The extraction of antioxidants from *Chlorella vulgaris* for cosmetics

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Abstract. *Chlorella vulgaris* is microalgae that contain chlorophyll as antioxidants, which has been widely used as a functional food. Antioxidants from *Chlorella vulgaris* also have potential as active ingredients in the cosmetics industry. Nowadays, consumers prefer natural cosmetics because they aren’t harmful to the skin. This study aimed to obtain chlorophyll antioxidants from *Chlorella vulgaris* and applied it to the cosmetics. The extraction process of antioxidants from *Chlorella vulgaris* was performed by maceration method, with *Chlorella vulgaris* concentration variable: 0.01; 0.05 and 0.1% in water as solvent. The antioxidant extracts powdered by spray drying method, with addition of 100 g/L maltodextrin. The observation was conducted on the characteristics of water content, ash content, carbohydrate content, protein content, antioxidant activity, and powder morphology. The extracts powder applied to cosmetics in the form of creams and lotions, the characterized for the antioxidant activity and microbial content. The best antioxidant capacity of extract powder was obtained on *Chlorella vulgaris* concentration 0.1%, which was 11.83 mg Vit C per 100 g sample. The results of antioxidant capacity in cosmetics as cream and lotion were 4.95 mg Vit. C per 100 g sample (IC₅₀ 719.75 mg/ml) and 4.73 mg Vit. C per 100 g sample (IC₅₀ 660 mg/ml), respectively. Both of cream and lotion weren’t shown any microbial contamination. Based on this study, *Chlorella vulgaris* with its antioxidant capacity can be developed as active ingredients for various kinds of cosmetics.

1. Introduction

Many species of microalgae are valuable as cosmetics raw materials because of the diversity on biologically active compounds in them. The example of microalgae that have the potential to be developed as cosmetics compound is *Chlorella vulgaris*. *C. vulgaris* is a unicellular, eukaryotic organism and a spherical microscopic cell with 2–10 μm diameter and contains several compounds like protein, vitamins, and pigments¹². This microalgae contains green pigment compounds called chlorophyll³. *Chlorella* has antioxidant compounds that are relatively strong, and it has an EC₅₀ value of less than 50 ppm⁴. Antioxidants from *C. vulgaris* also have potential as active ingredients in the cosmetics industry. It’s a rich source of protein, which reaches 42 to 58% proteins per dry weight of biomass¹ and contain essential amino acids which supplement human RNA and DNA; help in tissue growth; and repair or restructure and smooth out the surface of the skin, particularly with scared skin or aging skin in cosmetics⁵.

Chlorophyll is a bioactive compound with antioxidant and anti-mutagenic properties which widely used in cosmetics, pharmaceutical, and food industries⁶. Chlorophyll has five types known as a, b, c, d, and f; whose maximum wavelengths of absorption when solved in methanol are 665, 652, 630, 696, and 707 nm, respectively⁷. Chlorophylls can absorb light in the red and blue regions, as a result, it emits a...
green color. It can be used as a natural coloring agent and have antioxidant, antibacterial, and deodorizing properties. A compound with antioxidant activity can donate its hydrogen atom to DPPH as free radicals, which are characterized by the occurrence of purple to pale yellow. Antioxidant activity of Chlorella compounds that rich in protein, contributed by functional groups or the residue of amino acids. The antioxidant activity as IC$_{50}$ of C. vulgaris in acetone was 57.25 ppm, which can function as an scavenger of free radicals and able to minimize the concentration of the ROS. Antioxidants such as chlorophyll in cosmetics can work as oxidizing agents that provide glowing skin by preventing skin damage. An antioxidant also helps in skin tightening, reduction of wrinkles, and reduces inflammation. Antioxidants from Chlorella have a weakness on its instability and sensitivity to heat, oxygen, pH, illumination, and acid degradation that can change its color. One of the technology to increase the stability of chlorophyll is encapsulation method. The encapsulation process can be performed by spray drying using maltodextrin as a matrix encapsulant. Spray dryers commonly used for antioxidants encapsulation because of the fast method, low process, low temperature needed and can produce variance sized powder. Maltodextrin used as an encapsulant because it has low solubility in water, high viscosity, and relatively low process, able to protect the bioactive compound from degradation. This research aimed to obtain chlorophyll antioxidants from C. vulgaris and applied to cosmetics in the form of creams and lotions.

2. Materials and Methods

2.1. Materials
Chlorella vulgaris was obtained from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. The cultivating system of C. vulgaris was conducted on open pond. Maltodextrin was procured from Bratachem. Chemicals used in this study including acetic acid, ethanol, K$_2$SO$_4$, CuSO$_4$, H$_3$BO$_3$, NaOH, H$_2$SO$_4$, and HCl were purchased from Merck. Distilled water was obtained from Ikapharmindo Putramas, while methanol and hexan were purchased from J.T. Baker. DPPH reagent and ascorbic were obtained from Sigma-Aldrich. Material for cosmetics aquades, methyl parabens, virgin coconut oil, cetyl alcohol, propylene glycol, sodium lauryl sulfate, propylene parabens, glycerin, trienolamin, stearate acid, cetyl alcohol, and lanolin.

