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ANALYSIS OF THE ANTIOXIDANT POTENTIAL OF BROWN SEAWEED *Turbinaria decurrens* FROM THE SINAR BAHAGIA COASTAL WATERS, SIMEULUE ISLAND, INDONESIA

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Abstract

Turbinaria decurrens, a brown seaweed native to the coastal waters of Sinar Bahagia, Simeulue Island, Aceh, has antioxidant properties. This study aimed to evaluate the antioxidant potential of brown seaweed *T. decurrens* extracts. The research method includes phytochemicals, total phenol content and antioxidant activity assay with DPPH method with different solvents included methanol, ethyl acetate, and n-hexane solvents. Phytochemical screening demonstrated the existence of flavonoids, saponins, phenolics, and steroids in all extracts, indicating a substantial repository of secondary metabolites. The DPPH radical scavenging experiment was used to measure the antioxidant activity. The methanol extract showed the strongest activity (IC_{50} = 22.1±0.47 mg/mL), followed by ethyl acetate (IC_{50} = 36.7±2.52 mg/mL) and n-hexane (IC_{50} = 46.68±1.79 mg/mL). The total phenolic content was highest in the methanol extract (2.43 mg GAE/g), followed by ethyl acetate (1.50 mg GAE/g) and n-hexane (0.73 mg GAE/g). A strong positive correlation (R2 = 0.95) between total phenol content and antioxidant activity indicates that phenolic compounds are the dominant contributors to the radical scavenging capacity of *T. decurrens* extract. *Turbinaria decurrens* extract with methanol as the solvent showed a relatively higher phenolic content and antioxidant potential than those of other solvents. In conclusion, *T. decurrens* extracts demonstrated phenolic content and antioxidant potential, with methanol yielding a comparatively higher total phenolic content and activity among the tested solvents. These observations suggest opportunities for its use in nutraceutical or functional food applications, pending further validation studies. Future studies should explore specific phenolic isolation, in vivo evaluations, and broader solvent optimizations.

Keywords: bioactive compound, macroalgae, oxidative stress, phenolic, phytochemical

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Analisis Potensi Antioksidan Rumput Laut Cokelat *Turbinaria decurrens* dari Perairan Pesisir Sinar Bahagia Kepulauan Simeulue, Indonesia

Abstrak

Turbinaria decurrens merupakan rumput laut cokelat di perairan pesisir Sinar Bahagia, Kepulauan Simeulue, Aceh, yang memiliki sifat antioksidan. Studi ini bertujuan untuk mengevaluasi potensi antioksidan ekstrak rumput laut cokelat *T. decurrens*. Metode penelitian meliputi skrining fitokimia, penentuan kadar fenol total, dan uji aktivitas antioksidan dengan metode DPPH menggunakan pelarut yang berbeda meliputi pelarut metanol, etil asetat dan n-heksan. Skrining fitokimia menunjukkan keberadaan flavonoid, saponin, fenolik, dan steroid pada semua ekstrak, yang mengindikasikan adanya cadangan metabolit sekunder yang substansial. Hasil ekstrak metanol menunjukkan aktivitas yang paling kuat (IC_{50} = 22,1±0,47 mg/mL), diikuti oleh etil asetat (IC_{50} = 36,7± 2,52 mg/mL) dan n-heksana (IC_{50} = 46,68±1,79 mg/mL). Kadar fenol total, tertinggi pada ekstrak metanol (2,43 mg GAE/g), diikuti oleh etil asetat (1,50 mg GAE/g) dan n-heksana (0,73 mg GAE/g). Korelasi positif yang kuat (R2 = 0,95) antara kandungan total fenol dan aktivitas antioksidan menunjukkan adanya kontribusi dominan senyawa fenolik terhadap kapasitas penangkapan radikal pada ekstrak *T. decurrens*. Ekstrak *T. decurrens* dengan pelarut methanol menunjukkan kandungan fenolik dan potensi antioksidan yang relatif lebih tinggi dibandingkan pelarut lain. Temuan ini mengindikasikan peluang pemanfaatan ekstrak tersebut dalam aplikasi nutrasetikal atau pangan fungsional. Penelitian mendatang dapat mengeksplorasi isolasi senyawa fenolik spesifik, evaluasi in vivo, serta optimasi pelarut yang lebih luas.

Kata kunci: fenolik; fitokimia; makroalga; senyawa bioaktif; stres oksidatif

INTRODUCTION

Seaweeds are evolutionarily ancient, non-vascular photoautotrophs that lack true roots, stems, and leaves, which are characteristic of higher plants. Taxonomically, they are grouped into three major divisions based on their dominant photosynthetic pigments: Chlorophyta, which contains chlorophylls a and b; Phaeophyta, characterized by fucoxanthin as the primary accessory pigment; and Rhodophyta, distinguished by phycoerythrin and other phycobiliproteins. These pigment profiles not only define their classification but also influence their ecological distribution and light-harvesting adaptations in marine environments (Kang *et al*., 2014). There are many marine algae in the brown algae group (phylum Ochrophyta, class Phaeophyceae). The hue can be anything from yellow to dark brown, which is where the term is derived. Red seaweed (Rhodophyta) is the largest seaweed group. Finally, there are not as many green seaweeds (Chlorophyta) as there are brown and red seaweeds. The skin may be dark green or greenish yellow (Swamy, 2011).

