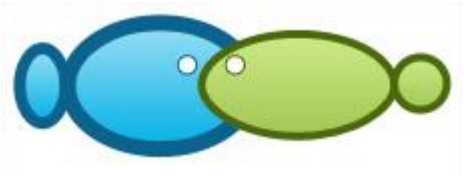

Biomineral characterization and phytochemical profile of green algae *Halimeda macroloba* and *Halimeda opuntia* from coastal waters of Tanjung Merah, Bitung City, North Sulawesi, Indonesia

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Abstract. This study aims to determine the mineral composition in green algae *Halimeda macroloba* and *H. opuntia*. The scanning electron microscope analysis and energy dispersive were used to show the morphology of the particles and the mineral composition contained in these algae. The scanning electron microscope analysis showed the particle morphology of *H. macroloba* and *H. opuntia*. Enlargement of *H. macroloba* nanoparticle images was carried out on a scale of 1,000x, 5,000x, 10,000x, 20,000x, and 200,000x, while enlargement of *H. opuntia* was 1,000x, 5,000x, 10,000x 20,000x, and 50,000x. Specimens analysis using energy dispersive showed that the two green algae *H. macroloba* and *H. opuntia* contained the following biomineral compound elements O>Na>Ca>Cl and O>Ca>C, respectively. *H. macroloba* has 24 major phytochemical compounds and at least five of the highest peaks were heptadecanoic acid, 16-methyl-, methyl ester, n-hexadecanoic acid, hexadecanoic acid, methyl ester, 1-chloroundecane, and eicosanoic acid. *H. opuntia* has 55 major phytochemical compounds and at least five of the highest peaks were phytol, neophytadiene, 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E), ethyl iso-allocholate, hexadecanoic acid, and ethyl ester.
Key Words: mineral, particle, scanning, seaweeds, spectrometer.

Introduction. The ecology and taxonomy of *Halimeda* were described by Hillis-Colinvaux (1980), Calumpong & Meñez (1997), Trono (1997), and Barton (1900). These studies explained more about morphological forms than anatomical forms. This is possible due to advances in microscope technology. Hillis (1959) recorded 23 species of *Halimeda* in Indo-Pacific region. Most of these species are spread in Indonesia. Based on the Snellius II Expedition results in 1984 and later identified in the Herbarium Leiden Rijk, 16 species of *Halimeda* were found in central and eastern Indonesia. Description of macroalgae species including *H. opuntia* and *H. macroloba* in Indonesia were carried out by Atmadja et al (1996), Kepel et al (2012) in Manokwari, and Kepel & Baulu (2013) in West Southeast Maluku.

In North Sulawesi waters, *H. macroloba* and *H. opuntia* were found in Tongkaina waters, Manado (Parera et al. 2015), Mokupa waters, Minahasa (Wowor et al. 2015), Tongkaina waters, Manado (Kepel et al. 2018a), Blongko waters, South Minahasa (Kepel et al. 2018b), Bahoi, North Minahasa (Baino et al. 2019), Mantehage Island (Kepel et al. 2019a), Minahasa Peninsula in wet season (Kepel et al. 2019b), and Minahasa Peninsula in dry season (Kepel et al. 2020). There are studies on *H. opuntia* (Mantiri et al. 2018) in polluted waters: in Totok Bay waters and Blongko waters that are polluted with arsenic, chromium and copper, and on *H. opuntia* (Tombokan et al. 2020) in Tanjung Merah waters, Kora-kora waters, and Talawaan Bajo waters polluted with cadmium, chromium and mercury. There are studies of antioxidant bioactivity and chlorophyll concentration in green algae *H. opuntia* from Totok Bay (Mantiri et al. 2019).

This study aimed to determine the mineral composition in green algae, respectively *H. opuntia* and *H. macroloba* because the mineral composition has never been studied, and it was investigated only in *Tricleocarpa fragilis* (Singkoh et al. 2019). The scanning electron microscope analysis and energy dispersive were used to show the morphology of the particles and the mineral composition contained in these algae.

Material and Method. Samples of green algae *H. macroloba* and *H. opuntia* (Figure 1) were taken from the coastal waters of Tanjung Merah, Matuari District, Bitung City, North Sulawesi Province, Indonesia (Figure 2). These algae grow naturally in this area. The samples were packaged in plastic bags and then placed in a cool box.

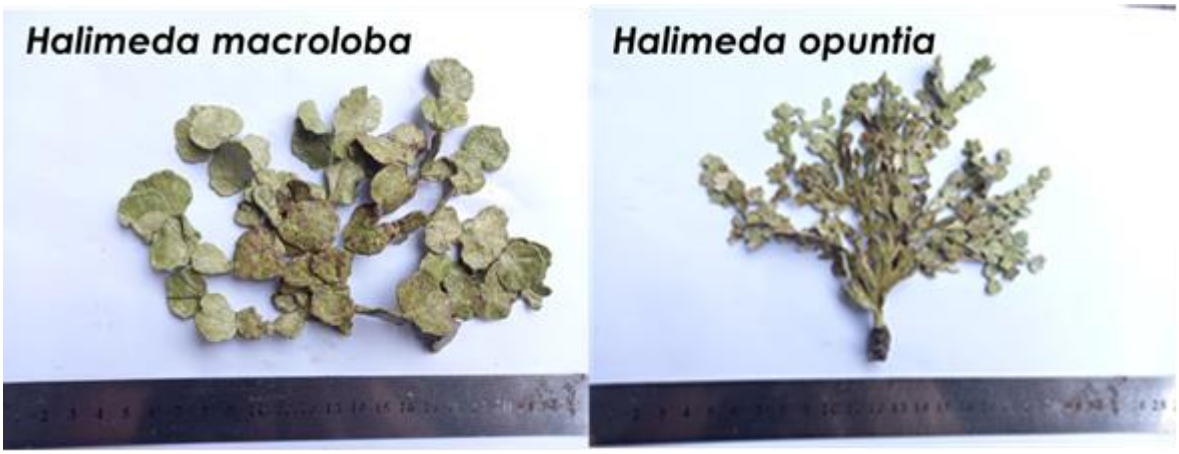


Figure 1. Green algae from coastal waters of Tanjung Merah.
(Source: photos taken by authors, 2021)

