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Phytochemical Profiling of *H. durvillei* Ethanol Extract and the Potential Activity as Antibacterial Agents on Fish

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Abstract

The investigation into seaweed species for the extraction of bioactive compounds has gained significant importance due to its wide-ranging potential applications. This study aimed to assess the metabolite profiles and phytochemical content of *Halymenia durvillei* ethanol extract along the coastline of Banda Aceh, Indonesia. The methodology proposed in this research encompassed three main steps: analysis of phytochemical composition, evaluation of antioxidant scavenging activity, and in vivo metabolite profiling utilizing gas chromatography-mass spectrometry (GC-MS), Fourier-transform infrared (FTIR) spectroscopy, and the 2,2-diphenylpicrylhydrazyl (DPPH) assay. The utilization of DPPH for assessing antioxidant potential revealed significant scavenging activity, with inhibition values reaching 47.22%. GC-MS analysis demonstrated its efficacy in swiftly and accurately monitoring both individual components and overall chemical composition of *H. durvillei* ethanol extract. The study identified 26 phytochemical compounds within *H. durvillei*. Subsequently, the ethanol extract underwent FTIR spectroscopic analysis, revealing prominent peaks at 3352 cm^{-1}, 1654 cm^{-1}, and 1019 cm^{-1}, indicating the presence of alkanes, alkenes, and hydroxyl groups, with other connections ranging from weak to medium. Based on the GC-M S results and literature studies, there are numerous bioactive compounds contained in *H. durvillei* ethanol extract that have potential as antibacterials in humans and animals, including fish. Consequently, *H. durvillei* exhibits promise as a source of antibacterial agents, particularly in the context of aquaculture for fish.

Keywords: Antibacterial, Antioxidant activity, *H. durvillei*, Phytochemical profiling, Macroalgae

1. Introduction

Indonesia, situated amidst the Pacific and Indian Oceans, bridging the continents of Asia and Australia, boasts a remarkable array of biodiversity owing to its unique geographical position. Among this biodiversity are macroalgae, with Indonesia being home to approximately 1,500 species of these marine organisms. Algae, categorized within the kingdom Protista, exhibit plant-like characteristics, featuring thallus-shaped structures and chlorophyll pigments that facilitate photosynthesis [1]. The intertidal zone, the stretch of shoreline between high and low tides, serves as a habitat for these algae [2]. The intertidal areas exhibit diverse environmental conditions, fostering a high level of organismal diversity. Macroalgae are commonly found as epiphytes on various substrates such as rocks, sandy soil, wood, mollusk shells, and other plants within these intertidal habitats [3].

Macroalgae, a biological asset with significant developmental prospects, is commonly found in intertidal coastal regions. Nonetheless, this organism is highly susceptible to fluctuations in natural environmental factors like wind, waves, currents, and seasonal variations, all of which contribute to alterations in macroalgae habitats.

Furthermore, anthropogenic influences such as domestic and solid waste, urban community endeavors, intensified shipping activities in bay areas, urban development projects, and aquatic community engagements tend to impact the diversity and growth of macroalgae [4].

Algae can be classified into four primary categories based on their pigmentation: brown algae (Ochrophyta), green algae (Chlorophyta), red algae (Rhodophyta), and blue-green algae (Cyanobacteria). Among these, green, red, and brown algae are typically larger in size and are commonly referred to as macroalgae. Red algae is algae that has a red pigment, this is due to the presence of phycoerythrin pigment reserves contained in the algae. Moreover, red algae contain various pigments, including chlorophyll, carotenoids, and phycocyanins [5]. On the other hand, brown algae are characterized by their larger size compared to green and red algae, with brown pigmentation derived from phycoxanthin compounds, prevalent in these algae species.

Red macroalgae exhibit antioxidant potential due to their composition of photosynthetic pigments and additional accessory pigments, including chlorophyll-a, α-carotene, β-carotene, phycobilins, neoxanthin, and zeaxanthin, making them promising candidates as anticancer agents [6]. Furthermore, Rhodophyta species demonstrate efficacy in combating antibiotic-resistant bacteria. Various bioactive compounds such as alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, terpenoids, acetogenins, and aromatic compounds have been isolated from red algae. Notably, extracts from Eucheuma cottonii algae possess broad-spectrum antibacterial properties, inhibiting the growth of both gram-negative and gram-positive bacteria [6]. A minimum inhibitory concentration of 3.9 - 15.62 g/mL has been reported to be used by the red algae *Portieria hornemannii* to demonstrate the antibacterial effectiveness of silver nanoparticles (AgNPs) against Fish pathogens (Vibrio bacterial variants) [7]. In addition, 1.0 mM of sodium dodecyl sulfate (SDS) with 0.1 mM of biosynthesized AgNPs by *Corallina elongata* has higher antibacterial efficacy than other concentrations evaluated against both *Micrococcus leutus* and *Escherichia coli* ATCC 8739 [8].

