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CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF KALAKAI (*Stenochlaena palustris*) LEAVES EXTRACT IN NANOSTRUCTURED LIPID CARRIER SYSTEM

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ABSTRACT

Exposure to ultraviolet light can cause damage and death of skin cells through multiple mechanisms, including the formation of free radicals that can cause hyperpigmentation, erythema, sunburn, photo-aging, and even skin cancer. Kalakai (*Stenochlaena palustris*) is a typical Kalimantan plant with the ability to be a high antioxidant. However, it is still very rarely utilized. Kalakai leaves contain polyphenolic groups that function as free radical antidotes, as well as flavonoid compounds that can stabilize radical compounds. Various technology-based drug delivery systems have been developed to improve therapeutic effectiveness, including nanotechnology. Nanostructured lipid carrier (NLC) is the second-generation lipid-based carrier designed to overcome the limitations of previous-generation lipid-based carriers. This system consists of a mixture of and unstructured due to their different constituent parts. This research will develop a formula for kalakai leaf extract in a nanostructured lipid carrier system using the emulsification-sonication method. Based on the data, the characteristics of kalakai leaf extract in a nanostructured lipid carrier system that meet the standards are F1 (5%) and F2 (10%). Among the three formulas, F3 showed the highest IC50 value compared to F1 and F2, which is 14,967 ± 0,240 with powerful antioxidant activity, followed by F2 with an IC50 value of 24,186 ± 1,797, and F1 with IC_{50} value of 65,504 ± 5,041.

Keywords: Kalakai leaves; Stenochlaena palustris; nanostructured lipid carrier; emulsification-sonication; antioxidant; DPPH

INTRODUCTION

Skin can experience damage and cell death due to exposure to ultraviolet light through various mechanisms, including forming free radicals. Free radicals can damage multiple types of cells and cause radical chain reactions, resulting in oxidative stress that can trigger multiple diseases (Liu et al., 2018).

Kalakai (*Stenochlaena palustris*) is one of the typical Kalimantan plants that can be used as a source of natural antioxidants. Kalakai leaf contain polyphenolic groups that are active as free radical antidotes.

Kalakai is a ground fern that is between 5-10 meters long, with rhizome roots that are tall, strong, flat, square, bare or scaly, and grow slowly or epiphytically (hitchhiking on other plants) with the main root in the soil. Kalakai is a fern or fern-like plant that lives wildly in scrub forests, swamps, and freshwater (Adawiyah, 2019; Dewi & Bekti, 2023).

Kalakai, especially the leaves, are reported to contain bioactive substances such as flavonoids, phenolics, alkaloids, saponins, tannins, and terpenoids, which function as potent antioxidants and have the potential as cytotoxic in cancer therapy (Syamsul et al., 2019).

Previous research has shown that using smaller particle sizes and polar solvents at the appropriate temperature and time can enhance the antioxidant activity of kalakai (Wijaya et al., 2017). Nanotechnology is one of the new technological developments made at the nanoscale to improve the bioavailability and solubility of drugs. Nanostructured lipid carrier (NLC) are the second generation of lipid-based nanocarrier formed from a mixture of liquid and solid lipids that are unstructured due to different constituent parts. This delivery system is designed to overcome the limitations of the previous generation of lipid-based nanocarrier (Olawoyin, 2018; Beloqui et al., 2016).

Sonication-emulsification is one of the methods of forming nanostructured lipid This method creates a homogeneous emulsion using high-frequency ultrasonic waves with two or more immiscible liquid phases. This method can break particles and produce more stable emulsions using powerful ultrasonic forces. This method can also produce tiny-sized particles, up to tens of nanometers, depending on the constituent materials. Generally, the particle size of NLC is up to 10 to 1000 nm (Annisa et al., 2016; Listiyana et al., 2020).

Therefore, developing new antioxidant preparations based on natural ingredients with effective delivery methods is crucial. This study aims to develop a kalakai leaf extract formula in nanostructured lipid carrier system with emulsification-sonication method.

METHODS

Materials

Kalakai (*Stenochlaena palustris*) leaves collected from Desa Sungai Puting, Candi Laras, Tapin, South Kalimantan. Glycerol monostearate, virgin coconut oil, tween 80, ethanol 70%, chloroform, ammonia, sulfuric acid, Mayer reagent, Wagner reagent, Dragendorff reagent, $FeCl_3$ 1%, anhydrous

acetic acid, 2,2-diphenyl-1-picrylhydrazyl, quercetin.