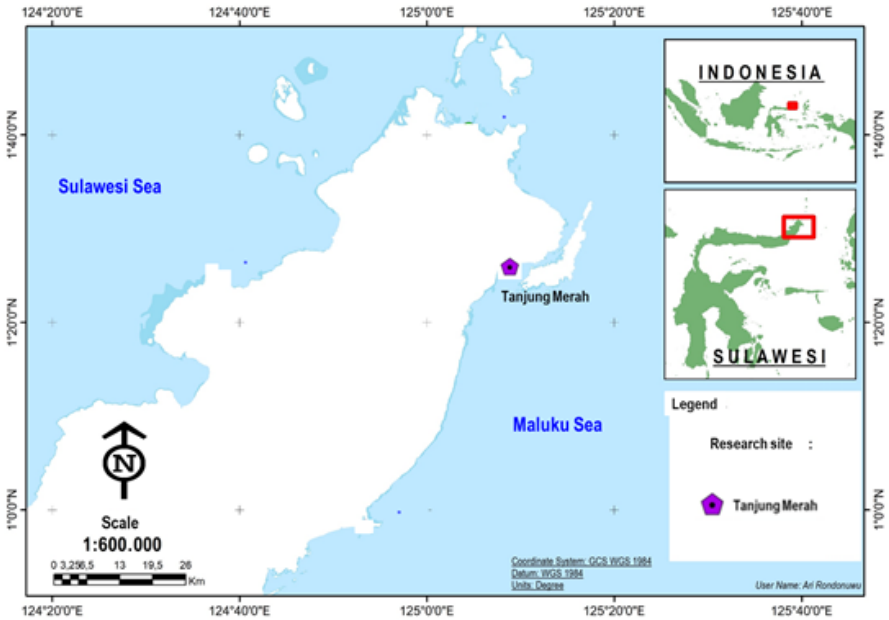


Figure 2. Map of research location in Tanjung Merah, Bitung City.
(Source: Topographical Map of Indonesia, Geospatial Information Agency, 2016)

Preparation of algae flour. The green algae were washed, soaked, rinsed, and then drained. Freshly cleaned algae were ground to powder using a grinder and dried for about 18 hours to reduce the water content. After that, the two species of green algae were grounded again and sieved to get the flour. Analysis of two species of green algae flour was carried out in the Laboratory of Minerals and Advanced Materials (Central Laboratory), State University of Malang. Observation of nanoparticles was done through

Scanning Electron Microscope (SEM) while the main composition and chemical compounds of the flour were analyzed by the Energy Dispersive Spectrometer (EDX).

Preparation of ethanol extract. These green algae were rinsed, drained, dried with a temperature of 40ºC, weighed, and then sieved. The product from the blender was extracted using 96% ethanol solvent by the maceration method for three days. It was then filtrated using filter paper. In the next step, to obtain ethanol extract, the product was filtrated and evaporated using Rotary Evaporator, then evaporated again using an oven at a temperature of 40ºC until the extract was thick. Analysis of two species of green algae ethanol extract was carried out in the Laboratory of Integrated Research and Testing, University of Gadjah Mada. Then, phytochemical extraction with LC-HRMS was carried out in the Central Laboratory of Life Science, State University of Malang. The procedure was as follows: the sample that weighed 10 grams was soaked in 95% ethanol for 3 days. The extract was prepared by dissolving in hexane, the supernatant was taken and dissolved in acetonitrile solution: methanol (50:50) plus BHT 0.01%, then analyzed. Samples were analyzed by LC-HRMS. Firstly, the sample went through liquid chromatography to separate the components present in sample. These components or molecules were then analyzed using mass spectrometry. The molecule can go through an ionization process that can be done in various ways. However, one of the ionization techniques the most commonly used is electrospray ionization (ESI). The liquid sample is pumped through the capillary and converted into very small droplets. Next, drops are converted to the gas phase using heat and nitrogen. In this process, the electric charge of the droplet will move to the molecule to be detected. Molecules can be positively or negatively charged and can be detected by the machine according to the desired setting. Next, the gas chromatography-mass spectrometry (GC/MS) results were checked in the laboratory and tested with the following procedure: (1) sample preparation; (2) derivatisation; (3) injection (inject the solution mixture into the GC column via the heated injection port; GC/MS is not suitable for analysis of labile compounds at high temperatures because it will be decomposed at the start of the separation); (4) GC separation (the mixture was carried by a carrier gas, usually helium at a certain flow rate passes through a GC column heated in a heater; GC column has an inert/stationary phase coating liquid); (5) MS detector (qualitative aspects: more than 275,000 mass spectra of compounds that do not known to be identified with computerized references and quantitative aspect: by comparing the standard curve of the compound known, the quantity of the unknown compound can be known); and (6) scanning (mass spectra were recorded regularly at 0.5-1 second intervals for separation of GC and stored in the instrument data system for use in analysis; the mass spectra in the form of a fingerprint can be compared with a reference; column: HP-5MS UI).