One of the resources found in the intertidal zone of the Ulee Lheue Waters in Banda Aceh is macroalgae. 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 or ± 80 ha and is located at an altitude of 0.8 above sea level. Macroalgae, commonly referred to as seaweed, is a marine resource with considerable economic value and significant benefits for both humans and the environment. Its usefulness to humans extends to its utilization as a food source, a key component in cosmetics, and as a medicinal ingredient. Additionally, macroalgae play a beneficial role in marine ecosystems by generating organic compounds through photosynthesis. Despite its potential, macroalgae remain underutilized by coastal communities in Aceh due to limited scientific research into its capabilities.

Halymenia durvillei is the genus *Halymenia* which is included in the class Florideophyceae. Various studies have explored the antibacterial properties and potential toxicity of *H. durvillei* red algae [9,10]. *H. durvillei* is a marine macroalgae that dominates the coastal area of Banda Aceh, specifically on the coast of Ulee Lheue, Banda Aceh City, Indonesia. The waters of Ulee Lheue Beach have biodiversity, including coral reefs, fish, mangrove plants and macroalgae. The aim of this study was to explore the profile of the macroalgae bioactive compounds *H. durvillei* from the coast of Banda Aceh, Indonesia. Therefore, identification is needed to obtain complete information to support the use of *H. durvillei* extract as a natural antibacterial that is safe and does not leave negative effects on fish, people, and the environment.

2. Materials and methods

2.1 Study area

Using the exploration approach, *H. durvillei* were obtained from the coastal region of Ulee Lheue Beach in Banda Aceh City, Indonesia (Figure 1). At a depth of about 2 m, *H. durvillei* was collected at the Ulee Lheue Beach intertidal zone. After removing epiphytes, dirt, and foreign objects with clean water, necrotic sections were also removed from the samples. *H. durvillei* were meticulously cleansed in both fresh and salt water. The acquired *H. durvillei* was then morphologically recognized by using the morphological descriptions of numerous earlier investigations. Additionally, the research will be conducted at Syiah Kuala University's Marine Chemistry Laboratory, which is part of the faculty of marine affairs and fisheries.

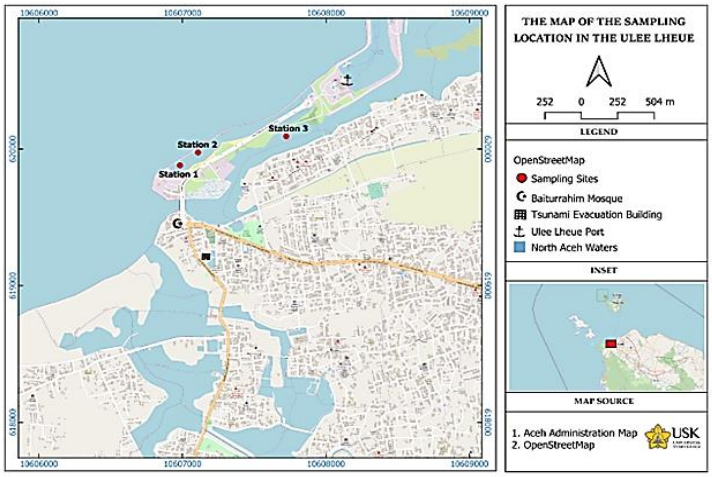

Figure 1 *H. durvillei* sampling locations: station 1 (5°33'35.0"N 95°17'02.9"E), station 2 (5°33'38.0"N 95°17'07.1"E), and station 3 (5°33'41.9"N 95°17'26.9"E).

2.2 Materials and Tools

The material used in this research is simplicia *H. durvillei*. The chemicals used for analysis were ethanol, DPPH (2,2-diphenyl-2-picrylhydrazil), HCl 2N, 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, $FeCl_3$. Then the tools used were centrifuge, rotary vacuum evaporator (Buchi R-300), microplate (Nunc), glassware, micro pipette, water bath, micro pipette, 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 H. durvillei Extraction

For two days, the clean *H. durvillei* were allowed to dry in the shade. According to the species identified, the dried *H. durvillei* were split into two containers and then blended. Up to 50 g of Simplicia were weighed and placed in an Erlenmeyer glass. Using ethanol, maceration was done at a 1:5 ratio. During 72 hours are spent storing the combination. Simplicia contains organic chemicals that can be extracted through soaking. 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 for use. Phytochemical analysis, total phenol content, antioxidant activity, spectroscopy FTIR, GC-MS, and spectroscopy UV/Vis were then performed on the *H. durvillei* ethanol extract.

