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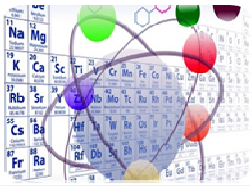

Exploration of Lipid Content in *Sargassum binderi* Algae from the North Coast of Manokwari West Papua

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ABSTRACT

The aim of this research is to determine the potential of biodiesel from the algae Sargassum binderi from the North Coast of Manokwari by determining the lipid components contained in the algae. Lipid extraction was carried out using the soxhletation method with n-hexane solvent. Lipid percentage was determined via GCMS analysis. The research results obtained 4 lipid components consisting of hexadecanoic acid (palmitic acid), 9,12-hexadecadienoic acid, 9-octadecenoic acid (Z) (oleic acid) and octadecanoic acid (stearic acid) with a percentage of 18.12%, 4.75%, 19.4% and 4.29% respectively. The total percentage of lipid components extracted was 46.56%, almost half of the total extract.

Keywords: algae, gas chromatography mass spectrometry, lipid, *Sargassum binderi*, soxletation

1. INTRODUCTION

Marine algae, also known as seaweed, is a marine biological resource that widely used, especially by coastal communities. Algae are divided into three major classes, namely Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae).[1] Algae contain compounds including alkaloids, flavonoids, saponins, phenols, steroids and triterpenoids which have bioactivity such as antioxidants antibacterial and antimicrobial.[2-7]

Apart from bioactive compounds, algae also contain lipid components which are useful as raw materials for biofuel production (biodiesel, biogas, and bioethanol). Lipids can be converted into biodiesel through esterification and transesterification reactions catalyzed by acids or bases.[8-17] Therefore algae is an

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important source of biomass for the development of Renewable Energy in Indonesia. This is an alternative solution to deal with the threat of an energy crisis due to dependence on fossil energy.

The Papua region itself is rich in flora and fauna sources that have not been fully explored, including marine biota. The ocean of the Bird's Head Seascape, West Papua Province, are one of the ocean with the highest biodiversity in the world which can provide genetic resources for various purposes, namely commercial, economic and ecological.[18,19] Therefore, more research is needed to explore, identify, understand, and ultimately utilize living organisms in the ocean. So far there has been few research regarding marine biota, including those from Manokwari waters. One of the marine biota that is often found in the coastal areas of Manokwari is brown algae. Based on the description above, the aim of this research is to determine the potential of the brown algae *S. binderi* (from the North Coast of Manokwari) as raw material for biodiesel production. Therefore, initial exploration was carried out through chemical composition analysis using GC-MS on the crude extract of the algae.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The materials used in this research were samples of *S. binderi* algae, n-hexane (C_6H_{14}), aluminum foil and filter paper. The tools used were general laboratory glassware, soxhlet apparatus, vial, oven, digital scale, rotary evaporator and gas chromatography mass spectrometry (GCMS, QP2010S Shimadzu).

2.2. Research Procedure

2.2.1 Algae Collection

The brown algae *S. binderi* were collected from North Coast of Manokwari (0°44'45" S, 133°59' E). The algae were brought back to the laboratory in cooler box.

2.2.2 Determination of Moisture Content

Determination of moisture content was carried out on wet algae samples. The wet sample is first cut into small pieces. The algae sample pieces were then weighed 10 g (W) in a chemical glass and then placed in an oven with a temperature 105ºC to evaporate the water. Evaporation is carried out to obtain a fixed weight where this fixed weight is the dry weight (D) of the sample. The procedure was carried out in triplicate to determine the average dry weight. The percentage of moisture content is calculated using Equation (1).

$$Moisture\ Content\ (\%) = \frac{W\text{-}D}{W} \times 100\% \qquad (1)$$

W – Wet Weight of Algae (g)
D – Dry Weight of Algae (g)

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2.2.3 Soxhlet Extraction

An illustration of a soxhlet apparatus is shown in Figure 1. The conditions for the extraction process using the soxhletation method are as follows: Wet sample weight, 50 g (SW); n-hexane volume, 440 mL; temperature, 69ºC; extraction time, 5 hours. The extract was then separated from the n-hexane solvent using a rotary evaporator. After that, the extract was weighed (EW) and the extract yield was calculated using Equation (2). Before being analyzed by GCMS, the extract was placed in a vial wrapped in aluminum foil and stored in a freezer to avoid degradation of the compounds structure.

$$\text{Yield (\%)} = \frac{\text{EW}}{\text{SW}} \times 100\% \qquad (2)$$

EW – Extract Weight (g)
SW – Sample Weight (g)