Results and Discussion. The results of SEM analysis showed the particle morphology of *H. macroloba* and *H. opuntia*. Enlargement of *H. macroloba* nanoparticle images was conducted on a scale of 1,000x, 5,000x, 10,000x, 20,000x and 200,000x. Figure 3 shows the shape and size of nanoparticles obtained through observations using SEM. Based on SEM data, it can be seen that the nanoparticles of *H. macroloba* were not uniform, porous at the edges and sizes tended to vary.

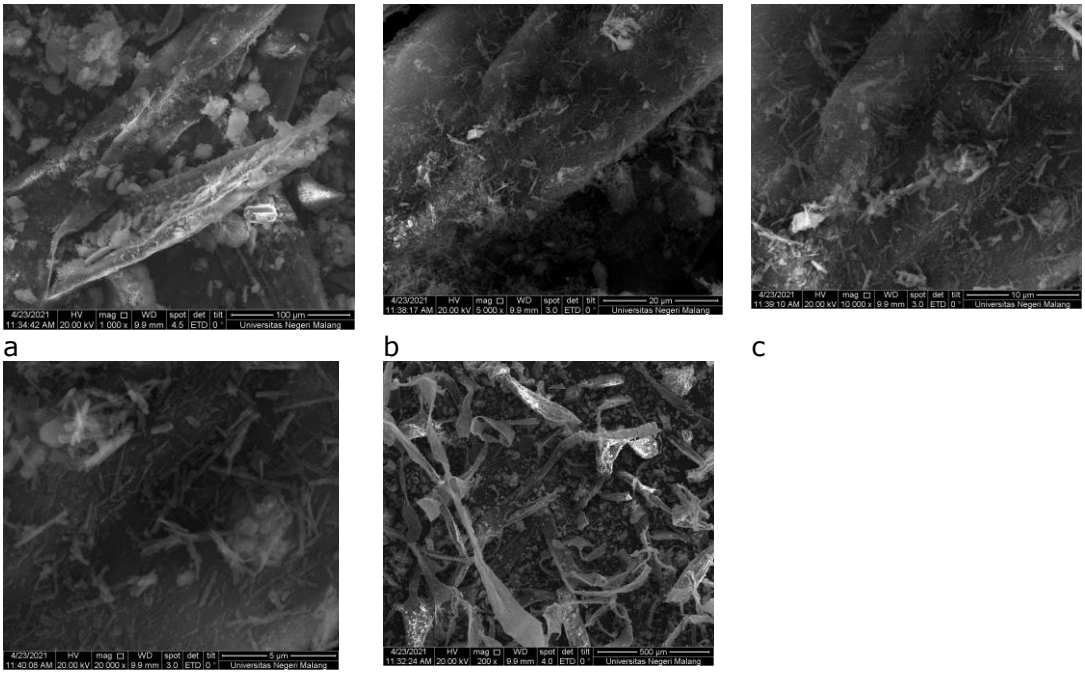


Figure 3. Morphological analysis of *Halimeda macroloba* using SEM (a). 1,000x, (b) 5,000x, (c) 10,000x, (d) 20,000x and (e) 200,000x magnification.
(Source: authors' personal archive and photos taken by authors, 2021)

Enlargement of *H. opuntia* nanoparticle images was carried out on a scale of 1,000x, 5,000x, 10,000x, 20,000x and 50,000x. Figure 4 shows the shape and size of nanoparticles obtained through observations using SEM. Based on SEM data, it can be seen that the nanoparticles of *H. macroloba* were not uniform, porous at the edges and sizes tended to vary.