2.4 Bioactive Components of H. durvillei Ethanol Extract

The phytochemical test was a preliminary test to qualitatively determine the content of active compounds such as alkaloids, flavonoids, phenol hydroquinones, steroids, triterpenoids, saponins and tannins.

2.5 Functional Group Analysis

A total of 0.0020 g of *H. durvillei* powder and 0.1980 g of KBr were weighed and then pulverized and printed to form thin (transparent) plates. The mixture were read using the FTIR (Bruker alpha). Furthermore, the resulting chromatogram is compared with the IR table.

2.6 Analysis of Total Phenol Content

The total phenolic content was assessed employing a method with slight adjustments [10]. Gallic acid standards were made by dissolving 5 mg of gallic acid in distilled water using a 25 mL measuring flask. Following that, standards at concentrations of 2, 4, 6, 8, and 10 ppm were made from this solution. Then, 20 mg of the extract were diluted in 25 mL of ethanol solvent to obtain the total phenol content. The mixture was then agitated to achieve homogeneity, then 0.5 mL of the solution's extract were combined with one mL of the 50% Follin Ciocalteu reagent, and the mixture was then allowed to stand for five minutes. Following the addition of one mL

of 5% Na_2CO_3, the mixture was homogenized for an hour in the dark. At a wavelength of 725 nm, the absorbance value was calculated using a UV-VIS spectrophotometer.

2.7 Antioxidant Activity by DPPH Method

The modified method involves assessing antioxidant activity through the utilization of the DPPH (1-diphenyl-2-picrylhydrazyl) assay at a concentration of 0.1 mM [9]. In ethanol, *H. durvillei* of crude seaweed extract were dissolved at concentrations of 2, 4, 6, 8, and 10 ppm. A positive control with an absorbance of 0.108 was ascorbic acid. The proportion of free radical inhibition determined by the following formula serves as an indicator of *H. durvillei* antioxidant activity (Equation 1).

$$Inhibition\ (\%) = \frac{Blank\ absorbance - Sample\ Absorbance}{Blank\ absorbance} x\ 100\% \quad (1)$$

2.8 Water Quality Measurement

The following characteristics of the water quality were noted: temperature, salinity, pH, dissolved oxygen, phosphate, and nitrate. Using a Nansen tube, *H. durvillei* of seawater were collected both near the bottom and at the surface layer. Measurements of pH, salinity, and temperature were done on-site. A GMK-910T thermometer was used to measure the temperature of the seawater, an Atago hand refractometer was used to evaluate salinity, and a HANNA HI9024 series pH metre was used to test pH. It was decided what the amounts of dissolved oxygen were using the Winkler titration method. Using a Shimadzu 1700 UV–VIS spectrophotometer, the spectrophotometric approach served as the foundation for the phosphate and nitrate analysis.

2.9 GC-MS Analysis

GC-MS 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 x 250 µm. Identification of chemical components was carried out by comparing the fragmentation pattern of the mass spectra of GC–MS results with the reference fragmentation pattern (library) of NIST12.LIB, WILEY229.LIB, and NIST62.LIB. The selected compounds are compounds based on literature searches that have a similarity index or SI (Similarity Index) greater than 90 and taking into account the compatibility of these compounds with the composition and properties of the original sample. The peak area of the GC chromatogram shows the relative concentration of a compound to the *H. durvillei* extract that evaporates during GC-MS operation.

3. Results

3.1 Water Quality Parameters

The physical and chemical quality of the aquatic environment has a significant impact on the existence of *H. durvillei* ethanol extract. Table 1 lists the findings of measurements made of a number of environmental factors in the coastal waters of Ulee Lheue, including salinity, temperature, phosphate and nitrate levels, pH, and dissolved oxygen (DO). According to Minister of Environment Decree No. 51/2004, these measurements are made in conformity with environmental factors.

Table 1 Table of 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	Dissolved Oxygen (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 No. 51/2004

3.2 Classification and Morphology of H. durvillei

The *H. durvillei* that have been collected are then identified for species classification and morphology. The results of the identification are presented in Table 2.

