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*Original research article*

# Evaluation of Bioactive Composition and Phytochemical Profile of Macroalgae *Gracilaria edulis* and *Acanthophora spicifera* from the Banda Aceh Coast, Indonesia

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**ABSTRACT**

This study aimed to examine the metabolite profiles and phytochemical content of *Gracilaria edulis* and *Acanthophora spicifera* along the coast of Banda Aceh in Indonesia. The three attributes observed by this study were phytochemical composition, antioxidant scavenging, and in vivo metabolite profile by the use of Gas Chromatography-Mass Spectrometry (GC-MS), antioxidant activity test, and Fourier Transform Infrared (FTIR) characterization, respectively. Extracts from both *G. edulis* and *A. spicifera* demonstrated high scavenging capability, with inhibition values of 42.59% and 39.81%, respectively. In both species, the GC-MS methodology was shown to be a quick, sensitive, and trustworthy way to monitor individual components and the entire chemical composition. In *G. edulis*, there were 29 phytochemical compounds, whereas in *A. spicifera*, there were 26 compounds. The ethanol extracts of *G. edulis* and *A. spicifera* were then subjected to an FTIR spectrum analysis. These findings at 3352 $cm^{-1}$, 1654 $cm^{-1}$, and 1019 $cm^{-1}$ proved that alkanes, alkenes, and hydroxyl groups were present, corresponding to several compounds in the GC-MS measurement results. *G. edulis* and *A. spicifera* are potential antibacterial agents; the bioactive compounds conferring this activity are heptadecane, hexadecanoic acid, and other ester derivatives.

**Keywords:** *Acanthophora spicifera*; Antibacterial; Antioxidant activity; Bioactive compounds; *Gracilaria edulis*

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## 1. Introduction

Indonesian waters store various biological resources that can be used as a source of food and economic activity that can improve human welfare. One of the biological resources that is very abundant in Indonesian waters is macroalgae. Indonesia has no less than 628 types of macroalgae out of the 8000 types of macroalgae found throughout the world [1].

Macroalgae are benthic organisms that grow in shallow water and have the ability to carry out photosynthetic activities. The ability of macroalgae to carry out photosynthesis has an impact on the role of macroalgae as a source of primary productivity in waters [2]. Macroalgae are very vulnerable to environmental changes and ecological pressures that can affect their existence. Environmental influences such as substrate, water movement, temperature, salinity, tides, light, pH, nutrients, and water quality can cause damage and even species extinction [3]. Macroalgae are low-level plants that grow on certain substrates such as coral, mud, sand, rocks, and other hard objects. Apart from inanimate objects, macroalgae can also attach to other plants epiphytically. The growth of macroalgae, which depends on the substrate, is directly influenced by sedimentation. Macroalgae also serve as a food source for several herbivorous organisms, so they have quite an important ecological role. Generally, macroalgae live in intertidal areas which have quite a high variety of environmental factors compared to other parts of the marine ecosystem [4].

Macroalgae, better known as seaweed, have functions from a biological, ecological and economic perspective [5]. Ecologically, the macroalgae community plays a beneficial role in the surrounding environment, namely as a shelter for certain fish species, a spawning place, and a place to find natural food for fish and herbivorous animals [6-8]. From an economic perspective, macroalgae is a very good commodity to develop considering its chemical contents. Macroalgae are widely used, both in the form of raw material for all parts of the plant and in processed form. In Indonesia it is usually used as vegetables, medicine, and sweets [4]. Finally, from a biological perspective, macroalgae greatly contribute to increasing primary productivity, pollutant absorption, organic material production, and oxygen levels. The use of macroalgae has now been widely developed in various industrial fields, namely as a food source, source of alginate compounds, heavy metal adsorbent, source of bioactive compounds for pharmaceutical development, bioethanol and biodiesel producer, organic fertilizer, and also has the potential to be used as a base material to replace plastic [5]. Demand for macroalgae commodities is now increasing. To meet these needs, we do not only depend on natural production potential, but we must also develop and improve macroalgae cultivation.

Ulee Lheue Beach is one of the beaches in the city of Banda Aceh, precisely in the province of Aceh which has quite good marine biodiversity. Banda Aceh, a province of Aceh, has a land area of 61.36 $km^2$ and vast potential coastal resources. Macroalgae are a potential marine biodiversity source that are commonly found along the coast of Aceh. Due to the dearth of scientific research on the potential of macroalgae, this plant is not optimally utilized by the coastal communities of Aceh. Marine macroalgae are abundant in the coastal regions of Banda Aceh, where they are predominantly affixed to dead coral rocks, making them susceptible to ultraviolet radiation. The waters of Ulee Lheue beach are located in Meuraxa District, which is 3 km from the center of Banda Aceh City. The area of Ulee Lheue Village is 7,258 $km^2$ ± 80 hectares and is located at an altitude of 0.8 above sea level.