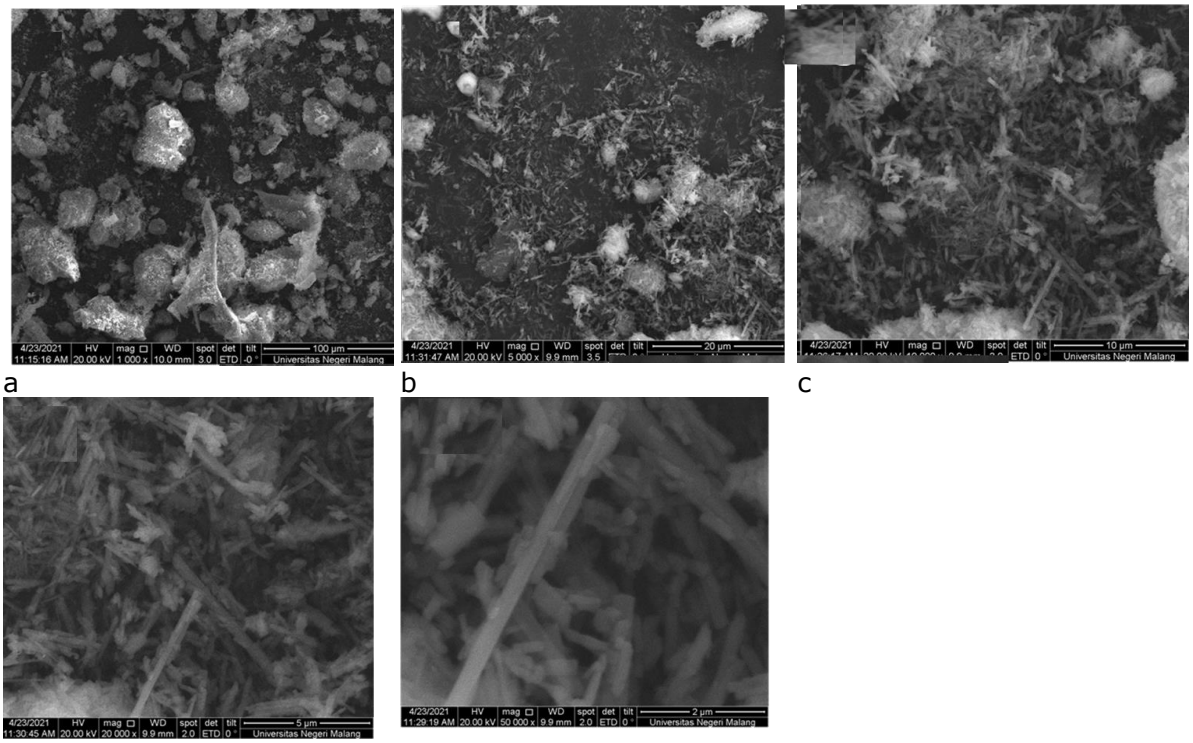
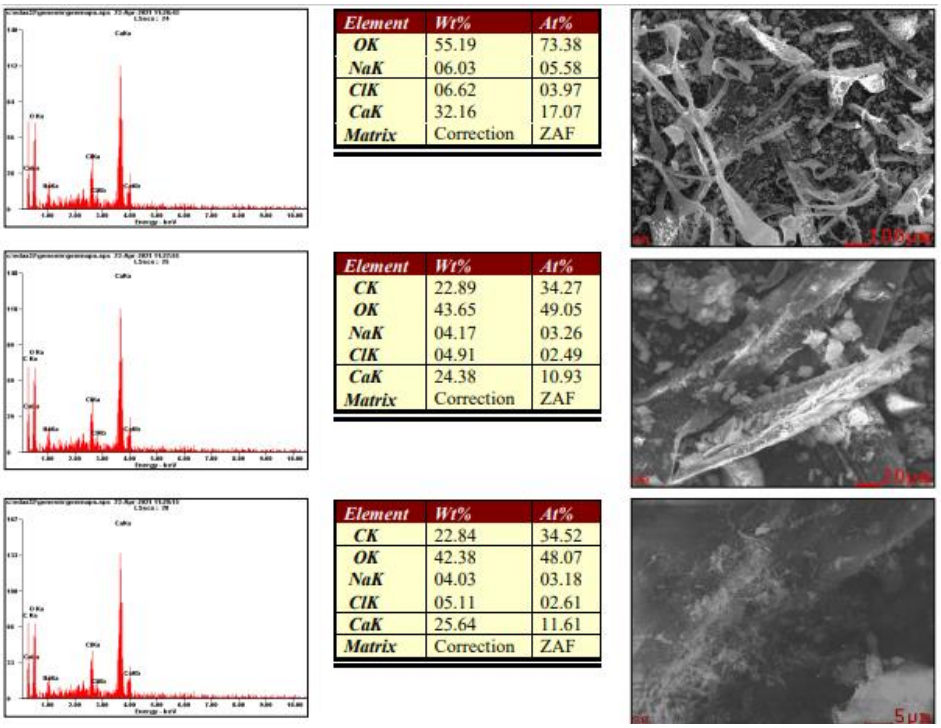


Figure 4. Morphological analysis of *Halimeda opuntia* using SEM (a). 1,000x, (b) 5,000x, (c) 10,000x, (d) 20,000x, and (e) 50,000x magnification.
(Source: authors' personal archive and photos taken by authors, 2021)

Green algae *H. macroloba* contains elements of biomineral compounds dominated by C (carbon) of more than 40%. From the specimens analyzed using EDX, the average elements of biomineral compounds present in *H. macroloba* are shown in Figure 5. In addition to the carbon element, *H. macroloba* is also composed of O (oxygen) 55.19%, Na (sodium) 6.03%, Ca (calcium) 32.16%, and Cl (chlor) 6.62% (photos above), O (oxygen) 43.65%, Na (sodium) 4.17%, Ca (calcium) 24.38%, and Cl (chlor) 4.91% (photos in the middle), and O (oxygen) 42.38%, Na (sodium) 4.03%, Ca (calcium) 25.64%, and Cl (chlor) 5.11% (photos below).



Element	Wt%	At%
OK	55.19	73.38
NaK	06.03	05.58
ClK	06.62	03.97
CaK	32.16	17.07
Matrix	Correction	ZAF

Element	Wt%	At%
CK	22.89	34.27
OK	43.65	49.05
NaK	04.17	03.26
ClK	04.91	02.49
CaK	24.38	10.93
Matrix	Correction	ZAF

Element	Wt%	At%
CK	22.84	34.52
OK	42.38	48.07
NaK	04.03	03.18
ClK	05.11	02.61
CaK	25.64	11.61
Matrix	Correction	ZAF

Figure 5. Energy dispersive spectroscopic analysis of *Halimeda macroloba* using SEM (Source: authors' analyses and photos taken by authors, 2021)
Explain what each picture represents

Green algae *H. opuntia* contains elements of biomineral compounds dominated by C (carbon) of more than 40%. From the specimens analyzed using EDX, the average elements of biomineral compounds present in *H. opuntia* are shown in Figure 6. In addition to the carbon element, *H. opuntia* is also composed of O (oxygen) 47.29%, Ca (calcium) 30.30%, and C (carbon) 22.41%.

