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Submission date: 13-Jan-2022 07:20PM (UTC+0700)

Submission ID: 1739993168

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To cite this article: S Y Lumbessy *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **681** 012017

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Identification of chlorophyll pigment on *Gracilaria Salicornia* seaweed

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Abstract. *Gracilaria salicornia* is one of the various types of seaweed that can be commercialized because it has the potential as an antioxidant due to its high chlorophyll-a pigment. The purpose of this study is to the identification of chlorophyll compound of *G. Salicornia* seaweeds. This identification has been carried out using Thin Layer Chromatography (TLC) method. Four compounds have been found, namely xanthophyll, chlorophyll b, chlorophyll a, and pheophytin. Xanthophyll was identified with the yellow spot on TLC plates, in which the Rf is 0.55. Chlorophyll b is a yellow-green spot with Rf is 0.60, the Rf of Chlorophyll a is 0,68 with the color blue-green, whereas pheophytin is indicated as a black spot with the Rf 0.73. Chlorophyll a have the maximum wavelength at 430 nm in the blue spectral and 662 nm in the red spectral.

1. Introduction

Seaweed has the potential to be developed as a functional food product because of its bioactive components, which is very important for health. The utilization of seaweed as a functional food will increase the added value of seaweed and can provide solutions to the provision of healthy food at affordable prices. One of the bioactive components in seaweed is its natural pigments such as chlorophyll [1]. The results of Pangestuti and Kim's (2011) and Wardani et. al., (2017) research show that chlorophyll and some of its derivatives such as pheophytin from seaweed have biological activities as antioxidants, anti-inflammatory, and neuroprotective [2,3].

One of the various types of red seaweed (Rhodophyceae), which has a high chlorophyll content, is *Gracilaria salicornia* [4]. Some research wrote that, naturally, *G. Salicornia* seaweed has a relatively high chlorophyll-a content, approximately 7.03 µg / l and has potential as an antioxidant compound and has an aromatic amino acid content that is quite high in the form of threonine [5–7]. Based on the data above, *G. Salicornia* seaweed has the potential to be used as a raw material for functional food products that can provide health benefits besides the nutritional benefits they contain.

To optimize the utilization of *G. Salicornia* seaweed as a functional food raw material, research on chlorophyll pigments and their compounds are needed. Therefore, this study aims to the identification of chlorophyll derivative compounds in *G. Salicornia* seaweed.

2. Methods

The material used in this research was *G. Salicornia* seaweed originating from the waters of Molok, Gerupuk, West Nusa Tenggara.



2.1. Sample Preparation

Sample preparation of *G. salicornia* is done by cleaning the sample, expanding the surface, and reducing the water content. Cleaning is done by removing dirt to the thallus walls, which can inhibit the extraction process. The expansion of the sample surface is done by cutting the sample to a size of 0.5 - 1.0 cm so that the water content in the material is more easily evaporated. Then the sample is aerated at room temperature and in a light-tight room to reduce the sample's water content. After that, samples were mashed using a blender and sieved to obtain the sample powder.

2.2. Pigment Extraction

About 100 g of samples were extracted by using a mixture solution of methanol: acetone (7: 3 v/v). During the extraction, calcium carbonate was added as a neutralizing agent. The extraction was done as quickly as possible to avoid further oxidations or enzymatic degradations. Then, the extract was filtered, and the residue was re-extracted until the color of residue became pale as an indicator of complete pigment extraction. The resulting extract was partitioned with diethyl ether. The diethyl ether layer was dried with nitrogen gas.

2.3. Isolation of Chlorophyll Compounds

The chlorophyll compounds were isolated from *G. Salicornia* crude extracts by column chromatography with silica gel as stationary phase and hexane: ethyl acetate ± 200 ml as a mobile phase. The mobile phase is continuously enhanced of polarity with a volume of ± 200 ml from the ratio hexane: ethyl acetate (8: 2 v / v), hexane: ethyl acetate (7: 3 v/v), hexane: ethyl acetate (6: 4 v / v), up to the hexane: ethyl acetate (5: 5 v/v). The fraction that is thought to be a green color band (chlorophyll) is collected in a test tube. The purity of pigments was analyzed with TLC and UV-Vis spectrophotometer.

2.4. Thin Layer Chromatography (TLC)

Silica gel Plates were activated in an oven at 100°C for 2 hours. A mixture of hexane: diethyl ether: acetone (5: 3: 2, v / v) was used as the mobile phase. Spots from the pigment extracts (*G. Salicornia*) were applied 2cm from the bottom of the silica gel plate using a capillary tube with proper spacing, and the silica gel plate was allowed to develop (separation of pigments) for approximately 10 minutes in the chamber with a mobile phase. Remove the TLC plate from the chamber when the solvent front is approximately 1.0 cm from the top of the TLC plate. The rate at which a pigment moves up the plate is reported as an R_f value, which is defined as the ratio of the distance moved by the spot to the distance moved by the solvent. Determine the R_f values for each of the pigments you observe using the formula provided below.

$$R_f = \frac{\text{distance moved by a solute (pigment)}}{\text{distance moved by solvent}}$$

2.5. Ultraviolet and Visible (UV – Vis) Spectrophotometry

UV – vis absorption of pigment dissolved in acetone 100% was recorded in a spectrophotometer Varian Cary 50 at a wavelength of 350 – 800 nm [8].

