

# Tyrosinase inhibitory activity and physicochemical properties of microalgae extract (*Spirulina platensis*)-loaded nanostructured lipid carriers gel

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**ABSTRACT:** Microalgae (*Spirulina platensis*) belongs to the family Cyanobacteria contains phycocyanin which is efficacious as a tyrosinase inhibitor. The purpose of the study was to make gel of Nanostructured Lipid Carriers (NLC) from the Microalgae extract (*Spirulina platensis*). Extracts were made by maceration with 70% ethanol solvent and evaluation of the enzyme tyrosinase inhibitory activity. The extract was made by NLC with solvent evaporation method and characterized which included particle size, polydispersity index, zeta potential and Transmission Electron Microscope (TEM). NLC extract was made into gel preparations by adding gelling agent, and evaluation included organoleptic homogeneity, viscosity and flow properties, pH, and the enzyme tyrosinase inhibitory activity. The enzyme tyrosinase inhibitory activity of Microalgae extract (*Spirulina platensis*) with the result  $IC_{50}$  in the amount of 150.22  $\mu\text{g/mL}$ . Five of NLC formulas was made based on the characterization obtained the best formula is NLC formula 2, which includes particle size of 119.6 nm, polydispersity index of 0.356, zeta potential of -37.8 mV, and spherical shape. The evaluation results of the gel were semi-solid, brown, typical smell, homogeneous, viscosity of 19500 cPs, plastic thixotropic flow properties, and pH 5.06. The enzyme tyrosinase inhibitory activity of NLC gel Microalgae extract (*Spirulina platensis*) with the result  $IC_{50}$  in the amount of 173.38  $\mu\text{g/mL}$ . It can be formulated into a physically and chemically stable gel preparation and has activity as a tyrosinase inhibitor.

**KEYWORDS:** Gel;  $IC_{50}$ ; Microalgae; NLC; *Spirulina platensis*; Tyrosinase inhibitor.

## INTRODUCTION

Ultraviolet radiation from sunlight activates the tyrosinase enzyme in skin tissue, accelerating melanin production. Tyrosinase enzyme *inhibitors*, including hydroquinone, azelaic acid, mercury, and kojic acid, have been widely utilized by the cosmetic industry for skin lightening. However, some of these compounds have dangerous side effects related to carcinogenesis and mutagenesis, such as kojic acid, which has inhibitory and stabilizing effects and is the most effective in preventing skin hyperpigmentation. The danger of kojic acid at high concentrations is hepatocarcinogenic, which will cause *erythema* and allergic contact dermatitis [1].

Therefore, natural ingredients are central to the development of cosmetic products as a strategy to avoid side effects and hazards that arise from long-term use on the skin. Numerous studies have examined active compounds in skin lightening preparations, including the active compound phycocyanin. Phycocyanin pigments are known to contain phytonutrients that act as free radical scavengers. The structure of the phycocyanin pigment is a tetrapyrrole molecule that can diminish some reactive oxygen species by donating hydrogen atoms [2]. The natural colorant found in *S. platensis* is phycocyanin pigment, yielding 18-20% of the total dry biomass during the extraction process [3]. Phycocyanin is a natural blue pigment that has been reported to exhibit anti-melanogenesis effects, inhibiting melanin synthesis. Concentrations of 50 and 100  $\mu\text{g/mL}$  demonstrate inhibitory activity on the tyrosinase enzyme, reducing melanin formation. A concentration of 50  $\mu\text{g/mL}$  can lower melanin formation from 75.7% to 65.7%, while 100  $\mu\text{g/mL}$  can reduce melanin formation from 56.2% to 47.5% [4]. The antioxidant activity of phycocyanin pigments from *Spirulina sp.*, using distilled water as the solvent, has an  $IC_{50}$  value of 110.80  $\mu\text{g/mL}$  [5], and when using a phosphate buffer solvent (pH 7), it has an  $IC_{50}$  value of 186.76  $\mu\text{g/mL}$  [5].

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This study used microalgae extract (*Spirulina platensis*) and made it into *Nanostructured Lipid Carriers* (NLC) by solvent evaporation. NLC consists of solid and liquid lipids as a carrier, where the active ingredients are entrapped. The lipid carrier was chosen because it can protect the entangled active ingredients, thereby increasing their bioavailability and delivery to the skin. Unlike SLNs, NLCs have at least one liquid lipid in addition to solid lipid [6],[7]. The partial substitution of solid lipids by liquid lipids promotes increased structural disorganization of the apolar nucleus, favoring the encapsulation of nonpolar drugs and preventing drug expulsion [6],[7]. Its ability to incorporate high amounts of lipophilic drugs combined with high tolerability has made NLC a promising system for drug delivery, which has been the subject of research by different research groups [7]-[9].

