Biomineral characterization and phytochemical compound of brown algae *Turbinaria decurrens* Bory de Saint-Vincent, 1828 and *Turbinaria ornata* (Turner) J. Agardh, 1848 from coastal waters of Kora-kora, Minahasa Regency, North Sulawesi, Indonesia

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Abstract. This study aims to determine the mineral composition of brown algae *Turbinaria decurrens* Bory de Saint-Vincent, 1828 and *Turbinaria ornata* (Turner) J. Agardh, 1848. 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 *T. decurrens* and *T. ornata*. Specimens analyzed using energy dispersive showed that *T. decurrens* contained biomineral compound elements: O, Na, Mg, Al, Si, S, Cl, K, Ca, Cu, and *T. ornata* contained biomineral compound elements: O, Na, Mg, Al, Si, S, Cl, K, Ca. Analysis by Gas chromatography-mass spectrometry (GC-MS) showed that *T. decurrens* has 43 phytochemical compounds while *H. opuntia* also has 43 phytochemical compounds.
Key Words: seaweed, mineral, particle morphology, Scanning Electron Microscope (SEM), Energy Dispersive X-ray spectroscopy (EDX).

Introduction. A genus of *Turbinaria* is found in tropical marine waters which grows on rocky substrates. *Turbinaria* species are consumed by herbivorous fishes and echinoids in tropical areas (Peter 1986). *Turbinaria* has many species, among others *T. decurrens* and *T. ornata.* Brown algae *T. decurrens* was discovered by Bory de Saint-Vincent, 1828 and *Turbinaria ornata* was discovered by J. Agardh, in 1848. Brown algae *T. decurrens* and *T. ornata* belong to kingdom Chromista, subkingdom Harosa, infrakingdom Heterokonta, phylum Ochrophyta, subphylum Phaeista, infraphylum Limnista, superclass Fucistia, class Phaeophyceae, order Fucales, family Sargassaceae, and genus *Turbinaria* (Guiry & Guiry 2025a; Guiry & Guiry 2025b).

T. decurrens has thalli erect, tough, dark to yellowish brown, with coarse, branched holdfasts; leaves fleshy and usually occur from lower intertidal to upper subtidal areas, strongly attached to rocks in wave-exposed habitats, whereas *T. ornata* has thalli erect and tough, dark brown, attached to the rocky substrate by coarse branched holdfasts and thrives mostly on rocky reef areas exposed to strong water turbulence (Calumpong & Meñez 1997; Trono 1997; Trono 1998). *T. decurrens* is widely distributed in the tropical waters of the Southeast Asia region and *T. ornata* is widely distributed in the warm waters of the tropics (Trono 1998). *T. ornata* is a perennial macroalga. It is widely distributed in tropical and subtropical areas of the Indian Ocean and throughout the western and southern Pacific Ocean (Rohfritsch et al 2007).

Description of macroalgae species including *T. decurrens* and *T. ornata* in Indonesia has been carried out by Atmadja et al (2019), in Manokwari (Kepel et al 2012), and in West Southeast Maluku (Kepel & Baulu 2013). In North Sulawesi coastal waters, *T. decurrens* and *T. ornata* was found in Tongkaina (Kepel et al 2018a), Blongko (Kepel et al 2018b), Kora-kora (Kepel & Mantiri 2019), Minahasa Peninsula in the wet season

(Kepel et al 2019), Minahasa Peninsula in the dry season (Kepel et al 2020), coastal waters at different heavy metals concentrations in Minahasa Peninsula (Tombokan et al 2020), Bombuyanoi Island (Patra et al 2021), Ondong (Kandati et al 2021), Tateli and Mokupa (Turangan et al 2024), Tongkaina (Rafii et al 2024) and coastal waters of Likupang Marine Station, Tongkaina and Kora-kora (Kepel et al 2024).