2.2. Extraction of C. vulgaris
Separation of Chlorella vulgaris from the medium was preceded by centrifugation on 17.000 rpm/min, the supernatant is Chlorella vulgaris. C. vulgaris diluted on distilled water with various concentration 0.01; 0.05; 1 and 0.1% w/v, respectively. C. vulgaris was extracted with maceration for 1 hour, then separated for its filtrate and supernatant, by centrifugation on 6000 rpm for 15 minutes. The filtrate was used as chlorophyll content.

2.3. Production of chlorophyll powder using maltodextrin
The chlorophyll solution with variation concentration 0.01; 0.05 and 0.1% were blended with maltodextrin 100 mg/l in each solution. Then, chlorophyll powder was reduced using spray drier that operated at condition inlet 120 to 130 °C, outlet 42 to 44 °C, blower 1.01m$^3$/min to 1.04 m$^3$/min and atomizing 200 to 230 kPa.

2.4. Determination of antioxidant activity
Antioxidant content was analyzed by Ascorbic acid Equivalent Antioxidant Capacity (AEAC), as the capacity of sample as antioxidant which react with DPPH as free radical. The samples were mixed with methanol DPPH then homogenized and stored for 120 minutes. The absorbance was recorded at 517 nm. Distilled water was used as blank and ascorbic acid was served as standard.
2.5. Analysis of water content and ash content
Water content and ash content were analyzed by using gravimetric method according to SNI 01-2891-1992 point 5.1 and 6.1, respectively. Water content was determined by weighing 1 g sample and dried it at 105 °C for 3 hours. Samples were placed in the desiccator until stable, and then the weight was determined. Subtract the weight before with after drying to get water content (%). Ash content was determined by weighing 2 g sample and furnace it at 600 °C for 2 hours. The ash content (%) was calculated based on the loss of weight before and after treatment.

2.6. Analysis of total fat, protein, and carbohydrate content
Protein content was analyzed by biuret method (Kjeltec) according to SNI 01-2891-1992. Total fat was determined by using soxhlet-hydrolysis method, using hexane. Carbohydrate percentage was measured using this formula: Carbohydrate = 100 – (%water content + %ash content + %protein + %lipid).

2.7. Analysis of morphology
Chlorophyll-maltodextrin rheology was determined by using SEM, the sample was coated with gold (Au) and observed using Scanning Electron Microscope (SEM) at 500x and 4000x magnifications on 20 kV voltages.

2.8. Production of chlorophyll cream
The ingredients used for making the cream consist of the water phase and the oil phase. Ingredients as water phase were distilled water and methyl parabens, which were heated above a water bath at 65 to 70 °C. Ingredients as oil phase were virgin coconut oil, cetyl alcohol, propylene glycol, sodium lauryl sulfate and cetyl stearil alcohol, which were melted at 65 °C – 70 °C while stirring using a hot plate magnetic stirrer at 200 rpm. Then, the oil phase was added to the water phase, while stirring it by using a homogenizer at 200 rpm for 15 minutes, to prevent air bubbles formation. If both phases were already well mixed, increased to 800 rpm for 10 minutes to form a cream base. After the cream base formed, extract alga (chlorophyll powder) was added, stirring for another 5 minutes until the cream homogeneously mixed.

| Ingredients                  | Composition (%) |
|------------------------------|-----------------|
| Extract alga                 | 4               |
| Distilled water              | 40              |
| Methyl parabens              | 0.18            |
| Propylene glycol             | 5               |
| Cetyl stearil alcohol        | 4.5             |
| Cetyl alcohol                | 5               |
| Sodium lauryl sulfate        | 0.5             |
| VCO                          | add until reach 100 |

2.9. Production of lotion
The ingredients used in making the lotion consist of the water phase and the oil phase. Ingredients served as water phase were distilled water, glycerin, triethanolamine, and methyl parabens, which were heated above a water bath at 65 to 75 °C. Ingredients employed as oil phase were stearate acid, cetyl alcohol, lanolin, and propylene parabens, which were melted at 65 to 75 °C. After all phases dissolved, the water phase was added to the oil phase while constantly stirring to form an emulsion. Then, chlorophyll powder was added in the emulsion and mixed until homogeneously.
Table 2. Formulation of lotion.

| Ingredient         | Composition (%) |
|--------------------|-----------------|
| Extract alga       | 3               |
| Stearic acid       | 6               |
| Methyl parabens    | 0.02            |
| Lanolin            | 3               |
| Triethanolamine    | 3               |
| (TEA)              |                 |
| Cetyl alcohol      | 6               |
| Prophylene parabens| 30.18           |
| Glycerin           | 3               |
| Aquadest           | Add until reach 150 |

