Antioxidant activity of *Porphyridium cruentum* water extracts for cosmetic cream

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**ABSTRACT.** *Porphyridium cruentum* is one of the microalgae that has antioxidant capacity. These antioxidants are useful for the cosmetics and pharmaceutical industries. The purpose of this study was to obtain antioxidants from *P. cruentum* which will be used in cosmetic products as a cream. *P. cruentum* was extracted by maceration method for one hour with water as solvent (5 g/L) and encapsulated using maltodextrin with a spray dryer. The variable concentration of maltodextrin was 50 g/L, 100 g/L, and 150 g/L. Characterizations observed for encapsulated microalgae extracts were the antioxidants capacity and proximate analysis; hence for cosmetic cream were the antioxidants capacity and microbiological contaminant. The best result was *P. cruentum* extract encapsulated with maltodextrin 50 g/L which gave antioxidant capacity as AEAC 7.07 mg vit. C/100 g sample and IC₅₀ 484.09 mg/mL. The cosmetic cream with addition encapsulated *P. cruentum* extract gave antioxidant capacity as AEAC on 8.12 mg Vit. C/g sample and IC₅₀ 29.08 mg/mL. The microbial and heavy metals contaminant for cream were below the threshold according to BPOM regulation. Based on these results, the *P. cruentum* has the potential to be used in the cosmetics industry.

1. **Introduction**

*Porphyridium cruentum* is one of the unicellular red alga which belongs to Rhodophyta, which lives naturally on neutral water and seawater [1]. *Porphyridium cruentum* lives solitary and colony. As colony *P. cruentum* capsulated with mucilage, a sulfated polysaccharide soluble in water [2–4]. The dominant pigment in *P. cruentum* is phycoerythrin and phycocyanin, which contain phycobilin. *P. cruentum* have chlorophyll a and d, and lack of chlorophyll b. All of these pigments contributed to photosynthesis [4,5]. Phycobilisome (PBS) located on the outer surface of thylakoid; therefore, several mechanisms performed to extracting the PBS. The membrane cell of *P. cruentum* constructed by two layers, the first layer is pectic, and the second layer is cellulosic microfibrils [2,5].

Biomasses compositions of *P. cruentum* are 32.1% (w/w) carbohydrate; 34.1% protein; Fatty acid consist of 1.6% for hexadecenoic acid; 0.4% of linoleic acid; 1.3% of arachidonic acid; and 1.3% for eicosapentaenoic acid. Minerals on 100 g dry biomass are Ca (4960 mg), K (1190 mg), Na (1130 mg), Mg (629 mg), and Zn (373 mg). *P. cruentum* also contains tocopherol, flavonoid, alkaloïd, phenol hydroquinone, and carotenoid [2]. With those compositions, *P. cruentum* has pharmaceutical properties like an antibiotic, antihyperglycemic, and antioxidant [3,6,7]. *P. cruentum* also has been already used as a cosmetic compound for conditioning agents (Asta technology), moisturizing agent, self-tanning agent, anti-acne agent, and anti-redness (Green-tech); and anti-wrinkle agent or anti-aging, film former and moisturizing agent (Givaudan active beauty) [8].

Antioxidant properties have a beneficial role in the cosmetic area by combating the damage caused by a free radical agent (Radical Oxygen Scavenger and Radical Nitrogen Scavenger). The
accumulation of uncontrolled free radicals can lead to severe diseases like skin cancer [9]. Free radicals also can lead the skin to wrinkle; therefore, the antioxidant activity correlates with anti-aging. Antioxidant properties of *P. cruentum* have several mechanisms to neutralize free radical, like hydrogen transfer (proton donating ability). Algae pigments have potential as an electron-directing agent in trans-membrane migration of electrons against oxidative stress by metal reduction [10]. Phenolic compounds have redox properties, which engage in absorbing and neutralizing free radicals, quenching singlet, and triplet oxygen or decomposing peroxides [9]. Antioxidant activity of protein contributed by functional groups or the residue of amino acids [11]. Polysaccharide contributed to antioxidant activity by combining several factors like the oligomeric, sulfate, and glycoprotein components of the polymer [7]. Those bioactive compounds were unstable and sensitive to the process condition. One of the technologies that can be used to protect the bioactive compound from the uncontrolled condition was encapsulation.

