

Determination Of Optimum Pigment Extraction Time From *Gracilaria Tikyahiae* Using Ultrasound Assisted Extraction Method (UAE)

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Abstract - Red macroalga (*Gracilaria tikyahiae*) contains primary and secondary metabolites which have bioactive compounds for human health such as pigment. For this time, photosynthetic pigments such as chlorophyll and carotenoids are extracted using conventional methods (i.e. maceration, soxhletation and so on) with some timed weaknesses and solvents. This study modified the extraction process method using ultrasound assisted *extraction* (UAE) method with acetone: methanol solvent (ratio (7:3) v/v). The purpose of this study is to determine the optimum time to obtain the highest percentage of pigment extract. This study showed that 30 minutes as the optimum extraction time with extract percentage namely 27.032%. Furthermore, the pigment content of *Gracilaria tikyahiae* has chlorophyll (0.00289 mg/g), chlorophyll b (0.01845 mg/g), total chlorophyll (0.1287 mg/g) and carotenoid 0.0034 (µg/g).

Keywords; *Gracilaria tikyahiae*; *Ultrasound Assisted Extraction*; *Chlorophyll*; *Carotenoid*; *macroalgae*

I. INTRODUCTION

Macroalgae, also known as seaweed, have been widely cultivated by seaweed farmers along the coast in Indonesia [1]. Macroalgae produce different types of secondary metabolites with various functions, so they have the potential as a promising source of pharmaceutical agents [2]. Potential anticancer compounds of seaweed include chlorophyll, carotenoids, phenolic acids, mycosporine such as amino acids (MAA), flavonoids, alkaloids, saponins, polysaccharides sulfate [3]. One type of seaweeds that can be cultivated and used as industrial raw materials is *Gracilaria* sp.. Indonesia is the second largest producer of *Gracilaria* sp. in 2013 with a total seaweed production of 975 tons [4].

Gracilaria is a genus of red macroalga which found in Indonesian oceans. Because, it can live in the tropics and subtropics ocean and grow mainly in shallow sea waters. It has a rapid production rate compared to others, which is about 7-13% and the growth rate can increase up to 20% every day. The chemical content of *Gracilaria corticata* is protein (22.84 g/100 g), fat (7.07 g/100 g), carbohydrates (8.30 g/100 g), polysaccharides (49.64 g/100 g) [5]. Chlorophyll and carotenoids; constituents of photosynthetic pigments present in algae,

have a positive effect on inflammation, oxidation and wound healing [6]. Chlorophyll acts directly as a free radical reducer and potentially protects lymphocytes against oxidative DNA damage by free radicals [7].

Some pigments have been isolated from *Gracilaria* sp. as ficoeritrin [8], anthocyanins [9], ficobilin [10]. During this time, pigment extraction method still used conventional methods (such as maceration and so on). This method requires too much time and consumes too much solvent.

Based on its weakness, alternative extraction techniques are needed, like using ultrasonic method [11]. Ultrasound Assisted Extraction (UAE) method is an extraction technique by delivering ultrasonic waves on the material to be performed extraction [12]. Ultrasonic extraction methods have some advantages which compared to other extraction methods for instance: consuming less solvents and obtaining more extract percentages. In addition, ultrasonic methods are safer and faster [13]. The purpose of the study is to modify the extraction method using the Ultrasound Assisted Extraction (UAE) method and to determine the optimum extraction time.

RESEARCH METHODS

2.1 Materials

The materials used in this study are red macroalga (*Gracilaria tikyahiae*), hexane (p.a), acetone (p.a), methanol (p.a), calcium carbonate (CaCO₃), aquadest.

2.2 Procedures

2.2.1 Collecting Macroalgae

Macroalgae red (*Gracilaria tikyahiae*) obtained from Losari Beach located in the West Makassar City, South Sulawesi Province, Indonesia. Astronomical location Losari Beach 5°8'47"S and 119° 24' 43"E. Fresh macroalga is collected in the morning without regardless of plant age. Macroalga is cleaned by water to remove dirt such as and some epiphytes and stored in cooler boxes to maintain freshness of macroalga.

