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Antioxidant Activity of Unsaturated Fatty Acid Isolated From The Green Algae (*Halimeda opuntia*)

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Abstract

Halimeda is a genus of green macroalgae found in Indonesian waters, including the waters around Ternate Island. Coastal communities typically consume algae as a food source. However, over time, knowledge about algae has expanded, and people have become more aware of their nutritional content. This study aimed to determine the antioxidant activity and fatty acid composition of green algae from waters off Ternate Island. Among the extracts tested, the methanol extract showed the highest antioxidant activity compared with the hexane and chloroform extracts. The methanol extract was then partitioned using hexane and chloroform, yielding three fractions. Among these fractions, the chloroform fraction (FrCl) showed the highest antioxidant activity (22.13%). To characterize the chemical profile of the chloroform fraction (FrCl), the fraction was analyzed using FTIR and UV-Vis spectroscopy. The FTIR spectrum showed an absorption band at 3010 cm^{-1} , consistent with C-H stretching in unsaturated compounds, and a band at $975\text{--}965\text{ cm}^{-1}$, which may indicate an out-of-plane vibration associated with an alkene. Please verify the assignment of the $975\text{--}965\text{ cm}^{-1}$ band before identifying it as a cis double bond, as this region is often associated with trans-disubstituted alkenes. Furthermore, UV-Vis analysis showed an absorption peak at approximately 390 nm in the chloroform fraction (FrCl). GC-MS analysis of FrCl tentatively identified five fatty acids: pentadecanoic acid, hexadecanoic acid, octadecanoic acid, tetradecanoic acid, and cis-15-tetracosenoic acid.

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1. Introduction

Green algae are a diverse group of organisms widely distributed throughout Indonesian waters, including around Ternate Island. They are commonly found in intertidal areas and reef flats, where they grow alongside other seaweeds. Approximately 7,000 species of green algae have been described, many of which have economic value and are traded as important commodities (Kasanah *et al.*, 2015; Matin *et al.*, 2024). One species commonly found in Indonesian waters is *Halimeda opuntia*, a member of the genus *Halimeda*. It has an upright thallus composed of branching segments, with a triangular appearance near the base of the macroalga (Balboa *et al.*, 2013; Barbosa *et al.*, 2014). The thallus of *Halimeda opuntia* is approximately 6–10 cm tall and has holdfasts that attach it to the substrate (Balboa *et al.*, 2013; Barbosa *et al.*, 2014). Coastal communities commonly consume algae as vegetables, and growing awareness of their nutritional value has increased interest in their use as food (Karaki *et al.*, 2014; Haniya *et al.*, 2015; Podungge *et al.*, 2018).

Green algae typically have a water content of 80–90%, while their protein and fat contents are relatively low. Despite its low fat content, algae may provide fatty acids that

are important for health (Podungge *et al.*, 2018). Algal lipids contain omega-3 and omega-6 fatty acids, which are components of cell membranes in the brain, nerves, retina, blood plasma, and reproductive organs (Podungge *et al.*, 2018; Matin *et al.*, 2024). Dried seaweed has been reported to contain 128–1,629 μg of omega-3 and 188–1,704 μg of omega-6 fatty acids per 100 g (Matin *et al.*, 2024; Diab *et al.*, 2024; Dimopoulou *et al.*, 2024).

Green algae, including *Halimeda opuntia*, contain bioactive metabolites that may contribute to antioxidant activity (Matin *et al.*, 2024; Diab *et al.*, 2024; Dimopoulou *et al.*, 2024). Reported secondary metabolites in *Halimeda* include alkaloids, flavonoids, terpenoids, tannins, and saponins (Balboa *et al.*, 2013; Barbosa *et al.*, 2014; Venepally *et al.*, 2017; Matin *et al.*, 2024; Diab *et al.*, 2024; Dimopoulou *et al.*, 2024). Antioxidants can act through different mechanisms. Chain-breaking antioxidants donate hydrogen atoms or electrons to free radicals, helping interrupt radical-chain reactions and limit lipid oxidation. Preventive antioxidants act through other mechanisms, such as reducing the formation of reactive species or limiting their effects (Djapiala *et al.*, 2013; Kasanah *et al.*, 2015; Basir *et al.*, 2017;

Rumengan *et al.*, 2015; Podungge *et al.*, 2018; Bedoux *et al.*, 2014; Safafar *et al.*, 2015; Handra *et al.*, 2019; Yoga *et al.*, 2022; Venkateswarlu *et al.*, 2007). This study aimed to evaluate the antioxidant activity of an algal isolate collected from Ternate Island and tentatively identify compounds in the active fraction.

