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Phytochemical Composition, Antioxidant Activity, and Bioactive Compound Profiling of *Sargassum sp.* Ethanol Extract: Potential for Antimalarial Application

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Abstract
Sargassum sp., a brown macroalga abundant in Indonesia's coastal ecosystems, has garnered attention for its diverse bioactive compounds with pharmacological potential. However, its application as a natural antimalarial and antioxidant agent remains underexplored. This study aimed to investigate the secondary metabolites in ethanol extracts of Sargassum sp. and evaluate their antioxidant and potential antimalarial activities. Ethanol extraction was conducted using the maceration technique, followed by phytochemical screening, FT-IR, and GC-MS analyses to characterize the extract. The results revealed the presence of saponins, terpenoids, tannins, alkaloids, and flavonoids. FT-IR and GC-MS confirmed compounds associated with antioxidant and antimalarial properties. The extract exhibited notable antioxidant activity with an IC_{50} value of 26.16 ppm. These findings highlight the potential of ethanol-extracted Sargassum sp. as a promising source for the development of safer, resistance-limiting antimalarial and antioxidant agents.

Keywords: *Sargassum sp*, Phytochemical Screening, FT-IR Analysis, GC-MS Profiling, Antioxidant Activity.

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1. INTRODUCTION

Marine macroalgae, especially brown seaweeds of the genus Sargassum (Class Phaeophyceae), are critically significant both biologically and economically in tropical coastal ecosystems, including the coral reef environments of Indonesia. Over 150 species of Sargassum are globally distributed, with several species prevalent in Indonesian waters, including S. binderi, S. cinereum, S. duplicatum, S. plagyophyllum, S. echinocarpum, S. polycystum, and S. crassifolium (Diachanty et al., 2017). These species are physically defined by cylindrical thalli, arboreal branching, sword-shaped blades, and unique air vesicle (Pamungkas et al., 2013).

Sargassum, a diverse genus of brown seaweeds, has been historically valued for its nutritional and medicinal properties. Rich in proteins, vitamins, and beneficial secondary metabolites, Sargassum serves as a functional food and a source of bioactive compounds. Its applications span various industries, including food, health, and agriculture, highlighting its versatility and importance (Nazarudin et al., 2021). Alginate derivatives from Sargassum are widely recognized for their multifunctional applications across various industries, particularly in pharmaceuticals, cosmetics, and food. These derivatives serve as stabilizers, emulsifiers, and gelling agents due to their unique physicochemical properties, which include biodegradability, non-toxicity, and the ability to form gel matrices in the presence of calcium ions (Prastuty et al., 2024; Pragatheeswari & Raajeswari, 2024). In addition to its nutritional and industrial significance, mounting data indicates that Sargassum possesses a diverse array of pharmacological properties, encompassing antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, antiviral, and antimalarial actions (Saraswati et al., 2019).

In recent years, there has been an increasing interest in marine derived chemicals as innovative antimalarial medicines, especially due to the global issue of Plasmodium resistance to current treatments. Marine sponges, including Haliclona sp., Xestospongia sp., and Petrosia spp., have produced promising antiplasmodial metabolites (Murtihapsari et al., 2021). Various seaweeds, such as Padina australis, Laurencia nidifica, Sargassum plagyophyllum, and Eucheuma spinosum, have demonstrated antioxidant and antibacterial properties (Podungge et al., 2017; Sidauruk et al., 2021). These findings underscore the underexploited potential of marine macroalgae, such as Sargassum, in pharmacological research.

Marine algae-derived natural antioxidants are noteworthy for their capacity to neutralize reactive oxygen species (ROS) and their synergistic effects in addressing oxidative stress-related diseases, including parasitic infections like malaria (Menaa et al., 2021; Pangestuti et al., 2017). Oxidative stress is pivotal in the pathophysiology of malaria, indicating that substances with both antioxidant and antiplasmodial *characteristics may provide superior treatment advantages.* Although several studies have reported the pharmacological activities of *Sargassum* species, such as antioxidant, anti-inflammatory, and antibacterial effects, the exploration of its antimalarial potential remains limited. A few recent investigations have demonstrated in vitro antiplasmodial activity in certain *Sargassum* extracts, however, comprehensive studies focusing specifically on ethanol extracted secondary metabolites with both validated antioxidant and antimalarial properties are still scarce (Nilamsari et al., 2023). Additionally, the role of solvent polarity, particularly the use of ethanol in selectively extracting bioactive compounds such as flavonoids, terpenoids, and phenolics has often been overlooked in prior research.