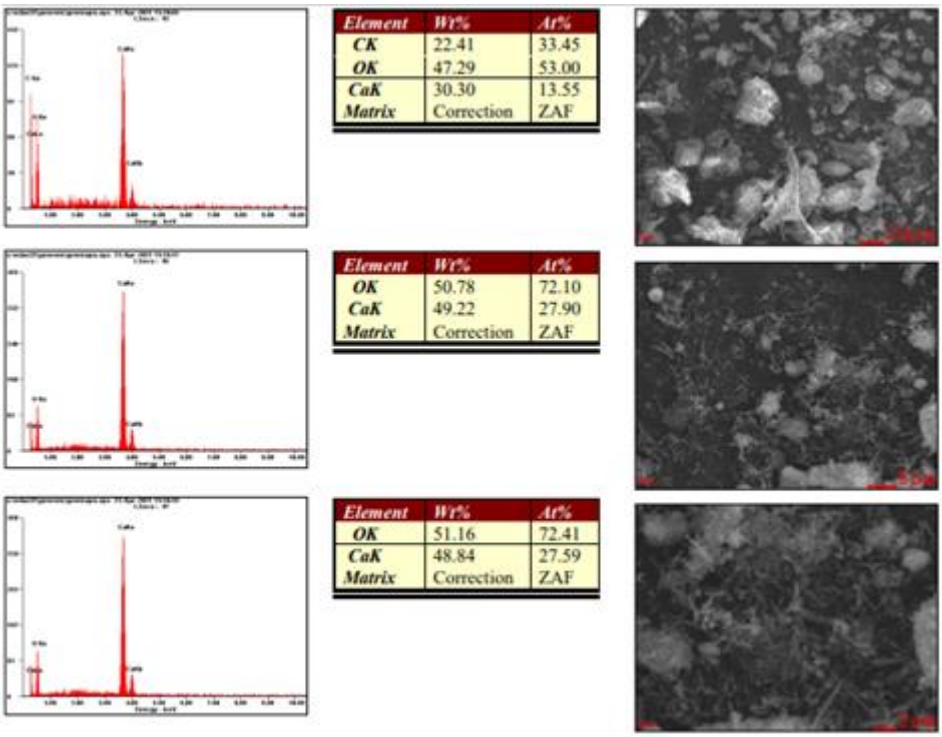


Figure 6. Energy dispersive spectroscopic analysis of *Halimeda opuntia* using SEM (Source: authors' analyses and photos taken by authors, 2021)

Castro-Gonzalez et al (1996) showed that the mineral composition of edible seaweed *Ulva lactuca* was Ca>P>Fe. Taboada et al (2010) mentioned that the mineral composition of edible seaweed *Ulva rigida* was Mg>Na>K>Ca>Fe>P>Mn>Zn>Cue. Ganapathy Selvam & Sivakumar (2014) shown that the order of chemical elements from epidermal portion of the leaf of Seaweed Liquid Fertilizer (SLF) treated *Arachis hypogea* and control was as follows: Ca>P>N>Na>K>Mg>Mn>S>Fe>Zn, Ca>N>P>Na>Mg>Mn>K>Zn>S>Fe respectively. The high value of calcium obtained in the leaf is understandable due to its involvement in the formation of cell wall layer. Reka et al (2017) mentioned the results of energy dispersive spectrum analysis of element constituents in cell wall of the selected seaweeds and showed the presence of different chemical elements in the cell wall of *Ulva lactuca*, *U. reticulata*, *Stoechospermum marginatum*, *Acanthophora spicifera*, *Gracilaria corticata* and *G. edulis*. The order of the nine chemical elements from epidermal portion of the *U.* lactuca was C>O>Mg>S>Si>Ca>Na>Al>K>Cl>Se. The weights of different elements were given as percentage (%) values. Among the nine elements, the maximum contribution were: carbon 47.49%, oxide 43.96%, magnesium 2.66%, sulphur 2.54%, silicon 1.45%, sodium 0.69, calcium 0.79, potassium 0.10, and selenium 0.01%. Algae *A. spicifera* showed the eight elements in the following order O>C>K>Cl>S>Ca>Na>Mg and in *Gracilaria corticata* and *G. edulis* the order was C>O>Cl>K>S>Na>Si>Fe>Al>Mg and O>C>K>Cl>S>Ca>Na>Si>Mg>Al, respectively. Maximum amount of oxide was detected in *A. spicifera* (30.97%), followed by *G. edulis* (29.43%). Magnesium share was 1.17% in *A. spicifera*, 0.08% in *G. corticata*, and 0.84% in *G. edulis*. Calcium was found to be 5.82% in *A. spicifera* and 5.42% in *G. edulis*. *G. corticata* had 0.25% of iron. Singkoh et al (2019) mentioned that red algae *T. fragilis* contained elements of biomineral compounds dominated by C (carbon) of more than 40%. This red algae is also composed of O (oxygen) 39.86%, Ca (calcium) 14.5%, (and many minerals in low quantity) Pb (lead) 3.53%, Pt (platinum) 3.13%, S (sulfur) 1.5%, Ni (nickel) 0.34%, K (potassium) 0.19%, Fe (iron) 0.19%, Co (cobalt) 0.19%, Zn (zinc) 0.16%, Mg (magnesium) 0.15%, Na (sodium) 0.14%, Al (aluminum) 0.12%, Mn (manganese) 0.09%, Cr (chromium) 0.05%, Se (selenium) 0.04 %, and P (phosphorus) 0.02%.