## ▪ MATERIALS AND METHODS

### Materials

Microalgae powder (*Spirulina platensis*) was obtained from the Live Feed Laboratory, Ministry of Marine Affairs and Fisheries Directorate General of Aquaculture, Center for Brackish Water Aquaculture in Jepara, Kojic Acid (Thornhill, Canada), L-DOPA (SIGMA- Aldrich), *Tyrosinase from Mushroom* (SIGMA-Aldrich), potassium dihydrogen phosphate (Dow Chemical Pasific, Singapore), sodium hydroxide (Dow Chemical Pasific, Singapore),, cetyl alcohol (Lanette→ 16, Care chemicals), oleic acid (Dow Chemical Pasific, Singapore),, tween 80 (Kao Corporation), propylenglycol (Dow Chemical Pasific, Singapore), carbomer 940 (Aquepec HV 505 HC, Sumitomo Seika Chemicals), triethanolamine (SIGMA-Aldrich), ethanol (Merck) and purified water (PT. Brataco).

### Microalgae powder extraction and phytochemical screening

Extraction of microalgae powder was performed using maceration method [10]. The extraction starts by mixing powder of *Spirulina platensis* with ethanol (70%) in the ratio of 1:2. The filtrate was concentrated using a vacuum evaporator and followed by phytochemical screening using the Farnsworth method to identify alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, essential oils, quinones, and coumarins.

### Optimization of microalgae extract NLC preparation

Two optimizations were carried out: optimization of solid and liquid lipids mixture (cetylalcohol and oleic acid) and optimization of lipid phase and surfactant mixture composition [8],[9]. Optimization of the solid and liquid lipids mixture was carried out by comparing the results of solid and liquid lipids fusion in the concentration range of 90-10%, then a consistency test using a penetrometer was carried out on the lipid fusion mixture. Optimization of lipid and surfactant mixture concentration used 2% and 5% lipid mixture variation, 3%-5% surfactant variation, and 6%-8% propylenglycol variation.

### Preparation of NLC of microalgae extract (*Spirulina platensis*) by solvent evaporation method

NLC of microalgae extract (*Spirulina platensis*) was prepared by solvent evaporation method [8],[9]. Solid lipid (cetyl alcohol) and liquid lipid (oleic acid) were melted in a water bath at 70 °C. Microalgae extract (*Spirulina platensis*) dissolved in acetone (Mass 1) was added to the melted lipid. Propylenglycol, surfactant (Tween 80), and purified water were heated at 70 °C (Mass 2). Mass 1 was incorporated into the mass 2 mixture by stirring with a *magnetic stirrer* at 600 rpm. The emulsion was stirred with ultra-turrax at 24,000 rpm for 15 minutes, then the resulting nanoemulsion was cooled with water at 2-5 °C with rapid stirring, and nanoparticles were produced. Characterization of NLC included particle size using Malvern Zetasizer 2000 (Malvern, UK), polydispersity index, zeta potential, and nanoparticle morphology using *Transmission Electron Microscope* (TEM).

### Preparation of microalgae extract (*Spirulina platensis*) NLC gel

The NLC gel was prepared using previous method [1]. Carbomer 940 was dispersed in pure water at 30 times its weight, triethanolamine was added and homogenized with a *stirrer* until a gel base (mixture A) was formed. Weighed NLC of Microalgae extract (*Spirulina platensis*) was added to mixture A until homogenized. Propylenglycol, methyl paraben, and the remaining pure water were added and then homogenized with a stirrer at 500 rpm for 15 minutes, referring to previous studies. The gel was evaluated for organoleptic, pH, homogeneity, viscosity, and flow properties.