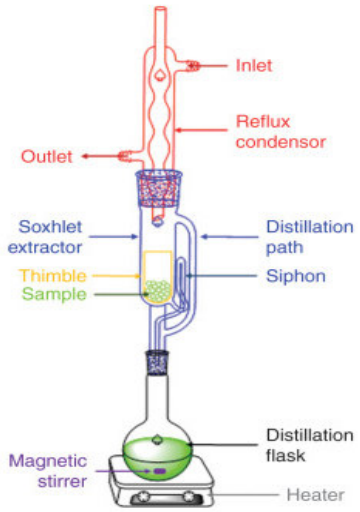

Figure 1. Soxhlet Apparatus Illustration

2.2.4 GC-MS Analysis

GC-MS compound analysis was carried out using a GCMS-QP2010S Shimadzu equipped with an Agilent DB-5MS UI column (length 30 m, diameter 0.25 mm and film thickness 0.25 µm). The instrument was set to an initial temperature of 70ºC and an injection temperature of 300ºC with a column flow rate of 0.50 mL/minute, helium carrier gas at a pressure of 13.7 kPa with a flow rate of 3 mL/minute and a linear speed of 25.9 cm/second. All compounds were identified by comparison of mass spectra and component retention index data found in the literature and spectrum databases stored in the GC-MS library. Then the compound composition data detected by GC-MS is displayed descriptively using a table.

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3. RESULTS AND DISCUSSION

3.1. Moisture content

Table 1 shows the moisture content values of the *S. binderi* algae. Moisture content was determined through triplicate measurements. It was found that the measured moisture content was relatively high, 85%. The algae samples were dried in an oven at 105°C to evaporate the water.

Table 1. Moisture Content Measurement Results (%)

No	Beaker	Sample Weight (g)	Total Weight (g)	Constant Weight (g)	Water Weight (g)	Water Weight (%)	Average water weight percent (%)
1	A	10	40,30	31,77	8,53	85,30	85
2	B	10	42,42	33,88	8,54	85,40	
3	C	10	40,60	32,07	8,53	85,30	

3.2. Extraction Results

Figure 2 shows the flow diagram of research carried out starting from the initial stage, sampling, to the analysis stage using GCMS. The preparation stage does not involve samples drying and grinding to minimize loss of volatile compound and to prevent degradation of the chemical structure of the lipids which are the target components. Based on polarity, lipids are divided into two, namely non-polar lipids and polar lipids.

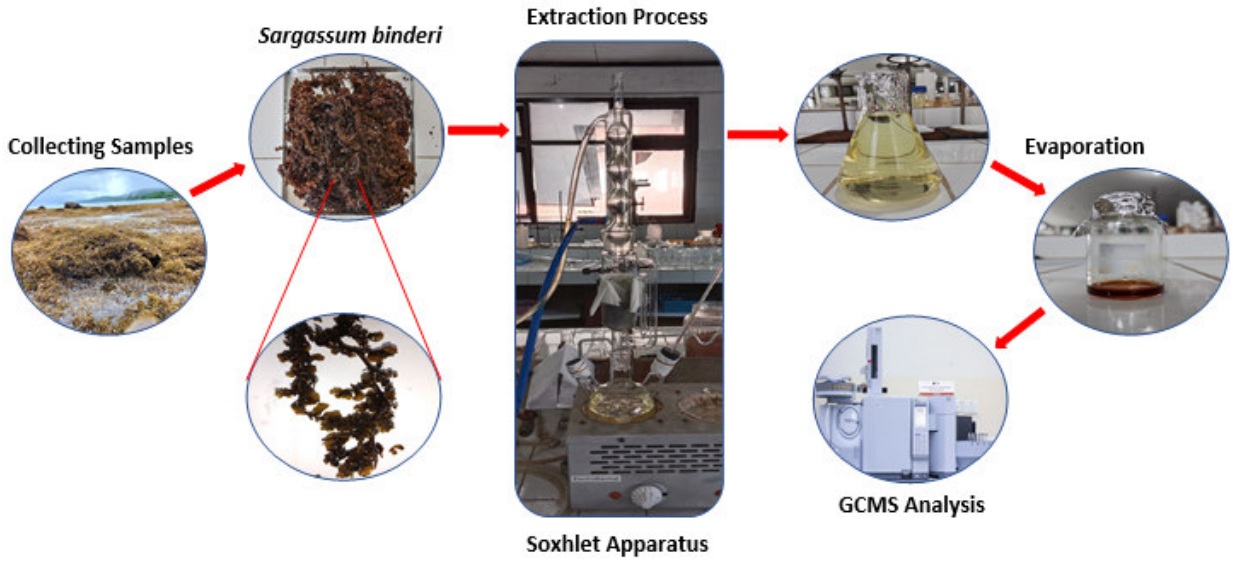

Figure 2. Research Flow Diagram

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Non-polar lipids generally used as raw material for biodiesel production. N-hexane is a non-polar solvent so it is used in this soxhletation method to extract lipid components based on the "like dissolves like" principle. Ramluckan et al stated that lipids can be extracted with high yields using n-hexane solvent. [20]