James and Martin first introduced gas chromatography technique in 1952 (Sparkman et al. 2011). Gas Chromatography Mass Spectrometry (GCMS) is one of the

chromatographic techniques that can only be used to detect volatile compounds. The criteria for yawning are that it can evaporate at high vacuum and low-pressure conditions and can be heated (Darmapatni et al. 2016). The basis of separation using gas chromatography is dispersion sample in the stationary phase while the gas as the mobile phase elutes the stationary phase. GCMS is a combination of two tools, namely gas chromatography and mass spectrometry. GCMS is used to detect masses between 10 m/z to 700 m/z (Fassenden & Fessenden 1982). Most analyzes with GC-MS can be divided into two groups, namely: qualitative and quantitative. Both analyzes use mass spectrometer as a detector (Munson 1991). The GC-MS reads the spectra contained in the two combined methods. The number of peaks on graph determines the number of phytochemical compounds in GC spectra. Based on time data retention that is already known from the literature, which compounds are present in the sample can be known. The next step is to enter the suspected compound into a mass spectroscopic instrument. This can be done because one of the functions of gas chromatography is to separate compounds from a sample. After that, the results of the obtained graphs or spectograms consisting of different compounds were shown through the peaks of different wavelengths. The information obtained from these two techniques combined in the GC-MS instrument is the result of the individual spectra. The most important information obtained for spectra GC is the retention time for each compound in the sample. As for the MS spectra, information can be obtained regarding the relative molecular mass of the sample compound.

Green algae *H. macroloba* has several compounds, as evidenced by the results of the analysis with GC-MS in the integrated research and testing laboratory, at State University of Malang. The chromatogram of *H. macroloba* described 24 major phytochemical compounds (Figure 7) and at least 5 of the highest peaks were selected (Table 1).

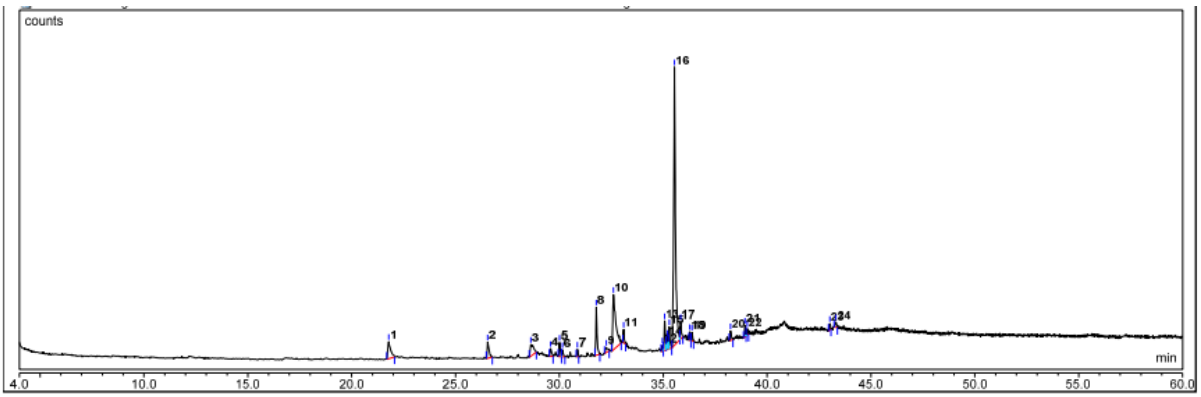


Figure 7. GC-MS chromatogram of *H. macroloba*
(Source: authors' analyses, 2021)

The phytochemical compounds produced from *H. macroloba* generated the highest compound, namely heptadecanoic acid, 16-methyl-, methyl ester ($C_{19}H_{38}O_2$), molecular weight 298 and relative area 46.92% (Table 1). This is followed by n-hexadecanoic acid ($C_{16}H_{32}O_2$), hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), 1-chloroundecane ($C_{11}H_{23}C_1$), and eicosanoic acid ($C_{20}H_{40}O_2$) (Table 1).

Table 1.
The phytochemical compound, chemical formula, molecule weight, and relative area of *H. macroloba*

No.	*Compound*	*Chemical formula*	*Molecular weight (g/mol)*	*Relative area (%)*
1	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	46.92
2	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.60
3	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.35
4	1-Chloroundecane	$C_{11}H_{23}C_1$	190	4.41
5	Eicosanoic acid	$C_{20}H_{40}O_2$	312	3.95

(Source: authors' analyses, 2021)

1) Heptadecanoic acid, 16-methyl-, methyl ester. The compound of heptadecanoic acid, 16-methyl-, methyl ester can be found in methanolic leaf extract identified through GC-MS analysis of *Cirsium arvense*. All parts of *C. arvense* have antifungal potential against phytopathogenic fungus *Macrophomina phaseolina*. The leaf extract showed the highest antifungal activity, followed by the stem extract (Banaras et al 2017).