This study aimed to determine the mineral composition of brown algae, *T. decurrens* and *T. ornata*. Scanning electron microscope analysis and energy dispersive were used to show the morphology of the particles and the mineral composition of these algae.

Material and Method. Samples of brown algae *T. decurrens* and *T. ornata* (Figure 1) were taken from the coastal waters of Kora-kora, East Lembean District, Minahasa Regency, 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. Brown algae, *Turbinaria decurrens* (left), and *Turbinaria ornata* (right) from coastal waters of Kora-kora (Source: author's personal archive and photos taken by authors 2024).

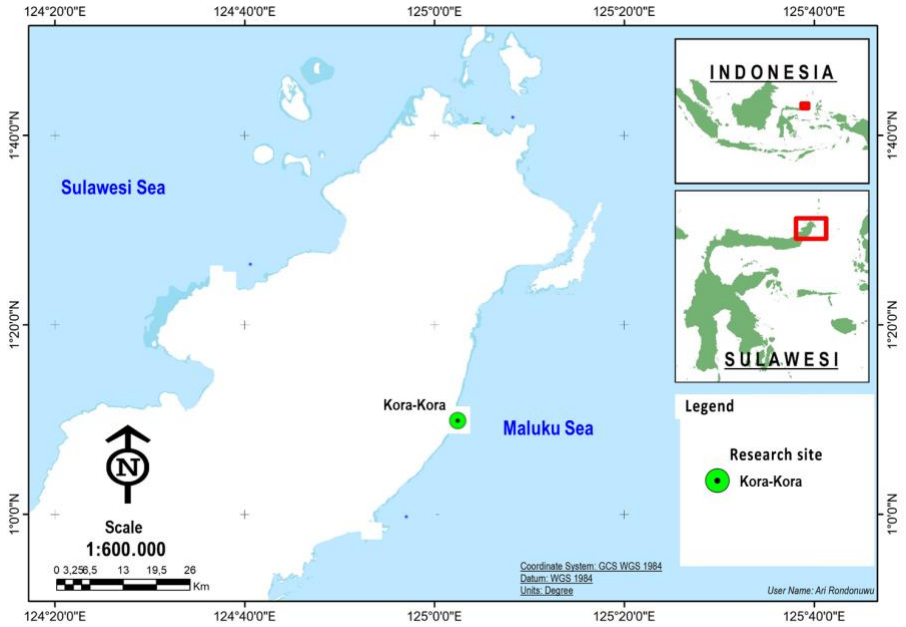


Figure 2. Map of research location in Kora-kora, Minahasa Regency.