2.10. Microbial contamination analysis
Microbial contamination analysis of bacteria refers to ISO 21149:2006, yeast and mould refers to ISO 16212:2008, Pseudomonas aeruginosa refers to 22717:2015, Candida albicans refers to ISO 18416:2015, and Staphylococcus aureus refers to ISO 22718:2015. Enumeration and detection for bacteria, yeast and mould were analyzed by total plate count method (TPC) which involves enumeration of microbial colonies on a non-selective agar medium on mesophilic aerobic condition. Detection of P. aeruginosa, C. albicans and S. aureus were performed by checking the absence of microbial target growth after enrichment. First step was the enrichment by using a non-selective broth medium. The second step (isolation) was performed on a selective medium followed by identification tests. P. aeruginosa, C. albicans and S. aureus can cause skin or eye infections.

3. Result and Discussion

3.1. Extraction of C. vulgaris and production of chlorophyll powder
The bioactive compound on C. vulgaris is chlorophyll, which is known to has antioxidant properties and has a sensitivity and unstable characteristic. The extraction method was performed by maceration with distilled water as solvent, for one hour. Extraction chlorophyll by maceration method was also performed by Aryanti, Nafiunisa, and Wilis (2016). The research needs 3-hour maceration to extract chlorophyll from Pleomele angustifolia leaves. Maceration method can keep the bioactive compound from heating because it was done without heating, only by stirring. The maceration method also commonly used because it’s fast, environmentally friendly, and effectively cost, so it easy to expanded it in the industrial scale. The production of chlorophyll powder was using a spray dryer, with maltodextrin as a coating. In this research, spray dryer was commonly used for antioxidant as the process will be on low temperature condition. Maltodextrin has a glucose chain that effects on moisture and ash content. The ash content decrease because the addition of maltodextrin will increase of evaporation.

3.2. Moisture and ash content
The moisture content of chlorophyll powder ranged between 5.34 to 5.58 g per 100 g (figure 1). The highest moisture content was on the powdered C. vulgaris 0.05%, and the lowest on C. vulgaris 0.01%. The moisture content of all C. vulgaris powders were below 10%. The moisture content indicates the quality of a product (powder), according to the Indonesian Pharmacopoeia or Materia Medika Indonesia, the water content in a product should be <10%. According to the previous research, encapsulation chlorophyll by spray drying produces a moisture content below 10%.

The ash content, ranged between 0.17 to 0.26 g per 100 g, thus increase with the addition of concentration of C. vulgaris (figure 2). Spray dryer reducing water content and water activity. Maltodextrin has a glucose chain that effects on moisture and ash content. The ash content decrease because the addition of maltodextrin will increase of evaporation.
3.3. Carbohydrate, protein and total fat content

The carbohydrate content of chlorophyll powder ranged between 93.5 g/100 g until 93.95 g/100 g (figure 3). The highest carbohydrate content was powdered _C. vulgaris_ 0.05%. The addition of maltodextrin increased the carbohydrate content because its component is carbohydrate.

The highest protein content was powdered _C. vulgaris_ 0.1% with protein content 0.69 g per 100 g (figure 4). The addition concentration of _C. vulgaris_ increases the protein content because it’s rich in protein, contributed by functional groups or the residue of amino acids. The protein content of _C. vulgaris_ reaches 42–58% proteins per dry weight of biomass.

Total lipid content on microalgae in open pond cultivating system is 3.18%, which usually present in the form of glycerol and fatty acids. Total fatty acid content on microalgae in open pond cultivating system is 0.9 to 22.6%, which majority compound are palmitic acid (C16:0) 22.6% and stearic acid (C18:4) 21.4%. _C. vulgaris_ was macerated with water as polar solvent, due to hydrophobic of fatty acids that interact with non-polar or non-polar-polar solvents (biphasic system), the fat content was not detected on _C. vulgaris_ various concentration. Lipid can be extracted using n-hexane-methanol which can break the bond between lipid and protein.