This study fills the gap by conducting phytochemical screening, FTIR, and GC-MS analyses of ethanol extract from Sargassum sp. sourced from Indonesian waters, with the objective of characterizing its secondary metabolite profile and assessing its antioxidant capacity as an indicator of potential antimalarial activity. The utilization of ethanol, a semi-polar solvent, is anticipated to enhance the extraction of phenolics, flavonoids, and terpenoids compound classes recognized for their biological activity. This project aims to yield essential data for the creation of marine algae-derived antimalarial medicines that are safer and less susceptible to resistance.

2. RESEARCH METHOD

Materials and Equipment. This study utilized a rotary evaporator, separating funnel, test tubes, test tube racks, sieves, and filter paper as laboratory equipment. The biological material used was *Sargassum* sp., while the solvents and reagents included ethanol (96%, analytical grade), methanol (analytical grade), ethyl acetate (analytical grade), hydrochloric acid (37%,

analytical grade), Mayer's reagent, Dragendorff's reagent, 1% ferric chloride ($FeCl_3$), chloroform (analytical grade), glacial acetic acid (≥99.7%, analytical grade), concentrated sulfuric acid (H_2SO_4, 95–98%), magnesium powder (metallic, fine powder), and DPPH (2,2-diphenyl-1-picrylhydrazyl, ≥95% purity). All solvents and reagents were obtained from certified chemical suppliers and were of analytical grade to ensure result consistency and reproducibility. This study employed an in vitro experimental design to evaluate the phytochemical composition and antioxidant potential of ethanol extract derived from *Sargassum* sp.

Sample Preparation and Extraction. Recent specimens of Sargassum sp. were meticulously rinsed with purified water to exclude sand, salt, and epiphytes. The purified seaweed was further fragmented into smaller pieces and shade-dried for about four days under observation, followed by oven drying at 35–40°C to eliminate residual moisture. The desiccated substance was pulverized with a blender into a fine powder to yield the crude medicine (simplisia). The extraction was conducted via the maceration technique. One hundred fifty grams of dry powder were immersed in 1.5 liters of each solvent (ethanol, methanol, and ethyl acetate) at a 1:10 (w/v) ratio for three consecutive 24-hour periods with intermittent stirring. The macerates were filtered, and the solvents were evaporated at decreased pressure with a rotary evaporator to get concentrated extracts (Adderley et al., 2023).

Phytochemical Analysis. Phytochemical analyses were performed on the ethanol extract of Sargassum sp. to identify the presence of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and saponins. The screening procedures adhered to conventional protocols outlined by Tasmin et al. (2014), as detailed in Table 1.

Table 1. *Phytochemical test procedures*

Test	Procedure	Indicator
Saponins	0.5 mL extract + 5 mL distilled water, shaken vigorously for 30 seconds	Persistent froth formation
Terpenoids	0.5 mL extract + 0.5 mL glacial acetic acid + 0.5 mL concentrated H_2SO_4	Reddish or yellow color change
Tannins	1 mL extract + 3 drops of 1% $FeCl_3$ solution	Formation of dark blue or greenish-black color
Alkaloids	0.5 mL extract + 5 drops chloroform + 5 drops Mayer's reagent	White or brownish precipitate
Flavonoids	0.5 mL extract + 0.5 g Mg powder + 5 mL concentrated HCl (added dropwise)	Red or yellow coloration, foaming

Source: *Tasmin et al., 2014*

Mayer's reagent was prepared by dissolving 1 g of potassium iodide (KI) in 20 mL distilled water and adding 0.271 g of mercuric chloride ($HgCl_2$) until completely dissolved.