2. Material and Methods

2.1 Place and Time

This study was conducted from April to October 2025. Samples were collected from three stations in the coastal waters around Ternate Island: Station I off Kastela Village, Station II off Sasa Village, and Station III off Gamalama Village. The specimens were identified at the Bioecology Laboratory, Faculty of Fisheries and Marine Sciences, Khairun University, using taxonomic references appropriate for marine macroalgae. Antioxidant activity was

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tested at the Phytochemistry Laboratory, Faculty of Medicine, Khairun University.

Spectrophotometric analyses were conducted at the Chemistry Laboratory, Brawijaya University. The materials used included *Halimeda opuntia*, distilled water, ethanol (C₂H₅OH), methanol (CH₃OH), n-hexane, DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrochloric acid (HCl), ferric chloride (FeCl₃), magnesium (Mg), chloroform, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, glacial acetic acid, and sulfuric acid (H₂SO₄). Equipment included a rotary vacuum evaporator, analytical balance, freeze dryer, cabinet dryer, UV spectrophotometer, UV lamp, autoclave, centrifuge, vacuum apparatus, glassware, test tubes, graduated cylinders, droppers, and micropipettes. The analytical instruments used for sample characterization were an FTIR spectrometer, a UV-Vis spectrophotometer, and a gas chromatograph-mass spectrometer (GC-MS)..

