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Preliminary Study the Potency of Macroalgae in Yogyakarta: Extraction and Analysis of Algal Pigments from Common Gunungkidul Seaweeds

Riswi Haryatfrehni*, Shinta Candra Dewi, Afra Meilianda, Selvi Rahmawati, Ihda Zuyina Ratna Sari

Faculty of Biology Universitas Gadjah Mada, Jl. Sekip Utara Yogyakarta 55281, Indonesia

Abstract

Study on pigment for industrial purposes becomes a fascinating area so motivates the exploration of pigment resource candidates. The research aimed to get preliminary data about types of macroalgal pigment which were possibly produced by Gunungkidul seaweeds. Research conducted in March, 2013 in Porok Beach, Gunungkidul to selected seaweed samples. Pigment extraction was done using spectrophotometric method to determined non polar and polar pigments. The results showed common seaweeds in Gunungkidul which member of Chlorophyta had chlorophyll a, chlorophyll b, and carotoid whereas Rhodophyta contained chlorophyll a and phycoerythrin.

Keywords: Gunungkidul; macroalgae; pigment; spectrophotometry

* Corresponding author. Tel.: +62 852 2867 7400;.
E-mail address: r.haryatfrehni@gmail.com

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1. Introduction

Generally macroalgae is divided into Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) based on the pigmentation. The difference among algal pigments is an environmental adaptation which is needed to optimize light trapping for photosynthesis process in different depths. There are three types of macroalgal pigment namely chlorophyll, carotenoid, and phycobilin (Fig.1). Chlorophyll and carotenoid are non-soluble water pigments whereas phycobilin is a group of protein which dissolves in water\textsuperscript{1,3}.

Chlorophyll is a major pigment in most of photosynthetic organisms, especially chl \(a\). Chlorophyll exists in different kind for each organism, such as chl \(a\), chl \(b\), chl \(c\), chl \(d\), etc, each of which are distinguished by various substituents but the main component, which is the porphyrin ring, remains the same. Other pigments act as accessory pigments to expand the absorbed light spectrum and protect the photon-harvesting system. Carotenoid is isoprenoid polyena consisting eight units of isoprena (C5). Based on the structure, carotenoid is divided into carotene and xanthophyll. Xanthophyll or carotenol is derivative of oxidized carotene which is more polar than carotene, so it can be dissolved in certain polar solvents\textsuperscript{2,4}. Phycobilin has tetrapyrol ring and belongs to chromophore group that is usually found in red algae and cyanobacteria. There are two common kinds of phycobilin, phycoeritrin and phycocyanin. Phycobilin has more unstable structure so it can be easily damaged by light, oxygen, heat, or chemical substances such as urea and piridine\textsuperscript{2,5}.

Fig.1. Chemical structure of macroalgal pigments: (a) chlorophyll \(a\); (b), phycoerythrobilin (c) \(\beta\)-carotene\textsuperscript{2}
Chlorophyta is known to contain chl \(a\) and chl \(b\) as the dominant pigments, Rhodophyta has chl \(a\) and pycobilin, whereas Phaeophyta has fucoxanthin and chl \(c_6\). Several studies have verified the benefits of pigments for human prosperity especially for food, industry, and pharmaceutical. Manufactures have tried to produce natural dyes such as chlorophyll and some carotenoids to replace synthetic dyes that have been known to have carcinogenic effect. Chlorophyll could induce damaged tissue retrieval, enhance immunity, improve blood circulation and digestion system, and treat malignant diseases. Chlorophyll has been developed for cancer photodynamic therapy (PDT) because of its character as photosensitizer\(^8\). Chlorophyll maximum absorbance is between 600 nm to 700 nm so it can produce enough energy to form a substantial yield of reactive oxygen species. Carotenoid is recognized as excellent antioxidant agent and precursor for vitamin A\(^1\), whereas phycobilin is potential as cellular marker that useful for immunodiagnosis and also has immunomodulation performance\(^12,13\).