3.4. Morphology by SEM

Morphology was analyzed by SEM with magnification of 500x and 4000x (figure 5). The morphology of the powder displayed a spherical structure, separate dispersedly, and has a smooth form. These results revealed a homogeneous fluorescence distribution across all-spherical particles. The homogeneous distribution of fluorescence demonstrates the mono-dispersity of chlorophyll inside each sphere. In line with the addition of concentration _Chlorella_, the form is anisotropic wavy form and tends to rupture. Based on previous studies, emulsions microencapsulation is better than powder encapsulation because the size of particles can be controlled until less than 10% variance. Furthermore, the encapsulation could increase the stability of chlorophyll as an antioxidant.
3.5. Antioxidant content

The antioxidant content of *C. vulgaris* powder was increasing with the concentration addition of *C. vulgaris*. The antioxidant content was not detected on powders with concentration *C. vulgaris* 0.01 and 0.05%. The highest antioxidant content was 11.83 mg Vit. C/100 g sample, produced by powdered *C. vulgaris* 0.1% (figure 6). The antioxidant activity test was conducted by in vitro using the free radical reduction method, by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The powder with a concentration of *C. vulgaris* 0.1% can donate its hydrogen atom to DPPH as free radicals, which are characterized by the occurrence of purple to pale yellow. *C. vulgaris* has the highest antioxidant activity compared to other Chlorella strains using DPPH method.

The antioxidant activity linked to bioactive compounds, such as chlorophylls. The antioxidant from *Chlorella* has a weakness, for its un-stability and sensitivity to heat, oxygen, pH, illumination, and acid degradation that can change its color. The encapsulation method using spray drying enhanced the stability of chlorophyll. The encapsulant like maltodextrin able to protect antioxidant and bioactive compound release, which induced by the heat on the spray drying process. Beneficially of using spray drying process are water evaporation occurs on a very wide surface, shorten the time for drying process, and increase the thermal stability of bioactive compound because the addition of maltodextrin.

*C. vulgaris* is a species that is often used in cosmetics because it has a bioactive compound like chlorophyll, which as an antioxidant able to minimize the concentration of the ROS. Chlorophyll as antioxidant in cosmetics has a function as an oxidizing agent which providing glowing skin by preventing skin damage. Antioxidants also help in skin tightening process, reduction of wrinkles, and reduces inflammation. In this research, chlorophyll powder with the highest antioxidant activity applied to cosmetics in form of cream and lotion. Cream cosmetics has antioxidant content was 4.95 mg Vit C / 100 g sample with antioxidant activity 719.75 mg/ml and antioxidant content on lotion was 4.73 mg Vit C / 100 g sample with antioxidant activity 660 mg/ml (figure 6 and table 3). Both cream and lotion have antioxidants content and activity which was not different descriptively. In this research, the antioxidant capacity wasn’t influenced by the type of cosmetic, whether its cream or lotion.
Figure 6. Antioxidant capacity of *C. vulgaris* powder and cream and lotion.

Table 3. Antioxidant Capacity of Cosmetics.

| Cosmetics  | IC₅₀ (mg/mL) |
|------------|-------------|
| Cream      | 719.75      |
| Lotion     | 660         |

3.6. Antioxidant content

The microbial contamination in cosmetics was analyzed on Total Plate Count, yeast and mold, *P. aeruginosa*, *C. albicans*, and *S. aureus*, according to the Regulation of the Republic of Indonesia Drug and Food Supervisory Agency (2014) concerning Amendments to BPOM Regulation Number HK.03.1.23.07.11.6662 in 2011. Based on the analysis of microbial contamination (table 4), both of cosmetic, cream and lotion were not containing microbial contamination. It concludes that the cream with the composition above is safe to be used.

Table 4. Microbial contamination of cosmetics.

| Parameter        | Unit      | Result          |
|------------------|-----------|-----------------|
| Total Plate Count| CFU/g     | < 1.0 x 1₀⁹     |
| Yeast and Mould  | CFU/g     | < 1.0 x 1₀⁹     |
| *P. aeruginosa*  | Per g     | Negative        |
| *C. albicans*    | Per g     | Negative        |
| *S. aureus*      | Per g     | Negative        |

3.7. Antioxidant content

*C. vulgaris* has a green color with the bioactive compound chlorophyll. The color from chlorophyll already used as a natural pigment in cosmetics. Chlorophyll also used on skin aging supplements and cosmeceuticals to support collagen repair mechanisms. The chlorophyll powder product from the spray drying process with maltodextrin has a green color. From the organoleptic evaluation for cosmetics (table 5), both of cosmetics cream and lotion has a similar color with the powder. The color was stable until 8 weeks. The cosmetics (cream and lotion) were not having aroma or odor, and the texture still smooth until 8 weeks. The lotion was more liquid than cream. The homogeneity was excellently stable until 8 weeks, without bilayer formation or agglomeration. The cosmetics were relatively stable.
4. Conclusion
The bioactive compound from *C. vulgaris* was extracted by the maceration method, and then encapsulated by spray drying method. The best powder based on its antioxidant capacity, was *C. vulgaris* 0.1% which gave 11.83 mg Vit C per 100 g sample. The powders have a spherical structure, separate dispersedly, and smooth form. The best powdered *C. vulgaris* was applied to cosmetics as cream and lotion form. The antioxidant content in cosmetics type creams was 4.95 mg Vit C / 100 g sample with antioxidant activity 660 mg/ml. The antioxidant capacity of microalgae for biotechnological applications.

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