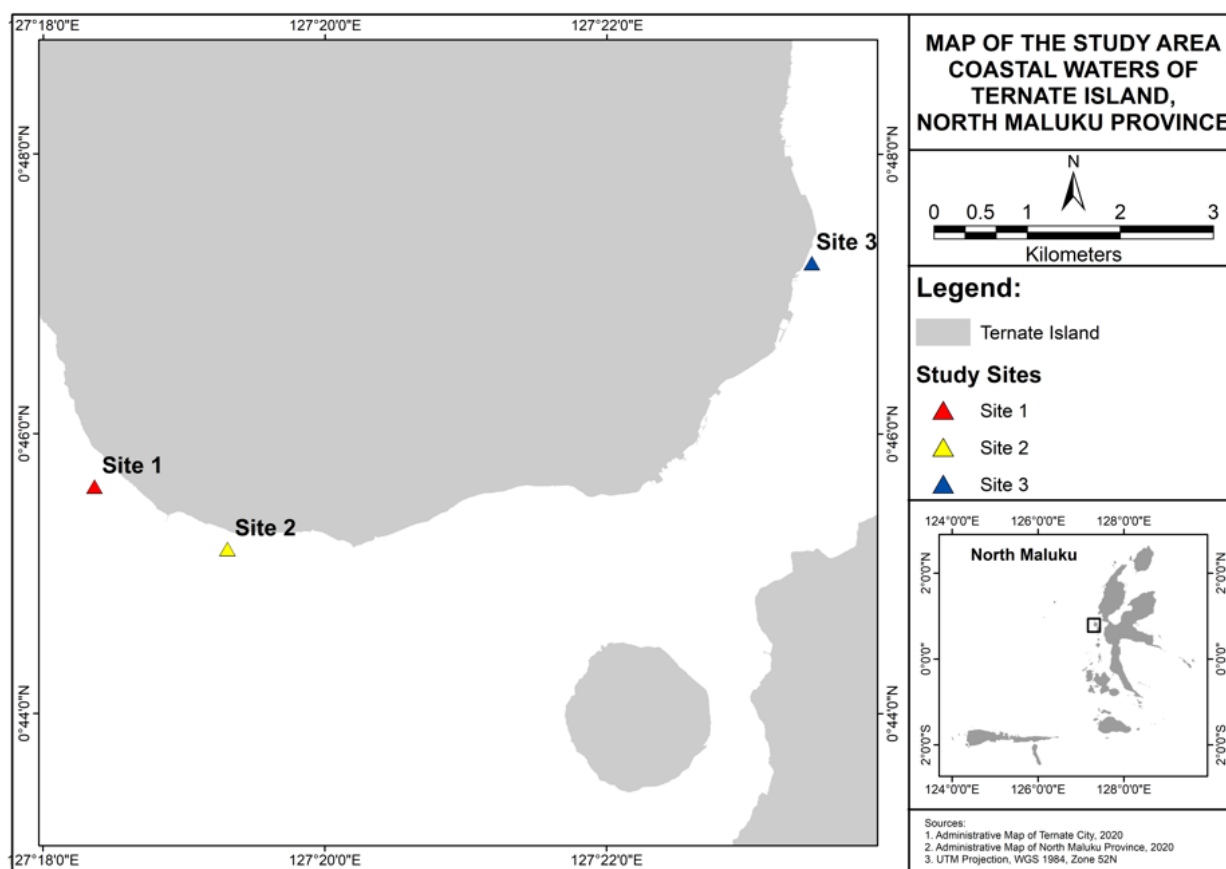


Figure 1. Map of the sampling locations

2.2 DPPH Antioxidant Activity Test

A DPPH solution (0.004%, w/v) was prepared by dissolving 0.004 g of DPPH crystals in ethanol and making up the volume to 100 mL in a volumetric flask. The sample stock solution (100 ppm) was prepared by dissolving 2.5 mg of sample in ethanol and making up the volume to 25 mL; the solution was shaken and filtered. A 50 ppm working solution was prepared by transferring 5.0 mL of the 100 ppm stock solution into a 10 mL volumetric flask and diluting to the mark with ethanol. For the assay, 1.0 mL of the 50 ppm working solution was mixed with 2.0 mL of the DPPH solution, shaken, and allowed to react for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer at $\lambda_{\text{max}} = 516.2$ nm. Antioxidant activity was assessed as the decrease in DPPH absorbance after addition of the sample, and the percentage of free-radical scavenging was calculated for each sample concentration.

$$\% \text{Immersion} = \frac{A_{\text{control}} - A_{\text{Sample}}}{A_{\text{control}}} \times 100\%$$

2.3 Infrared Spectrometer (FTIR) Measurement

A 0.2 mg sample was ground thoroughly with 200 mg of KBr and pressed into a thin pellet. The pellet was analyzed using a PerkinElmer FTIR 100 spectrometer at the Chemistry Laboratory, Brawijaya University, and its spectrum was recorded. Please verify the sample-to-KBr ratio: 0.2 mg of sample per 200 mg of KBr is considerably lower than commonly used proportions, so confirm that the reported sample mass is correct.

2.4 UV-Vis Spectrometer Measurement

The UV-Vis spectrum of the chloroform fraction (FrCl) was recorded from 200 to 400 nm. A 0.3 mg sample of FrCl was dissolved in 3 mL of the solvent used for the analysis. Then, 0.3 mL of this solution was diluted to a final

volume of 3 mL with the same solvent. The spectrum was recorded using a Milton Roy Spectronic 3000 Array spectrophotometer at the Chemistry Laboratory, Brawijaya University.

2.5 Fatty Acid Content Analysis

Fatty acid composition was analyzed using a GC-MS system (GCMS-QP2010S, Shimadzu) equipped with an Agilent J&W DB-1 column (30 m) and helium as the carrier gas. The oven temperature was held at 60 °C for 5 min and then increased to 300 °C over 41 min. Before analysis, the sample was derivatized with boron trifluoride–methanol (BF₃–methanol) to form fatty acid methyl esters. Verify the detector description: the GCMS-QP2010S uses a mass spectrometer as its detector, so the flame thermionic detector

(FTD) should not be attributed to this GC-MS system unless it was used separately.

3. Results

3.1 Yield Measurement

Assuming the specimens were *Halimeda opuntia*, as stated in the title, abstract, and methodology, the paragraph can be revised as follows: *Halimeda opuntia* samples collected from three stations around Ternate Island were assigned the sample code AT (Ternate Algae) (Figure 2). Five samples were collected. The dry weights recorded at Stations 1, 2, and 3 were 410.27 g, 138 g, and 213 g, respectively. The samples were extracted by maceration using methanol, hexane, and chloroform. The reported extraction yields were 26.97%, 22.665%, and 29.020%, respectively

Table 1. Results of Sample Yield Measurement

No	Extract	Dry Weight (g)	Extract Weight (g)	Extraction yield (%)
1	Methanol	410.27	1.521	26.97
2	Hexane	138	0.609	22.67
3	Chloroform	213	0.734	29.02



a



b

Figure 2. Samples of *Halimeda opuntia* algae collected in the waters of Ternate Island (a. Image acquired above the sea surface dan b. Image acquired in the sea)

3.2 The antioxidant activity

The antioxidant activity values for the methanol, n-hexane, and chloroform extracts were 136.234, 130.408, and 115.278, respectively (Table 2). Based on these results, samples AT7 and AT8 were selected for further partitioning

with n-hexane, chloroform, and methanol. Define the sample codes AT7 and AT8 at their first mention in the manuscript. The extraction yields should be reported separately in the relevant table: based on the listed weights, they were 0.37% for methanol, 0.44% for n-hexane, and 0.34% for chloroform.