*G. edulis* and *A. spicifera* belong to the same class Florideophyceae which is included in the division Rhodophyta. *G. edulis* and *A. spicifera* are marine macroalgae that dominate the coastal area of Banda Aceh, specifically on the coast of Ulee Lheue, Banda Aceh City. Many local people use the area for recreation

and fishing. Ulee Lheue Beach is also a popular beach for tourists. Ulee Lheue Beach also has an embankment made of piles of rocks which act as breakwaters, so big waves rarely occur on this beach. The waters of Ulee Lheue Beach have good biodiversity, including coral reefs, fish, mangrove plants, and macroalgae.

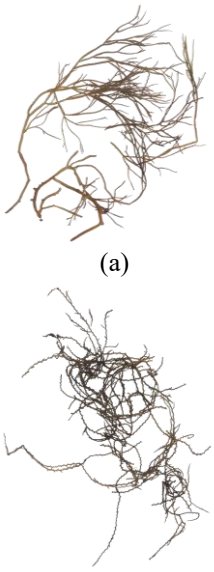
This research aimed to determine the content of metabolite compounds found in the leaves of the *G. edulis* and *A. spicifera* plants, their antioxidant activity, and their potential biological properties. The benefit of this research is that it can provide additional knowledge in the field of medicine and applications in chemical synthesis.

## 2. Materials and Methods

### 2.1 Macroalgae sample collection and preparation

Macroalgae samples were obtained from the coastal region of Ulee Lheue Beach in Banda Aceh City. Macroalgae samples were collected in the intertidal zone of Ulee Lheue Beach at a depth of about 2 meters. The macroalgae species taken were *G. edulis* and *A. spicifera*. After removing epiphytes, dirt, and foreign objects with clean water, necrotic sections were also removed from the samples. Algal samples were meticulously cleansed in both fresh and salt water.

Sampling was carried out by free collection; three individuals of each species were taken and then stored in a freezer. Samples were then morphologically characterized using the morphological descriptions of numerous earlier investigations. Research was conducted at the Marine Chemistry Laboratory of the Faculty of Marine and Fisheries at Syiah Kuala University. The macroalgae samples that were collected were then identified for species classification and morphology. The results of the identification are presented in Fig. 1 and Table 1.



(a) (b)

**Fig. 1.** Morphology of (a) *G. edulis* and (b) *A. Spicifera.*

**Tabel 1.** Classification and morphology of macroalgae samples.

| Classification | Plant (a) | Plant (b) |
|---|---|---|
| Kingdom | Protista | Protista |
| Division | Rhodophyta | Rhodophyta |
| Class | Florideophyceae | Florideophyceae |
| Order | Gracilariales | Ceramiales |
| Familia | Gracilariaceae | Rhodomelaceae |
| Genus | Gracilaria | *Acanthophora* |
| Species | *Gracilaria edulis* (S.G. Gmelin) P.C. Silva, 1952 | *Acanthophora spicifera* (M.Vahl) Børgesen, 1910 |

### 2.2 Materials

The materials used in this research are Simplicia *G. edulis* and *A. spicifera*. The chemicals used for analysis were ethanol, DPPH (2,2-diphenyl-2-picrylhydrazil), HCl 2 N, ascorbic acid, Kjeldahl tablets, 2% $H_3BO_3$, bromine cresol indicator, 40% NaOH, concentrated $HNO_3$, $HClO_4$, HF, $NaBH_4$, distilled water, Meyer's reagent dissolved in 60 mL of distilled water, 0.5 g KI B solution, Dragendroff reagent, Wagner reagent, ether, and $FeCl_3$. The tools used were centrifuge, rotary vacuum evaporator (Buchi R-300), microplate (Nunc), glassware, micro pipette, water bath, Thermo Scientific ISQ LT Single Quadropole Mass Spectrometer, Aquamate

8100 UV-VIS spectrophotometer, Thermo Scientific Trace 1310 Gas Chromatograph, and Fourier Transform Infrared (FTIR) (Bruker alpha).

### 2.3 Macroalgae sample extraction

For two days, the cleaned macroalgae were allowed to dry in the shade. According to the species identified, the dried samples were split into two containers. Samples were then milled on a commercial mill and vacuum packed. The biomass was stored in our laboratory facilities under dry and dark conditions, avoiding direct contact with sunlight. Up to 50 g of sample were weighed and placed in an Erlenmeyer glass. Using ethanol, maceration was done at a 1:5 ratio. The resulting mixture then spent 72 hours in storage. The mixture is then condensed using a rotating vacuum evaporator after being filtered using regular filter paper. The resulting extract paste was then kept at 4°C until use. Phytochemical analysis, total phenol content, antioxidant activity, spectroscopy FTIR, GC-MS, and spectroscopy UV/Vis were then performed on the macroalgae extract.