Table 2 Classification and morphology of *H. durvillei.*

Species	Morphology	Classification		
	*H. durvillei* has a thallus shape with flat and soft branches, smooth like gelatin (gelatinous), up to 30 cm high. The thallus type is like cartilage (cartilagoneus) with a round stem (stipe) (terete). Thallus is red and orange. Branching alternates irregularly on both sides of the talus (pinnate alternate) and the branching model is pinnate. The lower thallus usually widens and tapers towards the top, while the edge of the thallus is serrated and has a pointed tip. The root form (holdfast) is a disc that is attached to the substrate.	Kingdom	:	Protista
		Divisio	:	Rhodophyta
		Class	:	Florideophyceae
		Ordo	:	Halymeniales
		Familia	:	Halymeniaceae
		Genus	:	*H.*
		Species	:	*H. durvillei* Bory de Saint-Vincent, 1828

3.3 Bioactive Components

The initial step in identifying the kinds of bioactive chemicals present in plants is phytochemical analysis. To forecast active components that are advantageous to the human body, knowledge of active components is crucial. The plants evaluated can be in dose form, fresh, dried, powdered, or extract form. Using color changes or precipitates produced in response to the provided reagents, the *H. durvillei* extract was subjected to qualitative testing of their bioactive components. Table 3 lists the *H. durvillei* ethanol extract bioactive components that are present.

Tabel 3 Phytochemical test results for sample ethanol extracts of macroalgae *H. Durvillei.*

No	Secondary metabolite	Test Results
		H. durvillei
1	Flavonoids	+
2	Tanin	-
3	Polyphenol	+
4	Kuinon	-
5	Steroid	+
6	Triterpenoids	-
7	Saponin	+
8	Alkaloid	
	a) Mayer	-
	b) Wagner	+
	c) Dragendroff	+

3.4 Antioxidant Activity and Total Phenol

The extract's ability to prevent antioxidants from working was evaluated using the 50% inhibitory concentration (IC_{50}) value. This figure represents the *H. durvillei* ethanol extract concentration required to reduce 50% of the DPPH free radical activity. The results of the antioxidant activity test are shown in Table 4 and Figure 2, which showed that the *H. durvillei* ethanol extract and regular vitamin C had different effects.

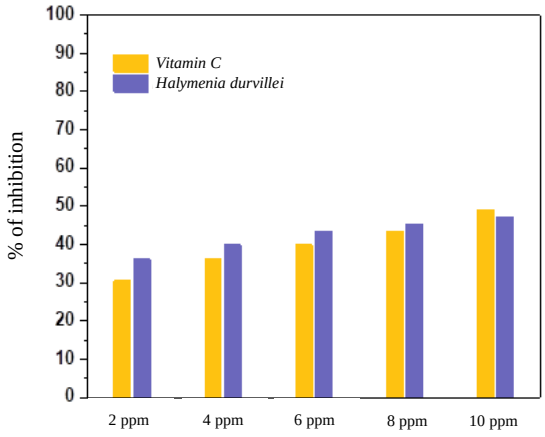

Figure 2 *H. durvillei* ethanol extracts' capacity to scavenge DPPH radicals.

Table 4 Antioxidant activity test results and total phenols of *H. durvillei* ethanol extract.

No	Species	Total Phenolic (mg GAE/g)	*Antioxidant activity* (IC_{50})(mg/L)
1	*H. durvillei*	10.52 ± 1.38	11.47
2	*Vitamin C*	-	10.58

3.5 Infrared (IR) and UV Vis Spectrum Characterization

FTIR was used to estimate the chemical bonds or functional groups found in the ekstrak etanol macroalga *H. durvillei*. Then, the UV-Visible absorption spectrum of *H. durvillei* extract was recorded between wavelengths of 300 to 900 nm By analysing the infrared absorption spectra in Table 5, the bonds were found. The FTIR and UV Vis spectra of the macroalgae *H. durvillei*, which are ethanol extract, are displayed in Figure 3.

