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Identification of Chlorophyll a from Green Seaweed (Chlorophyta) Caulerpa Racemosa by High Performance Liquid Chromatography (HPLC)

Kartini Z.*[1], TA. Rochmaniar[2] and W. Eko[1]

[1.] *Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Jawa Timur, Indonesia*
[2.] *Student, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Jawa Timur, Indonesia*

*Corresponding author's e-mail: kartinizailaniek@yahoo.com

ABSTRACT: Caulerpa racemosa is one of green seaweed (Chlorophyta). Generally, green seaweed consists of chlorophyll and carotenoid. However, technique identification of chlorophyll a was not well observed, so writer want to exploit the use of High Performance Liquid Chromatography (HPLC) to determine the chlorophyll a. The Purpose of this research was to detect the content of chlorophyll a and the result of HPLC to determine the chlorophyll inside Caulerpa racemosa. Hexane and acetone (7:3 v/v) was used as mobile phase, silica gel F-254 as a stationary phase. Retardation factor (Rf) of chlorophyll was 0, 42 with blue green color spot. Identification of chlorophyll a using Spectrophotometer UV-Vis UV 1601, and acetone was used as a solvent, result for blue zone (B/soret): 429, 5 nm) and red zone (Qx: 615, 0 nm; Qy: 662, 0 nm). Whereas identification chlorophyll a using High Performance Liquid Chromatography (HPLC) was detect at 38, 1 minute retention time. Chlorophyll a yield was 0,089 % ± 0,000001.
Key Words: Chlorophyll a, Caulerpa racemosa, HPLC

SHORT ARTICLE
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INTRODUCTION

Seaweed has been classified according to thallus colour for more than 200 years. Different seaweed groups are characterized by specific sets of pigment, chlorophyll a being the most abundant, while the other photosynthetic pigments are considered as accessory [1].

Two chlorophylls are important today as food colorants, chlorophyll a and chlorophyll b [2]. Chlorophyll has exceptional healing properties that have been very well researched. These include building the blood, promotes blood flow and the removal of waste by dilating blood vessels thus potentially increases tumour blood flow and oxygen supply and assisting the removal of cell debris, rejuvenating our cells, counteracting the side effects of radiation treatment, removing drug deposits from the body, counteracting toxins and de-activating many carcinogens, and supporting our immune system by combating unhealthy colonies of bacteria, yeasts and fungi in the body [3]

Madura Island, specifically in Sumenep there is a lot of green seaweed Caulerpa racemosa but they didn't use it so much because this species containing many chlorophyll and carotenoid. Identification technique of chlorophyll a in a qualitative manner is rare, so we need to use High Performance Liquid Chromato-graphy (HLPC) to compared chlorophyll a with standard of chlorophyll a.

MATERIALS AND METHODS

Sample of green seaweed were collected from Cabiye Village, Talango district, Sumenep, Madura Island. Sample obtained were first washed with fresh water to remove dirts such as sand, rock and mud then it were drained. All samples were packed in black polyethylene bags and put in cool box before transported to laboratory for further analysis.

Pigment extraction from this sample was following method as described by Pangestuti et al. [4]. The pigment extracts obtained were dried using nitrogen gas and collected in bottle coverage with aluminium foil and stored in freezer compartment. The pigment extract were then analyzed using TLC to identify the spot color and Rf value using stationary phase Silica gel F-254 and mobile phase mix solvent of hexane and acetone (7:3 v/v). Pigment at a guess is chlorophyll a analyzed using spectrophotometer UV-Vis to find out wave length then this pigment analyzed using HPLC LC-20AD, Shimadzu-Kyoto with Shim-pack VP-ODS C-18 column and photo diode array detector following method as described by Hegazi et al. [5]. Chlorophyll content was measured using Spectrofotometer UV-Vis UV-1700 Pharmaspec, Shimadzu-Kyoto.

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RESULTS AND DISCUSSION

Analysed of pigment separation by TLC method was based on spot colour and Rf value. At least, there are 5 pigments of green seaweed that can be separated according to the amount of the spot namely yellow, grey, blue green, green, and orange where grey indicator of pheophitin *a*, blue green was chlorophyll a, green was chlorophyll b and orange was carotenoid. It is same as with Jeffrey et al. [6] that chlorophyll *a* was blue green, carotenoid was orange and pheophitin *a* was grey.

Thereafter, the pigment of chlorophyll *a* analysed by HPLC in which chlorophyll a was obtained as compared to standard chlorophyll. Standard analysis of chlorophyll a, pure extract chlorophyll a and between chlorophyll a with pure extract *Caulerpa racemosa* using HPLC found at 430 nm showed in Fig. 1.

Chlorophyll a content of Caulerpa racemosa was as presented in Table 1.

Table 1. Chlorophyll *a* content of *Caulerpa racemosa*

Green Algae	Gram Sample	Rendemen (%)	Deviation Standard
Caulerpa racemosa	83.33	0.089 %	0.000001

Chlorophyll a from Caulerpa racemosa contain 0,089 % ± 0,000001 and HPLC method can identified chlorophyll a quality because there is standard system.

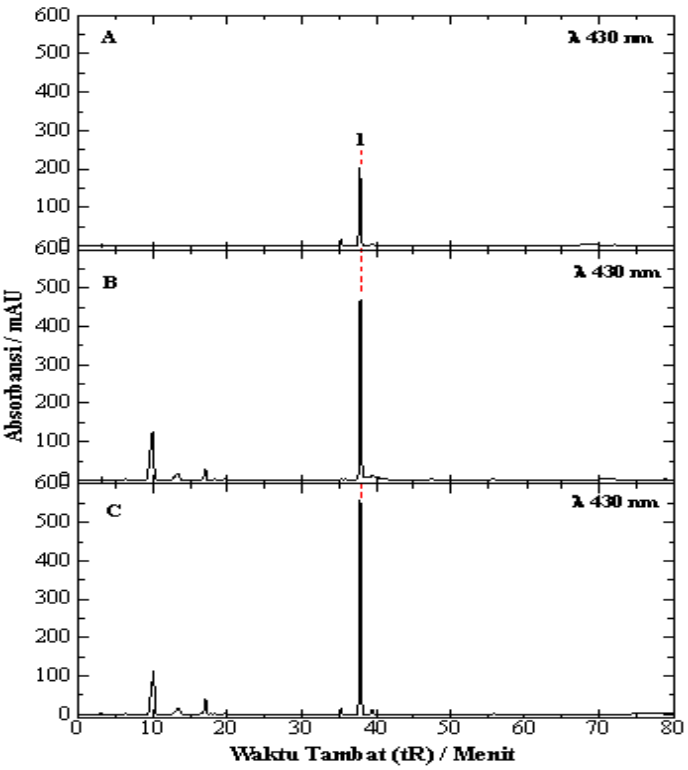

Fig 1. Result of isolating and spiking using HPLC-DAD method Chromatography Chlorophyll a : (A) Chlorophyll a Standard (B) Result of isolating *Caulerpa rasemosa* chlorophyll a (C) Co-chromatoghraphy between chlorophyll *a* standart and isolating *Caulerpa racemosa* chlorophyll a

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