Table 2. DPPH Antioxidant Activity of Green Algae Extracts

No	Extract	Concentration (ppm)	Absorbance	Antioxidant Capacity
1	Control	—	0.875	—
2	Methanol	50	0.866	136.234
3	Hexane	50	0.524	130.408
4	Chloroform	50	0.556	115.278

Based on the antioxidant assay, the methanol extract showed higher activity than the hexane and chloroform extracts. The methanol extract was partitioned using water, n-hexane, and chloroform, yielding six fractions. Three fractions—FrA, FrH, and FrCl—were tested for antioxidant activity; at 50 ppm, FrCl showed the highest activity (22.13%), while Fr3A showed the lowest (20.21%). FrCl was

selected for further FTIR, UV-Vis, and GC-MS characterization. Interpret these findings and compare them with relevant published studies in a separate Discussion section, citing appropriate sources. Before finalizing, clarify the names of all six fractions and confirm whether Fr3A was among the three fractions tested, since the fraction names in the current text do not align.

Table 3. Antioxidant activity of the three main fractions selected from the six fractions obtained during fractionation

No.	Extract	Concentration (ppm)	Absorbance	Antioxidant Activity (%)
1	Control	–	0.827	–
2	FrAq	50	0.12	20.87
3	FrHx	50	0.222	21.32
4	FrCl	50	0.083	22.13

3.3 FrCl Identification Using a Spectrophotometer

3.3.1. Fourier Transform Infra-Red (FTIR)

The results of the characterisation of unsaturated fatty acids with FTIR show an absorption peak of 3010 cm^{-1} originating from the stretching vibration of the C=CH cis group, as well as a peak in the region around $975\text{--}965\text{ cm}^{-1}$ indicating the presence of cis double bonds (Figure 3). An

increase in peak intensity here shows higher levels of unsaturated fatty acids, such as linoleic acid. $975\text{--}965\text{ cm}^{-1}$: The peak in this region also represents the presence of double bonds in fatty acids, often associated with stretching vibrations of cis double bonds that give unique characteristics to unsaturated fatty acids.