According to the Ministry of Marine Affairs and Fisheries (KKP), Indonesia produced 10.80 million tons of seaweed in 2024. This is 10.82% higher than the previous year. By the end of October 2024, 18.26 million tons of fish and seaweed were produced, with 8.02 million tons from cumulative production. According to KKP statistics, cultivated species are the most common. *Kappaphycus alvarezii* was the most common, followed by *Gracilaria* spp. and *Eucheuma spinosum*. The targets are 11.65 million tons by 2025 and 14.01 million tons by 2029, respectively. At the moment, 175,967 hectares are being grown, which is 11.65% of the 1.51 million hectares that might be grown (Kementerian Kelautan dan Perikanan RI, 2026). After China, Indonesia is the second-largest producer of seaweed in the world. In 2021, it had a 12.3% share of the world's market, worth US$345 million (WHO, 2022). Basyuni et al. (2024) found that Indonesia has at least 325 different types of seaweed. There were 103 types of green seaweed (Chlorophyceae), 167 types of red seaweed (Rhodophyceae), and 55 types of brown seaweed (Phaeophyceae).

Aceh is one of the provinces in Indonesia with the potential to grow seaweed. Aceh is a province in Indonesia with a large land area of 57,956 km² and a long coastline of 2,817.90 km in length. This large ocean area is an excellent place to start looking for and developing seaweed resources. Aceh's coastal

environment is highly diverse, with different geomorphological features that directly affect seaweed distribution. More specifically, the eastern littoral zone is mostly composed of muddy substrates, whereas the western coastline is mostly composed of sandy and rocky substrates. Simeulue Island is one of the Aceh regions and has much more seaweed growth than other areas because many types of seaweed prefer sandy and rocky substrates. The differences in coastlines are a major factor in where seaweed can be grown and how much it can yield in the region. Brown seaweed *Turbinaria* sp. is one of the phaeophyceae that can be found in Aceh, especially on Simeulue Island.

Seaweed-derived bioactive compounds exhibit a broad spectrum of pharmacological properties in vitro and in animal models, including antioxidant, anti-inflammatory, antidiabetic, antihypertensive, lipid-lowering, neuroprotective, and anticancer activities. Among these, sulfated polysaccharides such as fucoidans from brown seaweed, porphyran from red seaweed, and ulvans from green seaweed along with phlorotannins predominantly found in brown seaweed, are particularly recognized for and have attracted considerable attention due to their immunomodulatory, antiviral, anticoagulant, and antimicrobial effects. However, robust clinical evidence substantiating these bioactivities in humans remains limited and warrants further investigation (Qin, 2020; Matos *et al*., 2024).

Major marine polysaccharides include alginates and fucoidans from brown seaweed, carrageenans and agarans from red seaweed, and ulvans from green seaweed. These compounds exhibit significant functional properties and serve as thickeners, gelling agents, and stabilizers in the food, pharmaceutical, and cosmetic industries. Beyond their technological applications, marine polysaccharides exhibit diverse bioactive properties in experimental models, including anti-inflammatory and gastroprotective effects, highlighting their dual role as functional ingredients and potential therapeutic agents (Choudhary *et al*., 2021). Brown seaweeds such as Ecklonia, Laminaria, and Undaria contain phenolic chemicals, particularly phlorotannins, which have substantial antioxidant, antibacterial, antiviral, antidiabetic, and anticancer activities. In addition, carotenoids (such as fucoxanthin), sterols, peptides, and unique lipids are additional bioactives that contribute to the reported metabolic, neuroprotective, and cardiometabolic benefits associated with brown seaweed consumption(Peñalver *et al*., 2020).

Antioxidants mitigate oxidative stress by inhibiting or retarding the oxidation reactions that produce reactive oxygen and nitrogen species (ROS/RNS), thereby protecting critical biomolecules, including lipids, proteins, and DNA, from oxidative damage. Their mechanisms of action are broadly categorized into two classes: (1) direct antioxidant activity, which involves scavenging free radicals and terminating chain propagation in oxidation reactions, and (2) indirect antioxidant effects, which involve the modulation of cellular redox signaling pathways, including the suppression of pro-oxidant enzymes and upregulation of endogenous antioxidant defense systems (Hunyadi, 2019; Chaudhary *et al*., 2023; Chandimali *et al*., 2025). Natural antioxidants found in seaweeds, primarily phenolic compounds, pigments, and sulfated polysaccharides, exhibit strong radical-scavenging capacity and the ability to modulate redox-sensitive signaling pathways, both in vitro and in vivo. Owing to their high concentrations of phlorotannins, carotenoids such as fucoxanthin, and sulfated polysaccharides such as fucoidan, brown seaweeds generally display stronger antioxidant activity than green and red seaweeds (Begum *et al*., 2021; Kumar *et al*., 2021; Khan *et al*., 2022).