Compound Characterization. *The ethanol extract was further analyzed to identify bioactive functional groups using* Fourier Transform Infrared Spectroscopy (FT-IR) was conducted to identify functional groups in the ethanol extract of *Sargassum* sp., using a scan range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. Gas Chromatography-Mass Spectrometry (GC-MS) was employed to determine the chemical profile of individual components. The GC-MS analysis was performed using a capillary column (e.g., HP-5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness), with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed as follows: initial temperature at 60 °C (held for 2 min), increased to 280 °C at a rate of 10 °C/min, and held for 10 min. The injection volume was 1 µL in splitless mode, and mass spectra were recorded in the range of 40–600 m/z. These methods are commonly used for identifying and characterizing secondary metabolites in marine algae.

Antioxidant Activity Assay. The antioxidant activity of the ethanol extract of *Sargassum* sp. was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The extract was dissolved in methanol to obtain concentrations of 50, 100, 150, 200, and 250 ppm. Each concentration was mixed with 200 µL of 0.1 M acetate buffer (pH 5.5) and 100 µL of 5×10^{-4} M DPPH solution. The use of acetate buffer at pH 5.5 in this study was intended to create a mildly acidic environment that supports the stability and activity of key secondary metabolites, particularly phenolic and flavonoid compounds, which are known to be sensitive to pH changes. A slightly acidic condition helps maintain their structural integrity and enhances their reactivity, especially in antioxidant assays where electron transfer plays a crucial role. Additionally, pH 5.5 falls within the effective buffering range of acetic acid, ensuring consistent pH throughout the experiment. This condition also approximates certain biological microenvironments, such as the acidic compartments within parasitic organisms, thereby providing a more physiologically relevant setting for preliminary evaluation of the extract's antimalarial potential. The mixture was incubated at room temperature (37°C) in the dark for 30 minutes, and the absorbance was measured at 517 nm using a UV–Visible spectrophotometer. In this study, data from the DPPH assay were analyzed by calculating the percentage of radical scavenging activity at various extract concentrations, followed by linear regression analysis to estimate the IC_{50} value, using statistical software. The IC_{50} is defined as the concentration of extract required to inhibit 50% of DPPH radicals. All measurements were performed in triplicate, and results are reported as mean ± standard deviation (SD) to ensure statistical reliability (Marliana et al., 2018).

3. RESULTS AND DISCUSSION

Phytochemical Analysis of Sargassum sp. Ethanol Extract

The phytochemical analysis identified five principal groups of secondary metabolites in the ethanol extract of Sargassum sp.: saponins, terpenoids, tannins, alkaloids, and flavonoids, as detailed in Table 2.

Table 2. Phytochemical screening of ethanol extract of *Sargassum* sp.

No.	Compound	Results
1.	Saponin	+
2.	Terpenoid	+
3.	Tanin	+
4.	Alkaloid	+
5.	Flavonoid	+

***Note**: (+) indicates the presence of the compound.*

The qualitative phytochemical screening of the ethanol extract of *Sargassum* sp. indicated the presence of several classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids. The presence of these metabolites was confirmed by characteristic color changes or precipitate formation: a brownish coloration for alkaloids (using Mayer's reagent), persistent froth for both saponins and flavonoids, a dark blue-black solution for tannins (with ferric chloride), and a yellowish-red color for terpenoids (in the Liebermann–Burchard test).

These findings are consistent with previous studies similar profiles for ethanol extracts of *Sargassum* sp. and *Padina* sp., showing the presence of saponins, terpenoids, tannins, alkaloids, and flavonoids (Silvinia, 2021). Mulyadi also identified β-carotene, flavonoids, tannins, and saponins in *Sargassum* sp. Extract (Mulyadi et al., 2019). Riwanti and Izazih, using

96% ethanol, observed positive results for alkaloids, flavonoids, saponins, steroids, terpenoids, and tannins (Riwanti & Izazih, 2019). Likewise, Manteu et al confirmed the presence of flavonoids, steroids, saponins, and alkaloids in *Sargassum polycystum* and *Padina minor* (Manteu et al., 2018).