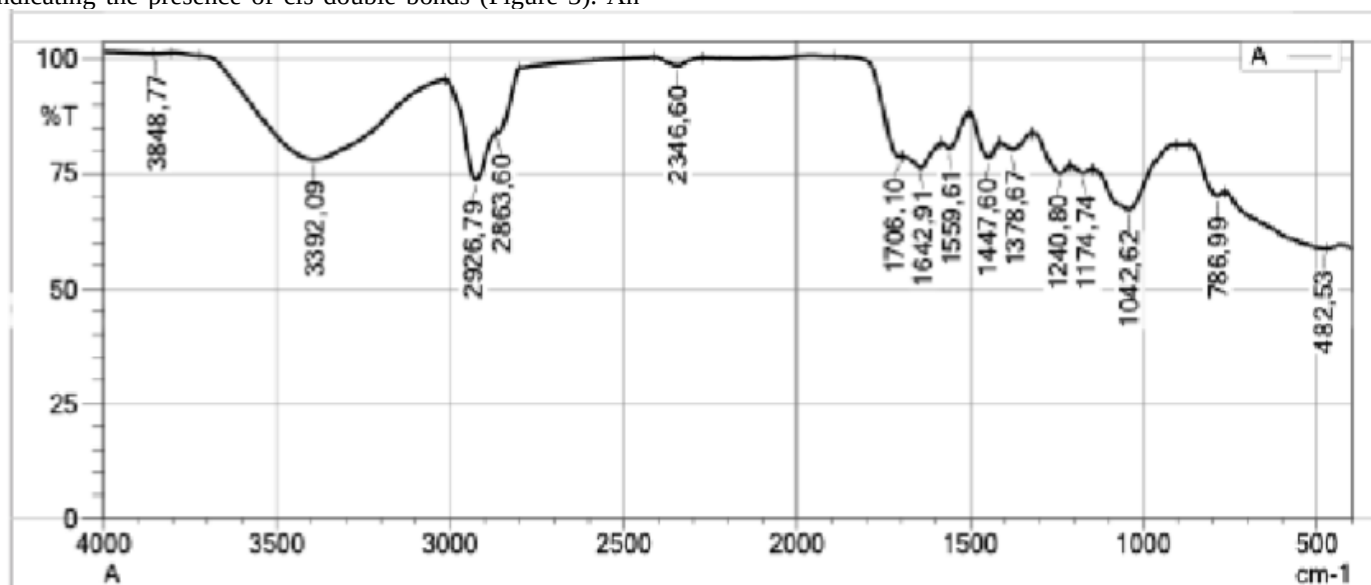


Figure 3. FTIR spectrum of FeCl sample

3.3.2. Ultraviolet-Visible UV-VIS

Characteristics of chemical compounds, including unsaturated fatty acids, are reflected in their unique UV-Vis absorption spectra, which can be used for identification and quantitative analysis. Unsaturated fatty acids can be absorbed by UV-Vis spectrophotometers (Figure 4). In this study, UV-

Vis analysis of the chloroform fraction (FrCl) showed an absorption peak at approximately 390 nm. This result describes the spectrum of the fraction and does not, by itself, establish that the peak originates from unsaturated fatty acids or is specific to antioxidant activity; the compounds identified in FrCl by GC-MS are reported separately.

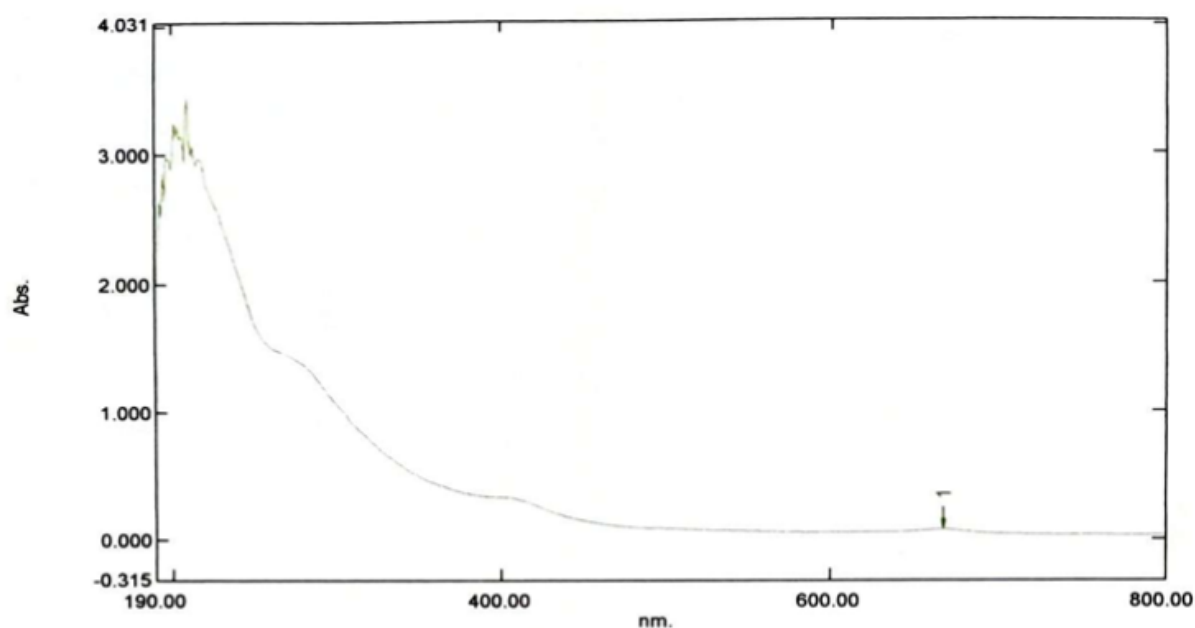


Figure 4. UV-VIS spectrum of FeCl sample

2.1. Identification of Fatty Acid Compounds in the FrCl Isolate by GC-MS

GC-MS analysis of the chloroform fraction (FrCl) tentatively identified five fatty acids: pentadecanoic acid, hexadecanoic acid, octadecanoic acid, tetradecanoic acid, and cis-15-tetracosenoic acid. The first four are saturated fatty acids, while cis-15-tetracosenoic acid is unsaturated: pentadecanoic

acid, hexadecanoic acid, octadecanoic acid, tetradecanoic acid, and cis-15-tetracosenoic acid (Table 4). Based on their structures, pentadecanoic, hexadecanoic, octadecanoic, and tetradecanoic acids are saturated fatty acids, whereas cis-15-tetracosenoic acid is an unsaturated fatty acid. Thus, the compounds detected should not be collectively described as unsaturated fatty acids.