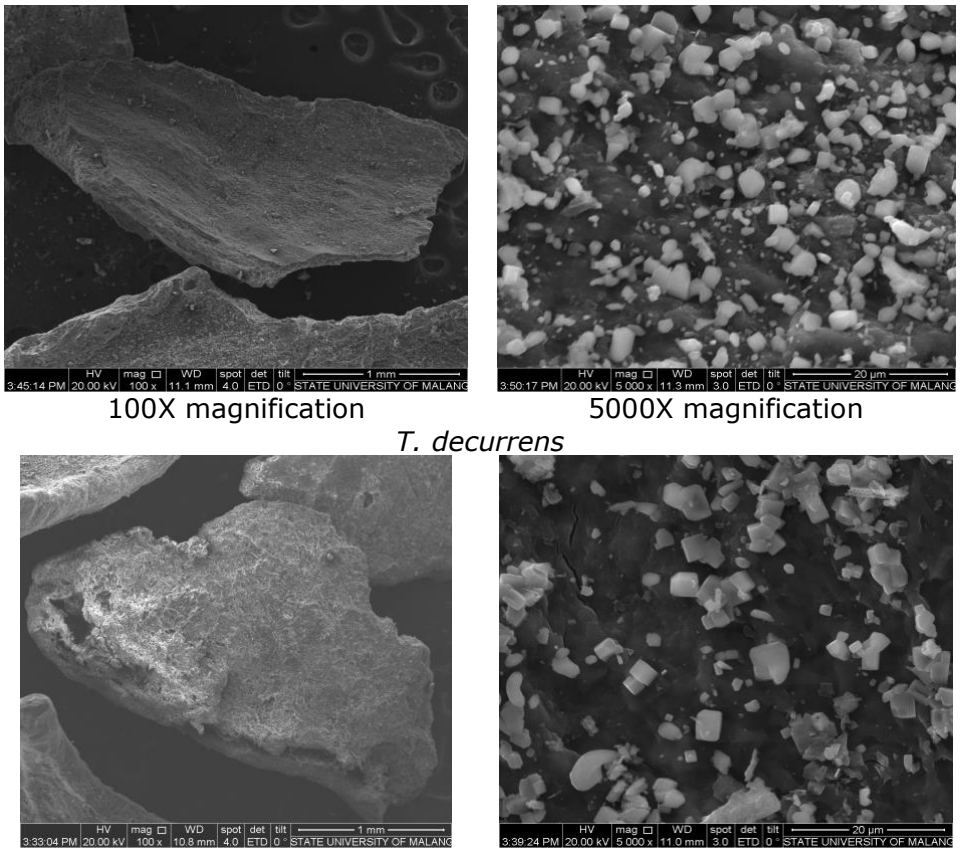
Preparation of algae flour. The two species of brown algae were washed and then soaked in fresh water for one night to remove dirt, then rinsed under running water so that the algae were clean and drained. Freshly cleaned algae were ground to powder using a grinder and dried for about 18 hours to reduce the water content. After that, the two species of brown algae were respectively ground again and then sieved to get the flour. Analysis of two species of brown 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 a 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 brown algae were rinsed under running water so that the algae were clean and then drained. Freshly cleaned algae were weighed and then dried using an oven at a temperature of 40ºC. Dried algae were weighed. After that, the two species of brown algae were respectively ground and then sieved. The product from the blender was extracted using 96% ethanol solvent by maceration method for 3 days. Then filtrated using filter paper. To obtain ethanol extract, it is filtrated and evaporated using a Rotary Evaporator, then evaporated again using an oven at a temperature of 40ºC until the extract is thick. Analysis of two species of brown algae ethanol extract was carried out in the Laboratory of Integrated Research and Testing, University of Gadjah Mada. Then, phytochemical extraction with liquid chromatography-high resolution mass spectrometry (LC-HRMS) was carried out in the Central Laboratory of Life Science, State University of Malang. The procedure is as follows: the sample was weighed 10 grams and 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 analyzed by LC-HRMS first go through liquid chromatography to separate the components present in the sample. These components or molecules are continued to 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 to be detected can be positively or negatively charged and can be detected by the machine with the accordingly desired setting.

Next, the gas chromatography-mass spectrometry (GC-MS) results were checked in the laboratory and testing with the following procedure: sample preparation, derivatization, and 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), 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), 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 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

Scanning electron microscopy (SEM). Microphotographs of electron displacement from the cross-sectional area of the lateral branches of *T. decurrens* and *T. ornata* (Figure 3) were obtained at magnifications of 100X and 5000X. SEM is based on the transfer (or rastering) of the electron beam across the sample and the detection of electrons ejected from the surface. The incoming electrons are scattered back from the atomic surface species or spread to the sample material. SEM is used primarily for the topographic characteristics of material surfaces.



100X magnification 5000X magnification

T. decurrens

100X magnification 5000X magnification

T. ornata

Figure 3. SEM micrograph of *T. decurrens* and *T. ornata*.

The topographic characteristics of each alga at a magnification of 100X showed that *T. decurrens* had a flat cell surface, and *T. ornata* had an uneven cell surface. Meanwhile, at a magnification of 5000X of each alga, the cell surface shape looks almost mostly like a cube. The surface shape of algae cells in different species, such as the study by Singkoh et al (2019) showed that the morphology of *Tricleocarpa fragilis* particles at magnifications of 200x, 1000x, and 10,000x nanoparticles is not uniform, porous at the edges, and tends to vary in size.