### 2.4 Determination of total phenolics content

Total phenolics content (TPC) in *G. edulis* and *A. spicifera* extracts were analyzed spectrophotometrically using Folin-Ciocalteu reagent [9]. Specifically, 7.9 mL of distilled water and 0.1 mL of extract were transferred in glass vials and the mixture was homogenized by vortexing. Afterwards, 0.5 mL of Folin-Ciocalteu reagent was added and the solution was homogenized. Next, 1.5 mL (20% w/v) $Na_2CO_3$ solution was added. The final mixture was incubated for 30 min in a water bath at 40 °C and its absorbance was subsequently measured at 765 nm using an Aquamate 8100 UV-VIS spectrophotometer and was compared to a gallic acid calibration curve. The measurements were performed in triplicate.

### 2.5 Determination of antioxidant activity ($IC_{50}$)

Antioxidant activity was assessed using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay [10]. Briefly, 100 µL of *G. edulis* and *A. spicifera* extracts were added to a 3 mL methanolic solution of DPPH (0.03% w/v). Following this, the absorbance of the mixture was recorded at 515 nm after incubation for 20 min at room temperature. The $IC_{50}$ values reported in the present study denote the concentration of sample that is required to scavenge 50% of DPPH free radicals. All measurements were performed in triplicate.

### 2.6 GC-MS analysis

GC-MS was done with PerkinElmer Clarus 680 GC with SQ8 MS, with Columns Elite-5MS, and stationary phase 5% diphenyl, 95% dimethyl polysiloxane. The measurements use the following parameters: initial temp 40°C for 3 min, ramp 3°C/min to 115°C, hold 10 min, ramp 2°C/min to 140°C, hold 8 min, ramp 3°C/min to 210°C, hold 5 min, Inj 210°C, Volume 0 µL, Split 30:1, Carrier Gas He, Solvent Delay 3.00 min, Transfer Temp 210°C, Source Temp 210°C, Scan: 45 to 500 Da, Column 30.0 m × 250 µm

## 3. Results and Discussion

### 3.1 Water quality parameters

The physical and chemical quality of the aquatic environment had a significant impact on the presence of macroalgae. Table 2 lists the measurements for a number of environmental factors in the coastal waters of Ulee Lheue, including salinity, temperature, phosphate and nitrate levels, pH, and dissolved oxygen (DO).

**Table 2.** Water quality measurements and quality standards.

| No | Parameters | Measurement Results | Standard Value* |
|---|---|---|---|
| 1 | Temperature (°C) | 29.8-30 | 28-30 |
| 2 | Salinity (‰) | 29.4-31.5 | 33-34 |
| 3 | pH | 7.81-7.96 | 7-8.5 |
| 4 | DO (mg/L) | 7.85-8.2 | >5 |
| 5 | Phosphate (mg/L) | 0.1-0.46 | 0.015 |
| 6 | Nitrate (mg/L) | 0.2-0.5 | 0.008 |

*Decree of the Minister of Environment Indonesia No. 51/2004

### 3.2 Bioactive components

The initial step in identifying the kinds of bioactive chemicals present in plants is phytochemical analysis. In order to predict which bioactive components are advantageous to the human body, knowledge of active components is crucial [5]. The plants evaluated can be in dose form, fresh, dried, powdered, or extract form. All macroalgae samples were subjected to qualitative testing of their bioactive components using observations of color change or precipitate formation in response to the provided reagents. Table 3 lists the bioactive components that were found present in the macroalgae.

**Tabel 3.** Phytochemical test results for sample ethanol extracts of macroalgae *G. edulis* and *A. spicifera*.

| No | Secondary metabolite | *G. edulis* | *A. spicifera* |
|---|---|---|---|
| 1 | Flavonoids | + | + |
| 2 | Tanin | + | - |
| 3 | Polyphenol | + | + |
| 4 | Kuinon | - | - |
| 5 | Steroid | + | + |
| 6 | Triterpenoids | - | - |
| 7 | Saponin | - | - |
| 8 | Alkaloid | | |
| | a) Mayer | - | - |
| | b) Wagner | - | + |
| | c) Dragendroff | + | + |

### 3.3 Antioxidant activity and total phenol content

*G. edulis* and *A. spicifera* ethanolic extracts exhibited high antioxidant activity with $IC_{50}$ values of 11.47 mg/L. Antioxidant activity is characterized as high if the $IC_{50}$ value is less than 50 mg/L, moderate if it is between 50-100 mg/L, weak if it is between 150-200 mg/L, and extremely weak if it is beyond 200 mg/L [10]. The ability of the extract to function as a proton donor is strongly suggested by a low $IC_{50}$ value. The hydroxyl groups found in phenolic compounds are responsible for the high scavenging ability.

In this investigation, *G. edulis* and *A. spicifera* extract showed antioxidant activity at all concentrations, including at concentrations much lower than that of the standard vitamin C. At 10 g/mL, macroalgae extract showed more efficacy with a 47.22% inhibition (Fig. 2). *G. edulis* and *A. spicifera* antioxidant activity displayed strong ABTS+ radical scavenging activity and a high capacity for copper reduction, with $IC_{50}$ values of 106 g GAE/mL and 20.44 g GAE/mL, respectively [9].