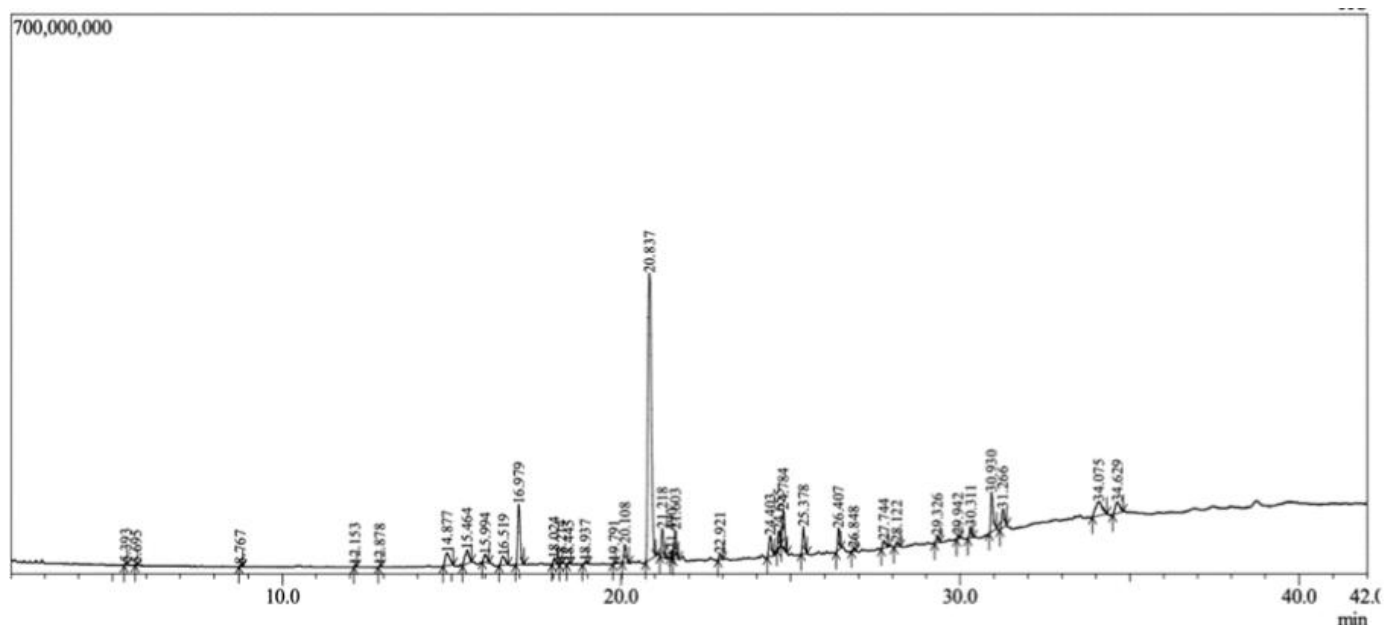


Figure 5. LC-MS chromatogram of compounds in the algal sample. Description: The sample was analyzed using a Shimadzu LC-MS system equipped with a 2.0×150 mm ODS column and a photodiode array (PDA) detector. The acetonitrile (ACN) concentration was increased from 10% to 100% over 30 minutes and then maintained at 100% for 20 minutes. The flow rate was 0.2 mL/min. Confirm that this is a separate LC-MS method; it should not be presented as the GC-MS method used to identify fatty acids. Specify the other mobile-phase component if required for reproducibility.

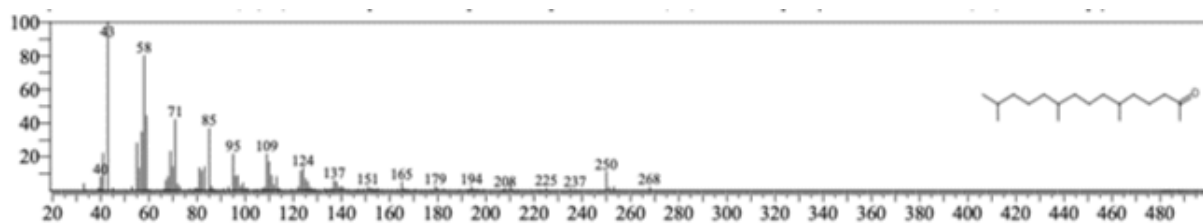


Figure 6. Peak MS no: 14 Retention Time 18.937 minutes

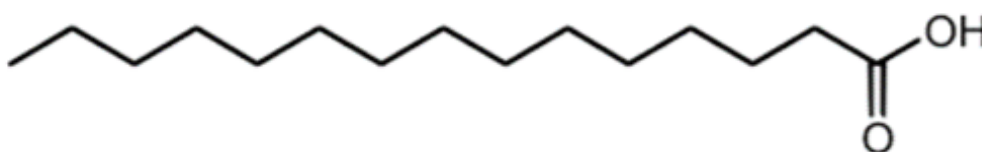


Figure 7. Prediction of the Chemical Compound Structure of Peak No. 14 (BM. 268)