## Tyrosinase inhibitory activity of extract and NLC Gel preparation of microalgae extract (*Spirulina platensis*)

A 70  $\mu$ L of sample solution, 110  $\mu$ L of L-DOPA solution, and 30  $\mu$ L of tyrosinase enzyme solution were loaded into a 96-well *microtiter plate*. Incubated for an optimum incubation time of 20 minutes at 37 °C. The mixture was measured for absorbance using a *microplate reader* at the optimum wavelength. The measured absorbance is the absorbance of dopachrome formation. The percentage of tyrosinase inhibition was calculated according to the following formula

$$\% \text{ Tyrosinase inhibition} = \frac{B - S}{B} \times 100\%$$

Description: B = control absorbance minus control blank ( $B_1 - B_0$ ); S = absorbance of sample minus blank sample ( $S_1 - S_0$ )

IC<sub>50</sub> was calculated using absorbance linear regression equation, sample concentration (in logarithm) as x-axis and percent inhibition (% inhibition) as y-axis. From the equation  $y = a + bx$ , the IC<sub>50</sub> value can be calculated. Kojic acid was used as a positive control.

## RESULTS

### Microalgae powder extraction and phytochemical screening

The yield of Microalgae extract (*Spirulina platensis*) obtained was 16.73%, and the DER-native was 5.98. Phytochemical screening of *Spirulina platensis* extract obtained compounds of flavonoids, saponins, and steroids. The formation of color in the amylalcohol layer indicates the presence of flavonoid compounds. The formation of stable foam indicates the presence of saponin group compounds.

### Optimization of microalgae extract NLC preparation

The optimization of the solid and liquid lipids mixture yielded penetration results ranging from 0 to 360 mm. The penetration results from 0 to 63 mm indicate the consistency of the lipid mixture, which is solid, while the results from 78 to 360 mm demonstrate the consistency of the solid lipid mixture, which is semi-solid.

**Table 1.** Optimization of solid lipid mixture of cetyl alcohol and oleic acid.

Material	Run (%)							
	1	2	3	4	5	6	7	8
Cetyl alcohol	90	80	70	60	50	40	30	20
Oleic acid	10	20	30	40	50	60	70	80
Penetrometer scale (mm <sup>-1</sup> )	0	0	17	21	26	29	36	120
Penetration result (mm)	0	0	51	63	78	87	108	360

The concentrations of solid and liquid lipids utilized cetyl alcohol and oleic acid in a ratio of 60:40. The optimization of the concentration of the solid lipid and surfactant mixture involved variations of 2% and 5% lipid mixtures, 3% to 5% surfactant variations, and 6% to 8% propylene glycol variations. Based on the optimization process, a lower concentration of the lipid mixture at 2% produced a liquid NLC, while the 5% lipid mixture resulted in a viscous mixture.

**Table 2.** Optimization of solid lipid, surfactant, and co-surfactant concentration.

Material	Run (%)											
	1	2	3	4	5	6	7	8	9	10	11	12
Solid lipid (Cetyl alcohol & Oleic acid) (60:40)	2	2	2	2	2	2	5	5	5	5	5	5
Surfactant (Tween 80)	4	4	4	3	4	5	4	4	4	3	4	5
Propylenglycol	6	7	7	7	8	7	6	7	8	7	7	7
Acetone	6	6	6	6	6	6	6	6	6	6	6	6
Pure water ad	80	80	80	80	80	80	80	80	80	80	80	80
Results	L	L	L	L	L	S	S	S	S	S	S	S

Description: L = Liquid, S = Solid

### Preparation of NLC of microalgae extract (*Spirulina platensis*) by solvent evaporation method

A 2% extract of Microalgae (*Spirulina platensis*) was used in the optimization results, which were conducted by mixing a variation of lipid mixture concentration at 2% with differing amounts of Tween 80 and propylene glycol, as shown in Table 3.

**Table 3.** Evaluation of NLC of microalgae extract (*Spirulina platensis*).

Formula	Propylenglycol : Tween 80	NLC parameters		
		Particle size (nm)	Polydispersity Index	Zeta potensial (mV)
1	6:4	154.4	0.369	-38.9
2	7:7	119.6	0.356	-37.8
3	7:4	159.3	0.423	-39.7
4	7:5	157.8	0.260	-39.0
5	8:4	145.6	0.375	-40.9

NLC morphology was characterized on nanoparticles with the smallest particle size, namely Formula 2.



**Figure 1.** Transmission Electron Microscope (TEM) test results.

Nanoparticle morphology analyzed using TEM revealed spherical nanoparticles with a particle size of 100 nm. This particle size is nearly identical to the results from the particle size test conducted with PSA, which measured 119.6 nm.