Since the invention of pigment advantages, pigments have been used in many applications. Pigment necessity is still supplied mostly by microalgae manufacturers, for instance Chlorella as source of chlorophyll, diatom for carotenoids, and Porphyridium for phycobilin. Recently, trend of industry demand of natural product is increasing which encourages pigment source exploration. Indonesia is a tropical country in Southeast Asia which possesses long coastal area but the utilization of marine resources, especially pigment, is yet optimized. Gunungkidul, a district of DIY province, has several beaches with high organism diversity. Macroalgae are abundantly found in intertidal zone of the coast but there is still few study on the macroalgae potency. Local residents have made an effort to produce seaweed chips, mainly Ulva and Enteromorpha in household scale whereas seaweed has many profitable features. This research aimed to determined pigment of common seaweeds in Gunungkidul, DIY in order to understand potency of macroalgal pigments for small to industrial purposes.

2. Materials and methods

2.1. Materials

Six species from Chlorophyta and Rhodophyta which were abundantly found were used as samples. Each sample was taken completely from substrate for identification. The materials used were sea sands, cold acetone 90 % (analytical grade), and cold distilled water. Sea sand was used for maceration, cold acetone was used for non polar pigment extraction while cold distilled water for polar pigment extraction.

2.2. Instrumentations

Instrumentations which were used in this research were zip-lock plastics and ice box for storage, semi-analytic balance, mortar and pestle for thallus maceration, flacon bottles for pigment extract storage, micro pipettes, glassware, micro tubes, centrifuge, aluminum foil, and spectrophotometer UV-VIS Shimadzu Genesys 10 UV scanning.

2.3. Sampling and preparation

Sampling was done in March, 2013 in Porok Beach, Gunungkidul, DIY (8°08’02.73”S 110°33’17.47”T). Samples were stored in zip-lock plastics and were kept in ice box. Samples were cleaned and identified. Flacon for sample storage were wrapped using aluminum foil.

2.4 Pigment extraction

Extraction was performed in the laboratory with low light intensity and temperature below 25 °C. Non polar pigment extraction was performed both for Chlorophyta and Rhodophyta. Five g of clean macroalgae samples of each species were cut into small pieces. Samples were put into mortar then 2 g sea sands were added followed by 6 ml of cold 90 % acetone. It was grinded for 4 min until the solution turned green then poured into flacon. Previous step was repeated by adding 5 ml of 90 % acetone into earlier samples then macerated again for 3 min and poured into the same flacon. One mililiter extract was span using centrifuge for 5 min, and was placed into micro tube.
Polar pigment extraction was performed only for Rhodophyta. Five g of clean macroalgae were cut into small pieces and put into mortar then 2 g of sea sands and 10 ml cold distilled water were added. It was macerated for 3 min until 4 min then 5 ml of cold distilled water was added. Maceration was done for 1 min to 2 min until the solvent turned red. Pigment extract in solvent was poured and filtered into flask then was treated the same way as non-polar pigment.

2.5 Spectrophotometer method

Spectrophotometric method was performed for pigment determination of macroalgae extract. Initially, spectrophotometer was calibrated using 90 % acetone for non-polar pigment determination, while distilled water was used for polar pigment determination. One ml extract was placed into quartz cuvette and solvent was added. Absorbances were recorded from wavelength 400 nm to 750 nm with interval 25 nm. In every wavelength measurement, calibration ought to be done. Absorbance values were converted into line graph for analysis.

3. Result and discussion

3.1. Common seaweed in Gunungkidul

Macroalgae that were found in Porok Beach were *Ulva fasciata*, *Chaetomorpha crassa*, *Enteromorpha intestinalis*, *Gracilaria verrucosa*, *Acanthophora spicifera*, and *Laurencia cartilaginea* as shown in Fig. 2 and 3.