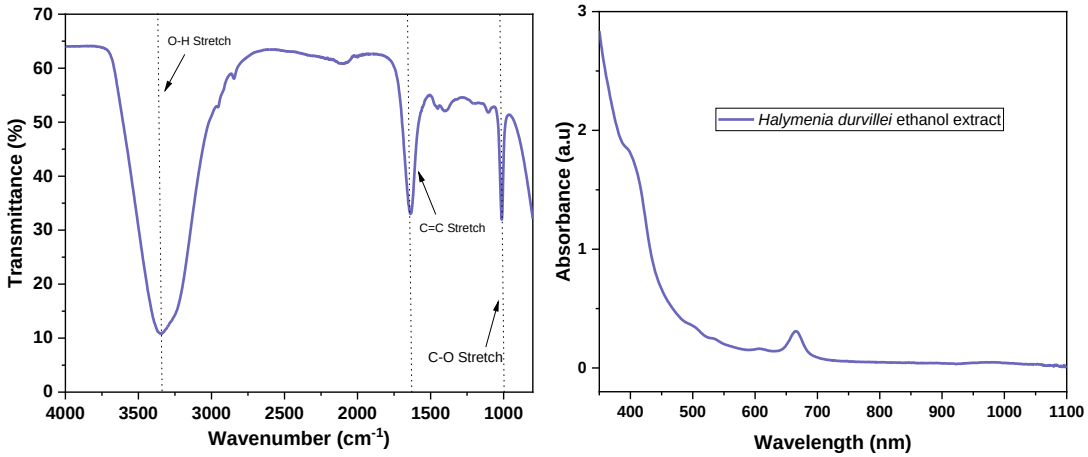

Figure 3 FTIR (a) and UV–VIS (b) spectra of *H. durvillei* ethanol extract. All extracts were analysed at 1 mg/mL.

Tabel 5 FTIR functional groups and spectral peak values of *H. durvillei* extract.

No	Frequency Range (cm^{-1})	Wave Number (cm^{-1})	Possibility 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]

3.6 GC-MS analysis

The chromatogram of the macroalgae *H. durvillei*'s ethanol extract produced 26 peaks as a result. Figure 4 and Table 6 show the chemical component profiles that were found for the ethanol extract of the macroalgae *H. durvillei*, respectively. Heptadecane was the chemical component in the macroalgae extract that had a higher percentage area value than the others. Therefore, the macroalgae *H. durvillei*'s ethanol extract's primary ingredient is this peak. Heptadecane is said to have antibacterial properties. Another study on antioxidant and anti-inflammatory properties provided a molecular analysis of dietary heptadecane for the anti-inflammatory modulation in the elderly kidney [14]. In the volatile fraction of *Synechococcus sp*. strain GFB01, in the absence of branched-chain alkanes, heptadecane was the dominant compound. Heptadecane and pentadecane are related as the main compounds in cyanobacteria from freshwater and marine settings, respectively. The antibacterial capabilities of cyanobacteria from *Synechococcus sp*. are the main topic of the study.

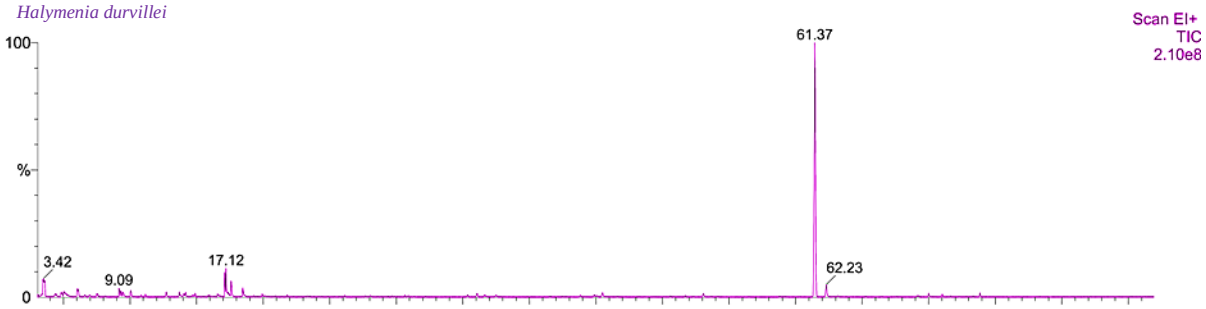

Figure 4 Chromatogram profile of ethanol extract of the macroalgae *H. durvillei.*