One class of antioxidant found in food is phenolic chemicals. Phenolic chemicals have been shown to be powerful antioxidants, able to stifle free radicals, and to bind metal ions. Although phenol compounds are chemical substances with the ability to act as antioxidants, phenol compounds are not the main source of antioxidant activity. Antioxidants include pentacyclic triterpene chemicals, vitamin C, pigments like chlorophyll, sulfur compounds, and nitrogen. As shown in Table 4, the two types of extracts had a TPC that ranged from 4.48 ± 0.04 to 4.87 ± 0.04. The presence of phenolic chemicals in the ethanol extract is indicated by the significant antioxidant activity of the extract.

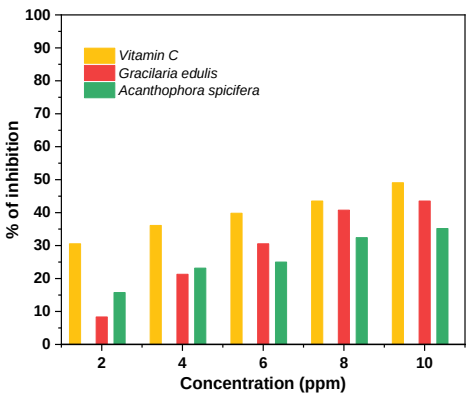



**Fig. 2.** Macroalgae ethanol extracts' capacity to scavenge DPPH radicals.

**Table 4.** Antioxidant Activity Test Results and total phenols of Macroalgae extract.

| No | Species | Total Phenolic content (mg GAE/g) | *Antioxidant activity* ($IC_{50}$)(mg/L) |
|---|---|---|---|
| 1 | *G. edulis* | 6.85 ± 0.08 | 10.70 |
| 2 | *A. spicifera* | 4.34 ± 0.07 | 15.85 |
| 3 | *Vitamin C* | - | 10.58 |

### 3.3 Infrared spectrum characterization

Strong bonds were found at 3352 $cm^{-1}$, 1654 $cm^{-1}$, and 1019 $cm^{-1}$; the others ranged from being weak to being middling. These findings showed that hydroxyl groups, alkanes, and alkenes were present (Fig. 3). Similar infrared (IR) vibration peak patterns were visible in both extracts. This vibration mode represents the alkane chain in the compounds contained in both extracts. The FTIR spectrum at 1654 $cm^{-1}$ was assigned as the C=C from alkenes, this vibrational mode has a medium character so it can be confirmed that the extracts also contain C=O carbonyls [11]. Moreover, the peak around 3677 – 3012 $cm^{-1}$ was assigned as O–H hydroxyl groups [12], which may be related to several polyphenol derivatives identified in Table 5. In addition, C–H was read as stretching in the area 2972-2936 $cm^{-1}$ and 2878-2831 $cm^{-1}$, as well as bending in the area 1420-1358 $cm^{-1}$. The measurable C=C vibrational mode can be derived from flavonoids; flavonoids are polyphenols characterized by two benzene rings joined by a linear carbon chain. Apart from that, the C–H vibration of benzene was also read at around 2950-3180 $cm^{-1}$ [13]. By utilizing FTIR spectrophotometry to identify benzenoid chemicals, the results of the phytochemical screening, which revealed the presence of flavonoids and phenols, were confirmed.

### 3.4 UV visible spectrum characterization

A prominent absorption peak was visible in the UV-Vis spectrum of the *G. edulis* and *A. spicifera* extract at $\lambda_{max}$ = 660 nm (Fig. 3). The electron that was stabilized by the flavylium cation on the oxygen atom is responsible for the peak's electronic transition. It can be determined that the electronic transition between the electrons surrounding benzene was responsible for the minor peak that also appeared at $\lambda_{max}$ = 358 nm and for the relatively modest intensity of this transition [11]. This information suggests that the extracts of *G edulis* and *A. spicifera* contain a polyphenol ring.

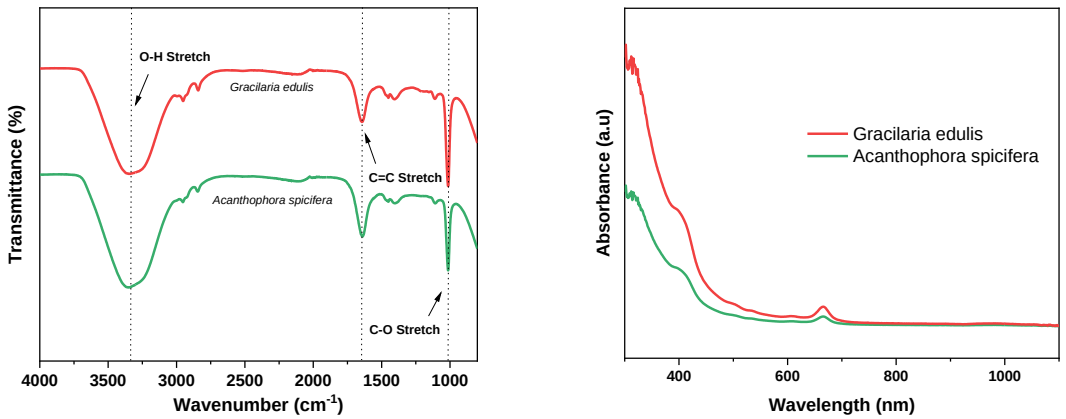



**Fig. 3.** FTIR (a) and UV–VIS (b) spectra of *G. edulis* and *A. spicifera* ethanol extract. All extracts were analysed at 1 mg $mL^{-1}$.