Energy dispersive X-Ray spectroscopy (EDS). The EDS spectrum depicts x-rays of various macro and micro elements in the form of an energy spectrum which in turn helps in the identification of concentrations of elements such as sodium, magnesium, silicon, phosphorus, sulfur, chloride, potassium, calcium, manganese, iron and zinc. EDS spectrum of *T. decurrens* and *T. ornata* algae (Figure 4), and elements of *T. decurrens* and *T. ornata* (Table 1).

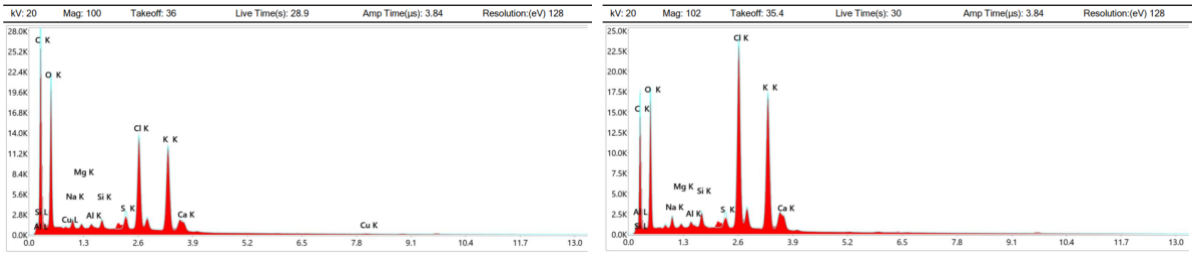


Figure 4. Spectrum EDX elemental analysis of *T. decurrens* (left) and *T. ornata* (right).

Table 1

EDX elemental analysis of *Turbinaria decurrens*

Characteristic elements	*Element concentration*			
	T. decurrens		*T. ornata*	
	Weight (%)	*Atomic (%)*	*Weight (%)*	*Atomic (%)*
Carbon (C)	60.0	70.0	52.8	65.4
Oxygen (O)	29.7	26.0	29.3	27.2
Sodium (Na)	0.5	0.3	0.6	0.4
Magnesium (Mg)	0.1	0.1	0.1	0.1
Aluminium (Al)	0.1	0.1	0.2	0.1
Silicone (Si)	0.2	0.1	0.4	0.2
Sulfur (S)	0.6	0.2	0.5	0.2
Chlorin (Cl)	3.6	1.4	7.5	3.1
Potassium (K)	4.5	1.6	7.7	2.9
Calcium (Ca)	0.6	0.2	1.0	0.4
Copper (Cu)	0.1	0.0	-	-

Brown alga *T. decurrens* and *T. ornata* contain elements of biomineral compounds dominated by C of 60% and 52.8%, and O 29.7% and 29.3%. In addition to the carbon element, *T. decurrens,* and *T. ornata are* also composed of Na (sodium), Mg (magnesium), Al (aluminum), Si (silicone), S (sulfur), Cl (chlorine), K (potassium), Ca (calcium), and Cu (copper) contain only in *T. decurrens*. The process of photosynthesis in algae greatly influences the existence of atoms in organisms.

Algae can absorb and store carbon in thallus with a high percentage of weight. As in the algae *T. fragilis* has a carbon element greater than 40%, followed by oxygen 39.86% (Singkoh et al 2019). However, there is also the algae *Sargassum wightii* which contains elements of biomineral compounds dominated by sulfur (S) which is the highest at 21.90%. The brown algae of the specimen analyzed using EDS, other elements of biomineral compounds consisted of chloride (Cl) 19.29%, calcium (Ca) 3.72%, potassium (K) 2.27%, manganese (Mn) 0.30%, iron (Fe) 15.75%, sulfur (S) 21.90%, magnesium (Mg) 2.33%, sodium (Na) 2.96%, and silicon (Si) 9.89% (Deepika 2019).