The variation in phytochemical content reported across studies may be attributed to differences in solvent polarity, geographic origin of the seaweed, harvesting season, and environmental conditions. Ethanol, being a polar solvent due to its hydroxyl group (–OH), is known to effectively extract polar and moderately polar secondary metabolites such as flavonoids, alkaloids, and tannins (Pangestuti et al., 2017). Solvent polarity plays a crucial role in the efficiency and specificity of bioactive compound extraction, with polar solvents generally yielding higher phytochemical content from polar-rich matrices like brown seaweeds (Kadam et al., 2015; Azmir et al., 2013).

Alkaloids are nitrogen-containing secondary metabolites with well-documented pharmacological activity, including potent antimalarial effects. Their mode of action often involves interference with parasite DNA replication, enzyme inhibition, or disruption of membrane potential (Widyawaruyanti et al., 2011). Flavonoids, widely distributed in marine algae, also exhibit antimalarial activity through inhibition of nutrient uptake, hemoglobin catabolism, and heme detoxification pathways in *Plasmodium* parasites (Widyawaruyanti et al., 2011; Kaur et al., 2009).

Terpenoids, another major class of metabolites identified in this study, have been shown to penetrate erythrocyte membranes due to their lipophilic structure, thereby impairing parasite invasion and growth. Some terpenoids may also inhibit protein biosynthesis in malaria parasites (Attemene et al., 2018). Saponins, known for their surfactant properties, may also contribute to antiplasmodial activity by disrupting parasite membrane integrity (Pattanayak et al., 2010).

The synergistic or additive effects of these secondary metabolites are likely responsible for the therapeutic potential of *Sargassum* extracts. As Ntie-Kang emphasized, natural products containing diverse scaffolds such as alkaloids, terpenoids, flavonoids, coumarins, phenolics, xanthones, steroids, and lignans represent valuable leads for antimalarial drug development (Ntie-Kang et al., 2014).

The type and polarity of solvent used during extraction significantly influence the quantity and class of phytochemicals obtained. Ethanol, being semi-polar, facilitates the extraction of both polar and moderately non-polar compounds. According to Pangestuti, more polar solvents tend to yield higher concentrations of phenolic and flavonoid compounds, which contribute significantly to antioxidant and antimalarial activity (Pangestuti et al., 2017). Therefore, the use of ethanol in the current study is justified by its ability to extract a broad spectrum of bioactive compounds relevant to pharmaceutical applications.

FT-IR Spectroscopy Analysis of *Sargassum* sp. Ethanol Extract

FT-IR (Fourier Transform Infrared) spectroscopy was employed to identify the functional groups present in the ethanol extract of *Sargassum* sp., which are indicative of its secondary metabolite content. The FT-IR spectrum (Figure 1) showed multiple absorption peaks corresponding to characteristic vibrations of various functional groups, suggesting the presence of diverse bioactive compounds.