**Tabel 5.** FTIR functional groups and spectral peak values of *G. edulis* and *A. spicifera* extracts.

| No | Frequency Range ($cm^{-1}$) | Wave Number ($cm^{-1}$) | Possible Functional Groups | Compound Analysis Results | Reference |
|---|---|---|---|---|---|
| 1 | 3677-3012 | 3352 | N–H stretch/ C–O stretch / O–H bend | Alcohol | [11] |
| 2 | 2972-2936 | 2951 | C–H stretch | Alkanes | [11] |
| 3 | 2878-2831 | 2849 | C–H stretch | Alkanes | [12] |
| 4 | 1757-1525 | 1654 | C=C stretch | Alkenes | [12] |
| 5 | 1420-1358 | 1396 | C–H bend | Alkanes | [13] |
| 6 | 1054-972 | 1019 | C–C(O)–C stretch | C–O From Alcohols | [13] |

### 3.5 GC-MS analysis

The chromatogram results on the ethanol extract of the macroalgae *G. edulis* and *A. spicifera* showed 27-30 peaks. The GC chromatogram profile of the ethanol extract of the macroalgae *G. edulis* and *A. spicifera* can be seen in Fig. 4, and the chemical component profiles obtained can be seen in Table 6 and Table 7.

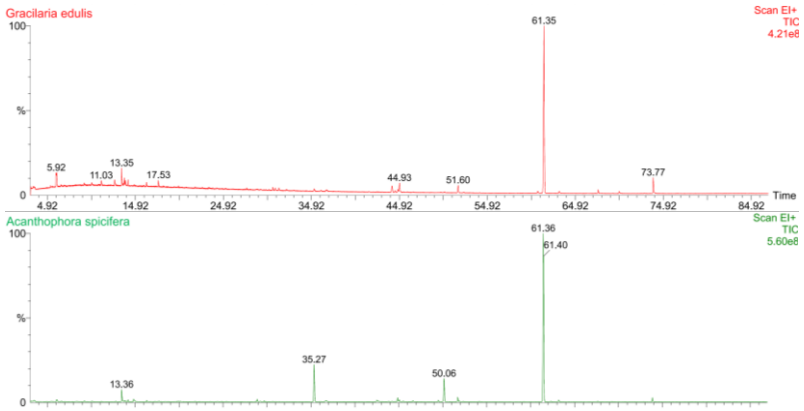



**Fig. 4.** Chromatogram profile of ethanol extract of the macroalgae *G. edulis* and *A. spicifera.*

**Table 6.** Percentage chemical composition of ethanol extract from *G. Edulis.*

| No | RT | Compound Name | Height | Area % | Reported Biological Activity |
|---|---|---|---|---|---|
| 1 | 3.349 | Heptanal, 2-methyl- | 6,693,977 | 0.436 | Antibacterial, Antimicrobial, Antifungal [30] |
| 2 | 5.923 | Hexanal | 35,943,040 | 2.587 | Antibacterial, Antimicrobial, Antifungal [31] Anti-inflammatory [32] Anticancer [33] |
| 3 | 9.950 | Octadecanal, 2-bromo- | 10,943,025 | 0.740 | - |
| 4 | 11.029 | Hexanoic acid, methyl ester | 16,403,781 | 1.004 | Antibacterial, Antimicrobial, Antifungal [34] |
| 5 | 12.547 | 2-heptenal, (Z)- | 18,942,494 | 1.196 | - |
| 6 | 13.346 | Aniline | 48,230,452 | 1.914 | - |
| 7 | 13.644 | 1-Propanol, 3-methoxy-2,2-bis(methoxymethyl)- | 24,210,108 | 1.026 | - |
| 8 | 13.778 | 2-octen-1-ol, (Z)- | 17,867,560 | 0.723 | Antibacterial, Antimicrobial [35] |
| 9 | 13.918 | Phenol, 4-[2-[2-(chloromethyl)-1,3-dioxolan-2- | 6,966,482 | 0.376 | - |
| 10 | 14.076 | Cyclotetrasiloxane, octamethyl- | 19,915,192 | 0.891 | - |
| 11 | 14.805 | 2H-pyran, tetrahydro-2-(12-pentadecynyloxy)- | 8,321,884 | 0.463 | - |
| 12 | 15.511 | [(1R,5S,6S)-3-(hydroxymethyl)-5-[(Z)-2- | 7,169,101 | 0.396 | - |
| 13 | 15.885 | 10-octadecenoic acid, methyl ester | 7,162,862 | 0.465 | Anti-inflammatory [36] |
| 14 | 16.165 | 1-hexanol, 2-ethyl- | 14,551,722 | 0.736 | - |
| 15 | 17.245 | Deoxyspergualin | 6,314,126 | 0.366 | - |
| 16 | 17.531 | 2-octenal, (E)- | 20,360,878 | 0.854 | - |
| 17 | 30.545 | Furan, 2-pentyl- | 8,707,950 | 0.371 | - |
| 18 | 35.226 | 1-undecanol | 6,929,942 | 0.368 | Anti-inflammatory [37], Insecticidal [38], Antimicrobial [39] |
| 19 | 36.661 | [5-Ethenyl-3-hydroxy-4-(3-hydroxyprop-1-en- | 4,934,894 | 0.373 | - |
| 20 | 44.079 | Cyclotridecane | 16,025,555 | 0.934 | - |
| 21 | 44.767 | Pentadecane | 7,461,964 | 0.392 | - |
| 22 | 44.931 | Pentanoic acid, 5-hydroxy-, 2,4-di-t- | 23,317,390 | 1.293 | Anti-inflammatory [40], Antidiabetic [41] |