Inorganic elements considered to be essential for normal body functions include (i) the major elements, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and chloride (Cl), (ii) the trace elements, cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), iodine (I), manganese (Mn), molydenum (Mo), selenium (Se) and zinc (Zn), and (iii) the 'newer' trace elements, arsenic (As), lead (Pb), lithium (Li), nickel (Ni), silicon (Si), vanadium (V), and possibly fluorine (F) and tin (Sn) (Fairweather-Tait & Hurrel 1996). The value of edible seaweeds in human nutrition is based on their richness in several minerals, like sodium (Na), magnesium (Mg), phosphorous (P), potassium (K), iodine (I), iron (Fe), and zinc (Zn) (Circuncisão et al 2018). Seaweeds contain the essential minerals and trace elements required for human nutrition, and macroalgal ash content is typically high (Reddy et al 2023). According to Ortega et al (1993), mineral content in marine algae is higher than that of land plants and animal products. Publications of Abbas et al (1992) and Basson and Abbas (1992) indicated that marine seaweeds contained high amounts of K, Ca, Mg, Fe, Zn, Mn, and Cu. Matanjun et al (2009) mentioned these seaweeds contained 12.01–15.53% macro-minerals (Na, K, Ca,

and Mg) and 7.53–71.53 mg.100 g trace minerals (Fe, Zn, Cu, Se, and I). Osman et al (2011) mentioned moisture contents in green alga *Caulerpa serrulata* contained large amounts of Na, Ca, K, and Fe, moderate amounts of Zn, and were lower in Cu, Cd, Ni, and Mn. Pb was measured in brown alga *Padina tetrastromatica*. Anantharaman et al (2010) found *Ulva reticuata* showed the maximum content of mineral composition of chromium (Cr), copper (Cu), and magnesium (Mg) and the lowest level of mineral content of cobalt (Co), copper (Cu), magnesium (Mg), manganese (Mn), lead (Pb) and zinc (Zn) were present in *Halimeda tuna*.

Gas chromatography-mass spectrometry (GC-MS). Gas chromatography (GC) is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase. In gas chromatography, the components of a sample are dissolved in a solvent and vaporized to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column. Gas chromatography is one of the sole forms of chromatography that does not utilize the mobile phase for interacting with the analyte. The stationary phase is either a solid adsorbent, gas-solid chromatography (GSC), or a liquid on an inert support, gas-liquid chromatography (GLC). In the early 1900s, Gas chromatography (GC) was discovered by Mikhail Semenovich Tsvett as a separation technique to separate compounds. The gas chromatography technique was first introduced by James and Martin in 1952 (Sparkman et al 2011). The only chromatographic technique that can be used to detect volatile compounds is Gas Chromatography Mass Spectrometry (GCMS). The criteria for evaporation are that it can evaporate under high vacuum and low-pressure conditions and can be heated (Darmapatni et al 2016). The basis of separation using gas chromatography is the 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 (Fassenden & Fessenden 1982). Most analyses with GCMS can be divided into two groups, namely qualitative and quantitative. Both analyses use a mass spectrometer as a detector (Munson 1991).

The results of *the* GC-MS analysis show that the brown alga *T. decurrens* has phytochemical compounds. The chromatogram of *T. decurrens* describes 43 phytochemical compounds (Figure 5). Based on the areas, only 8 prominent compounds were taken. The pharmacological activity of the 8 highest compounds from *T. decurrens* is shown in Table 2.