Macroalgae have gained significant attention as potential marine resources with antioxidant properties. These organisms are categorized into three major phyla: Chlorophyceae, Rhodophyceae, and Phaeophyceae, based on their distinct photosynthetic pigment profiles (Anggadiredja *et al*., 2006). Brown seaweeds are rich in bioactive secondary metabolites,

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such as polyphenols and phlorotannins, which effectively neutralize free radicals via antioxidant mechanisms, contributing to their potential as natural sources of oxidative stress inhibitors (Hermund *et al*., 2016). *Turbinaria decurrens* is distributed species within a brown seaweed (Phaeophyceae) found in the waters surrounding Simeulue Island, Indonesia. Erniati *et al.* (2023a) reported that the brown seaweed (phaeophyceae) group has the most species, followed by the green seaweed (Chlorophyceae) and red seaweed (Rhodophyceae) groups, which are widely distributed in the waters off the west coast of Aceh. The measured water quality parameters show that the environment is good for seaweed growth. Brown seaweed is the most common type of seaweed on the west coast of Aceh. Taxonomically, the genus Turbinaria belongs to the family Sargassaceae, which is an ecologically significant group of marine macroalgae. *T. decurrens* is commonly found inhabiting rocky substrates and coral reef ecosystems, where it contributes to benthic structural complexity and enhances primary productivity, underscoring its ecological importance in tropical marine environments.

Turbinaria decurrens, a brown seaweed (Phaeophyceae) widely distributed across tropical and subtropical marine ecosystems, remains relatively underexplored, despite its potential as a source of bioactive compounds. Members of the genus Turbinaria are known to produce structurally diverse secondary metabolites, and *T. decurrens* has been reported to yield substances exhibiting antimicrobial, anti-inflammatory, antiviral, cardioprotective (Rushdi *et al*., 2021), antioxidant, and anti-diabetic properties (Ismail *et al*., 2020a). These findings underscore its pharmaceutical relevance and highlight the need for more comprehensive investigations, particularly focusing on its antioxidant potential and the characterization of its bioactive compounds.

Brown seaweed are known to contain wide range of bioactive compounds with notable antioxidant potential. One such species with distinctive bioactive constituents is *Turbinaria decurrens*. *T. decurrens* is a member of the phylum Phaeophyta and can grow and thrive in the coastal waters of Simeulue Island, Indonesia. Several seaweed species extracted from Aceh coastal waters have been previously reported, including *Sargassum* sp. and *Padina australis* (Gazali *et al*., 2018; Gazali *et al*., 2022), *Halimeda* sp. (Gazali *et al*., 2019a, 2019b; Gazali *et al*., 2023; Husni *et al*., 2024), *Chaetomorpha* sp. (Gazali *et al*., 2019; Gazali *et al*., 2020), *Caulerpa racemosa* (Gazali *et al*., 2022), *Boergesenia forbesii* (Gazali *et al*., 2023), and *Halimeda tuna* (Gazali *et al*., 2024) were also investigated. Collectively, these studies indicate that seaweeds from the Aceh coastal waters represent promising natural resources for functional materials, pharmaceuticals, nutraceuticals, and cosmetic applications.

However, there is currently a lack of scientific evidence regarding the antioxidant potential of brown seaweed *T. decurrens*, particularly in specimens collected from the Sinar Bahagia Coastal Waters, Simeulue Island, Indonesia. Differences in habitat environmental conditions and life cycle stages of seaweed can significantly influence the composition and concentration of bioactive compounds present. *T. decurrens* contains is known to contain bioactive constituents that may play an important role in antioxidant activity; however, comprehensive evaluation remains limited. Therefore, this study aimed to evaluate the antioxidant activity of extracts obtained from the brown seaweed *T. decurrens* collected from the Sinar Bahagia coastal waters, Simeulue Island. The findings of this study are expected to provide scientific evidence supporting the potential of *T. decurrens* as a natural antioxidant source, thereby contributing to further research and development in the field of safe biopharmaceuticals and related functional applications.

MATERIAL AND METHODS

Sample Collection and Identification

Brown seaweed *T. decurrens* were collected from the intertidal zone near Sinar Bahagia, West Simeulue Subdistrict, Simeulue Island, Aceh Province, Indonesia (2.4719622°S, 95.5856264°E) in October 2022. The samples were thoroughly rinsed with freshwater to eliminate adherent

particulate matter and extraneous debris. Subsequently, the rinsed specimens were air-dried under ambient laboratory conditions until a constant mass was achieved. Specimens were taxonomically identified using classical morphological criteria, with diagnostic features of the thallus, including vesicles, holdfast structure, blade morphology, primary and secondary branching patterns, stipe characteristics, and receptacle architecture systematically examined. Species identity was further validated by cross-referencing with authoritative taxonomic databases, namely AlgaeBase (www.algaebase.org) and the World Register of Marine Species (WoRMS), as well as peer-reviewed literature (Zainee *et al.*, 2018; Erniati *et al.*, 2023), ensuring accurate and current taxonomic assignment.