| 23 | 51.595 | 3-hexadecene, (Z)- | 19,066,370 | 1.016 | - |
| 24 | 61.353 | Heptadecane | 416,722,240 | 23.908 | - |
| 25 | 63.110 | Methyl tetradecanoate | 5,790,150 | 0.414 | - |
| 26 | 67.522 | 1-octadecanol, methyl ether | 9,899,891 | 0.396 | - |
| 27 | 73.772 | Hexadecanoic acid, methyl ester | 39,777,956 | 1.847 | Antibacterial [42], Antimicrobial [43], Antifungal [44], Anticancer [45] |

**Table 7.** Percentage chemical composition of ethanol extract from *A. spicifera*.

| No | RT | Compound Name | Height | Area % | Reported Biological Activity |
|---|---|---|---|---|---|
| 1 | 3.414 | Cyclohexanol, 4-methyl-, trans- | 3,922,844 | 0.339 | |
| 2 | 3.460 | Cyclobutylsilane | 3,745,156 | 0.262 | |
| 3 | 6.028 | Hexanal | 6,840,707 | 0.772 | Antibacterial, Antimicrobial, Antifungal [31] Anti-inflammatory [32] Anticancer [33] |
| 4 | 9.127 | 3-Methoxy-1-pentene | 3,370,299 | 0.250 | - |
| 5 | 9.979 | Heptanal | 2,773,838 | 0.199 | - |
| 6 | 13.364 | Aniline | 40,476,960 | 2.680 | - |
| 7 | 14.105 | Cyclotetrasiloxane, octamethyl- | 6,259,958 | 0.313 | - |
| 8 | 14.736 | N,N'-di-t-butylethylenediamine | 8,668,446 | 0.866 | - |
| 9 | 16.206 | 1-Hexanol, 2-ethyl- | 4,611,784 | 0.203 | - |
| 10 | 28.795 | cis-5,8,11,14,17-eicosapentaenoic acid | 8,235,078 | 0.500 | Antibacterial, Antimicrobial, Antifungal [46] Anti-inflammatory [47] Anticancer [48] |
| 11 | 29.180 | 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H- | 2,097,209 | 0.203 | - |
| 12 | 29.600 | Undecanal | 3,447,791 | 0.203 | - |
| 13 | 35.273 | Cyclotetradecane | 123,475,240 | 9.196 | - |
| 14 | 36.562 | 1,2-15,16-diepoxyhexadecane | 4,074,355 | 0.781 | - |
| 15 | 42.422 | Solstitialin A | 5,710,474 | 0.639 | Antibacterial, Antimicrobial, Antifungal [49] Anti-inflammatory [50] Anticancer [51] |
| 16 | 42.650 | 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1- | 2,460,026 | 0.266 | |
| 17 | 44.120 | 9-octadecenoic acid, 2,2,2-trifluoroethyl ester | 1,922,718 | 0.220 | |
| 18 | 44.791 | Pentadecane | 12,497,874 | 0.929 | |
| 19 | 44.966 | 2,4-Di-tert-butylphenol | 5,127,232 | 0.358 | Antibacterial, Antimicrobial, Antifungal [52] Anti-inflammatory [53] Anticancer [54] |
| 20 | 45.410 | 2-tridecen-1-ol, (E)- | 2,938,572 | 0.232 | - |
| 21 | 46.525 | Dodecanoic acid, methyl ester | 4,177,100 | 0.355 | Antibacterial, Antimicrobial, Antifungal [55] |
| 22 | 49.419 | Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- | 5,577,315 | 0.442 | - |
| 23 | 50.061 | 1,7-hexadecadiene | 77,256,600 | 6.288 | - |
| 24 | 51.614 | 1-Hexadecanol | 15,780,826 | 1.223 | Antibacterial, Antimicrobial, Antifungal [56] |
| 25 | 51.830 | 2-decylfuran | 3,269,138 | 0.278 | - |
| 26 | 52.262 | Ethanol, 2-(octadecyloxy)- | 2,026,180 | 0.207 | - |
| 27 | 54.065 | Tridecanoic acid, methyl ester | 2,200,534 | 0.223 | - |
| 28 | 61.360 | Heptadecane | 558,622,528 | 50.802 | - |
| 29 | 63.099 | Methyl tetradecanoate | 6,371,086 | 0.590 | |
| 30 | 73.779 | Hexadecanoic acid, methyl ester | 14,576,226 | 0.946 | Antibacterial [42], Antimicrobial [43], Antifungal [44], Anticancer [45] |