2) n-Hexadecanoic acid. Hexadecanoic acid, also known as palmitic acid, functions as an anti-bacterial and cholesterolemic (Dineshkumar & Rajakumar 2015). According to Jegadeeswari et al (2012) the compound hexadecanoic acid or palmitate has antioxidant, anti-androgenic, anti-fungal, anti-tumor, anti-bacterial, hemolytic, pesticide, and lubricant. On extract methanol of *Justicia gendarussa* combination treatment of IAA and BAP, identified by GCMS with an area of 2.69% (Wahyuni et al. 2017). The compound of hexadecanoic acid can also be found in the GCMS analysis against ethanol extract of *Pleiospermium alatum*, (Parthipan 2015), also in *Marsilea quadrifolia* leaf extract (Gopalakrishnan & Udayakumar 2014). Hexadecanoic acid of 1.61% was also identified by GCMS in *Solanum melongena* extract (Vanitha 2016). Hexadecanoic acid (ethyl ester), also known as acid palmitate ethyl ester, can be used as a flavoring, fragrances, lubricants, cosmetic additives, have activity antibacterial and hypercholesterolemia (Gideon 2015). This compound can lower cholesterol in the blood (Hema et al. 2011). It inhibits the cyclooxygenase (COX) II enzyme and produces anti-selective inflammation (Belakhdar et al. 2015).

3) Hexadecanoic acid, methyl ester. Hexadecanoic acid methyl ester or metil palmitate belongs to fatty acid group with capacity as anti-bacteria by disrupting bacterial cell wall and cell membrane. This compound was reported by Balakhdar et al (2015), one of the most abundant compounds in extract of *Thesium humile*. Hema et al (2011) found this compound as an excellent antioxidant and anti-inflammatory constituent. Kavitha and Uduman Mohideen (2017) also found this compound had capacity as antioxidant, hypocholesterolemic, and antiandrogenic. Pinto et al (2016) also found this compound had capacity as antimicrobial activity, particularly on fungi, of compounds belong to fatty acid methyl ester (including hexadecanoic acid, methyl ester). Padmini et al. (2010) also found the ability of this compound to work synergically with various active compounds and increase its anti-bacterial activity.

4) 1-Chloroundecane. 1-Chloroundecane is used as an intermediate in the chemical industry under strictly controlled and rigorously contained condition. It has a low toxic potential but causes irritation to the skin. This compound was detected in arial parts of *Pupalia lappacea* (Selvan et al 2014).

5) Eicosanoic acid. Arachidic acid, also known as eicosanoic acid, is a saturated fatty acid with a 20-carbon chain. It belongs to the class of organic compounds known as long-chain fatty acids. These are fatty acids with an aliphatic tail that contains between 13 and 21 carbon atoms. Arachidic acid is a very hydrophobic molecule, practically insoluble in water, and relatively neutral. This compound found in starfishes (Bruno et al 1992). It also found in *Nannochloropsis* sp. (Ermavitalini et al 2019). The eicosanoic acid has an antifungal activity, isolated from the endophytic fungus *Mycosphaerella* sp. against *Cryptococcus neoformans* and *C. gattii* (Pereira et al 2016).

Green algae *H. opuntia* has several compounds, this is evidenced by the results of the analysis with GC-MS in the integrated research and testing laboratory, State University of Malang. Chromatogram of *H. opuntia* described 55 major phytochemical compounds (Figure 8) and at least five of the highest peaks were selected (Table 2).

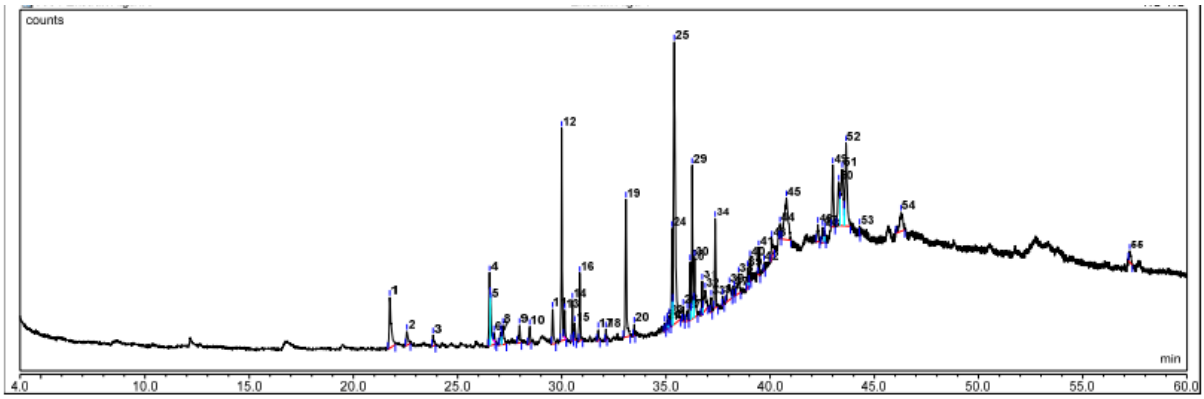


Figure 8. GC-MS chromatogram of *H. opuntia*
(Source: authors' analyses, 2021)

The phytochemical compounds produced from *H. opuntia* have produced the highest compound, namely phytol ($C_{20}H_{40}O$), molecular weight 296 and relative area 16.52 (Table 2). This is followed by neophytadiene ($C_{20}H_{38}$), 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E) ($C_{27}H_{44}O_3$), ethyl iso-allocholate ($C_{26}H_{44}O_5$), and hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) (Table 2).