### Tyrosinase inhibitory activity test of extract and NLC gel preparation of microalgae extract (*Spirulina platensis*)

Tyrosinase hydroxylates L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), which is subsequently oxidized to o-quinone. This compound is further converted to melanin. Tyrosinase, also known as polyphenol oxidase, is a copper-containing oxidase and a key rate-limiting enzyme [11],[12]. The test of tyrosinase enzyme inhibitory activity is carried out *in vitro* based on the measurement of orange-colored compounds, namely dopachrome, which results from the oxidation of L-DOPA by tyrosinase. The presence of inhibitors causes the production of dopachrome to decrease, which is indicated by a decrease in the intensity of the orange color. Based on the *lock and key* theory, enzymes can only bind to specific substrates, so the substrate used must be right because it will affect the results of the reaction. In previous research, preliminary test results were obtained, which stated that the optimum incubation time of enzyme inhibition was 20 minutes, 10 mM L-dopa substrate concentration, 75 U/mL tyrosinase enzyme concentration, and absorption measurement at a wavelength of 490 nm [13]. Four solutions were measured, namely, control, control blank, sample, and sample blank. The control solution consisted of phosphate buffer solution pH 6.8, L-DOPA, and tyrosinase enzyme, made as a comparison. The control blank, consisting of phosphate buffer solution and L-DOPA, and the sample blank, consisting of phosphate buffer solution, L-DOPA, and sample (without enzyme), were used as correction factors. The sample solution consisted of L-DOPA, sample enzyme, and tyrosinase enzyme. IC<sub>50</sub> measurements of kojic acid samples were carried out at five concentration series, namely 50, 20, 10, 5, and 2.5 µg/mL. This concentration series is used to create a linear

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equation to determine the  $IC_{50}$  value. Based on the average % inhibition of kojic acid in series 1, series 2, and series 3, the  $IC_{50}$  value of 13.25  $\mu\text{g/mL}$  was obtained. Measurement of  $IC_{50}$  of microalgae extract samples (*Spirulina platensis*) was carried out at five concentration series, namely 500, 400, 300, 200, and 100  $\mu\text{g/mL}$ . Based on the % inhibition of microalgae extract (*Spirulina platensis*) on average in series 1, series 2, and series 3, the  $IC_{50}$  value of 150.22  $\mu\text{g/mL}$  was obtained. Measurement of  $IC_{50}$  of NLC Gel samples of microalgae extract (*Spirulina platensis*) was carried out at five concentration series, namely 500; 400; 300; 200 and 100  $\mu\text{g/mL}$ . The  $IC_{50}$  value of 173.38  $\mu\text{g/mL}$  was obtained from the average % inhibition of *Spirulina platensis* extract NLC Gel across series 1, 2, and 3, giving lower tyrosinase enzyme inhibitory activity than the extract.

## DISCUSSION

### The extraction and phytochemical screening

The yield value obtained in this study is greater than the previous study, which obtained a yield value of 10.74% [14]. One of the most important parameters to be observed in the extract is its composition (native extract content that represents the extract without excipients) and the DER native (Drug Extraction Ratio native). DER native is the ratio between herbal drug and genuine extract (extract without excipients). Therefore, the DER native is the amount of material that can be extracted (dry residue-extractable matter) that is obtained with a proper extraction solvent and a validated extraction process [15]. These results of phytochemical screening are similar to previous studies that conducted phytochemical screening of *Spirulina platensis* taken from the Karimun Jawa sea, Indonesia [10]. Phytochemical screening of *Spirulina platensis* extract obtained compounds of flavonoids, saponins, and steroids, the results were similar to a previous study [16].

### The optimization of microalgae extract NLC

In this optimization process, a combination of solid and liquid lipids was used to form NLC. This combination is expected to enhance the incorporation of the active substance in NLC. The penetration results of various combinations ranged from 0 to 360 mm. The penetration results between 0 and 51 mm indicated the consistency of the solid lipid mixture, while results from 63 to 360 mm suggested the consistency of the semi-solid lipid mixture. A higher penetration result indicates that the mixture of solid and liquid lipids is becoming softer. From this optimization process, the concentration of solid and liquid lipids used in NLC consists of cetyl alcohol and oleic acid in a ratio of 60:40, allowing the matrix of the NLCs to accommodate a greater amount of API.

The optimization of the concentrations of mixed lipids (2% and 5%), surfactants (3-5%), and cosurfactants (6-8%) was conducted. Based on the results of this optimization, it was determined that a low concentration of the lipid mixture at 2% produced liquid NLC. Liquid NLC yields a smaller particle size than NLC with high viscosity.