Table 2. Extraction Results

Parameter	Extract
Extract color	Brownish red + white precipitate
Extract Weight (g)	0,23
Yield dry weight basis (%)	3,07

Initially, the results of soxhletation obtained a yellow extract. However, after the n-hexane evaporation process with a rotary evaporator, a concentrated extract was obtained which was brownish red in color and a slight white precipitate was observed. Yield dry weight basis is relatively small (< 5%), 3,07%. This can be understood considering that the water content of *S. binderi* algae is relatively high.

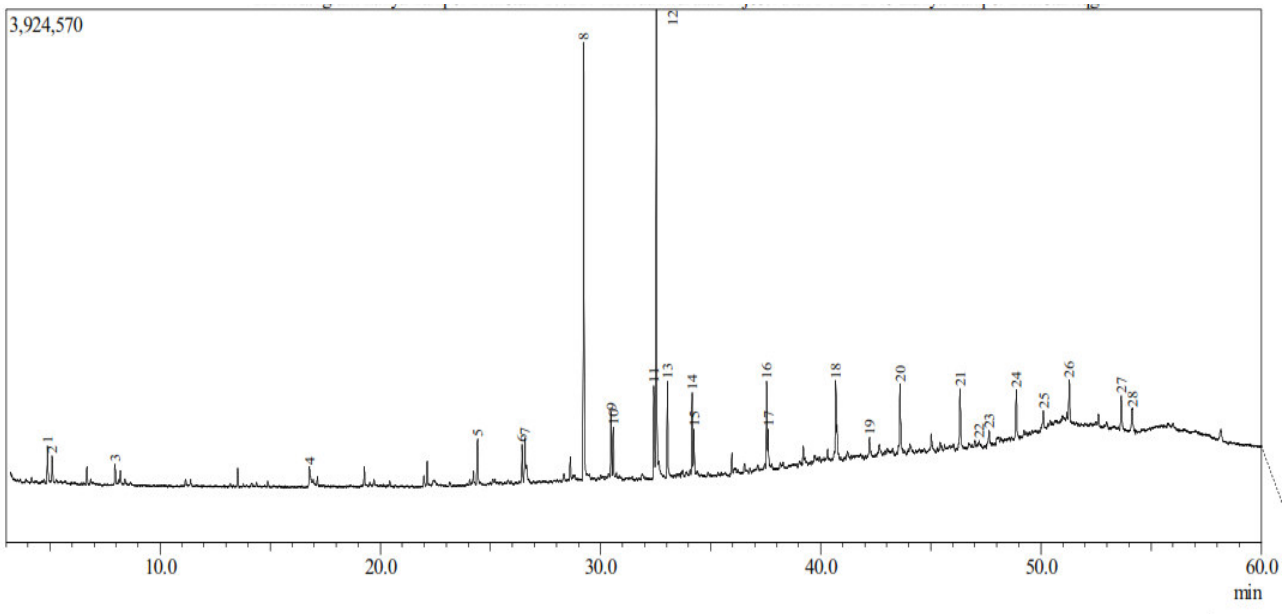

Figure 3. GCMS Chromatogram of *S.binderi* Extract from the Soxhletation Method

The GCMS chromatogram of *S. binderi* extract can be seen in Figure 3. Based on the chromatogram, 28 peaks were observed, of which 4 peaks represented the lipid component. The data for the 4 peaks is shown Table 3. The lipid components detected after the esterification process were hexadecanoic acid methyl ester, 9,12-hexadecadienoic acid methyl ester, 9-octadecenoic acid (Z)- methyl ester and octadecanoic acid methyl ester. The 9-octadecenoic acid (Z)- methyl ester compound detected at the retention time of 32,538 minute had the highest percentage 19,4% with a similarity level 96%.