Table 6 Percentage chemical composition of ethanol extract from *H. durvillei.*

No	Compounds	Retention Time (min)	Relative Area (%)	Molecular formula	Molecular weight	PubChem ID
1	Cyclohexanol, 4-methyl-, trans-	3.419	5.017	$C_7H_{14}O$	114.19	11524
2	Disulfide, dimethyl	4.312	0.397	$C_2H_6S_2$	94.2	12232
3	2-Pentenal, (E)-	4.797	0.927	C_5H_8O	84.12	5364752
4	Methanesulfonylacetic acid	4.948	0.569	$C_3H_6O_4S$	138.14	3732841
5	2-Chloroethyl methyl ether	5.013	0.743	C_3H_7ClO	94.54	12316
6	Hexanal	5.970	1.646	$C_6H_{12}O$	100.16	6184
7	Methyl valerate	6.874	0.328	$C_6H_{12}O_2$	116.16	12206
8	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-	7.475	0.507	C_9H_{14}	122.21	572141
9	Acetaldehyde, propylhydrazone	9.086	0.937	$C_5H_{12}N_2$	100.16	5366202
10	3-Methyl-3-hexene	9.238	0.650	C_7H_{14}	98.19	5352447
11	Heptanal	9.979	0.882	$C_7H_{14}O$	114.19	8130
12	Benzaldehyde	12.658	0.673	C_7H_6O	106.12	240
13	2-Vinylfuran	13.638	0.447	C_6H_6O	94.11	73881
14	Cyclohexanol, 3-methyl-	13.953	0.390	$C_7H_{14}O$	114.19	11566
15	Cyclotetrasiloxane, octamethyl-	14.105	0.465	$C_8H_{24}O_4Si_4$	296.61	11169
16	3-Methylbut-2-enoic acid, 3-methylphenyl ester	16.510	0.425	$C_{12}H_{14}O_2$	190.24	532355
17	Furan, 2-ethyl-	17.023	1.749	C_6H_8O	96.13	18554
18	Tetradecane	35.990	0.575	$C_{14}H_{30}$	198.39	12389
19	E-2-Tetradecen-1-ol	36.568	0.314	$C_{14}H_{28}O$	212.37	5353006
20	6α-Hydroxymethandienone	37.408	0.313	$C_{20}H_{28}O_3$	316.4	13241205
21	1,15-Pentadecanediol	45.427	0.740	$C_{15}H_{32}O_2$	244.41	518994
22	Octadecanal	53.002	0.554	$C_{18}H_{36}O$	268.5	12533
23	Heptadecane	61.365	45.598	$C_{17}H_{36}$	240.5	12398
24	13-Methyltetradecanal	62.235	2.207	$C_{15}H_{30}O$	226.4	11085507
25	2-Pentadecanone, 6,10,14-trimethyl-	69.921	0.429	$C_{18}H_{36}O$	268.5	10408
26	Pentadecanoic acid, 14-methyl-, methyl ester	73.778	0.449	$C_{17}H_{34}O_2$	270.5	21205

4. Discussion

4.1 Bioactive Components

The *H. durvillei* ethanol extract produced favorable outcomes for the alkaloid compounds in the Wagner and Dragendroff reagents but unfavorable outcomes for the Mayer reagent. Alkaloids contain antibacterial and anti-inflammatory qualities that aid in blood circulation, postpartum strength restoration, and uterine infection prevention [15]. They also help with pain management and blood circulation. The findings of the polyphenol test for *H. durvillei* ethanol extract was then positive. Due to their anti-inflammatory, anti-oxidant, anti-carcinogenic, and anti-cholesterol capabilities, phenolic compounds can lower the risk of a number of chronic diseases [4]. Bioactive substances play a significant influence in antioxidant activity and can be identified using phytochemical assays.

4.2 Antioxidant Activity and Total Phenol

H. durvillei ethanol extracts exhibit high 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 [16]. The ability of the extract to function as a hydrogen atom 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, *H. durvillei* extract shown activity at all concentrations that was much less than that of normal vitamin C. At 10 g/mL, *H. durvillei* ethanol extract shown more efficacy with a 47.22% inhibition. *H. durvillei* 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 [17].

One class of antioxidant found in food are phenolic chemicals. Phenolic chemicals have been shown to be powerful antioxidant sources, to stifle free radicals, and to bind metal ions. Phenolic chemicals are associated with antioxidant activity. 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, colors like chlorophyll, sulfur compounds, and nitrogen [14]. According to Table 4, the two types of extracts had a total phenolic content 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.

4.3 Infrared (IR) Spectrum Characterization

The peak at 1654 cm^{-1} was attributed to C=C bonds from alkenes, indicating the presence of C=O carbonyls [11]. Additionally, peaks between 3677 – 3012 cm^{-1} were identified as O–H hydroxyl groups [12], potentially associated with polyphenol derivatives as listed in Table 5. The stretching of C–H bonds was observed between 2972 – 2936 cm^{-1} and 2878 – 2831 cm^{-1}, with bending occurring between 1420 – 1358 cm^{-1}. These results are corroborated by the GC–MS analysis in Tables 6 and 7, indicating the presence of carbonyl derivatives. The measurable C=C vibration mode is indicative of flavonoids, characterized by two benzene rings connected by a linear carbon chain. Furthermore, benzene C–H vibrations were detected around 2950 – 3180 cm^{-1} [13]. Utilizing FTIR spectrophotometry to identify benzenoid compounds confirmed the results of phytochemical screening, which detected the presence of flavonoids and phenols.