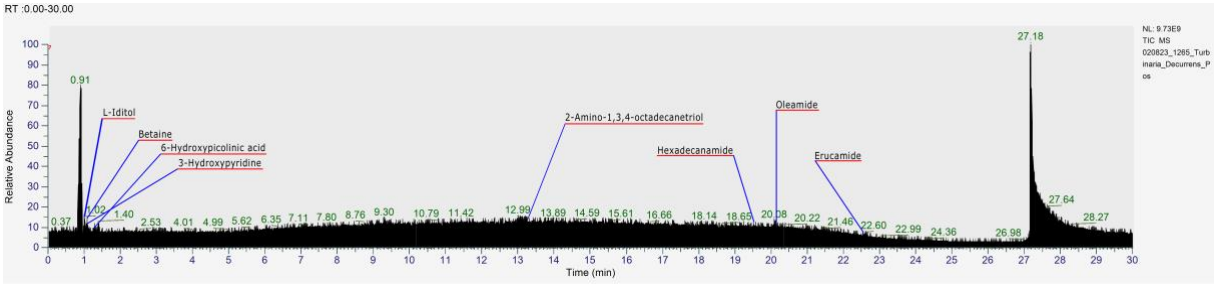


Figure 5. GC-MS chromatogram of *T. decurrens*.

Table 2

The pharmacological activity of 8 highest compounds from alga *Turbinaria decurrens*

No.	*Compound name*	*Molecule formula*	*Pub-Chem ID*	*Retention time (min.)/area (max)*	*Pharmacology activities*
1.	Oleamide	$C_{18}H_{35}NO$	5283387	20.093 / 1828095278.03771	Neuroprotective effects (Cravatt et al 1995; Bisogno et al 1997)
2.	L-Iditol	$C_6H_{14}O_6$	5460044	1.021 / 653860874.391749	Antioxidant (Islami et al 2014)
3.	3-Hydroxypyridine	C_5H_5NO	7971	1.089 / 548510596.980355	Antioxidant (Suzdal'tseva et al 1982), neuroprotective effect (Kolesnichenko et al 2020).
4.	Erucamide	$C_{22}H_{43}NO$	5365371	22.586 / 322275667.081599	antimicrobial (Xie et al 2021)
5.	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	248575	13.122 / 232087618.394408	Neuroprotective (Zeng et al 2024),
6.	Hexadecanamide	$C_{16}H_{33}NO$	69421	19.527 / 220908534.797369	Anti-inflammatory neuroprotective (Bao et al 2023)
7.	Betaine	$C_5H_{11}NO_2$	247	1.028 / 205882409.80995	Hepatoprotective, anti-inflammatory, cardioprotective (Arumugam et al 2021)
8.	6-Hydroxypicolinic acid	$C_6H_5NO_3$	242721	1.29 / 190324798.171815	Antimicrobial, antioxidant, anticancer, neuroprotective (Kumar et al 2018)

The phytochemical compounds produced from *T. decurrens* are:

Oleamide ($C_{18}H_{35}NO$). The oleamide compound (cis-9-octadecenamide) is a well-known additive lubricant from the amino group of fatty acids, besides erucamide ((Z)-docos-13-enamide) and stearamide (octadecanamide) (Farajzadeh et al. 2005; Lau & Wong 2000). Oleamide (oleic acid amide) is also an endogenous bioactive signaling molecule that acts in diverse cell types and consequently triggers different biological effects. Among its various functions, the best known is its sleep-inducing effect (Cravatt et al 1995; Bisogno et al 1997).

L-Iditol ($C_6H_{14}O_6$). The first report of L-iditol and taxane compounds was extracted from the angiosperm, *Yunnanopilia longistaminata*. Two compounds L-iditol and taxane were isolated from tender burgeon *Y. longistaminata*, and their structures were identified as Taxayunnansin B, Taxumairol E, and L-iditol based on NMR and MS spectra. This is a new plant source for L-iditol and taxanes (Liu & Liu 2008). In the brown algae *T. decurrens*, the total carotenoids it contains are closely related to the antioxidant activity (Islami et al 2014).