The encapsulation process can be done by several methods; one of the methods that generally used is spray drying. Encapsulation will convert the bioactive compound from liquid form to solid form (better mixing and prevent lumping), coating the bioactive compound by carrier material to prevent evaporation and to degradation of the bioactive compound by undesirable circumstances while on process production [12]. Encapsulation by spray drying was influenced by several factors like inlet and outlet temperature, aspirator rate, nozzle rate, pump rate, and materials concentration used for encapsulation [13].

Encapsulation with spray drying can maintain the antioxidant capacity of *P. cruentum* [14]. The encapsulated material has to have high emulsifier ability, able to maintain the flavor of a compound, accelerate the drying rate of a compound, and able to release the encapsulated compound. Maltodextrin was used as encapsulated materials because it is soluble in water, not toxic, and able to protect the bioactive compound from degradation [15]. This research aims to obtain antioxidants from *P. cruentum*, which will be applied to cosmetic products as cream.

2. Materials and methods

2.1. Materials

*Porphyridium cruentum* procured from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. The material used were maltodextrin (Bratachel), 2,2-diphenyl-1-picrylhydrazyl (Sigma), butylated hydroxytoluene (Sigma), ethanol (Merck), acetic acid (J.T. Baker), potassium sulphate (Merck), CuSO₄ (Merck), H₂BO₃ (Merck), sodium hydroxide (Merck), sulfuric acid (Merck), chloric acid (Merck), hexane (J.T. Baker), isopropyl alcohol (Merck), methylene chloride (Merck), diethyl ether (Merck), acetonitrile (Merck), HNO₃ (Merck), aquadest, and aquabidest (Ikapharmindo Putramas).

2.2. Instrumentation

The instruments used were homogenizer (Miyamoto Riken), spray dryer (Eyela), and centrifugation (Kokusan), Kjeldahl (Kjeltec FOSS), oven (Memmert), Atomic Absorbance Spectrophotometry (AAS) (Shimadzu), Soxhlet evaporator (Buchi), spectrophotometer UV-VIS (Shimadzu UV-1280), Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) and Scanning Electron Microscope (SEM) (Jeol JSM-6510LA).

2.3. Procedure

2.3.1. Extraction of *Porphyridium cruentum*. *P. cruentum* that has been separated by maceration media with water (1% w/v) 190 rpm, for 1 hour at room temperature. Separation with debris was done by centrifugation at 6000 rpm for 15 minutes.

2.3.2. Encapsulation of *Porphyridium cruentum* Extract. *P. cruentum* extracts were mixed with maltodextrin, as a matrix for spray-dried process, for 60 minutes. The ratio of variation on *P. cruentum* extract (mL): maltodextrin (g) were 20:1 (F50), 10:1 (F100) and 6.7:1 (F150). Spray dry running with
condition inlet 120°C - 130°C, outlet 41°C - 44°C, blower 1.01 m³/min – 1.04 m³/min and atomizing 200 kPa - 230 kPa.

2.3.3. Determination of antioxidant capacity. Antioxidant capacity were determined by Ascorbic acid Equivalent Antioxidant Capacity (AEAC) method using spectrophotometric on 517 nm, by measured the capacity of free radical content which reacted with 2,2-diphenyl-1-picrylhydrazyl. As blank and standard were aquadest and butylated hydroxytoluene, respectively [16].

2.3.4. Analysis of moisture content and ash content. Analysis of moisture content and ash content by gravimetric was regarding SNI 01-2891-1992 point 5.1 and 6.1, respectively [17].

2.3.5. Analysis of lipid, protein and carbohydrate content. Lipid content was determined by Soxhlet-hydrolysis method. The analysis was conducted by warping the sample by filter paper then Soxhlet with hexane. Determination of protein contents by biuret method (Kjeltec) referred to SNI 01-2891-1992 point 7.1. Carbohydrate content was determined by calculation. Carbohydrate = 100 – (%water content + %ash content + %protein + %lipid) [17].

2.3.6. Analysis of morphology. Rheology of encapsulated Porphyridium cruentum was determined using Scanning Electron Microscopic (SEM), by coating the sample with gold (Au) and observed it at 500x and 4000x times magnifications [18].