Extraction

Dried seaweed samples were manually fragmented into small pieces and subsequently homogenized into a fine powder using a stainless steel mechanical grinder. A total of 50 g of powdered biomass was subjected to maceration in 500 mL of analytical-grade methanol (Merck), ethyl acetate (Merck), and n-hexane (Merck) while maintaining a solid-to-solvent ratio of 1:10 (w/v) according to the method described by Andriani et al. (2019). The extraction was carried out in sealed containers under ambient conditions for a minimum of 72 h to ensure exhaustive compound solubilization. To ensure optimal and uniform extraction efficiency, the maceration mixture was manually agitated at regular intervals during the extraction period. The maceration process was performed in triplicate to maximize the compound recovery. The combined supernatants were subsequently concentrated under reduced pressure using a rotary vacuum evaporator (Heidolph WB 2000) at a controlled temperature of 40 °C, yielding an ethanolic crude extract. The resulting ethanolic crude extract was transferred to airtight containers and stored at 4 °C until further phytochemical and bioactivity analyses.

Phytochemical Assay

Phytochemical screening is a preliminary test used to qualitatively identify the presence of active compounds, such as alkaloids, flavonoids, hydroquinone phenols, steroids, triterpenoids, saponins, and tannins (Harborne, 1993).

Total Phenolic Concentration

The total phenolic content was measured according to the method described by Lawag *et al.* (2023), with modifications. A gallic acid standard solution was prepared by dissolving 5 mg of gallic acid in distilled water in a 25 mL volumetric flask. Standard solutions with concentrations of 10, 20, 30, 40, 50, 60, and 70 µg/mL were prepared from this stock solution. The total phenolic content assay was performed by dissolving 20 mg of each extract (methanol, ethyl acetate, and n-hexane extracts) separately in 25 mL volumetric flasks using their respective solvents, followed by homogenization using a shaker. Then, 0.5 mL of each extract solution was pipetted and mixed with 1 mL of 50% Folin-Ciocalteu reagent (FCR). The mixture was allowed to stand for 5 min, after which 1 mL of 5% Na_2CO_3 solution was added. The reaction mixture was then homogenized and incubated in the dark for 1 h. The absorbance was measured at a wavelength of 725 nm using a UV-Vis spectrophotometer (Spectrophotometer UV2500).

Antioxidant Assay

Antioxidant activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method at a concentration of 0.1 mM, following the modified method of Baliyan *et al.* (2022). Crude seaweed extracts were individually dissolved in n-hexane, ethyl acetate, and methanol to obtain concentrations of 10, 20, 30, 40, and 50 ppm, respectively. Subsequently, the mixture was vortexed and incubated at 37°C in a laboratory incubator (Lab Incubator Nesco DSI 300D) in the dark. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive control at concentrations of 1, 2, 3, 4 ppm, and 5 ppm. The percentage of DPPH free radical scavenging activity for each

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sample was determined using the following equation (Das *et al*., 2014):

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100$$

Where A is the absorbance of control and B is the absorbance of test sample.

Statistical Analysis

Data are presented as standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism software (version 10.2.3, GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) was used to assess the differences between groups. Following significant one-way ANOVA ($p<0.05$), Tukey's multiple comparisons test was applied as a post-hoc analysis to determine pairwise differences between groups, with significance set at $p<0.05$.

RESULT AND DISCUSSION

Turbinaria decurrens Morphology

Turbinaria decurrens is characterized by cylindrical, erect, and rough stipes (stems), which may exhibit limited branching. Its holdfast consists of a small, disc-shaped structure with radially arranged, root-like extensions spiraling around the main stipe (Figure 1). A key morphological feature is its leaf-like extensions (lamina), which are shaped like a triangular cone. Pneumatocysts (air bladders) are distinctively pyramidal. The receptacles (reproductive structures) are arranged in racemose clusters (Le Lann *et al*., 2008).

Turbinaria decurrens is a perennial brown seaweed found throughout the tropical Indo-Pacific region, including the coastal waters of Aceh, located at the northwestern tip of Sumatra in Indonesia. As a member of the family Sargassaceae within the class Phaeophyceae, this species is recognized for its unique morphology, ecological resilience, and significant contributions to reef-associated ecosystems. Within the diverse marine habitats of Aceh, including coral reefs and rocky substrates, *T. decurrens* exhibits notable morphological plasticity, habitat adaptability, and genetic variation. These characteristics make it a crucial component of the benthic marine biodiversity in the region.

Turbinaria decurrens is a brown seaweed with a tough, erect thallus comprising a central stipe and flattened, ruffled blades. These blades often appear cup-like or scrolled, particularly in younger fish. The thallus exhibits a leathery texture and olive-green to brown coloration, which results from high concentrations of fucoxanthin, the primary photosynthetic pigment found in brown seaweeds. In the ecologically diverse marine environment of Aceh, characterized by extensive coral reefs, rocky intertidal zones, and shallow subtidal platforms, *T. decurrens* is typically found in the mid-intertidal to shallow subtidal zones,

Figure 1 *Turbinaria decurrens* morphology

ranging from approximately 1 to15 meters. It attaches to hard substrates, such as coral rubble, dead coral skeletons, or rocky outcrops. The distribution and abundance of this species are strongly influenced by monsoon-driven currents and nutrient upwelling in this region.