![Fig. 2. Common species of Chlorophyta in Porok Beach: (a) Ulva fasciata; (b) Chaetomorpha crassa; (c) Enteromorpha intestinalis](image1)

![Fig. 3. Common species of Rhodophyta in Porok Beach: (a) Gracilaria verrucosa; (b) Acanthophora spicifera; (c) Laurencia cartilaginea](image2)

Research on diversity of macroalgae in Gunungkidul have been performed, however, more specific study in Porok was yet explored. *Enteromorpha, Ulva, Chaetomorpha* act as epiphyte on intertidal area which are able to absorb nutrient from other organism, therefore those species are dominant in Gunungkidul coastal area which is located in the south of Java Island. Located in Indian Ocean, the coast has strong wave, thus, macroalgae in this area
have small size and slender thallii as adaptation. The community of macroalgae is formed from various species which is strongly attached on death coral reef niches.

Previous monitoring in Sarangan Beach, Gunungkidul\textsuperscript{16} indicated species turnover in Gunungkidul coastline. Some genus tended to growth annually such as *Ulva*, *Cladophora*, and *Chaetomorpha* but other genus appeared in certain month such as *Gracilaria* and *Acanthophora*. Monitoring the availability of macroalgae species is needed since macroalgae is one of pigment sources. Eligible species should available abundantly and continuously exist in order to fulfill industrial demand on pigment. Gunungkidul is located in Indonesia which has two seasons, wet and dry seasons. Certainly several environmental factors changed regularly such as salinity, temperature, wave strength, and nutrient availability\textsuperscript{20,21} which might affect on species diversity. Some species might have small tolerance to environmental alteration suggesting this outcome could be different in other month. Species turnover is very noteworthy to understand the pattern of macroalgae appearance. Macroalgae abundance should be clarified by statistic data such as analysis of percentage cover or species density\textsuperscript{22,23}.

3.2. Pigments determination of macroalgae

This study was a preliminary research to found out types of macroalgal pigment using simple method without analyzing the quantity. Pigment extraction was performed by spectrophotometric method and distinguished by pigment polarity. Pigment absorb visible light which approximately ranged from 400 nm to 700 nm. Acetone is known as good organic solvent for chlorophyll analysis because it gives very sharp Chl absorption peaks\textsuperscript{24,25} although other research have showed that methanol and ethanol gave better result\textsuperscript{26,27}. Members of Chlorophyta exhibited absorption peaks in the range of 400 nm to 475 nm and 660 nm. *Enteromorpha intestinalis* absorbed highly at range 430 nm to 475 nm and 660 nm, *Ulva fasciata* had maximum absorption peak at range 430 nm to 475 nm while *Chaetomorpha crassa* were between 430 nm to 475 nm and 660 nm (Fig. 4).

![Absorption spectra of Chlorophyta](image)

**Fig. 4.** Absorption spectra of Chlorophyta were extracted with 90 % acetone

Pigment from Rhodophyta were also extracted using acetone. Pigment extracted from *Laurencia cartilaginea* shows absorption peak at around 400 nm, 430 nm, and 660 nm, *Gracilaria verrucosa* was undetected, while *Acanthophora spicifera* absorption peak were at between 410 nm to 450 nm and 660 nm, *Ulva fasciata* had maximum absorption peak at range 430 nm to 475 nm while *Chaetomorpha crassa* were between 430 nm to 475 nm and 660 nm (Fig. 5). Fig. 3 and Fig. 4 depict two absorption peaks in the range of 400 to 475 nm and about 660 nm. *Laurencia cartilaginea* (Rhodophyta) were extracted using distilled water resulting absorption peaks at 490 nm and 540 nm; *Gracilaria verrucosa* at 490 nm, 560 nm, and 690 nm; *Acanthophora spicifera* at 490 nm, 540 nm, and 680 nm.
Determination type of pigment was obtained by comparing the maximum absorption peak with the existing literature as can be seen in Table 1 and Table 2.