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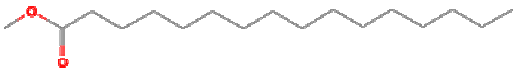
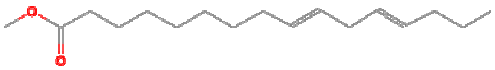
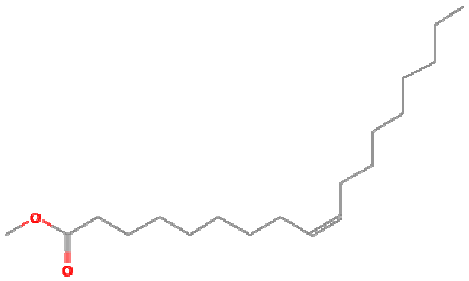
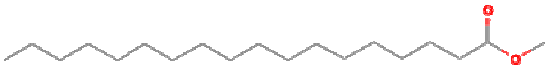
Table 3. Lipid components from GCMS Analysis

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Similarity	Compound Name
8	29,234	29,167	29,367	8783172	18,12	3216067	96	Hexadecanoic acid, methyl ester
11	32,419	32,333	32,483	2302193	4,75	688901	95	9,12-Hexadecadienoic acid, methyl ester
12	32,538	32,483	32,608	9403607	19,4	3440571	96	9-Octadecenoic acid (Z)-, methyl ester
13	33,037	32,95	33,167	2079457	4,29	707961	96	Octadecanoic acid, methyl ester

Table 4. Molecular Formula, Molecular Weight and Structural Formula of Lipid Components

Peak#	Molecular formula	Molecular Weight	Compound Name (Other name)	Structure Formula
8	$C_{17}H_{34}O_2$	270,4507	Hexadecanoic acid, methyl ester (Palmitic acid)	
11	$C_{17}H_{30}O_2$	266,4189	9,12-Hexadecadienoic acid, methyl ester	
12	$C_{19}H_{36}O_2$	296,4879	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid)	
13	$C_{19}H_{38}O_2$	298,5038	Octadecanoic acid, methyl ester (Stearic acid)	

The molecular formulas, molecular weights, and structural formulas of the 4 lipid components are shown in Table 4. These 4 components have long molecular chains (alkyl groups, R) with 16-18 carbon atoms. Therefore, these 4 components tend to be non-polar. The 3 components are better known as palmitic acid, oleic acid and stearic acid. Palmitic acid, oleic acid and stearic acid are classified as free fatty acids

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which can be converted into biodiesel through acid or base catalyzed esterification and transesterification reactions.

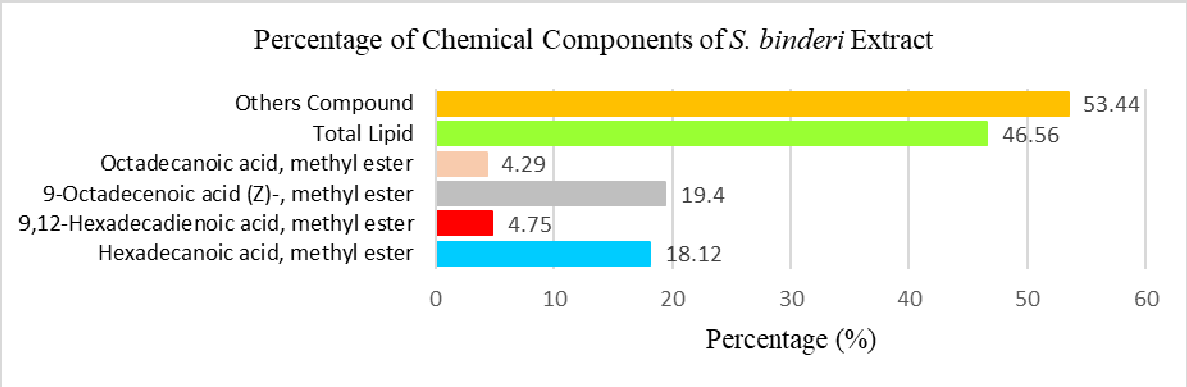

Figure 4. Percentage of Chemical Components of *S. binderi* Extract

The total percentage of lipid components was 46.56%, almost half of the total extract yield. So research related to the potential of *S. binderi* algae as raw material for biodiesel production is a prospect to be carried out in the future.

4. CONCLUSION

Based on the research results, 4 lipid components were obtained from the algae *S. binderi* which were extracted using the soxhletation method with n-hexane solvent. The lipid components consist of hexadecanoic acid (palmitic acid), 9,12-hexadecadienoic acid, 9-octadecenoic acid (Z) (oleic acid) and octadecanoic acid (stearic acid) with percentages of 18.12%, 4.75 %, 19.4% and 4.29% respectively. The total percentage of lipid components extracted was 46.56%. Moisture content of *S. binderi* algae is relatively high, 85%, which influences the low yield percentage 3.07% obtained.

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