3. Results and Discussion

3.1. Identification of Chlorophyll Compounds using TLC method

The chromatogram of TLC is shown in Figure 1. The pigments were separated, as can be seen from their spot color. The R_f value of each spot was calculated, and then they were compared with the literature. The identification of pigments from *G. Salicornia* extract by the above chromatography is shown in Table 1. Spot 1 showed black color and had an R_f value of 0.73. It was identified as pheophytin. Spot 2 was identified as chlorophyll because it was blue-green with R_f 0.68. Spot 3 was

yellow-green with Rf 0.60 and identified as chlorophyll b. Spot 4 showed yellow with Rf 0.55 and was determined to be xanthophyll.

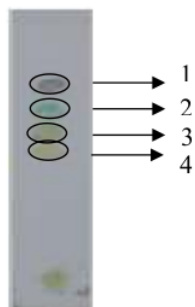


Figure 1. The chromatogram of TLC.

Table 1. Identification of *G. Salicornia* pigment extract by thin-layer chromatography method

Research Result				Literature		
No	Spot Colour	Rf	Identification	Spot Colour	Rf	Rf
1	Black	0.73	Phaeophyta	Black	0.75	[9]
2	Blue Green	0.68	chlorophyll <i>a</i>	Blue Green	0.68	[10]
3	Yellow Green	0.60	chlorophyll <i>b</i>	Yellow Green	0.54	[10]
4	Yellow	0.55	xanthophyll	Yellow	0.42	[10]

Photosynthetic pigments such as chlorophylls, xanthophylls and β -carotene are soluble in organic solvents [11]. The pigments extracted into the particular organic solvent can then be separated and presumptively identified with a thin layer of silica [12]. Chlorophyll *a* and *b* spots were separated into two zones during development in the chloroform-light petroleum solvent.

3.2. Isolation of chlorophyll *a* by column chromatography

The blue-green band corresponds to chlorophyll *a* was fractionated. The band was then identified with TLC and UV-Vis spectrophotometer. The chlorophyll-*a* purity test was carried out by TLC to see how many spots are produced, as the number of spots in chromatogram will inform the number of compounds. One blue-green spot was found on TLC of chlorophyll *a*, but no other pigment bands were seen. The chromatogram of purified chlorophyll is shown in Figure 2a.

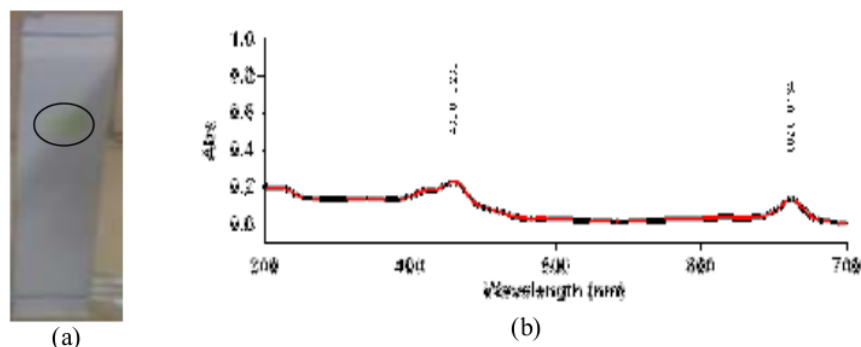


Figure 2. (a) Chromatogram of isolated chlorophyll *a* and (b) Chlorophyll *a* spectrum pattern.

The spot had an Rf value of 0.68, and its color was blue-green, and it was identified as chlorophyll-*a* in accord with literature using the same method. Further, the spectrum pattern and its maximum

wavelength of chlorophyll-*a* isolated were determined by UV-Vis spectrophotometer. The pattern of the spectrum is shown in Figure 2b. The pattern of the spectrum is shown in Figure 4b. The result showed that pattern and the maximum wavelength at 430 nm in the blue spectral and 662 nm in the red spectral. The red absorption maximum of Chl *a* shift from 660 to 665 nm, and the blue absorption maximum from 428 to 432 nm [13]. The absorption maxima of extracted pigments strongly depend on the type of solvent and, to some degree, on the type of spectrophotometer used.

4. Conclusion

TLC analysis of the pigments from *G. Salicornia* extract showed four spots; they are Phaeophytin, chlorophyll *a*, chlorophyll *b*, and xanthophyll. Chlorophyll *a* have the maximum wavelength at 430 nm, 616, and 661 nm.

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