3-Hydroxypyridine (C_5H_5NO). The existence of a 3-hydroxypyridine degradation pathway and utilization of 3-hydroxypyridine by *Agrobacterium* sp. It may have potential use for bioremediation of polluted environments with 3-hydroxypyridine (Zhao et al 2019).

Erucamide ($C_{22}H_{43}NO$). Oleamide and erucamide are used to reduce surface friction in industrial applications. This application is considered efficient and economical. The slip properties of fatty acid amides mixed with paraffin wax as the static sliding friction coefficient (μs) were measured. The μs values were significantly reduced by the addition of oleamide or erucamide (Getachew et al 2016).

2-Amino-1,3,4-octadecanetriol ($C_{18}H_{39}NO_3$). Many examples of synthetic methods toward D-erythro-sphingosine are reported, most of which require several steps to introduce the 2-amino-1,3,-dihydroxy group in the correct configuration. 3 D-ribo-phytosphingosine [(2S,3S,4R)-2-amino-1,3,4-octadecanetriol (Ha et al. 2009).

Hexadecanamide ($C_{16}H_{33}NO$). Hexadecanamide and hypoxanthine compounds have the potential to be used as antibiotic candidates, based on the results of the interaction between the ligand and the target protein. This is supported by several studies that have reported that hexadecanamide and hypoxanthine compounds can be used as antibacterials (Nurhikmah 2021).

Betaine ($C_5H_{11}NO_2$). Betaine compounds are widely distributed in animals, plants, and microorganisms, and rich food sources include seafood, especially marine invertebrates (≈1%); wheat germ or bran (≈1%); and spinach (≈0.7%). The main physiological role of betaine is as an osmolyte and methyl donor (transmethylation) (Craigh 2004).

6-Hydroxypicolinic acid ($C_6H_5NO_3$). The compounds of 6-hydroxypicolinic acid (6-OHpicH) and 2-hydroxynicotinic acid (2-OHnicH) exhibit enol-keto tautomerism both in the solid state and in solution due to the unstable mobility of the OH group, which is close to the nitrogen atom of the base and can be easily transferred there (Sun et al., 2004; Yue et al. 2004).

Brown algae *T. ornata* has phytochemical compounds, as evidenced by the results of the analysis with GC-MS. The chromatogram of *T. ornata* describes 43 phytochemical compounds (Figure 6). Based on the areas, only 8 prominent compounds were taken. The pharmacological activity of the 8 highest compounds from alga *T. ornata* is shown in Table 3.

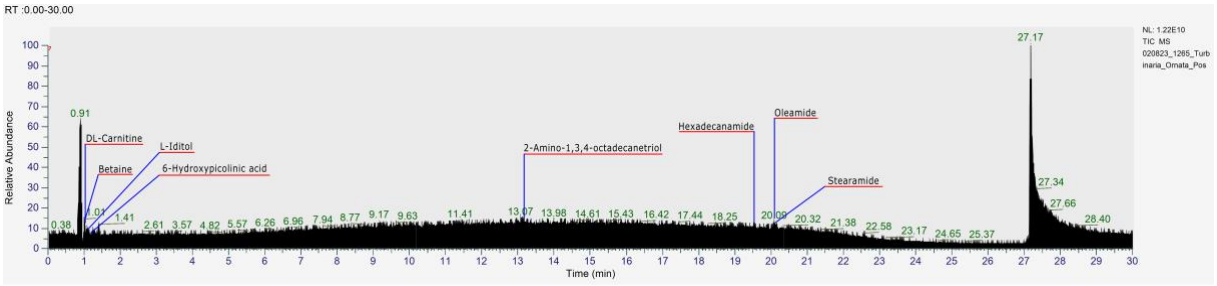


Figure 6. GC-MS chromatogram of *T. ornata*.