Both macroalgae extracts showed the same type of chemical component with a larger % area value compared to the others, namely heptadecane with a relative area of 22-50 %. So, this peak is the main component in the ethanol extract of the macroalgae *G. edulis* and *A. spicifera*. It has been reported that heptadecane has antimicrobial activity. Apart from being an antimicrobial, heptadecane can also be applied to improve the quality of wood biocomposite materials. The results obtained for heptadecane-impregnated wood show good energy storage/release capacity with phase change temperatures suitable for building applications [15].

However, several medium-intensity chemical components were identified, such as hexadecanoic acid, methyl ester in both *G. edulis* and *A spicifera*. Hexadecanoic acid, methyl ester with molecular formula $C_{17}H_{34}O_2$, reportedly possesses nematocidal, antimicrobial, pesticidal, antioxidant, insecticidal, and anti-androgenic effects, and is hypocholesterolemic [16]. As an antioxidant, studies on the activity of the $IC_{50}$ value against hexadecanoic acid methyl ester have been reported in ethanol extracts of the roots, stems, and leaves of the Song of India plant (*Dracaena reflexa*). The results showed a medium-level $IC_{50}$ value for leaf extract, $134.62 \pm 0.78$ [16].

Dodecanoic acid, methyl ester and 10-Octadecenoic acid, methyl ester have been discovered in a variety of plant species, including *Coronopus didymus*, *Ageratum conyzoides*, and *Cannabis sativa* [17, 18]. Methyl esters isolated from *Euphorbia kansui* showed anticancer activity by initiating growth inhibition and inducing apoptosis in tumor cells. In addition, previous studies have also

shown that fatty acid methyl ester extracts of *Arthrocnemum indicum*, *Suaeda monoica*, *Sesuvium portulacastrum*, *Salicornia brachiata*, *Excoecaria agallocha*, and *Suaeda maritima* possess antifungal and antibacterial activities [19]. A study of essential oils revealed the occurrence of 2-pentadecanone, 6,10,14-trimethyl- point towards the chemotaxonomic and phytogenic relationships between the examined Senna species. The oils were moderately active when tested for antibacterial effects against a variety of Gram-positive and Gram-negative bacteria, as well as fungi.

The antimicrobial molecule n-1-octadecanol, methyl ether has been isolated from *Acacia nilotica* by bioactivity-directed fractionation of ethyl acetate extract from the air-dried seeds and pods. Using *Acacia nilotica* extract with a concentration of 1000 µg/cm$^3$ from seed against *Salmonella typhi*, *Streptococcus faecalis*, *Escherichia coli*, *Candida krusei*, *Shigella dysenteriae*, and *Staphylococcus aureus*, zones of inhibition with diameters ranging from 9-29 mm resulted [20]. On the other hand, fish and fishery products are regularly reported to include *Staphylococcus aureus*, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), with prevalences ranging from 2 to 60% [21]. Because consuming staphylococcal enterotoxins produced by *Staphylococcus aureus* might result in food poisoning, the presence of *Staphylococcus aureus* in fish is problematic [22].

The current work has revealed the bioactive chemicals responsible for the endophytic fungi's antibacterial activity and their antibacterial potential in *Dillenia indica* L. Further, 2-tridecen-1-ol, (E)-was one of 40 chemicals found in the *Fomitopsis meliae* ethyl acetate extract after GC-MS analysis. With a zone of inhibition ranging from 15 to 29 mm, the ethyl acetate extract of *Fomitopsis meliae* demonstrated the best effectiveness against certain human pathogenic microorganisms. Dual culture assays were used to preliminary screen a total of 25 endophytic fungi for their antibacterial activity against human pathogenic bacteria, such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* [23]. Additionally, it has been noted that *Pediococcus acidilactici* extracts from curd milk produce bioactive tetradecane. *Escherichia coli*, *Listeria monocytogenes*, *Clostridium bifermentans*, *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* have all previously been evaluated for *Pediococcus acidilactici* activity using a disc diffusion assay, with an inhibitory zone at 183.15.14 ± 0.11 to 23.2 ± 0.91 mm [24].