2.3.7. Physical evaluation cream. Analysis of pH was using a pH meter that already calibrated using a buffer solution of pH 4 (four) and pH 7 (seven). The organoleptic analysis was examined based on the sensing process, to observe the physical stability of the cream on transformation in texture, color, and odor during storage time. The observations were done for 8 weeks, on room temperature [19].

2.3.8. Microbial and heavy metal contamination analysis. Analysis of Total Plate Count was determined by pour plate which referred to ISO 21149:2006 [20]. Analysis of mold and yeast were determined by pour plate that referred to ISO 16212:2008 [21]. Detection of microbial contamination by Pseudomonas aeruginosa, Candida albicans, and Staphylococcus aureus were according to ISO 22717:2015 [22], ISO 18416:2015 [23], and ISO 22718:2015 [24], respectively. Lead and cadmium were detected with ICP-OES; mercury and arsenic were detected with AAS.

3. Results and discussions

3.1. Ash and moisture content
The moisture content ranged from 6.24/100 g - 7.59 g/100 g, with the lowest result was F50. The ash content ranged from 14.77/100 g - 32.70 g/100 g, with the lowest result was F150 (figure 1). The highest for moisture content and ash content were F100. Fuentes [2] observed that the ash contents of Porphyridium cruentum were ranging from 16.8 g to 23.6 g. The moisture content of Porphyridium cruentum was low, with a mean of 3.24 g, a value in the range of general recommendations which was less than 10% [2]. Maltodextrin encapsulation will hold the bioactive compound of Porphyridium cruentum and prevent it from loss by high temperature. The water could cause the variable of moisture content to escape through the surface of a particle through diffusion, avoiding the crust of particle [25], based on its encapsulation form. The encapsulation shape of F100 was round and has less dents than F50 and F150, but the encapsulated shape and agglomerate formed was irregular, which tended to break, causing higher moisture and ash content.
3.2. Total fat, protein and carbohydrate

The *Porphyridium cruentum* was polar extracted by water, with fatty acid that consisted of a non-polar compound like hexadecenoic acid, linoleic acid, arachidonic acid, and eicosapentaenoic acid [2] might lead the low result or undetected of total fat. The protein content of *Porphyridium cruentum* was around 0.07 g/100 g - 0.34 g/100 g (figure 2). Research by Safi [28] concluded that *Porphyridium cruentum* didn’t need complicated extraction for the protein due to its fragility of pseudo-cell wall. Alternatively, several methods for cell disruption and extraction of *Porphyridium cruentum* has been developed using high-pressure cell disrupter [26] and pressurized liquid extraction (PLE) [27]. *P. cruentum* protein contribute 35% of biomass, the protein consists of 38.7% essential amino acids, and 61.0% non-essential amino acids, with optimum pH for extraction were 12-14 [28,29]. The low concentration of protein in this research might be due to several factors like the cell lysis before extraction because of the fragility of its cell wall and also can be due to the growing condition (pH medium, medium composition, and harvested time). The recommended time to harvested *Porphyridium cruentum* is 12-16 days, after that the *Porphyridium cruentum* tend to rupture and dissolve in the medium. Due to its diameter which ranging between 6 and 10 µm, the high-speed centrifugation was the optional step to separate biomass from the medium [30–32]. From SEM analysis, we concluded that rupture on F150 more than F50 causes the high protein concentration on F150. The carbohydrate of *Porphyridium cruentum* were around 30% [2], the addition of maltodextrin (C$_6$H$_{12}$O$_5$)$_n$H$_2$O that is starch hydroxylate (polymer of saccharide) will add the carbohydrate content.