Table 2.
The phytochemical compound, chemical formula, molecule weight and relative area of *H. opuntia*

No.	*Compound*	*Chemical formula*	*Molecular weight (g/mol)*	*Relative area (%)*
1	Phytol	$C_{20}H_{40}O$	296	16.52
2	Neophytadiene	$C_{20}H_{38}$	278	5.91
3	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)	$C_{27}H_{44}O_3$	416	5.61
4	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	5.37
5	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	5.00

(Source: authors' analyses, 2021)

1) Phytol. Phytol is a compound found abundantly in nature. It is a part of the chlorophyll molecule and produced by almost all photosynthetic organisms, including algae (de Souza & Nes 1969), plants (Ischebeck et al 2006), and bacteria (cyanobacteria) (Proteau 1998). It has a potential for antioxidant activity (Pejin et al. 2014; Santos et al 2013). It is recognized for its wide range of pharmacological effects on the nervous system, including anxiolytic and antidepressant (Pereira Costa et al 2014). Some phytol-derivatives (phytanol, phytanyl amine, and phytanyl mannose) exert immune-stimulating activity by induction of the expression of a range of chemokines and cytokines (Aachoui et al 2011a; Roy Chowdhury et al 2013) and modulation of immune responses through apoptotic/necrotic (Aachoui et al 2011b) effects on target tumor cells. Phytol has antinociceptive (Santos et al 2013) and anti-inflammatory activities (Silva et al 2014).

2) Neophytadiene. Neophytadiene, is a diterpenoid hydrocarbon that belongs to the group of compounds known as phytanes (Tejeda et al. 2001). Neophytadiene normally occurs in animal tissues and is derived mainly from plant origin, and is an important vegetable wax component (Post-Beittenmiller 1996). The GC-MS result of ethyl acetate extract leaves of sea poison (*Barringtonia asiatica*), dadap (*Erythrina lithosperma*),

gempol (*Nauclea orientalis*), and soursop (*Annona muricata*) contain neophytadiene (Hidayati & Nuringtyas 2016). Neophytadiene is a molecule isolated from a marine algae *Turbinaria ornata* in LPS-induced inflammation in both in vitro and in vivo conditions. This molecule is significantly confirming the anti-inflammatory potential (Bhardwaj et al 2020).

3) 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E). The 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E) is new chemical compounds. This is one of major phytochemical compounds identified in methanolic extract of *Ricinus communis* with unknown activity (Hussein et al 2016; Al-Gara'awi et al 2019), *Zingiber officinale* with unknown activity (Shareef et al 2016), *Citrullus colocynthis* as new chemical compound (Idan et al 2015). This compound is one of the major phytoconstituents of GC-MS phytochemical analysis of *Cnidoscolus aconitifolius* leaves (Chukwu et al 2020).

4) Ethyl iso-allocholate. The ethyl iso-allocholate is steroid derivative with antimicrobial activity (Malathi et al 2016). GC-MS analytical results for the dichloromethane and ethyl acetate fractions of *Tabernaemontana catharinensis* stem bark found ethyl iso-allocholate. This compound is present in the dichloromethane fraction. It is the ester of a bile acid and can act as an emulsifying agent so that water soluble digestive enzymes can digest fats and oils in the small intestine. This constituent may be responsible for the relief of constipation and indigestion (Boligon et al 2014). It was detected in *Feronia elephantum* leaf and bark extract. The ethyl iso-allocholate identified in the ethanol leaf extract of *F. elephantum* is an antimicrobial, diuretic and anti-inflammatory. Its activity in the ethanol leaf extract of *F. elephantum* is antimicrobial, diuretic, and anti-inflammatory and antiasthma (Muthulakshmi et al 2012). *Lepidagathis cristata* leaf extract was investigated for its potential bioactive compounds heptadecane, 9-hexyl and ethyl iso-allocholate are effective plant extract and as antifungal agent for plant and human pathogenic fungi (Abubacker & Devi 2015).

5) Hexadecanoic acid, ethyl ester. The hexadecanoic acid ethyl ester has another name of stearic acid that is commonly used as food supplements, cosmetics, and industrial products. This compound is used as a material in wax, plastic, and soap making in addition to soften rubber in the stearic acid industry. It has been reported to have activities as hypercholesterolemic, lubricant, antimicrobial, flavor, cosmetic, and perfumery (Gideon 2015). An antitumor activity of this compound was also mentioned (Tyagi & Agarwal 2017). This compound was identified as the active constituents present in aerial parts of *Fluggea leucopyrus* (Euphorbiaceae) by gas chromatography-mass spectrometry (GC-MS) analysis (Sudha et al 2013).

Conclusions. Green algae *H. macroloba* from coastal waters of Tanjung Merah in Bitung City is characterized by mineral elements content composed of O, Na, Ca and Cl. *H. opuntia* had O, Ca and C. *H. macroloba* has 24 major phytochemical compounds and at least five of the highest peaks were heptadecanoic acid, 16-methyl-, methyl ester ($C_{19}H_{38}O_2$), n-hexadecanoic acid ($C_{16}H_{32}O_2$), hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), 1-chloroundecane ($C_{11}H_{23}C_1$), and eicosanoic acid ($C_{20}H_{40}O_2$). *H. opuntia* has 55 major phytochemical compounds and at least five of the highest peaks were phytol ($C_{20}H_{40}O$), neophytadiene ($C_{20}H_{38}$), 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E) ($C_{27}H_{44}O_3$), ethyl iso-allocholate ($C_{26}H_{44}O_5$), and hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$). The results show a relationship between mineral content and the constituent elements of bioactive compounds from algae that contain oxygen. The discovery of minerals and biochemical compounds in the two green algal species is the initial stage to determine the active ingredients for pharmaceuticals, cosmetics, and nutraceuticals products from marine organisms. Knowing the various bioactive compounds in macroalgae allows to determine the benefits of these bioactive compounds. Depending on their benefits, these bioactive compounds can be further used in various products.

Conflict of interests. The authors declare no conflict of interest.

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