirring for 1 hour at 65°C. Next, the primary emulsion was added to a mixture of soy lecithin (emulsifier) and 5% sodium alginate (biopolymer), then mixed for 1 hour with light stirring without heating. Furthermore, 1 gram of primary emulsion mixture was added little by little to the aqueous phase in the form of deionized water with light stirring for 3 hours. Finally, the mixture was sonicated for 1 hour with a bath-type sonicator. The formula was made with three variations of extract concentration: Formula 1 (F1), Formula 2 (F2), and Formula 3 (F3). The NES formulations are presented in Table 1.

Table 1. Formula of Sungkai Leaves Extract (NES)

Ingredients	Formula NES		
	NES F1	NES F2	NES F3
Sungkai leaves extract (EDS)	260.0 mg	400.0 mg	530.0 mg
VCO	0.5 g	0.5 g	0.5 g
Tween 80	4.0 g	4.0 g	4.0 g
PEG 400	1,5 g	1.5 g	1.5 g
Soy Lecithin	0.5 g	0.5 g	0.5 g
Sodium Alginate	5.0 g	5.0 g	5.0 g
Water	Up to 200 mL	Up to 200 mL	Up to 200 mL

Characterization of Sungkai Leaf Extract Nanoemulsion (NES)

The NES formed was characterized by various parameters of physicochemical properties such as particle size, polydispersity index (PDI), zeta potential, pH, viscosity, percent transmittance, and physical stability tests. Particle size and particle size distribution were determined by dynamic light scattering (DLS) using a Particle Size Analyzer/PSA (Horiba SZ 100z). The zeta potential of the nanoemulsion was measured by Laser Doppler Electrophoresis (LDE) with a PSA apparatus. pH measurement using a pH meter with a pH calibration of 7 and 4 at room temperature (28 ± 2°C). NES viscosity values were measured with a TVi-10 viscometer with spindle number 1 and a speed of 50 rpm. Percent transmittance was measured using a UV-Vis spectrophotometer with a wavelength of 650 nm and deionized water as a blank (Listyorini et al. 2018). Physical stability testing using centrifugation at a speed of 1520 g for 15 minutes (Zhao et al. 2018).

RESULT AND DISCUSSION

Sungkai Leaf Extract

The 70% ethanol extract of sungkai leaves is obtained with a distinctive aroma, blackish brown color, viscous liquid form, and a yield of 18.60%. This yield in previous studies using 70% ethanol was 18.47% (Melisa and Yuliawati, 2022). This reveals that maceration of EDS with 70% ethanol is effective in withdrawing bioactive compounds from sungkai leaves, so the yield is more than 10%. The EDS characteristics of the organoleptic test results can be seen in Table 2.

Table 2. Characteristics of Sungkai leaf extract (EDS) organoleptic test results

Characteristics	Result
Color	Dark Chocolate
Aroma	Typically
Shape	Viscous liquid
Yield	18.60%

Based on the results of LC-MS analysis, EDS obtained at least seven compounds (Table 3). Five of these seven compounds are classified as flavonoids, while one is a group of coumarins and anthraquinones. Eupatilin (Figure 1) is the EDS's dominant compound (24.5%).

Table 3. Compound detected in Sungkai leaf extract

Retention time (min)	Molecular weight (m/z)	Compound name	Molecule formula	% Area	Group of compounds	Reported pharmacological activities
1.32	163.039	4-hydroxycoumarin	$C_9H_6O_3$	2.58	Coumarins	Anti-coagulation (Muhammad *et al.*, 2013)
5.32	579.173	Viteksin-2-O-ramnoxide	$C_{27}H_{30}O_{14}$	8.39	Flavonoids	Antioxidant (Lorizola *et al.* 2018); Antiapoptosis (Wei *et al.* 2014)
5.91	285.076	Physcion	$C_{16}H_{12}O_5$	14.71	Anthraquinone	Anti-cancer(Liu *et al.* 2019); Anti-cholesterol(Lee *et al.* 2019)
6.27	317.067	Isorhamnetin	$C_{16}H_{12}O_7$	3.36	Flavonoids	Anti-ateroklorosis (Luo *et al.* 2015)
6.81	301.072	Chrysoeriol	$C_{16}H_{12}O6$	4.15	Flavonoids	Anti-osteoporosis dan anti-fungal (Aboulaghras *et al.* 2022)
8.14	331.081	Quercetin dimethyl ether	$C_{17}H_{14}O_7$	5.96	Flavonoids	Anti-hypertension (Hussain *et al.* 2018)
9.19	345.097	Eupatilin	$C_{18}H_{16}O_7$	24.51	Flavonoids	Anti-cholesterol (Lee *et al.* 2019)

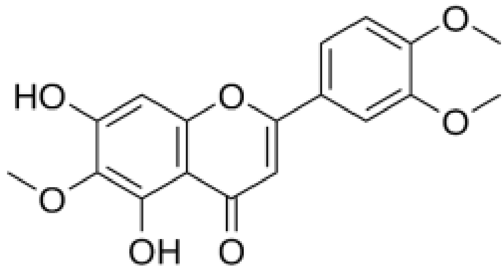

Figure 1. Eupatilin Structure

In this study, no quercetin was found, which had previously been reported in sungkai leaves by Dillasamola et al. (2021). However, quercetin is found in the form of quercetin dimethyl ether. Furthermore, betulinic acid was reported to be found in sungkai leaves by Muharni et al. (2021) and is also not found in the EDS. Differences in growing places or the age of the leaves used may cause this.