Phytochemical Results

Sami & Nur (2022) reported that the crude extract of the brown seaweed *T. decurrens* contains a diverse array of phytochemicals, with notable levels of carotenoids, particularly fucoxanthin, along with phenolic compounds, flavonoids, alkaloids, saponins, tannins, steroids, and glycosides. The specific composition and relative abundance of these constituents are strongly influenced by the extraction methodology and type of solvent employed.

The methanol, ethyl acetate, and n-hexane extracts of *T. decurrens* exhibited distinct phytochemical compositions and associated biological activities, which can be attributed to differences in solvent polarity and extraction efficiency for specific compound classes. Alkaloids, steroids, triterpenoids, phenols, saponins, and flavonoids are moderately polar to polar phytochemicals that are commonly found in methanolic extracts. Methanol has a medium dielectric constant, moderately high polarity, protic character, and strong ability to form hydrogen bonds. This makes it possible to successfully solvate and extract these chemicals from plant matrices while keeping extremely non-polar lipids and waxes out (Sijabat *et al*., 2023). Ethyl acetate extracts exhibit substantial phenolic content surpassing that of methanolic and n-hexane extracts in certain studies, along with measurable flavonoid levels. These extracts demonstrated antioxidant activity comparable to or lower than that of methanolic extracts, as evidenced by moderate IC_{50} values that reflect robust intermediate radical scavenging capacity. Owing to its intermediate polarity, ethyl acetate is particularly effective in enriching a range of moderately polar to non-polar phytochemicals (Sami & Nur, 2022). The n-hexane extract of *T. decurrens* typically contains alkaloids, steroids, triterpenoids, and phenols but is generally deficient in or contains only low levels of flavonoids and saponins. The nonpolar nature of n-hexane renders it especially efficient for the extraction of lipophilic compounds, such as sterols, fatty acids, and triterpenes (Sijabat *et al*., 2023).

Antioxidant Activity Results

The results demonstrated that the methanolic extract exhibited antioxidant activity with an IC_{50} = 22.1±0.47 mg/mL, followed by ethyl acetate extract with IC_{50} = 36.7±2.52 mg/mL and n-hexane extract with IC_{50} = 46.68±1.79 mg/mL using ascorbic acid as the positive control. Among the tested extracts, the methanolic extract displayed the highest antioxidant activity, as indicated by the lowest IC_{50} value, compared to the ethyl acetate and n-hexane extracts. The antioxidant hierarchy observed among solvent extracts

Table 1 Specific band assignments of hydrochar at various temperatures

Metabolites	Extracts			Comparative study*	Results
	n-Hexane	Ethyl Acetate	Methanol		
Saponin	-	-	+	-	Foam
Flavonoid	-	+	+	+	Orange color
Tannin	-	-	-	+	Blue back color
Alkaloid	-	+	-	+	-
Phenolic	-	+	-	+	Orange/red color
Steroid	+	+	+	-	Dark green
Terpenoid	-	-	-	+	-

*: Handayani *et al*., 2024; + = detected, - = not detected

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provides basic insights into phytochemical partitioning and bioactivity. Polar solvents are effective in separating polar compounds, such as phenolics and flavonoids, which are water-loving antioxidants. Moderately polar parts capture semi-polar molecules that work well together.

This makes them balanced but less powerful than the other models. Non-polar extracts, on the other hand, look for lipophilic substances that do not have much redox capacity. This pattern shows how solvents and extractants work together: solvents with high polarity make it easier to dissolve polyphenols that release hydrogen. This makes DPPH decolorization or ABTS cation quenching more effective. Different solubilities change the profiles of the extracts. This is why polar fractions are better than transitional ones, which are more dispersed. Seaweed extracts often exhibit these tendencies. More polar antioxidants lower oxidative stress by neutralizing reactive oxygen species (ROS). Consistent with these findings, aRecent study by Newehy *et al.* (2025) reported that the brown seaweed T. decurrens exhibits antioxidant activity, with different solvent extracts showing varying levels of efficacy depending on their phenolic and flavonoid content.