### The characterization of NLC of microalgae extract (*Spirulina platensis*)

Particle size measurements of NLC formulas 1, 2, 3, 4, and 5 indicated sizes ranging from 119.6 to 159.3 nm. The size of nanoparticles used in cosmetics varies from 10 to 1000 nm. The polydispersity index is a parameter that describes the particle size distribution of a nanoparticle system, with values ranging from 0 to 1. A polydispersity index value close to 0 indicates a uniform nanoparticle size distribution, while a value greater than 1 suggests that the sample has a very broad size distribution and may include larger particles or aggregates that can slowly sediment. Zeta potential measures the electric charge between colloidal particles. A higher zeta potential value effectively prevents flocculation. The measured zeta potential value is - 37,8 - 40,9 mV. The negative lower values of zeta potential are attributed to electrostatically stable particles in the colloidal system. To ensure a stable dispersion, the particles must carry the same charge, causing them to repel each other and avoid forming aggregates (sticking together). The greater the charge of a particle, the more stable it becomes. This stability results from the increased repulsion between similarly charged particles, which prevents them from merging. This indicates that there is no agglomeration among the particles from the NLC of microalgae extract (*Spirulina platensis*).



The NLC gel was prepared using Formula 2 of NLC extract, incorporating carbomer 940 as the gel base. The color of the NLC gel is a clear brown, influenced by the presence of the microalgae extract (*Spirulina platensis*). According to viscosity testing, the blank viscosity measured 65416.67 cPs, and the gel viscosity was 19500 cPs. This result revealed a difference in viscosity values between the blank gel and the NLC gel, viscosity and resistance decreased in NLC gel as the amount of NLC microalgae extract (*Spirulina platensis*) increased in the formula. Viscosity indicates a liquid's resistance to flow; the higher the viscosity, the greater the resistance. Flow properties were determined by creating a graph plotting force (dyne/cm) against rpm based on the data collected, and then the data was plotted with force (x) and rpm (y). The flow properties of the microalgae extract NLC gel are classified as plastic thixotropic.

#### **The activity of tyrosinase inhibitory of extract and NLC gel preparation of microalgae extract (*Spirulina platensis*)**

IC<sub>50</sub> measurements of kojic acid samples were carried out, and the IC<sub>50</sub> value of 13.25 µg/mL was obtained. So, it can be concluded that kojic acid provides high tyrosinase enzyme inhibitory activity. Measurement of IC<sub>50</sub> of Microalgae extract samples (*Spirulina platensis*) was carried out, and the IC<sub>50</sub> value of 150.22 µg/mL was obtained. Another previous report revealed that the tyrosinase inhibitory effect of *Spirulina platensis* demonstrated the IC<sub>50</sub> at 200 µg/ml [17], whereas the *S. platensis* extract in this study revealed the IC<sub>50</sub> of tyrosinase activity at 150.22 mg/ml, which is recognized as a better outcome. The tyrosinase enzyme inhibition activity test on gel preparations aims to determine the effect of gel formulations on tyrosinase enzyme activity. The IC<sub>50</sub> value of 173.38 µg/mL was obtained from the average % inhibition of *Spirulina platensis* extract NLC Gel across series 1, 2, and 3, giving lower tyrosinase enzyme inhibitory activity than the extract. This indicates the gel preparation formulation's effect on the gel's tyrosinase enzyme activity. The NLC gel of Microalgae extract (*Spirulina platensis*) contains Carbomer 940 as a *gelling agent*, which can result in a decrease in tyrosinase enzyme inhibitory activity.

#### **CONCLUSION**

Microalgae extract (*Spirulina platensis*) has tyrosinase enzyme inhibitory activity with an IC<sub>50</sub> value of 150.22 µg/mL, microalgae extract (*Spirulina platensis*) is made in the form of nanostructured lipid carriers (NLC) with particle size characterization of 119.6 nm, polydispersity index of 0.356, zeta potential of -37.8 mV, and spherical shape.

NLC of microalgae extract (*Spirulina platensis*) can be made into a physically and chemically stable gel preparation. This gel preparation has a semi-solid shape, light brown color, distinctive odor, homogeneous, plastic thixotropic flow properties, and a pH of 5.06. It also has tyrosinase enzyme inhibitory activity, with an IC<sub>50</sub> value of 173.38 µg/mL.

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