Furthermore, the macroalga sample is dried using the oven (60°C). Then, the sample is blended to obtain the powder macroalga and stored for a further step.

2.2.2 Determination of Macroalga

The macroalgae used are red macroalgae (*Gracilaria* sp.). Losari Beach, Makassar City, South Sulawesi Province, Indonesia will determine to know the species of macroalga used. Macroalgae (*Gracilaria* sp.) has been dissolved in ethanol solvents for species determination at the Center for Plant Conservation and Research of Botanical Gardens-LIPI, Ir. H. Juanda Street, Bogor, Indonesia.

2.2.3 Extraction of Macroalga (*Gracilaria tikyahiae*) by Ultrasound Assisted Extraction Method (UAE)

The extraction process of macroalgae pigments (*Gracilaria tikyahiae*) was carried out based on the Folch method [14] modified by converting the maceration method to the Ultrasound Assisted Extraction (UAE) method using a sonicator. 200g *Gracilaria* sp. powder dissolved with acetone: methanol ((7: 3) v / v), and added CaCO₃. Then, extracted using a sonicator with a time variation of 5, 10, 15, 20, 25, 30 minutes (triplo). Furthermore, the pigment extract obtained was evaporated by rotary evaporator.

2.2.4 Pigment Extract Percentage Determination

Determination of pigment content percentage from *Gracilaria tikyahiae* extract using UV-visible spectrophotometer through readings at certain wavelengths according to the type of pigment. The method analyzed chlorophyll levels using wavelengths of 663 and 645 nm, total chlorophyll using wavelengths of 645 and 663 nm and carotenoids using wavelengths of 480 and 510 nm [15]. Analysis of chlorophyll b levels using wavelengths of 645 and 663 nm [16]. The formulation of the calculation of pigment content can be seen in this case below [17].

$$\text{Chlorophyll a (mg/g)} = \frac{[12.7 (A \ 663) - 2.69 (A \ 645) V]}{(1000 \times W)}$$

$$\text{Chlorophyll b (mg/g)} = \frac{[22.9 \times (A \ 645) - 4.68 (A \ 663) V]}{(1000 \times W)}$$

$$\text{Total chlorophyll (mg/g)} = \frac{[20.2 (A \ 645) + 8.02 (A \ 663) V]}{(1000 \times W)}$$

$$\text{Carotenoids (\u00b5g/g)} = \frac{[7.6 (A \ 480) - 1.49 (A \ 510) V]}{(1000 \times W)}$$

Description:

A = Absorption at certain wavelengths

V = Total volume of pigment extracts

W = Example of weight used for extraction

II. RESULTS AND DISCUSSIONS

3.1 *Gracilaria tikyahiae*

Macroalgae (*Gracilaria tikyahiae*) were collected from Losari Beach, located in the West of Makassar City, South Sulawesi Province, Indonesia. As a visual observation, *Gracilaria tikyahiae* has a cylindrical thallus, smooth, brownish-red color and unclear roots, leaves and stems. Its length from 10 - 15 cm. Based on the determination's result, the macroalgae used is *Gracilaria tikyahiae*. This is similar to Novita's research [18], which states that *Gracilaria tikyahiae* has a slippery cylindrical

cylindrical shape, an extension of the thallus. Dinar et al., [19] also reported that the phenotype of the color of the seaweed thallus *Gracillaria* sp. is generally light brown with a percentage increase along with the increase in salinity and there is a kind of small thorn in its branching.



Figure 1. *Gracilaria tikyahiae*



Figure 2. Losari Beach

3.2 Pigment Extracts

Ultrasound Assisted Extraction (UAE) method is an extraction technique by delivering ultrasonic waves to the extraction sample. This method has several advantages: it uses less solvent volume, shortens extraction time, and is energy efficient. The utilization of ultrasonic waves in the extraction process has been widely used, for Oleoresin Ginger's extraction [20]; Mulberry Anthocyanin Extraction [21]; Antioxidant Extraction of Soursop Leaves [22]; Extraction of Kabocha Pumpkin Carotenoid Pigment [23]; Extraction of Oil from Microalgae [24] and Ultrasonic Assisted Extraction of Triterpenoid Saponins from Mangrove Leaves [25]. In this study, *Gracillaria tikyahiae* pigment extract extracted by *Ultrasound Assisted Extraction* (UAE) method had color variations based on their time extraction process (six time variations). There is a discoloration from the initial time (5 minutes) to 30 minutes i.e. yellow to dark green (Figure 4).