3.3. Antioxidant capacity

The antioxidant capacity, which represents by the inhibition value, describes the ability of a compound as antioxidant to scavenge DPPH as free radical. Purple chromogen of DPPH reduced by antioxidant or reducing compound to pale yellow hydrazine. The reaction mechanism is based on an electron transfer reaction, while the hydrogen atom transfer reaction is a marginal reaction pathway, on the type strong hydrogen-bond-accepting solvent [33]. The inhibition value ranged from 0.87 % – 4.80 % (figure 3). The highest antioxidant content, near the raw material, was on F50 which give 4.80 % on sample concentration 5%. Research by Hasanah [14], non-encapsulated and encapsulated *P. cruentum* on 6.25 ppm gives inhibition of 3.05% and 2.85 %, respectively. The capsulation was done with the addition of 75 g/L maltodextrin and observed with DPPH assay [14]. The addition of maltodextrin can maintain the antioxidant capacity of the bioactive compound on the encapsulated form. Encapsulation can prevent degradation because of light, oxygen, and slow down the evaporation rate. Encapsulation can be used in the spray drying process for the heat-labile compound [12,15].
From our previous research on the encapsulation of *Spirulina* on maltodextrin as cosmetic raw materials, the protein showed a significant result with the antioxidant activity (as inhibition capacity) [34]. Because of the high protein content in *Spirulina* around 60-70% (including PBS), this contribute to its antioxidant capacity. Phycocyanin and phycoerythrin contains linear tetrapyrols, with double bonds and reducing ability, that contributing to the total antioxidant activity [11,28]. But that trend wasn’t showed by *Porphyridium cruentum*. Thos can be due to *Porphyridium cruentum* which has multiple compounds that contribute to its antioxidant activity besides PBS, like polysaccharide. Polysaccharide on *Porphyridium cruentum* consists of xylose, glucose, galactose, arabinose, rhamnose, and mannose. The cell-wall complex also contains sulfate, glucuronic acid, and a non-covalently glycoprotein [35]. The polysaccharide of dry matter *Porphyridium cruentum* (2 mg/mL) give inhibition rate until 20%, detected with TBA assay [7].

The susceptibility of maltodextrin was unable to form an emulsion, unable to form crosslinks with bioactive compounds, and influenced by the viscosity. Therefore, with a high concentration of maltodextrin will lead lower antioxidant activity.

3.4. Analysis morphology
The morphological characteristic of encapsulated *Porphyridium cruentum* extract shown in figure 4. F50 and F100 had the particle with smoother and spherical surface rather than F150, which had dent
surface. F50 had isotropic globular form and separate dispersedly, rather than F100 which gave anisotropic globular form and agglomerate. F150 also had anisotropic wavy form and tended to rupture. The increase of maltodextrin concentration gave wavy particle and cracked surface. The increase in surface roughness might increase the distance between surface, thus reducing the adhesive force and eventually decrease the wall deposition [25].

Figure 4. SEM from encapsulated *Porphyridium cruentum* extract.

Table 1. The analysis result of cosmetic cream using encapsulated *Porphyridium cruentum* extract.

| Parameter            | Unit       | Result     | BPOM (2011)       |
|----------------------|------------|------------|-------------------|
| IC50                 | mg/mL      | 29.08      |                   |
| AEAC                 | mg Vit. C/100 g | 8.12     |                   |
| Sample concentration 10% | sample   |            |                   |
| Inhibition value     | %          | 10.22      |                   |
| Sample concentration 10% |          |            |                   |
| Total Plate Count    | CFU/g      | 5.9 x 10²  | < 10³             |
| Yeast and Mould      | CFU/g      | < 1.0 x 10¹| < 10³             |
| *P. aeruginosa*      | Per g      | Negative   | Negative          |
| *C. albicans*        | Per g      | Negative   | Negative          |
| *S. aureus*          | Per g      | Negative   | Negative          |
| Mercury (Hg)         | mg/kg      | n.d.       | < 1               |
| Lead (Pb)            | mg/kg      | n.d.       | < 20              |
| Arsenic (As)         | mg/kg      | n.d.       | (LOD 0.08)        |
| Cadmium (Cd)         | mg/kg      | n.d.       | (LOD 0.02)        |

n.d. = not detected
3.5. Encapsulated *Porphyridium cruentum* extract as cream for cosmetic

*P. cruentum* extract was applied as a cosmetic cream compound as natural antioxidant sources [36,37]. One of the antioxidants compound in *Porphyridium cruentum* is Phycoerythrin. According to Gianetti [38], the application of antioxidant substances in cosmetic formulations able to protect the skin against oxidative damage by UV radiation and prevent signs of aging [38]. Cosmetic creams with *Porphyridium cruentum* extract were analyzed for the antioxidant capacity and microbiological contamination according to BPOM. The analysis results for cosmetic cream with encapsulated *Porphyridium cruentum* extract were shown on table 1. The increasing of antioxidant activity on cream was supported by others compound and also the formation of emulsion. Emulsion can maintain antioxidant activity and prolonged the antioxidant stability [39].

