Antioxidant Activity of Chlorella vulgaris Used As an Antioxidant Cream

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Abstract. This study aims to analyze the antioxidant activity of Chlorella vulgaris for used as an antioxidant cream. The method used in this study is the DPPH method to measure the antioxidant activity of Chorella vulgaris cream based on the IC50 value and the BSLT method for determining toxicity for cosmetic use. The result was that Chlorella Vulgaris cream had an IC50 value of 127 μg / mL with an antioxidant activity of 61.90%. As a control cream without the addition of Chlorella Vulgaris showed an IC-50 value of 79.423 μg /mL with antioxidant activity of 3.46%. Chlorella Vulgaris cream has a very high antioxidant activity compared to the control cream without the addition of Chlorella Vulgaris. Chlorella Vulgaris cream can be used as an anti-aging cream based on the LC50 value is non-toxic and has a pH of 5.5 and after storage the pH is 6.5 according to the pH of the skin, so it is safe to apply.

Keywords: Chlorella vulgaris, Antioxidant, DPPH, anti-aging cream

1. Introduction
The development of the times is so fast along with the increasingly dense human activity that causes many cases of premature aging that occur at a relatively young age of around 20 years. This premature aging is caused by 2 factors, namely external factors, including pollution, cigarette smoke, sunlight, and the effects of an unhealthy lifestyle. And internal factors, including genetics, insufficient nutritional intake, and prolonged illness and loss of supporting tissue under the skin (subcutaneous tissue), stress, gravity, facial movements, obesity which can produce free radicals [1].

A free radical is an atom or molecule that is highly reactive with unpaired (unstable)electrons, by attacking and binding the electrons around it. Free radicals that are produced in excess in the skin will damage collagen in skin cell membranes, so that the skin lacks elasticity and causes wrinkles or aging [2].

Chemical compounds that can counteract free radical (peroxide) reactions on the skin are compounds that contain antioxidants [3]. Antioxidants can donate one or more electrons to free radicals, so that these free radicals can be reduced and do not damage body cells [4].

Antioxidant compounds can be found in nature, one of which has the active component is microalgae such as the phytoplanton Chorella vulgaris. Chlorella vulgaris contains secondary metabolites such as flavonoids and phenolic which have activity as an anti-oxidant [5] [6]. Chlorella vulgaris also has a high content of fatty acids which can act as antioxidant compounds [7].
Chlorella vulgaris, which has active components such as antioxidants, can be used as the main ingredient in cream preparations. Not only the ingredients used in the manufacture of anti-aging creams are dangerous, but the solvents used usually come from organic solvents such as n-hexane which can be toxic when applied to the skin [8].

Avocado seeds can be said to be a natural solvent that is safe and environmentally friendly, because they are still used as waste by the community and there is no use even though avocado seeds contain lots of vitamins and fatty acids [9].

A natural solvent alternative that is safe to use to extract secondary metabolites is a solvent from avocado seed oil using the sonication method. Sonication is an extraction method that uses sound energy which can increase the mass transfer rate in tissues and facilitate the transfer of active compounds from cells to the solvent [10].

2. Material and Methods

The tools and materials used in this research were Chlorella vulgaris powder, avocado seed oil, stearic acid, distilled water, glycerin, triethanolamine, propylene glycol, nipagin, nipasol, phantenol, beaker, stirring rod, magnetic stirrer, pestle and mortar, digital scales, cream pot, pipette, Brookfield viscometer, pH meter, FTIR Prestige-21 Shimadzu, and Gas Chromatography-Mass Spectrometry.

2.1 Viscosity and pH Test of Chlorella vulgaris cream

Viscosity was measured using the Brookfield Viscometer. The sample was put into a film tube, then let stand for 30 minutes at 25°C. The spindle used is spindle number 7 with a rotating speed of 50 rpm for 15 minutes. The viscosity value is listed on the screen.

for pH test, at first, standardization was carried out by dipping the pH meter electrode into a standard pH of 6.86 and washing it with distilled water. Then 1 gram sample was diluted with distilled water (1:10). The pH meter electrode part is inserted into the sample that has been diluted and the number that appears on the screen is the pH value.

2.2 Antioxidant Activity Test of Cream Preparations [11]

We weighed 0.0079 grams of DPPH, put it in a 50 mL volumetric flask and then added up to 50 mL of pro-analysis methanol and obtained DPPH with a concentration of 0.4 mM. Weighed 0.05 grams of pro-analysis vitamin C, put it in a volumetric flask and then added up to 5 mL of pro-analysis methanol as antioxidant control.