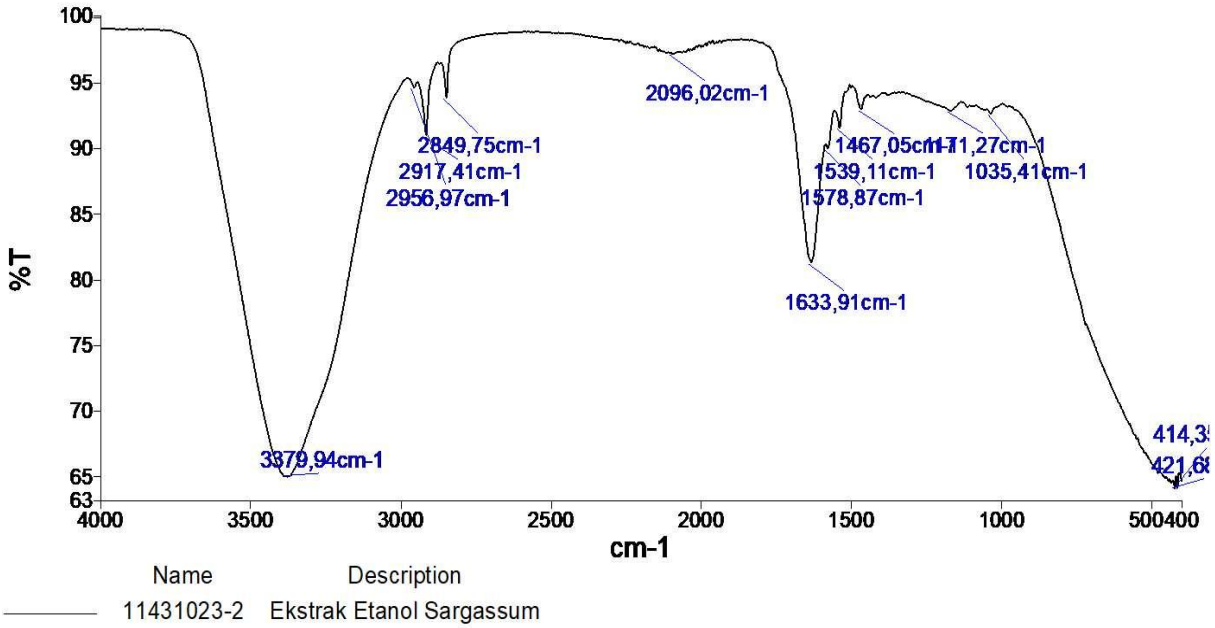


Figure 1. FT-IR Spectrum of Sargassum sp. Ethanol Extract

The detailed interpretation of the FT-IR spectrum is presented in Table 3**,** where 11 prominent absorption peaks were identified across the range of 3379.94 cm^{-1} to 1035.41 cm^{-1}. The peaks correspond to functional groups commonly found in phenolic compounds, flavonoids, alcohols, amides, ethers, and carboxylic acids.

Table 3. Functional Group Identification in FT-IR Spectrum of *Sargassum sp.*

Wavenumber (cm^{-1})	**Functional Group**	**Interpretation**
3379.94	O–H (alcohol, carboxylic acid)	Broad peak indicates hydroxyl group stretch
2956.97–2849.75	C–H (alkane, aldehyde)	Symmetric and asymmetric C–H stretches
2917.41	C–H (alkane)	Typical for CH_2 groups
2096.02	C≡C (alkyne)	Terminal alkyne stretch
1633.91	C=C (alkene), N–H (amide)	Stretching vibrations for unsaturated bonds
1578.87–1467.05	C=C (aromatic ring), ether	Aromatic skeletal vibrations
1171.27, 1035.41	C–O (alcohol, phenol, ether)	Strong band indicative of phenolic OH & ether C–O

The presence of O–H, C=O, C=C, C–O, and C–H functional groups strongly indicates that flavonoid-type compounds are present in the extract. These findings are consistent with previous reports by (Aranda-Ledesma et al., 2024; Dunky, 2023), who identified similar patterns in plant-based flavonoids using FT-IR spectroscopy. Flavonoids commonly contain these groups and often show bands in the range of 1000–1700 cm^{-1} due to aromatic and hydroxyl substitutions.

According to Sandamali, the detection of O–H, C=O, C=C, and C–O groups in pineapple peel extract corresponds to flavanon or dihydroflavonol subtypes (Sandamali et al., 2024). Flavanones are characterized by the absence of a double bond between C2 and C3 and are known to exhibit strong UV absorption and IR signals, particularly in the hydroxyl and aromatic regions (Alfaridz & Amalia, 2018).

Importantly, flavanones have been reported to exhibit potent antimalarial activity. Fatmawati demonstrated that flavanone compounds isolated from the stem bark of *Erythrina fusca* L. showed significant antiplasmodial activity against *Plasmodium falciparum* with IC_{50} values of 1.18 and 0.82 µg/mL, respectively (Fatmawati et al., 2018). These compounds are believed to exert their antimalarial effects through multiple mechanisms, including the disruption of parasite membrane integrity, interference with heme detoxification pathways, and inhibition of key enzymes involved in the parasite's redox metabolism and DNA replication. Flavanones may also induce oxidative stress within the parasite by promoting reactive oxygen species (ROS) accumulation, leading to parasite death. This supports the hypothesis that the flavonoid content in *Sargassum* sp., particularly of the flavanone subclass, contributes to its potential as an antimalarial agent by targeting essential biological processes in the malaria parasite.. Furthermore, the absorption peak at ~2096 cm^{-1}, corresponding to C≡C triple bonds, may indicate the presence of acetylenic compounds, which have also been reported to exhibit cytotoxic and antiparasitic activities (Bankova et al., 1983; Zhao et al., 2003).