4.4 UV Visible Spectrum Characterization

An prominent absorption peak was visible in the UV-Vis spectrum of the *H. durvillei* extract at λ_{max} = 660 nm. 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 shows at λ_{max} = 358 nm and for the relatively modest intensity of this transition [18]. This information suggests that the extract *H. durvillei* contains a polyphenol ring.

4.5 GC-MS analysis

Pentadecanoic acid, 14-methyl-, methyl ester has been discovered in a variety of plant species, including *Coronopus didymus, Ageratum conyzoides*, and *Cannabis sativa* [19,20]. 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 [21]. A study of essential oils revealed the occurrence of 2-Pentadecanone, 6,10,14-trimethyl- are revealing of 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 activity of n-Octadecanal has been isolated from *Acacia nilotica* by bioactivity-directed fractionation of ethyl acetate extract from the air dried seeds and pod. Using *Acacia nilotica* extract with a concentration of 1000 µg/cm^3 from seed against *Salmonella typhi, Streptococcus feacalis*, *Escherichia coli*, *Candida krusei*, *Shigella dysentriae*, and *Staphylococcus aureus* with a zone of inhibition diameter of 9-29 mm [22]. On the other hand, fish and fishery products are regularly reported to include *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), with prevalences ranging from 2 to 60% [23]. Because consuming staphylococcal enterotoxins produced by *S. aureus* might result in food poisoning, the presence of *S. aureus* in fish is problematic [24]. Apart from that, it has also been reported that *S. typhi* is also found in fish. However, Since it is spread by polluted water or careless handling, salmonella is not a biological pollutant that was first identified in fish. The ability to trace the microbial source in fish slaughterhouses is made possible by the fact that this bacterium can live in soil, water, and fish. This fact also sheds light on the nature of the contamination and the potential route of this bacterium's spread. In this case, the ethanol extract from *H. durvillei* has the potential to be used as an antibacterial in fish because it contains n-Octadecanal.

The current work has revealed the bioactive chemicals responsible for the endophytic fungi's antibacterial activity and their antibacterial potential in *Dillenia indica* L. Tetradecane (13.86%) 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 *F. 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*, *E. coli*, and *S. aureus* [25]. Additionally, it has been noted that *Pediococcus acidilactici* extracts extracted from curd milk produce bioactive tetradecane. *E. coli*, *Listeria monocytogenes*, *Clostridium bifermentans*, *Candida albicans*, *S. aureus*, and *P. aeruginosa* and have all been evaluated for *P. acidilactici* activity using a disc diffusion assay, with an inhibitory zone at 183.15.14 ± 0.11 to 23.2 ± 0.91 mm [26].

Cyclotetrasiloxane, octamethyl- was identified in the extract of the sea cucumber *Bohadschia sp*. Antibacterial activity. The test bacteria used in this study consisted of *E. coli*, *S. aureus*, *Vibrio eltor* and *B. subtilis*. The inhibition zone's diameter was calculated to be 7 - 13 mm [27]. Besides that, Antimicrobial activity of olive (*Olea europaea* L.) leaves extract has been reported to give 9-11 nm inhibitory zone with several bacterial species [28].

Lepidium sativum L. seed oils were extracted using various methods, and their chemical composition, antibacterial, and antioxidant activity were assessed. The benzaldehyde level was found to be (11.21%). The

diameter of inhibition zones towards *S. aureus* and *B. cereus* were 15.57 ± 0.46, 13.86 ± 0.37, and 15.06 ± 0.13 mm as well as 13.12 ± 1.16, 11.20 ± 1.01, and 13.94 ± 0.56 mm at 1.0 mg/mL concentration, respectively [29]. Antibacterial properties of heptanal obtained from *Pyrrosia tonkinensis*. The antibacterial tests revealed that *P. tonkinensis* essential oil has effective antibacterial properties against all the examined microorganisms.

The bioactive compound 1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl- was obtained from (*Pulicaria undulata L.*, *Pulicaria incisa Lam.*, *Artemisia herba-alba Asso*., *A. monosperma Delile*, *A. judaica L.* and *Achillea fragrantissima* [30]. 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 *E. coli*, *Burkholderia cepatia*, *S. aureus*, and *P. stutzeri*, respectively, in the antibacterial activity of Spirulina extract (25 g/mL). While *B. cepatia* exhibits the highest level of inhibition (15 mm), all tested bacteria were inhibited at concentrations of 50 and 100 g/mL [31]. According to reports, *B. cepacia* develops 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 improved. Apart from this, studies related to fish infection by *Escherichia coli* bacteria have been reported. For this reason, *H. durvillei* can be a candidate for antibacterial fish infection.