Characteristics of Sungkai Leaf Extract Nanoemulsion (NES)

The results of the nanoemulsion formed show the appearance of a clear yellow color, the distinctive aroma of sungkai leaves, and a liquid and homogeneous form (Figure 2). The three formulas showed aroma, shape, and homogeneity, which did not differ organoleptically (Figure 2). However, the color parameters reveals that the color concentration is more yellow sequentially from Formula 1, Formula 2, and Formula 3 (Figure 2). This color concentration is due to the increased concentration of EDS from formula 1 to formula 3. The yellow color of NES comes from tween 80, which is yellow. The liquid form of the three NES formulas is due to the addition of the water phase more than the other components making up the NES. Therefore, NES can be categorized as an oil-in-water (O/W) nanoemulsion type. The three NES formulas did not observe the separation of the water and oil phases. The homogeneity of the NES formula is due to the addition of tween 80 and PEG 400, which reduce the surface tension of VCO with deionized water so that phase separation does not occur. In addition, tween 80 and PEG, which have HLB respectively 15 and 13.1, are suitable for oil-in-water (O/W) nanoemulsion formulations.

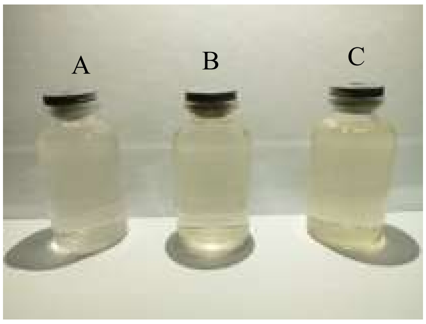

Figure 2. Sungkai leaf extract nanoemulsion formula (NES). A: NES Formula 1, B: NES Formula 2, and C: NES Formula 3.

The characteristics of the nanoemulsion made in this study are summarized in Table 4. Furthermore, the characteristics of each parameter are explained in each subsequent sub-chapter.

Table 4. Characteristics of the physicochemical properties of Sungkai leaf extract nanoemulsion (NES)

Formula	Particle Size (nm)	Zeta potential (mV)	PDI	pH	Viscosity (cP)	Physical Stability	Transmittance (%)
NES F1	270.4	-45.9	0.608	7.01±0.01	2.03±0.05	No separation	98.5±0.1
NES F2	578.4	-48.7	0.663	7.25±0.04	2.20±0.17	No separation	95.6±0.2
NES F3	510.2	-47.9	0.413	7.73±0.08	1.90±0.10	No separation	93.1±0. 2

Particle size and polydispersity index (PDI)

Table 4 reveals the particle size and particle size distribution of NES Formulas 1, 2, and 3. The results obtained from this study reveals that NES F1, NES F2, and NES F3 have an average particle size of 270.4 nm, 578.4 nm, and 510.2 nm, respectively. The smallest particle size was found on the NES F1. Particle size characterization is important to determine the formula that is formed, categorized as nanoemulsion. The size range of nanoemulsion particles is 20–600 nm (Choradiya and Patil 2021).The particle size distribution is described in polydispersity index (PDI) values. The closer the PDI value is to zero, the more particle size distribution shows homogeneity (Caballero and Davidov-Pardo 2021). The results of measurements with PSA show the PDI value of the NES formula around 0.413-0.608. Thus, the particle size distribution of NES is uniform.

Zeta potential

The zeta potential indicates the nanoemulsion particles' surface charge, which can affect the repulsive forces between the particles. Therefore, Zeta potential becomes important in predicting the stability of a nanoemulsion. The results in Table 4 indicate that the NES formula has stable criteria for all Formula 1, Formula 2, and Formula 3 because the value is less than -30 mV. Nanoemulation is stated to be stable if it has a potential zeta value of more than +30 mV or less than -30 mV (Gurpreet and Singh 2018).

Physical stability

The physical stability of NES was carried out by centrifugation to observe phase separation, creaming, and precipitation. The test results revealed that the three formulas did not have phase separation, creaming, and precipitation, indicating that the NES formula was physically stable. Stable nanoemulsions withstand gravity, flocculation, and creaming (Lee et al. 2018).

Viscosity and pH

NES viscosity is low, with a 1.9-2.2 cP (Table 4). The ratio of the water phase, which is more than the oil phase, makes NES a type of oil-in-water (M/A) nanoemulsion (Sondari and Tursiloadi 2018). Therefore, NES has a low NES viscosity value. Meanwhile, the final pH of NES was measured to be around seven after adding 0.01 N NaOH. In this study, 0.01 N NaOH was added to increase the pH to more than 6. The formation of nanoemulsions was influenced by pH, where pH 6.5-9.0 did not indicate flocculation, phase separation, and clumping (Son et al. 2019).

Percentage of transmittance

One of the important characteristics of nanoemulsion is that it is clear and transparent. Nanoemulsions with a transmittance percentage close to 100% show formulas in the clear and transparent category (Laxmi et al. 2015). The results obtained by the NES formula have a transmittance percentage of around 93% -98%, so it is classified as clear. The results reveal that the transmittance value negatively correlates with the EDS concentration. The lower the EDS concentration, the higher the transmittance percentage. The highest transmittance percentage was found in NES F1.

CONCLUSIONS

Based on the research results, it can be concluded that the sungkai leaf extract nanoemulsion (NES) has been well formulated based on the results of the characterization of NES with various physicochemical properties. The best NES is NES F1 (260 mg), with the smallest particle size and the highest transmittance percent. Thus, NES has the potential for the development of various treatment targets.

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