Table 1. Maximum absorbance of common macroalgae in Porok Beach

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximum absorbance on solvent (nm)</th>
<th>90 % Acetone</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulva fasciata</td>
<td>Chlorophyta</td>
<td>between 430 to 475</td>
<td>-</td>
</tr>
<tr>
<td>Chaetomorpha crassa</td>
<td>Chlorophyta</td>
<td>between 430 to 475 and 660</td>
<td>-</td>
</tr>
<tr>
<td>Enteromorpha intestinalis</td>
<td>Chlorophyta</td>
<td>between 430 to 475 and 660</td>
<td>-</td>
</tr>
<tr>
<td>Laurencia cartilaginea</td>
<td>Rhodophyta</td>
<td>400, 430, and 660</td>
<td>490, 540</td>
</tr>
<tr>
<td>Acantophora spicifera</td>
<td>Rhodophyta</td>
<td>between 410 to 450 nm and 660</td>
<td>490, 540, and 680</td>
</tr>
<tr>
<td>Gracilaria verrucosa</td>
<td>undetected</td>
<td>-</td>
<td>490, 560, and 690</td>
</tr>
</tbody>
</table>

Fig. 5. Absorption spectra of Rhodophyta were extracted with 90 % acetone

Fig. 6. Absorption spectra of Rhodophyta were extracted with distilled water
Table 2. Absorption spectra by photosynthetic pigments²⁸-³²

<table>
<thead>
<tr>
<th>Pigment type</th>
<th>Character peak of pigment absorption in acetone (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a</td>
<td>430 and 662</td>
</tr>
<tr>
<td>Pheo a</td>
<td>410 and 665</td>
</tr>
<tr>
<td>Chlid a</td>
<td>440 and 660</td>
</tr>
<tr>
<td>Chl b</td>
<td>457 and 645.5</td>
</tr>
<tr>
<td>Chl c</td>
<td>445 and 625</td>
</tr>
<tr>
<td>Chl d</td>
<td>690</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>between 400 to 500</td>
</tr>
<tr>
<td>Phycoerythrin</td>
<td>278, 308, 307, 497, 540 to 570</td>
</tr>
<tr>
<td>Phycoceanin</td>
<td>610 to 620</td>
</tr>
</tbody>
</table>

Appropriate candidate of pigment source from macroalgae should also contain considerably amount of pigments. Spectrophotometry is fairly accurate method for pigment determination, although shift in absorption peaks occur frequently. Furthermore, the absorption peaks of different pigments are sometime overlapping each other due to formation of degradation products such as chlorophyllides and pheophytin which have similar absorption spectra⁶,³³. These perfect combination may cause ambiguity in pigment determination. Thus, in this study, pigments were also determined based on literature. Absorption peak around 400 nm to 475 nm were expected as chl a and b. Peak at around 410 nm and 660 nm might be indicated as pheophytin a while at around 440 nm there was possibility to be chlorophyllide. Chlorophyta had slight peak at around 470 nm which might be carotenoid. Rhodophyta peaks indicated phycoerythrin though it was undetected in Gracilaria verrucosa which might be caused by low concentration. There was considerable peak at around 690 nm that was expected as chl d. Chl d is an exception of chlorophyll that is dissolve in water and has maximum absorbance at far red region³⁰.

Sample, solvent system, and spectrophotometer might complicate spectrophotometric method²⁵. Cell wall thickness affects the outcome of maceration. Thicker thallus takes longer time to obtain pigment extract. Gracilaria verrucosa has fleshy thallus which more difficult to be grinded and produced low concentration of extract. Solvent selection is very important issue for spectrophotometry since it determines the degree of affinity of solute and contributes in cell disruption. Combination of certain solvents might give better result of pigment absorbance. Distilled water can be replaced by phosphate buffer to prevent phycobilin degradation during extraction²⁴,³¹. From this preliminary study, follow up study is highly required in order to get reliable data about macroalgal pigment and its quantity which use reproducible method.

4. Conclusion

Macroalgae commonly found in intertidal of Porok beach, Gunungkidul were Ulva fasciata, Enteromorpha intestinalis, Chaetomorpha crassa which were member of Chlorophyta while Laurencia cartilaginea, Acanthophora spicifera, and Gracilaria verrucosa were Rhodophyta. Chlorophyta species contains chlorophyll a, chlorophyll b, and carotenoid while members of Rhodophyta have chlorophyll a and phycoerythrin. Further research using improved protocol are strongly recommended for more eligible determination of pigment source.

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References