Research Path

1. Collection and Authentication of Plant Material

Kalakai leaves were collected from Desa Sungai Puting, Candi Laras, Tapin, South Kalimantan. Botanical identification of plant was done at the Laboratory of Faculty of the Mathematics and Natural Science, Universitas Lambung Mangkurat.

2. Preparation of Kalakai Leaves Extract

Kalakai leaves were cleaned from dirt, drained, and chopped into small pieces. Then dried in oven at 50^{o}C and pulverized using a grinder. Soaked in ethanol 70% solvent in a 1:10 ratio for five days in a closed container and protected from the sun with occasional stirring. After five days, it was macerated again for two days. The extract was evaporated using a water bath at 60^{o}C until a thick extract was obtained (Ariyanti et al., 2020).

3. Phytochemical Screening

Kalakai leaf extract was subjected to phytochemical screening, including alkaloids, flavonoids, phenolics, saponins, tannins, steroids, and terpenoids (Harborne, 1987).

4. Nanostructured Lipid Carrier Formulation

The formulation of kalakai leaf extract in a nanostructured lipid carrier is shown in Table 1.

Table 1. Formulation of kalakai leaf extract in nanostructured lipid carrier

Materials	Formula (%)			Function
	F1	F2	F3	
Kalakai leaf extract	5	10	15	Antioxidant
Glycerol monostearate	6	6	6	Solid lipid
Virgin coconut oil	4	4	4	Liquid lipid
Tween 80	4	4	4	Surfactant
Aquadest	ad 100	ad 100	ad 100	Aqueous phase

The lipid phase (glycerol monostearate and virgin coconut oil) was melted until homogenous, and then add the extract to the lipid phase. The aqueous phase (Tween 80 and aquadest) was added to the mixture and stirred using homogenizer for 15 minutes at 6000 rpm. The NLC was then ultrasonicated for 5 minutes (Annisa et al., 2016; Rahayu et al., 2023).

This study is based on the referenced journals, with the expectation that the conditions outlined in the referenced work can be applied to the current research. While optimization is typically a crucial initial step,

the consideration of cost constraints has prevented its implementation in this case.

5. Characterization of Nanostructured Lipid Carrier Formulation

The nanostructured lipid carrier was characterized by organoleptic tests, pH tests, viscosity tests, particle size, and polydispersity index (PDI) values.

6. Stability Test

The stability test was done before the antioxidant activity test to ensure that the formula or extract stays stable under certain conditions, so its antioxidant activity can be measured accurately. The cycling test method is used to test the stability of the nanostructured lipid carrier system.

The NLC will be stored at 4^{o}C temperature for 24 hours, then at 40^{o}C temperature for another 24 hours. This is referred to as one cycle. The stability test will be conducted for six cycles. The physical evaluation, color, smell, pH, and viscosity of NLC should be observed in each cycles (Aqsyal & Mardiyanti, 2023).

7. Antioxidant Activity

2 ml of DPPH solution was mixed into each concentration and incubated in a dark place (Fauzi et al., 2021).

RESULTS AND DISCUSSION

1. Collection and Authentication of Plant Material

Kalakai leaves were obtained from Sungai Puting Village, Candi Laras Utara, Tapin, South Kalimantan.

Plant authentication was carried out at Universitas Lambung Mangkurat with test certificate number: 337/LB.LABDASAR/ XII/2023, showed that the plants used in this study were kalakai plants (*Stenochlaena palustris*) from the Blechnaeceae family.

2. Preparation of Kalakai Leaves Extract

The thick extract that was obtained was 128 grams. The characteristic of the thick extract is a blackish brown color with a typical kalakai leaf smell (**Figure 1**).

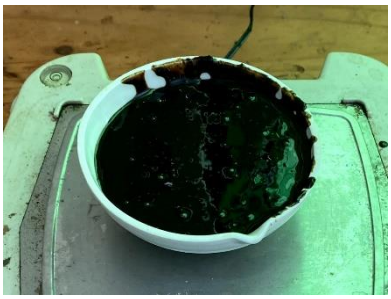

Figure 1. Kalakai leaf thick extract

3. Phytochemical Screening

Based on the results of phytochemical screening (Table 2), kalakai leaf extract showed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, and steroids compounds as indicated by color changes or the sediment formation. These compounds are considered to be powerful antioxidants that are beneficial as free radical scavengers

(Syamsul et al., 2019; Anggraeni & Erwin, 2015).