The high transmittance observed in the FT-IR spectrum (approaching 100%) suggests that the polymeric structures in the extract underwent stretching with minimal IR absorption, indicating low crystallinity or the presence of flexible chains consistent with alcohols, phenols, and glycosidic linkages found in polysaccharide-rich marine algae (Ojeda, & Dittrich, 2012). The FT-IR spectrum of the ethanol extract of *Sargassum* sp. confirms the presence of bioactive functional groups corresponding to flavonoids, phenolic acids, and other secondary metabolites. The dominant signals are consistent with flavanone-type flavonoids, which may play a critical role in the extract's antioxidant and antimalarial properties.

GC-MS Analysis of *Sargassum* sp. Ethanol Extract

The chemical profile of the ethanol extract of *Sargassum* sp. was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). The resulting chromatogram (Figure 2) displayed 10 major peaks**,** each corresponding to distinct chemical constituents identified by comparison with the WILLEY229.LIB and NIST2.LIB spectral libraries.

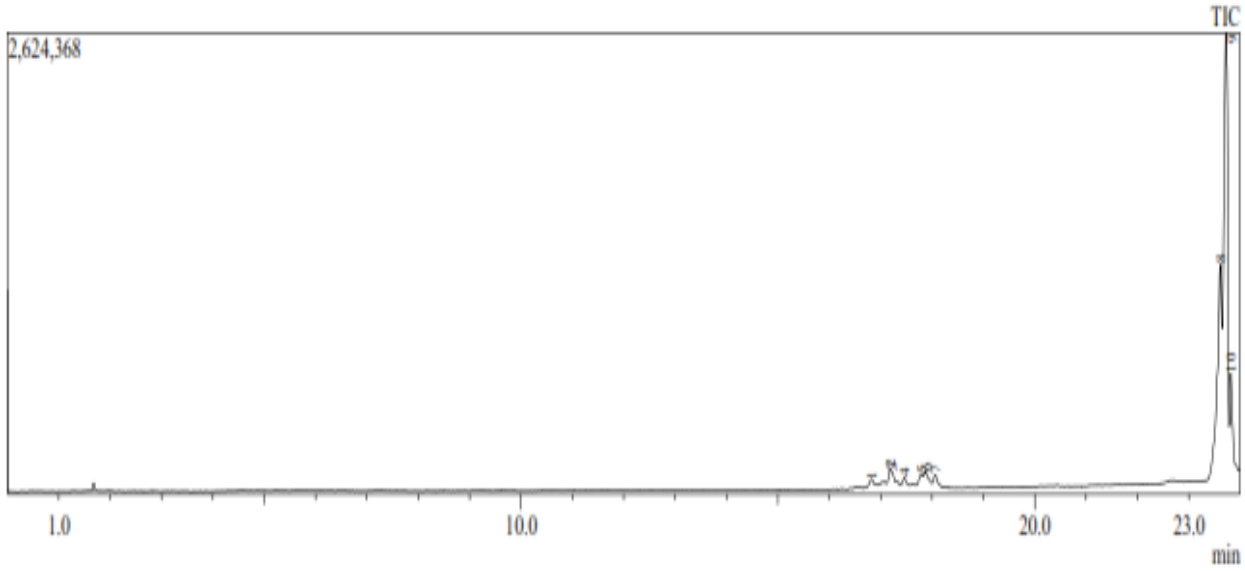


Figure 2. GC-MS Chromatogram of Sargassum sp. Ethanol Extract

The identification of compounds was based on their mass fragmentation patterns and similarity index (SI)**.** Table 4 summarizes the retention times (R. Time), molecular formulas, similarity indices, and relative peak areas of the identified compounds.

Table 4. GC-MS Identified Compounds in Sargassum sp. Ethanol Extract.