These results demonstrated that the methanolic extract possessed higher antioxidant activity with an IC_{50} value of 22.1±0.47 ppm than the ethyl acetate extract with an IC_{50} value of 36.7±2.52 ppm, followed by the n-hexane extract with an IC_{50} value of 46.68±1.79 ppm. The high polarity of methanol selectively extracts hydrophilic antioxidants because of the principle of like dissolves like. This results in higher bioactive yields than semi-polar ethyl acetate or non-polar n-hexane. In seaweed studies, this means that DPPH is stronger and IC_{50} values are lower. Methanolic extracts are the most effective at fighting free radicals because they dissolve polar phenolics and phlorotannins from brown seaweeds *Turbinaria decurrens* better than ethyl acetate and n-hexane extracts. This is directly linked to the study of solvent-based IC_{50} gradients. The DPPH method measures the efficacy of a substance as an antioxidant, and the IC_{50} value indicates its effectiveness (Sukweenadhi *et al.*, 2020). There are five groups of antioxidant activity based on the IC_{50} value: very strong (IC_{50} value <50 ppm), strong (IC_{50} value 50-100 ppm), medium (IC_{50} value 100-150 ppm), weak (IC_{50} value 150-200 ppm), and very weak (IC_{50} value > 200 ppm) (Molyneux, 2004). This study found that the methanolic extract had greater antioxidant activity than the ethyl acetate and n-hexane extracts.

The antioxidant potential of *T. decurrens* has been evaluated using well-established in vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS radical scavenging activities, total antioxidant capacity, and FRAP (Ferric Reducing Antioxidant Power) assays (Kataria *et al.*, 2025). Methanol, acetone, and aqueous extracts of *T. decurrens* consistently exhibit notable free radical scavenging activity; however, the acetone extract has been reported to demonstrate the highest antioxidant capacity among these solvents, with IC_{50} values as low as 1.06 mg/mL in the ABTS assay and 1.56 mg/mL in the DPPH assay, as well as a total antioxidant capacity of up to 4.3 mg ascorbic

Table 2 Antioxidant activity of Brown seaweed *T. decurrens*

Extracts	Antioxidant activity (ppm)	Comparative study (µg/mL)
Methanol	22.1±0.47[a]	340.06
Ethyl acetate	36.7±2.52[b]	180.54
n-Hexane	46.68±1.79[c]	502.25
Ascorbic acid	2.5±0.34[a]	1.72

Each value is presented as mean ± SD across the three treatments. Values marked with different letters (a-c) denote a significant difference between treatments at $p<0.05$, as determined by HSD-Tukey analysis.

acid equivalents per gram of dry extract. Furthermore, Methanol, acetone, and aqueous extracts of *T. decurrens* exhibited notable free radical scavenging activity; however, the acetone extract consistently demonstrated the highest antioxidant capacity among these solvents, with reported IC_{50} values as low as 1.06 mg/mL in the ABTS assay and 1.56 mg/mL in the DPPH assay, along with a total antioxidant capacity of up to 4.3 mg ascorbic acid equivalents per gram of dry extract (Ismail *et al*., 2020b).

Sami *et al.* (2019) have reported that the antioxidant properties of *T. decurrens* extracts exhibit a strong positive correlation with their phenolic and flavonoid contents, which are particularly enriched in acetone and methanol extracts due to the favorable polarity of these solvents for the efficient extraction such secondary metabolites .Kataria *et al.* (2025) also stated that The DPPH test showed that the methanolic extract of *T. decurrens* had antioxidant activity with an IC_{50} value of 1.56 mg/mL.

Total Phenol Content Results

Based on the results, the methanolic extract exhibited the highest total phenolic content, with a value of 2.43 mg GAE/g. The ethyl acetate extract showed a total phenolic content of 1.50 mg GAE/g, whereas the n-hexane extract showed the lowest total phenolic content of 0.73 mg GAE/g (Figure 2). The total phenolic content (TPC) results showed that *T. decurrens* extracts have a clear order dependent on the solvent used: methanol (2.43 mg GAE/g) > ethyl acetate (1.50 mg GAE/g) > n-hexane (0.73 mg GAE/g). This pattern is similar to the trend in antioxidant activity (IC_{50}: methanol 22.1±0.47 mg/mL < ethyl acetate 36.7±2.52 mg/mL < n-hexane 46.68±1.79 mg/mL). This implies a strong link between the number of phenolic compounds and their radical-scavenging ability. The high levels of phenolics in these extracts enable them to scavenge radicals. This is evidenced by the strong negative relationship between TPC (mg GAE/g) and DPPH IC_{50} values. The chemical basis for this interaction is that phenolic hydroxyl groups can donate hydrogen atoms to stabilize the DPPH radical.

There is a close link between TPC and antioxidants in terms of their action. Phenolics donate electrons and protons to DPPH and ABTS radicals, rendering them neutral. Ortho-dihydroxy structures stabilize phenolics via resonance delocalization. Statistical correlations (r>0.89) among seaweeds confirmed that TPC predicts IC_{50}, as a higher phenolic density increases