Observation on cosmetic creams was performed on pH and organoleptic. The pH value of cosmetic creams was still in the normal pH range. According to the Indonesian National Standard (SNI) 16-4399-1996 [40], the pH of the cream is in the range of skin pH, which is around 4.5 - 8.0. The cream pH can affect the stability and contamination of the cream, and also influence the skin as a topical cream. If the pH of cream not in between the range of skin pH, it will trigger skin irritation. According to the Regulation of the Republic of Indonesia Drug and Food Supervisory Agency (BPOM) concerning Amendments to BPOM Regulation Number HK.03.1.23.07.11.6662 in 2011, the limit of microbial contamination as Total Plate Count no more than 107 colonies/g; Yeast and Mold no more than 103 colonies/g [41]. The heavy metals content was undetected on the cream. Based on the analysis above, it can be concluded that encapsulated *Porphyridium cruentum* can be applied as natural antioxidant on cream as cosmetic.

Evaluation of cosmetic creams also observed organoleptically (table 2). Visually, the cream color with the addition of *Porphyridium cruentum* extract which contain phycoerythrin as natural antioxidant was white (figure 5). We assume the low concentration of phycoerythrin may affect the color of the cream, further analysis needed to strengthen the hypothesis. The color of cosmetic creams was stable until eight weeks, and shown no changes. The smell of the cream wasn’t given by rotten odor until the 8th week. This shows that the oil phase in the cream wasn’t undergoing oxidation. Discoloration and odor caused by oxygen from the air, can oxidize the fat or oil phase; besides that, light can also induce oxidation reactions [19]. The benefit for using natural antioxidant which also have antibacterial activity is the inhibition on microbial growth on the cream thus can be related by the reduction of concentration of preservative for the cream. *Porphyridium cruentum* extract which contains phenolic compound exhibited antioxidant and antimicrobial capacity, due its functional group. On pH range of the cream, it could maintain the stability of color, odor, texture and homogeneity until eight weeks.

### Table 2. Organoleptic observations on *Porphyridium cruentum* cream.

| Weeks | Color  | Odor    | Texture | Homogeneity |
|-------|--------|---------|---------|-------------|
| 1     | White  | No odor | Smooth  | Homogeneous |
| 2     | White  | No odor | Smooth  | Homogeneous |
| 3     | White  | No odor | Smooth  | Homogeneous |
| 4     | White  | No odor | Smooth  | Homogeneous |
| 5     | White  | No odor | Smooth  | Homogeneous |
| 6     | White  | No odor | Smooth  | Homogeneous |
| 7     | White  | No odor | Smooth  | Homogeneous |
| 8     | White  | No odor | Smooth  | Homogeneous |
Figure 5. *Porphyridium cruentum* cream on week 1 until 8 (a) and close view from *Porphyridium cruentum* cream on week 8.

4. Conclusion

The bioactive compound from *Porphyridium cruentum* could be extracted by maceration and encapsulated with maltodextrin by drying method with a spray dryer. The identification results on *Porphyridium cruentum* extracts indicated that it had capacity as antioxidant; the pinkish color on *Porphyridium cruentum* extract powder came from phycoerythrin. The best condition process obtained when *Porphyridium cruentum* extract encapsulated with maltodextrin 50 mg/L, with an antioxidant capacity as AEAC on 7.07 mg Vit. C/100 g sample and IC\textsubscript{50} 484.09 mg/mL.

Applications in cosmetic creams showed antioxidant capacity as AEAC on 8.12 mg Vit. C/100 g sample and IC\textsubscript{50} 29.08 mg/mL. Organoleptic analysis of cosmetic creams showed stable results for color, odor, texture, and homogeneity during eight weeks of storage at room temperature. Microbial and heavy metals contamination analysis were below the threshold according to BPOM regulations.

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