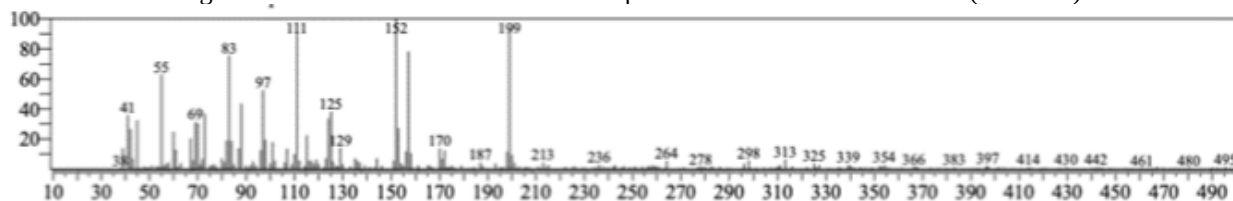


Figure 8. Peak MS no: 16 Retention Time 19,790 minutes

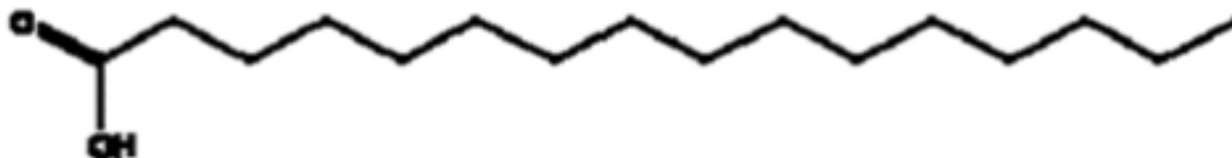


Figure 9. Prediction of the Chemical Compound Structure of Peak No. 16 (B. 188)

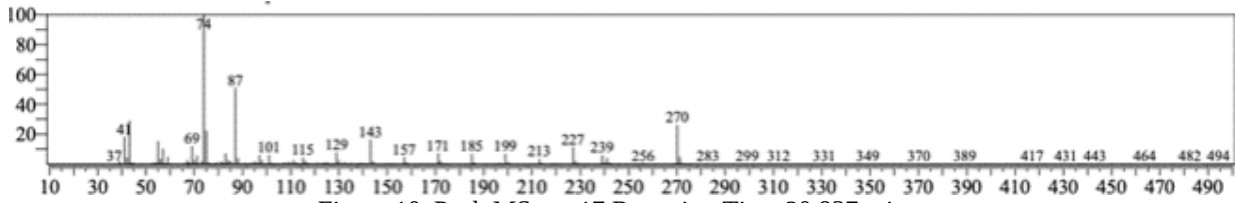


Figure 10. Peak MS no: 17 Retention Time 20.837 minutes

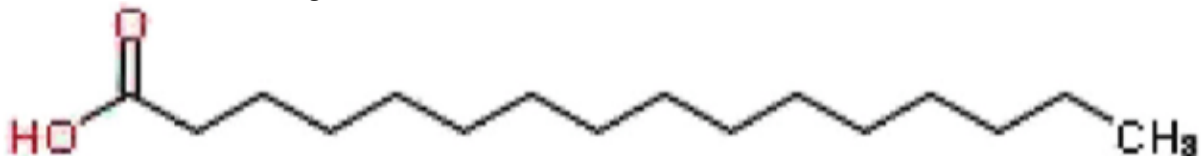


Figure 11. Predicted Structure of Silver Chemical Compound No. 17 (B. 270)



Figure 13. Predicted Structure of Silver Chemical Compound No. 28 (B. 330)