No	R. Time	Similarity Index	Compound Name	Molecular Formula	% Peak Area
1	16.817	75	Tetradecane, 2,6,10-trimethyl	C17H36	0.76
2	17.187	73	Dodecane, 2,6,10-trimethyl-	C15H32	1.16
3	17.225	71	8-Pentadecanone	C15H30O	0.67
4	17.461	70	Hexatriacontane	C36H74	0.86
5	17.799	74	Laurinsäure, 4-Octylester	C20H40O2	1.28
6	17.883	77	Dodecanoic acid, ethenyl ester	C14H26O2	2.18
7	18.061	72	Octadecane, 3-ethyl-5-(2-ethylbutyl)	C26H54	1.41
8	23.613	80	Dodecanoic acid, 1,2,3-propanetriyl ester	C39H74O6	30.17
9	23.719	73	Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester	C27H52O5	53.28
10	23.816	70	Octadecanoic acid, 2,3-bis[(1-oxotetradecyl)oxy]propyl ester	C49H94O6	8.23

The major constituents were fatty acid esters, predominantly Dodecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester (53.28%) and Dodecanoic acid, 1,2,3-propanetriyl ester (30.17%). These are derivatives of lauric acid (C12:0), which are commonly found in plant and algal lipids and are known for multiple biological properties including antibacterial**,** antioxidant**,** hepatoprotective**,** and enzyme inhibitory activities (El-Beltagi et al., 2022; Shannon & Abu-Ghannam, 2016; Dolganyuk et al., 2020; Zhang et al., 2022). Dodecanoic acid, 1,2,3-propanetriyl ester, also known as glyceryl trilaurate, is widely used in pharmaceuticals and cosmetics as a skin conditioning agent, and has been shown to exhibit antioxidant and antibacterial activity (Achi & Ohaeri, 2015; Matsue et al., 2019). Dodecanoic acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester is a glyceryl mono/diester that can act as a lipophilic carrier**,** potentially enhancing the bioavailability of other active compounds.

These fatty acid esters are not only consistent with the polar solvent (ethanol) used in extraction but also highlight the metabolic richness of *Sargassum* sp. in bioactive lipids, which are increasingly recognized for their role in malaria parasite inhibition, primarily through membrane disruption and enzyme inhibition pathways (Ntie-Kang et al., 2014; Lopes et al., 2021; Gnayem et al., 2025). The presence of minor constituents such as trimethylated alkanes (e.g., tetradecane, dodecane) and octadecanoic acid derivatives further supports the complex chemical makeup of the extract. While some of these hydrocarbons are non-polar and biologically inert, others may function as natural solvents**,** enhancers**,** or precursors in biosynthetic pathways of more active secondary metabolites.

Several of the identified compounds align with known antiplasmodial or supportive bioactivities: Fatty acid esters (e.g., lauric and stearic acid derivatives) have been shown to possess membrane-targeting antimalarial properties**,** disrupting parasite development (Carballeira, 2008; Ben Mamoun et al., 2010). The combination of fatty acids and flavonoids (as confirmed in FT-IR and phytochemical screening) suggests a synergistic mechanism**,** where flavonoids inhibit parasite metabolism, while lipids enhance delivery and membrane disruption (Soto-Sánchez, 2022; Mead & McNair, 2006). Overall, the GC-MS profile validates the phytochemical and FT-IR results**,** indicating that *Sargassum* sp. ethanol extract is rich in flavonoid-compatible functional groups and fatty acid esters with known bioactivities. These findings support the potential of this marine resource for further development as an antimalarial and antioxidant agent**.**

Antioxidant Efficacy of Sargassum sp. Ethanol Extract

The antioxidant capacity of the ethanol extract of Sargassum sp. was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a recognized method for assessing free radical inhibition by natural substances. Table 5 displays the findings of absorbance and % inhibition at various doses.