Cyclotetrasiloxane, octamethyl- was identified in the extract of the sea cucumber *Bohadschia* sp., conferring antibacterial activity. The test bacteria used in this study included *Escherichia coli*, *Staphylococcus aureus*, *Vibrio eltor*, and *Bacillus subtilis*. The inhibition zone diameter was calculated to be 7 - 13 mm [25]. Besides that, the antimicrobial activity of olive (*Olea europaea* L.) leaf extract has been reported to produce a 9-11 mm inhibitory zone with several bacterial species [26].

*Lepidium sativum* L. seed oils were extracted using various methods, and their chemical composition, antibacterial activity, and antioxidant activity were assessed. The dodecanoic acid, methyl ester level was found to be 11.21%. The diameter of inhibition zones towards *Staphylococcus aureus* was 15.57 ± 0.46, 13.86 ± 0.37, and 15.06 ± 0.13 mm and elder *Bacillus cereus* was 13.12 ± 1.16, 11.20 ± 1.01, and 13.94 ± 0.56 mm at 1.0 mg/mL concentration, respectively [27]. Antibacterial properties of heptanal obtained from *Pyrrosia tonkinensis* have also been studied. These antibacterial tests revealed that *Pyrrosia tonkinensis* essential oil has effective antibacterial properties against all the examined microorganisms.

The bioactive compound 2H-pyran, tetrahydro-2-(12-pentadecynyloxy)- was obtained from *Pulicaria undulata* L., *Pulicaria incisa* Lam., *Artemisia herba-alba* Asso., *Artemisia monosperma* Delile, *Artemisia*

*judaica* L., and *Achillea* fragrantissima [28]. Antibacterial and antitumoral activities of methanesulfonylacetic acid from spirulina platensis extracellular extract have been reported. Inhibition zones of 10, 8, 8, and 5 mm were observed for *Escherichia coli*, *Burkholderia cepatia*, *Staphylococcus aureus*, and *Pseudomonas stutzeri*, respectively, in the antibacterial activity of *Spirulina* extract (25 g/ml). While *Burkholderia cepatia* exhibits the highest level of inhibition (15 mm), all tested bacteria were inhibited at concentrations of 50 and 100 g/ml [29]. According to reports, *Burkholderia cenocepacia* has developed a niche for intramacrophage replication in zebrafish embryos. This is followed by bacterial spread and the development of a systemic infection. In addition to increasing the fish survival rates after exposure to *Burkholderia cepacia*, the antioxidant status, hepato-renal function, and gene expression were all improved. Apart from this, studies related to fish infection by *Escherichia coli* bacteria have been reported. For this reason, *G. edulis* and *A. spicifera* can be antibacterial candidates for fighting fish infection.

Based on the IR spectrum test, the ethanol extracts of the macroalgae *G. edulis* and *A. spicifera* showed an -OH vibrational mode that corresponds to several compounds present in the GC-MS measurement results, namely in *G. edulis.* These included 1-propanol, 3-methoxy-2,2-bis(methoxymethyl)-, 2-octen-1-ol, (Z)- , phenol, 4-[2-[2-(chloromethyl)-1,3-dioxolan-2-, 2H-pyran, tetrahydro-2-(12-pentadecynyloxy)-, [(1R,5S,6S)-3-(hydroxymethyl)-5-[(Z)-2-, 1-hexanol, 2-ethyl-, 1-hexanol, 2-ethyl-, [5-ethenyl-3-hydroxy-4-(3-hydroxyprop-1-en-, pentanoic acid, 5-hydroxy-, 2,4-di-t-, and 1-octadecanol, methyl ether. Meanwhile in *A. spicifera*, molecules recorded included cyclohexanol, 4-methyl-, trans-, 1-hexanol, 2-ethyl-, 2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5H-, 2,4-di-tert-butylphenol, 2,4-di-tert-butylphenol, ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-, 1-hexadecanol, and ethanol, 2-(octadecyloxy).

## 4. Conclusion

Our findings showed that *G. edulis* and *A. spicifera* ethanol extracts showed noteworthy in vitro activity. The two extracts were considered to have good performance in terms of their antibacterial and antioxidant properties. The extracts' high biological activity may be attributable to its high phenolic content. Several compounds with potential antioxidant and antibacterial activities were found in the GC-MS study. These compounds could help produce new medications that could help treat or prevent infectious diseases in people and animals. These macroalgae should be considered for use in drug discovery as innovative, sustainable sources for potential treatments, nutraceuticals, and large-scale pharmaceutical industrial uses. Therefore, more thorough research is necessary to fully understand the mechanisms of action of the extracts of *G. edulis* and *A. spicifera*, as well to catalogue their bioactive compounds, and assess the effects in biological systems in vivo through the use of animal models.

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