Table 2. Phytochemical screening of kalakai leaf extract

Constituents	Results
Alkaloids	(+)
Flavonoids	(+)
Phenolics	(+)
Tannins	(+)
Saponins	(+)
Steroids	(+)
Terpenoids	(-)

(+) positive, (-) negative

4. Characterization of Nanostructured Lipid Carrier Formulation

Kalakai leaf extract is formulated into three nanostructured lipid carrier system formulas using emulsification-sonication. The lipid used in this study combines glycerol monostearate (GMS) as a solid lipid with virgin coconut oil (VCO) as a liquid lipid. GMS has amphiphilic properties that can stabilize NLC emulsions (Azmi et al., 2020). VCO can also help reduce particle size because it contains fatty acids with shorter C atom chains and has high lipophilicity, allowing better interaction with active substances (Sriarumtias et al., 2017).

Tween 80 is a nonionic surfactant that can dissolve in water and has a high hydrophilic-lipophylic balance (HLB), where usually, HLB above ten can help stabilize the emulsion. Tween 80 will form a solid layer around the surface of nanoparticles and create a steric hindrance capable of producing particles with small dimensions and resistance to changes in pH and electrolyte concentration (Lüdtke et al., 2022; Damayanti et al., 2023)

The nanoparticle formation method used in this research is the top-down method, which breaks large particles into nanometer-sized particles (Harso, 2017). Ultrasonic waves play an essential role in helping to break large particle aggregates into smaller particles. Ultrasonic cavitation generates the shear solid forces required for nano-emulsification, resulting in vacuum bubbles that implode violently and asymmetrically and classify particles down to the nanometer scale (Pinesti & Lumintang, 2023).

The results of physical screening showed that the three formulas did not experience phase separation, so it can be concluded that the formulation was homogeneous with a brown color that got

darker as the concentration of the added extract increased (Figures 2).

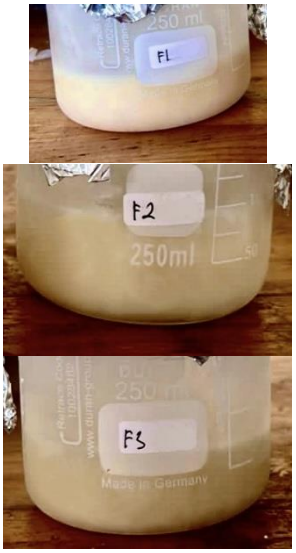

Figure 2. Physical screening of nanostructured lipid carrier (F1 = 5%, F2 = 10%, F3 = 15%)

Based on Table 3, F1 is the closest pH to neutral. The three formulas have met the requirements of a suitable pH for topical preparations in the range of 4.5 to 6.5 and oral preparations in the range of 5-7 (Depkes, 1979; Leboe, 2020).

The viscosity test results of the three formulas were 34.00 cPs, 40.00 cPs, and 80.00 cPs, respectively. The nanostructured lipid carrier systems' average viscosity was 32.00 to 127.00 cPs. Viscosity affects the mobility and ease of the carrier to release the active substance. Viscous systems tend to have more excellent resistance to release the active substance. In contrast, dilute systems have less resistance to release the active substance because the mobility of the active ingredient is increased (Annisa et al., 2016).

Table 3. Characterization result of nanostructured lipid carrier formulation

Test	Results		
	F1	**F2**	**F3**
Organoleptic:			
• Physical evaluation	Emulsion-like	Emulsion-like	Emulsion-like
• Color	Pale brown	Light brown	Brown
• Smell	Typical	Typical	Typical
pH	6	5	5
Viscosity	34,00 cPs	40,00 cPs	80,00 cPs
Particle size	972.9 nm	-	-
Polydispersity index	0.580	-	-

Based on the overall results, F1 was chosen as the most optimal formula to test particle size and polydispersity index value. The results show that the particle size of F1 is 972.9 nm, which already meets the requirements, whereas a good NLC particle size is 10 to 1000 nm (Annisa et al., 2016; Listiyana et al., 2020).

The polydispersity index (PDI) value shows the particle size distribution, which

ranges from 0 to 1. PDI values < 0.5 indicate uniform or homogeneous particle distribution, while values > 0.5 indicate that particles are not uniformly distributed (Taurina et al., 2017). However, in this study, the PDI value was 0.580, which means it has a broad particle size distribution.

The stirring speed is one-factor causing particle size and PDI values that are still relatively high. The homogenizer's higher speed and longer rotation will increase the contact intensity between molecules and reduce the particle size. In addition, the temperature used during the sonication process can also affect the particle size because the higher the temperature used during the sonication process, the smaller the particle size (Jusnita & Nasution, 2019).

5. Stability test

The three kalakai leaf NLC system formulas were subjected to accelerated stability testing using the cycling test method for six cycles (12 days). The results of physical form observations in the stability test are shown in Table 4.