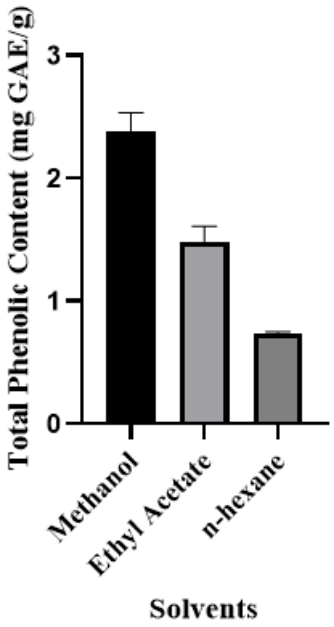

Figure 2 Total phenol content of brown seaweed *Turbinaria decurrens*

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capacity. The ~ 3.3-fold TPC edge of methanol over n-hexane explains its ~2.1-fold IC_{50} superiority, indicating a linear dose-response relationship (Pearson's r = 0.95 based on trends) (Lestario *et al.*, 2008). Furthermore, *the TPC of T. decurrens* is mild at 1.5–2.4 mg GAE/g, whereas methanol extracts of *T. ornata* can reach 3–5 mg GAE/g, and acetone fractions of *Gracilaria* sp can reach 45 mg/g. In ethanolic gradients, methanol/ethanol produced 9–12 mg GAE/g. Polar solvents are more effective (Sakthivel *et al.*, 2025).

In line with this finding, Sami *et al.* (2021) reported that the ethyl acetate extract of *T. decurrens* exhibited the highest total phenolic content (TPC), quantified at 4.8091 mg gallic acid equivalents (GAE) per gram of extract. This value was significantly higher than those obtained from the methanol and n-hexane extracts of the same algal material, underscoring the superior efficacy of semi-polar solvents, such as ethyl acetate, in extracting phenolic compounds from this species. Furthermore, the elevated phenolic content in the ethyl acetate extract showed a strong positive correlation with enhanced antioxidant activity, as evidenced by a DPPH radical scavenging IC_{50} value of 180.54 µg/mL. Chakraborty *et al.* (2013) stated that the observed positive correlation between extract composition and antioxidant capacity in *Turbinaria* sp. reflects a solvent polarity governs the selective partitioning of structurally distinct antioxidant classes, each contributing to radical scavenging through complementary mechanisms.

There was a positive relationship between the antioxidant activities of the methanolic and n-hexane extracts of *Turbinaria* sp. The higher the phenolic content or bioactivity in these fractions, the better they were at scavenging in all tests. This shows that phenolics/phlorotannins (methanol) and lipophilic antioxidants (n-hexane) are separated in different solvents. Methanol, a polar protic solvent with a high hydrogen-bonding capacity and dielectric constant ($\varepsilon \approx 33$), efficiently solubilizes hydrophilic phenolic secondary metabolites, most notably phlorotannin, which are unique polymeric phenolics. These macromolecules, composed of phloroglucinol (1,3,5-trihydroxybenzene) units linked via C–C or C–O–C bonds, possess dense arrays of electron-donating hydroxyl groups that facilitate potent radical neutralization via hydrogen atom transfer (HAT) and single-electron transfer (SET) pathways. Consequently, methanolic extracts typically exhibit a high total phenolic content (TPC) and strong activity in polar-phase assays, such as DPPH and $ABTS^+$ scavenging, where solubility and molecular accessibility are critical.

Correlation of Total Phenol Content and Antioxidant Activity

The correlation between total phenolic content and antioxidant activity, assessed using the DPPH method as a measure of free radical scavenging activity, was analyzed using linear regression to determine the strength of the relationship between total phenolics and antioxidant activity, as indicated by the IC_{50} value. Numerous studies have reported statistically significant correlations between the total phenolic content and antioxidant activity in *T. decurrens.* Notably, a very strong positive correlation ($R^2 = 0.945$) was observed between the phenolic content and DPPH radical-scavenging activity.

Phytochemical assays demonstrated that the phenolic and flavonoid compounds in *T. decurrens* have hydroxylated aromatic structures, which facilitate the movement of hydrogen atoms from one molecule to another. The Folin-Ciocalteu assay quantifies the number of redox-active phytochemicals, and the DPPH assay measures their ability to scavenge radicals. The strong connection between them shows that they both depend on the same molecular property: the ability of phenolic -OH groups to give up hydrogen atoms and stabilize the radicals that form through resonance. This structure-function relationship supports the use of TPC as a predictive marker of antioxidant potential in phytochemical studies of marine macroalgae.

This strong association provides compelling evidence that phenolic compounds are the primary bioactive constituents responsible for the antioxidant potential of *T. decurrens*. These results indicate

that approximately 95% of the variation in antioxidant activity can be explained by the contribution of phenolic compounds, while the remaining 5% may be attributed to other variables, likely associated with non-phenolic compounds that also possess antioxidant properties. Therefore, the presence of phenolic constituents is considered to contribute substantially to the overall antioxidant effect.