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3.	DL-Carnitine	$C_7H_{15}NO_3$	288	1.014 / 578076213.019972	Cardioprotective (Elantary & Othman 2024), neuroprotective (Latham et al 2021).
4.	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	248575	13.126 / 316140722.309441	Neuroprotective (Zeng et al 2024),
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7.	Stearamide	$C_{18}H_{37}NO$	31292	20.971 / 200712105.155459	anti-inflammation, antipruritic, antifungal, and antimicrobial (Riyardi 2020)
8.	6-Hydroxypicolinic acid	$C_6H_5NO_3$	242721	1.291 / 163424912.889371	Antimicrobial, antioxidant, anticancer, neuroprotective (Kumar et al 2018)

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DL-Carnitine (C7H15NO3). The primary function of DL-Carnitine is the transport of long-chain fatty acids through the cell membrane for oxidation in mitochondria (Marcello da Silva et al 2018).

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Stearamide ($C_{18}H_{37}NO$). Stearamide is a primary amide fatty acid. Stearamide can be produced on a large scale and is usually available in powdery granular form. Stearamide at room temperature is clear, with white crystals. Stearamide has a maximum temperature of 220°C and stearamide is widely used in applications such as rubber production (Syukri & Masyithah 2018).

6-Hydroxypicolinic acid ($C_6H_5NO_3$). The compounds of 6-Hydroxypicolinic acid (6-OHpicH) and 2-hydroxynicotinic acid (2-OHnicH) exhibit enol-keto tautomerism both in the solid state and solution, due to the unstable mobility of the OH group, which is close to the nitrogen atom of the base. and can be easily transferred there (Sun et al 2004; Yue et al 2004).

According to previous research show that *T. decurrens* contains a bioactive compound that is beneficial for health. This species is one of many species of brown seaweed that grows in Indonesian marine life and has been known to have cytotoxic activity (Zakariah et al 2018). Silver and gold nanoparticles of *T. ornata* show antimicrobial activity against human pathogens (Kayalvizhi et al 2014). The highest antibacterial activity in *Staphylococcus aureus* was found in whole extracts of *T. decurrens* (Dali et al 2013). The anticoagulation activity of *T. decurrens* was increased with an increase in the sulfate content in the fucoidan. The inhibition activity toward α-glucosidase enzyme, laminarin fraction of *T. decurrens* showed higher activity compared to fucoidan fraction, whereas the alginic fraction showed no inhibition activity and concluded that the brown algae show the antidiabetic property (Hardoko et al 2014). *T. ornata* possesses antioxidant, antimicrobial, antifungal, anti-inflammatory, and antidiabetic properties that could be used in the form of food, energy, medicine, and pharmaceutical industries (Rout & Kumar 2015). Isolation and identification of secondary metabolites of *T. decurrens* from ethyl acetate extract got a phenolic compound, gallic acid. Gallic acid has been tested for anticancer activity (Sami & Nur 2022). The in vitro growth-promoting effects of a fucoidan of *T. decurrens* in both monocot and dicot plants for the first time that fucoidan fractions can supplement tissue culture media to facilitate faster growth responses as a cost-effective alternative to commercial PGRs for plant micropropagation application (Kaniyassery et al 2023). In North Sulawesi waters, studies of biomineral characterization and phytochemical profile have been carried out in red alga *Tricleocarpa fragilis* (Singkoh et al 2019) and in green algae *Halimeda macroloba* and *Halimeda opuntia* (Kepel et al 2021).

Conclusions. Brown algae *T. decurrens* from coastal waters of Kora-kora in Minahasa Regency is characterized by mineral elements content composed of O, Na, Mg, Al, Si, S, Cl, K, Ca, and Cu while *T. ornata* had O, Na, Mg, Al, Si, S, Cl, K, and Ca. *T. decurrens* has 43 phytochemical compounds as well as *T. ornata.* The results show that there is a relationship between mineral content and the constituent elements of bioactive

compounds from algae that contain oxygen. Where the most elements that make up bioactive compounds are O and C.

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