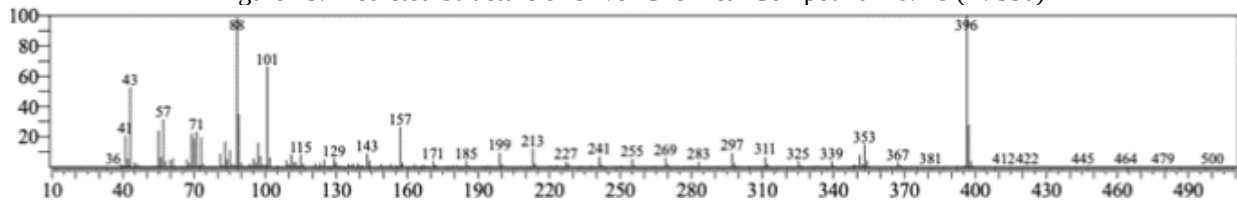


Figure 14. Peak MS no: 36 Retention Time 34.629 minutes

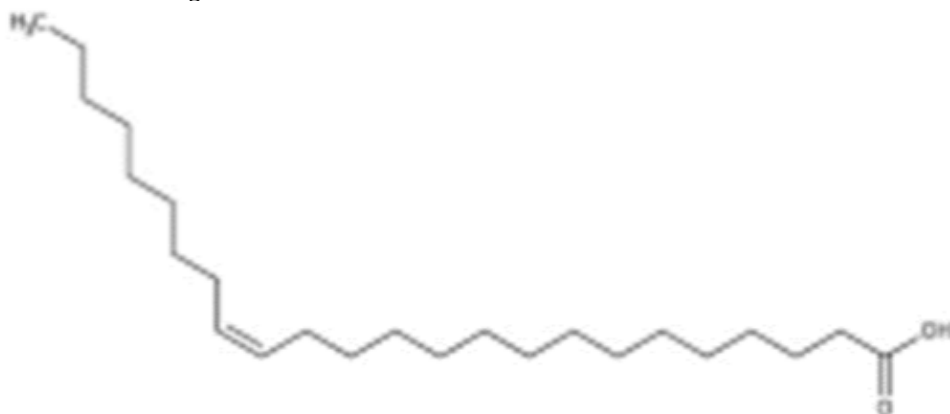


Figure 15. Prediksi Struktur Senyawa Kimia Peak No :36 (BM. 380)

Table 4. Chemical Compounds of Green Algae Identified by GC–MS Based on Dominant Peaks

No.	Peak	Retention Time (min)	Name of Chemical Compound	Molecular Weight (g/mol)
1	14	18.937	Pentadecanoic acid	268
2	16	19.79	Hexadecanoic acid	188
3	17	20.837	Octadecanoic acid	270
4	28	27.744	Tetradecanoic acid	330
5	36	34.629	cis-15-Tetradecenoic acid	380

4. Discussion

Green algae contain fatty acids in phospholipids and galactolipids; monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) may contain fatty acids with different degrees of saturation and chain lengths (Mishra *et al.*, 2016; Venepally *et al.*, 2017; Matin *et al.*, 2024; Diab *et al.*, 2024; Dimopoulou *et al.*, 2024). Petkov and Garcia (2007) reported that *Chlorella* species typically contain myristic acid (C14:0), hexadecadienoic acid (C16:2), and hexadecatrienoic acid (C16:3). Fatty acids from green algae have also been reported to have potential therapeutic activities, including antidiabetic, antidiarrheal, and antioxidant effects (Venugopal, 2010; Turupadang, 2018). The reported proximate composition of *Halimeda opuntia* includes 27.68% water, 33.70% ash, 7% fat, 0.25% protein,

and 31.37% carbohydrates (Vera *et al.*, 2011; Guven *et al.*, 2010; Omar *et al.*, 2012; Hanapi *et al.*, 2013; Perez *et al.*, 2016; Yoga *et al.*, 2022). The chromatograms and predicted compound identities are presented in Figures 4 and 15, while the chemical structures of the fatty acids are shown in Figures 7, 9, 11, and 13.

5. Conclusions

The results of this study are summarized as follows: The methanol extract showed the highest antioxidant activity among the extracts tested. FrCl showed the highest antioxidant activity among the fractions tested. FTIR analysis of FrCl showed absorption bands at 3010 cm^{-1} and 975–965 cm^{-1} , consistent with cis double-bond vibrations. UV-Vis analysis showed an absorption peak at approximately 390 nm.

GC-MS analysis of FrCl tentatively identified five fatty acids: pentadecanoic, hexadecanoic, octadecanoic, tetradecanoic, and cis-15-tetracosenoic acids.

Ethics approval

No permits were required.

Data availability statement

The database consisting of the results of this review can be accessed through the supplementary materials.

Author contributions

A.S and M.J.A. were responsible for conceptualization, data curation, methodology, resources, software visualization, as well as writing and editing. M.A.F. and S.S contributed to conceptualization, supervision, validation, formal analysis, as well as review & editing.

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Declaration of competing Interest

None

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