The cream preparation from the microalgae extract was weighed as much as 0.05 grams and dissolved in pro-analysis methanol up to 5 mL (5000 ppm mains solution). Several series of standards were created in concentrations of 20, 40, 80, 160 and 320 ppm. Entered 0.02; 0.04; 0.08; 0.16 and 0.32 mL into the volumetric flask. To each volumetric flask, 1 mL of 0.4 mM DPPH solution was added in pro-analysis methanol. The volume is sufficient to 5 mL, then the solution is shaken and stored at room temperature for 30 minutes, then the absorption is measured at a maximum wavelength of 515 nm. As a positive control, vitamin C was used as a comparison. Antioxidant activity was expressed in percent of DPPH immersion. The amount of antioxidant power is calculated by the formula:

\[
\text{Antioxidant} = \left( \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \right) \times 100\%
\]

Information:
Control: Solvent + DPPH
Sample: Solvent + DPPH + sample

The principle of decreasing the absorbance value is used to determine the antioxidant capacity of a compound. The results of the DPPH method are generally made in the form of IC50 (Inhibitor Concentration 50), which is defined as the concentration of the sample solution that will reduce DPPH activity by 50%. The greater the antioxidant activity, the smaller the IC50 value [12].
Specifically, a compound is said to be a very strong antioxidant if the IC50 value is <50 ppm, it is strong if the IC50 value is between 50-100 ppm, moderate if the IC50 value is between 101-150 ppm, and is weak if the IC50 value is >150 ppm [13]

2.3 FTIR Analysis

The anti-aging cream from Chlorella vulgaris was then analyzed using Prestige-21 Shimadzu FTIR (Fourier Transform Infra Red) to determine the presence of functional groups.

3. Result and Discussion

3.1 Viscosity and pH of Chlorella vulgaris cream

The production of anti aging cream mixing the ingredients that are soluble in the water phase into the ingredients that are soluble in the fat phase by heating and stirring [13]. oil phase (avocado oil and Chlorella vulgaris powder, stearic acid, triethanolamine) and water phase (aquades, nipagin, nипасол).

Chlorella vulgaris cream has a pH of 5.5 and after being stored for a month the pH becomes 6.5. This pH is still classified as safe for the skin because it is in accordance with the skin's pH which ranges from 4.5 to 6.5.

Viscosity is tested to determine the thickness of a cream. Chlorella vulgaris cream has a viscosity value of 15,200 Cps. this figure corresponds to the thickness of the cream to be applied to the skin so that it easily absorbs and enters the stratum corneum.

3.2 Antioxidant Activity of Chlorella vulgaris cream

The results showed that Chlorella Vulgaris cream had an IC50 value of 127 μg / mL with antioxidant activity of 61.90%. As a control cream without the addition of Chlorella Vulgaris showed an IC-50 value of 79,423 μg / mL with antioxidant activity of 3.46%. Chlorella Vulgaris cream has very high antioxidant activity compared to control creams without the addition of Chlorella Vulgaris.

| Cream | IC 50 (ppm) | Category |
|-------|-------------|----------|
| C     | 79423.3333  | very weak|

Chlorella vulgaris contains secondary metabolites such as flavonoids, phenolics, saponins, steroids and triterpenoids [5]. Flavonoids have the ability to scavenge free radicals and inhibit lipid oxidation [14] [15]. Phenolic compounds have the ability to donate hydrogen, so the antioxidant activity of phenolic compounds can be generated in the neutralization reaction of free radicals that initiates the oxidation process or at the end of the chain radical reactions that occur [16]. The antioxidant activity of phenol and flavonoid components by reducing free radicals depends on the number of hydroxy groups in the molecular structure [17]

3.3 FTIR

From the FTIR spectrum of control cream (without the addition of Chlorella vulgaris) with the spectrum of Chlorella vulgaris cream, it can be seen that there is an erosion in wave numbers 1219.01 and 1080.14. It is predicted that there is a reaction in the C-O bond
4. Conclusion

*Chlorella vulgaris* has proven antioxidant power when the control cream is added *Chlorella vulgaris* can increase the antioxidant value from fat to moderate. *Chlorella vulgaris* cream has a pH that matches the skin and its viscosity is able to absorb into the layer of the stratum corneum.

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