A strong negative linear relationship was observed between the antioxidant activity (IC_{50}) and total phenolic content ($R^2 = 0.9457$, slope = -0.2140). A direct and scientifically supported correlation exists between total phenol content (TPC) and antioxidant activity in the brown seaweed *T. decurrens*, wherein elevated phenolic concentrations correspond to enhanced antioxidant efficacy. Gazali *et al.* (2018) reported a positive correlation between total phenol content and antioxidant activity in the brown seaweed Sargassum sp., with $R^2 = 0.997$.This relationship is attributable to the presence of distinct functional groups characteristic of phenolic compounds, which can be identified and verified through Fourier-transform infrared (FTIR) spectroscopy and complementary spectroscopic analyses (Kumaran *et al*., 2024). Rafi *et al*. (2012) reported that phenolic compounds, as secondary metabolites in plants, possess significant antioxidant potential. This activity is attributed to the presence of hydroxyl (-OH) groups in their molecular structure. These hydroxyl groups can function as hydrogen atom donors when reacting with free radical species via an electron transfer mechanism, thereby interrupting radical chain reactions and inhibiting oxidation.

Sami *et al.* (2019) reported that numerous studies consistently showed that extracts with the highest total phenolic content (TPC), particularly those obtained using ethyl acetate as the extraction solvent, demonstrated the most potent antioxidant activity, as evidenced by the lowest DPPH radical scavenging IC_{50} values. For instance, the ethyl acetate extract of *T. decurrens* yielded a TPC of 4.8091 mg gallic acid equivalents (GAE) per gram of extract and a DPPH IC_{50} value of 180.54 µg/mL, reflecting its superior antioxidant efficacy. de Quirós *et al*. (2010) stated that phenolic compounds exhibit antioxidant activity and are known as phenolic acids found in seaweed, including catechin, epicatechin, EGCG, quercetin, among others. The relationship between the total phenolic content and antioxidant activity of T. decurrens extracts, as determined using the DPPH method, is presented in Figure 3. El-Shenody *et al.* (2019) stated that antioxidant

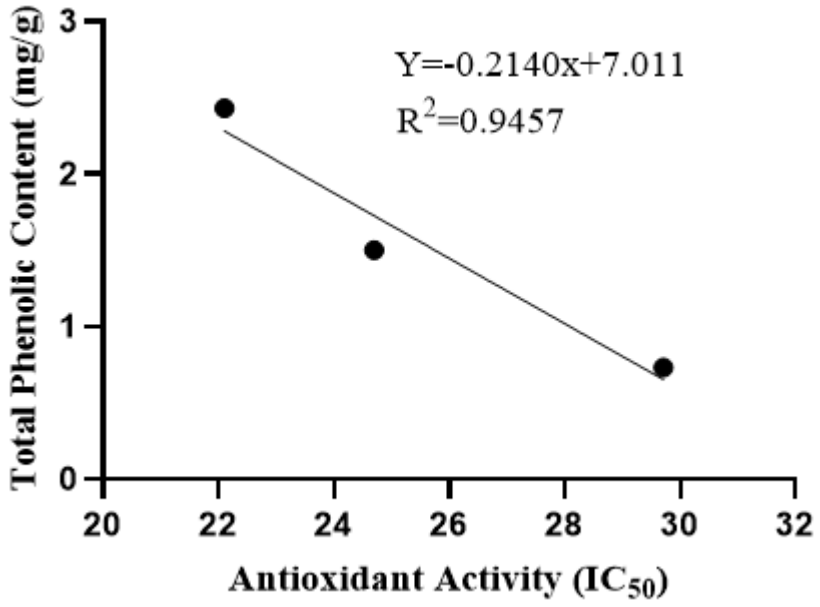

Figure 3 Correlation between total phenol content and antioxidant activity

activity exhibits a direct and inverse relationship with the IC_{50} value in the context of total phenolic content (TPC): specifically, higher concentrations of phenolic compounds in an extract correspond to lower IC_{50} values in DPPH radical scavenging assays.

This inverse correlation signifies that as the TPC increases, a smaller concentration of the extract is required to scavenge 50% of the free radicals, thereby reflecting enhanced antioxidant potency. Consequently, phenolic-rich extracts demonstrate greater efficacy in neutralizing oxidative species, underscoring the pivotal role of phenolic compounds as primary contributors to overall antioxidant capacity. Different solvents have different polarities: n-hexane is nonpolar, ethyl acetate is semipolar, and methanol is polar. Methanol and ethyl acetate are polar solvents that typically extract more hydrophilic phenolics, such as phenolic acids and flavonoids. This results in a higher TPC. In contrast, n-hexane prefers lipophilic molecules. Because ethyl acetate is an intermediate polar substance, it often maximizes the TPC by matching the solubility of numerous phenolics (Abu *et al*., 2017; Khiya *et al*., 2021).

CONCLUSION

Quantitative antioxidant assays consistently demonstrated that methanol extracts possessed the lowest IC_{50} values, indicating the highest antioxidant potency, which strongly correlated with their elevated total phenolic content. Methanolic extraction effectively concentrates phenolic compounds from *T. decurrens*, which are the primary contributors to its radical-scavenging activity and have a positive correlation with the total phenolic content. These findings provide a scientific basis for developing optimized, eco-friendly extraction protocols aimed at maximizing phenolic yield. The scalable production of bioactive methanolic extracts holds significant promise for applications in the nutraceutical and pharmaceutical industries. Future research should focus on the isolation and characterization of specific phenolic compounds responsible for antioxidant activity and the validation of these effects through in vivo studies.

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