4.6 Water Quality Parameters

The parameters of sea water temperature have a tolerance for macroalgal growth; water temperatures below 25 °C will result in reduced growth in the Gracilaria genus, and if the temperature is high, the thalus will turn pale yellowish and unhealthy. According to biology, low temperatures result in the cessation of biochemical activity in the thallus body, whereas high temperatures damage enzymes and destroy biochemical mechanisms in the macroalgal thallus. Ulee Lheue Beach in Banda Aceh City has water temperatures that range from 29.8-30 ºC, which is still considered typical for tropical waters. The temperature between 15°C-30°C is the ideal temperature range for macroalgae growth in the tropics. These waters' temperature, which should range from 28-32 ºC with deviations of up to 2 ºC from the natural temperature allowed, is still suitable for supporting marine biota life. Green, brown, and red algae can start to bloom at a temperature of 34.5 ºC [32].

Macroalgae require salinity to survive; high or low salinity will disrupt physiological processes. Saltwater salinities in these waterways range from 29.4-31.5 ‰, which is still favorable for macroalgal growth. Macroalgae typically inhabit waters with a salinity range of between 30-32 ‰, however many varieties may also survive in waters with a higher salinity range. The growth of seaweed might be hampered by salinity ranges that are too high or too low. The ideal salinity range for Eucheuma growth is between 28-34 ‰. These waters' salinity nevertheless falls within the range of typical coastal regions' salinities [33].

The pH of these waters, which ranges from 7.81-7.96, is still favorable for macroalgal life. In the pH range of 7 to 8, macroalgae can develop constantly. A pH range of less than 6.5 will inhibit growth even if a pH range of less than 9 is excellent for streams. The pH range of seawater, which is typically 7 to 8.5, is regarded as appropriate. These restrictions still apply to the pH values in this range.

One of the fundamental foundations for marine life and a sign of water fertility is dissolved oxygen. Dissolved oxygen concentrations in water masses are relative and typically range from 6 to 14 ppm. These waters have dissolved oxygen concentrations between 5.57 and 5.96 mg/L. While an oxygen level of 2 ppm is sufficient to support the existence of aquatic organisms, an oxygen content of 5 ppm with water temperatures between 20 and 30 ºC is generally considered to be relatively good for fish life [34].

One way to gauge a body of water's fertility is by looking at its high and low concentrations of phosphate and nitrate. According to observational findings, phosphate and nitrate levels varied between 0.1 and 0.46 mg/L and 0.2 and 0.5 mg/L, respectively. This number exceeds the benchmark for quality. Fertile waters have phosphate levels between 0.051-0.1 mg/L and moderately fertile waters have levels between 0.0021 and 0.05 mg/L [35]. There is a suspicion that land-based sources, like household waste, play a significant role in the increasing phosphate and nitrate levels in the streams north of Banda Aceh. Hydrooceanographic factors like currents also have an effect on the high phosphate and nitrate levels close to the northern waters of Banda Aceh because they cause nutrients in the sediment to be lifted into the water column. The likelihood of algal blooms can be affected by phosphorus and nitrate levels in Banda Aceh that are higher than the requirements for marine biota. Phytoplankton can absorb phosphorus up to a maximum of 5.51 mg/L before it enters the food chain.

5. Conclusion

In conclusion, our research revealed that the ethanol extract of *H. durvillei* exhibited notable in vitro properties. These evaluated extracts fared better in terms of both their antibacterial and antioxidant activities. The extract's high phenolic content may be the cause of its increased biological activity. The ethanol extract underwent FTIR spectroscopic analysis, revealing prominent peaks at 3352 cm^{-1}, 1654 cm^{-1}, and 1019 cm^{-1}, indicating the

presence of alkanes, alkenes, and hydroxyl groups, with other connections ranging from weak to medium. In the GC-MS study, several substances with possible antioxidant and antibacterial properties were discovered. These substances may contribute to the development of novel treatments or preventative measures for infectious illnesses in fish. It is recorded that there are around 9 bioactive compounds out of 26 which have the potential to act as antibacterials in fish, namely Methanesulfonylacetic acid, 1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-, Heptanal, Benzaldehyde, Cyclotetrasiloxane, octamethyl-, Tetradecane, Octadecanal , Heptadecane, and Pentadecanoic acid, 14-methyl-, methyl ester. Future plans call for the use of marine seaweed as an innovative, sustainable natural drug discovery method for treatments, nutraceuticals, and large-scale pharmaceutical industrial uses. To fully understand the mechanisms of action of the extracts of *H. durvillei*, as well as their bioactive components, and assess the effects in biological systems in vivo through the use of experimental animal models, more thorough research is necessary.

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7. References

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