Figure 4. *Gracillaria tikyahiae* Pigment Extract in Six Different Times

Based on this study, the time variation of the extraction process affects the pigment results of *Gracilaria tikyahiae*, (Figure 4). The highest yield in 30 minutes is 27.03% and has a dark green color. Compared to pigments extracted in 5 minutes has a yellow color and less yield of 10.81%. Too short the extraction time results in the dissolving of the compound is not optimum so that the material has not been extracted perfectly and vice versa, the longer the extraction time it will increase the amount of analytes extracted because the contact between solvent and dissolved substance will be longer, therefore the process of dissolving phenolic compounds will continue and stop until the solvent is saturated. [26].

3.3 Pigment Contents of *Gracilaria* sp.

Macroalgae *Gracilaria* sp. has phenolic compounds, pigments and flavonoids [27]. Differences in the content of chemical compounds of some types of seaweed may be influenced by several things such as different types / species, places of life, seasons and several other factors. This is similar to Elingsetyo et al.'s research [28], that the content of phytochemical compounds is influenced by various factors namely species, varieties, growth conditions, seasonal variations, processing and storage methods. Macroalgae have three main pigment types, namely chlorophyll, carotenoids, and ficocyanin. Each type of pigment has a variety of benefits, especially for health purpose [39].

In this study, pigment were analyzed using UV-Vis Spectrophotometry in wavelengths of 663 and 645 nm (for chlorophyll a, b and total) while 480 and 510 nm (for carotenoid pigments). The results showed the extraction time had a very significant effect on the pigment content of *Gracilaria tikyahiae*. The results of pigment extract can be seen in Table. 1-3.

Table 1. Yield Pigment Percentage of *Gracilaria tikyahiae* Using Ultrasound Assisted Extraction (UAE) Method

No	Time (minutes)	Initial weight (grams)	Extract Vol (mL)	Yield (%)
1	5	200	22	10,813%
2	10	200	28	13,762%
3	15	200	36	17,694%
4	20	200	45	22,117%
5	25	200	48	23,592%
6	30	200	55	27,032%

Table 2. Chlorophyll (a, b and total) Yield of *Gracilaria tikyahiae*

Time (Minutes)	Pigment	Pigment Content (mg/g)
5	Chlorophyll a	0,0008281
	Chlorophyll b	0,005274
	Total Chlorophyll	0,0367762
10	Chlorophyll a	0,00149058
	Chlorophyll b	0,0094932
	Total Chlorophyll	0,06619716
15	Chlorophyll a	0,00215306
	Chlorophyll b	0,0137124
	Total Chlorophyll	0,09561812
20	Chlorophyll a	0,00207025
	Chlorophyll b	0,013185
	Total Chlorophyll	0,0919405
25	Chlorophyll a	0,00231868
	Chlorophyll b	0,0147672
	Total Chlorophyll	0,10297336
30	Chlorophyll a	0,00289835
	Chlorophyll b	0,018459
	Total Chlorophyll	0,1287167

Table 3. Carotenoid Pigment Yield of *Gracilaria tikyahiae*.

Time (Minutes)	Pigment Content (µg/g)
5	0,000981
10	0,0017658
15	0,00214
20	0,0024525
25	0,0027468
30	0,0034335

The results of this study are similar to Sholihah's research [30] which explained that the higher the amplitude and the longer extraction time can increased the yield pigment extraction.

III. CONCLUSION

In conclusion, the optimum time of pigment extraction from *Gracilaria tikyahiae* is 30 minutes with yield percentage such as 27.032%. *Gracilaria tikyahiae* contains pigments namely chlorophyll a (0.00289 mg/g), chlorophyll b (0.01845 mg/g), total chlorophyll (0.1287 mg/g) and carotenoid pigment (0.0034 µg/g).

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