Table 4. Stability test

Test	Formula	Cycle						
		0	**1**	**2**	**3**	**4**	**5**	**6**
Physical evaluation	F1	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like
	F2	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like
	F3	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Emulsion-like	Phase separation	Phase separation
Color	F1	Pale brown	Pale brown	Pale brown	Pale brown	Pale brown	Pale brown	Pale brown
	F2	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown
	F3	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Smell	F1	Typical	Typical	Typical	Typical	Typical	Slightly rancid	Slightly rancid
	F2	Typical	Typical	Typical	Typical	Typical	Slightly rancid	Slightly rancid
	F3	Typical	Typical	Typical	Typical	Typical	Slightly rancid	Slightly rancid
pH	F1	6	6	6	6	6	6	6
	F2	5	5	5	5	5	5	5
	F3	5	5	5	5	5	5	5
Viscosity	F1	34,00	34,00	33,00	29,00	29,00	25,00	22,70
	F2	40,00	50,00	45,00	34,00	35,00	34,00	30,00
	F3	80,00	80,00	78,90	77,00	72,70	69,90	67,40

Based on the table, F1 and F2 did not separate and maintained their homogeneity for up to 6 cycles. At the same time, F3 had phase separation starting from the fifth cycle (Figure 3).

Figure 3. Separation phase of F3 after 6 cycles

However, it could return to homogeneity after light stirring. The three formulas did not change color. However, there has been a slight odor change since the fifth cycle.

Phase separation in F3 may indicate instability caused by the coalescence of the globules to become more prominent. The lack of surfactant concentration can cause this instability because the coating of the globules was not optimal. In addition, extreme temperatures can also result in separation because they can accelerate the reaction rate, so the number of collisions increases and surface tension increases (Husni et al., 2019)

The pH observation results show no change after six cycles, which is likely stable. The viscosity test results on the three formulas showed a decrease in viscosity after the cycling test compared to before. However, the viscosity decrease is not significantly different from before the test.

6. Antioxidant Activity Test

Antioxidant testing of the three formulas showed a colour change from purple to yellow, indicating that the three formulas contained antioxidants positively. The antioxidant activity test for the NLC system was also carried out using a UV-Vis spectrophotometer, which was measured at a wavelength of 515 nm. The DPPH wavelength range is generally between 515 to 520 nm (Tenda et al., 2023)

The comparison that acts as a positive control is quercetin 100 ppm diluted in several variations. Based on Table 5, the IC_{50} value obtained for quercetin was 0,012 ± 0,008. Among the three formulas, F3 showed the highest IC_{50} value compared to F1 and F2, which is 14,967 ± 0,240 with powerful antioxidant activity, followed by F2 with an IC_{50} value of 24,186 ± 1,797, and F1 with IC_{50} value of 65,504 ± 5,041.

Table 5. Antioxidant activity test results

Sample	IC_{50} value	Description
Quercetin	0,012 ± 0,008	Very strong
F1	65,504 ± 5,041	Strong
F2	24,186 ± 1,797	Very strong
F3	14,967 ± 0,240	Very strong

The results indicated that as the sample concentration increased, the IC50 value decreased. This is due to the reduction in DPPH absorbance, suggesting that the sample contains groups capable of releasing hydrogen atoms to react with DPPH free radicals. Higher concentrations increase the number of hydrogen atoms reacting with DPPH, leading to a reduction in DPPH, as evidenced by decreased absorbance and a color change to yellow (Hakim et al., 2021). Based on the data, F2 and F3 exhibited IC50 values below 50, indicating powerful antioxidant activity, while F1 had an IC50 value below 100, indicating strong antioxidant activity.

CONCLUSIONS

Based on the overall results, it can be concluded that kalakai leaf extract in a nanostructured lipid carrier system exhibits favorable characteristics for formulations F1 (5%) and F2 (10%) in terms of organoleptic evaluation, pH, viscosity, stability, and antioxidant activity. The antioxidant activity of the kalakai leaf extract in a nanostructured lipid carrier system was measured as follows: F1 had an activity of 65.504 ± 5.041, F2 showed 24.186 ± 1.797, and F3 exhibited 14.967 ± 0.240. The highest antioxidant activity was observed in F3 (15%).

Future research should focus on formulating kalakai leaf extract using different lipid carriers, surfactants, and methods. Furthermore, it is essential to characterize additional aspects of nanostructured lipid carrier systems, such as zeta potential, sorption efficiency, and particle morphology, to ensure that the data obtained is comprehensive and maximized.

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