Table 5. Antioxidant Activity of Sargassum sp. Ethanol Extract (DPPH Assay)

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (ppm)
DPPH blank	20	0.342	-	-
Sargassum sp. extract	100	0.322	5.99	26.16
	200	0.306	10.53	
	300	0.295	15.93	
	400	0.281	17.84	
	500	0.281	20.03	
	600	0.274	24.27	
	700	0.259	27.63	
	800	0.237	30.70	
	900	0.231	32.60	
	1000	0.208	39.33	

The observations in Table 5 demonstrate a distinct concentration-dependent enhancement in DPPH radical inhibition by the Sargassum sp. extract. As the concentration escalated from 100 ppm to 1000 ppm, the absorbance values systematically diminished, signifying enhanced neutralization of free radicals. The inverse relationship arises from antioxidant chemicals giving hydrogen atoms or electrons to stabilize DPPH radicals, hence diminishing their absorbance at 517 nm (Molyneux, 2004). The ethanol extract had an IC_{50} value of 26.16 ppm, indicating the quantity necessary to block 50% of DPPH radicals. Based on the classification scheme established by Tristantini, substances exhibiting an IC_{50} value of 50 ppm is regarded as a potent antioxidant concentration; Active concentrations range from 50 to 100 ppm; Concentrations of 101–250 ppm exhibit moderate activity, and Concentrations of 250–500 ppm are regarded as weak (Triastinurmiatiningsih et al., 2011).

Consequently, the ethanol extract of Sargassum sp. is classified as a powerful antioxidant, indicating significant potential for mitigating oxidative stress. The results align with the findings of Pangestuti, which indicated that brown seaweeds are abundant in polyphenols, flavonoids, and sulfated polysaccharides that enhance their antioxidant effects (Pangestuti et al., 2017). The polar characteristics of ethanol as an extraction solvent enhance the retrieval of antioxidant components, including flavonoids, phenolic acids, and terpenoids, as corroborated by the FT-IR and phytochemical screening results. This reinforces the idea that the antioxidant action is due to the synergy of these secondary metabolites.

The DPPH approach was chosen for its simplicity, speed, reproducibility, and low sample demand. Methanol served as a solvent for DPPH and sample dilution due to its non-interference with the redox reaction between antioxidants and DPPH radicals (Molyneux, 2004). The ethanol extract of Sargassum sp. exhibits significant antioxidant activity, with an IC_{50} value well below 50 ppm, classifying it as a potent free radical scavenger. This outcome underscores its potential application as a natural antioxidant for medicinal, nutraceutical, or cosmetic purposes. However, this study has certain limitations. Although ethanol, methanol, and ethyl acetate were initially used as extraction solvents, only the ethanol extract was subjected to detailed phytochemical analysis, compound characterization, and antioxidant testing. Ethanol was selected because of its efficiency in extracting both polar and semi-polar metabolites and its safety profile for pharmacological applications. Nevertheless, this choice restricts the

exploration of metabolites that may be preferentially soluble in methanol or ethyl acetate, thereby limiting the comprehensiveness of the chemical profile. Furthermore, the phytochemical analysis was restricted to qualitative screening without quantitative assays of total phenolic, flavonoid, or alkaloid content, limiting the correlation between metabolite levels and bioactivity. The antioxidant evaluation was also based solely on the DPPH assay, which may not fully represent biological efficacy. In addition, the GC-MS identification of active compounds remains preliminary without bioactivity-guided fractionation to confirm the major contributors to the observed antioxidant and potential antimalarial effects.

Future research should focus on isolating and purifying individual bioactive compounds from the ethanol extract to evaluate their specific mechanisms of action. Additional antioxidant assays, including ABTS, FRAP, and cellular antioxidant activity models, are recommended to validate and compare the extract's activity across different systems. Furthermore, in vitro antiplasmodial testing and in vivo evaluations are essential to establish the therapeutic relevance and safety profile of *Sargassum*-derived compounds for antimalarial drug development.

4. CONCLUSION

The ethanol extract of Sargassum sp., a brown macroalga found in Indonesian waterways, contains various bioactive secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids. These compounds have antioxidant and antimalarial properties, with FT-IR analysis revealing flavonoid structures. GC-MS profiling revealed 10 principal chemicals, with dodecanoic acid derivatives being the predominant components. The extract's significant antioxidant activity suggests potential applications in antimalarial treatment, particularly by mitigating oxidative stress associated with Plasmodium infection. Future studies should focus on in vivo validation and the isolation of specific active compounds to support the development of Sargassum-based therapeutics for use in clinical or preventive settings.

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