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# Bioactive compounds and DPPH antioxidant activity of underutilized macroalgae (*Sargassum* spp.) from coastal water of Makassar, Indonesia

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**Abstract.** *Widyaswari SG, Metusalach, Kasmiati, Amir N. 2024. Bioactive compounds and DPPH antioxidant activity of underutilized macroalgae* (Sargassum *spp.*) *from coastal water of Makassar, Indonesia. Biodiversitas 25: 162-168.* This study focuses on determining the chemical component and potential of two species of macroalgae (seaweed) *Sargassum* spp., i.e., *Sargassum polycystum* and *Sargassum ilicifolium* from the coastal waters of Makassar City, South Sulawesi, Indonesia. Several macroalgae are highly cultivated and exported; however, these species of *Sargassum* remain underutilized due to limited studies on their potential. Brown algae (Phaeophyceae), especially *Sargassum* spp., contain secondary metabolites known for their health-promoting properties, including antioxidant effects, inhibiting oxidative stress, lowering blood pressure, and improving the immune system. The investigation analyzes the nutritional components and bioactive substances in *Sargassum* spp. harvested from the Makassar City coastal waters, South Sulawesi, Indonesia. Nutritional content was analyzed using proximate analysis. Phytochemical screening was conducted based on the method by Harborne method. Antioxidant activity was performed using the DPPH method. Total phenolic/flavonoid content based on spectrophotometric method. The investigation reveals the primary chemical composition of *Sargassum polycystum* showing the presence of valuable secondary metabolites such as alkaloids, flavonoids, phenol hydroquinone, and reactive saponins. *Sargassum ilicifolium* contains flavonoids, phenol hydroquinone, tannins, and steroids. The $IC_{50}$ values for the antioxidant activity of *S. polycystum* and *S. ilicifolium* were 51.34 ppm and 51.25 ppm, respectively. The compounds in these seaweeds might be beneficial in promoting health. These findings suggest the possibility of underutilized seaweeds as a natural source of antioxidants in food and non-food industries.

**Keywords:** Brown algae, chemical compounds, natural antioxidant, seaweed, secondary metabolites

## INTRODUCTION

The Indonesian Archipelago possesses many seaweed resources (Pambudi et al. 2010; Rimmer et al. 2021). Seaweed, also known as macroalgae, has been known for its potential benefits in the food and non-food industries (Mabeau and Fleurence 1993; Vijayabaskar and Shiyamala 2011; Tiwari and Troy 2015; Zang et al. 2020), including cosmetics (Lourenço-Lopes et al. 2020) and nutraceuticals (Shannon and Abu-Ghannam 2019). Macroalgae are an abundant source of marine biodiversity, with more than 700 species identified in Indonesia (Weber-van Bosse 1913, 1921). Some macroalgae species are cultivated and exported, such as *Kappaphycus* sp. (Valderrama et al. 2015), *Gracilaria* sp. (Nivedita and Raghunathan 2016), *Caulerpa* sp. (de Gaillande et al. 2017), *Ulva* sp. (Chemodanov et al. 2019), and *Eucheuma* sp. (Nainggolan et al. 2022). However, some other species remain underutilized due to insufficient scientific studies on their potential benefits (Moreira et al. 2022).

Certain groups of macroalgae haven't been used optimally because there haven't been sufficient scientific studies (Gazali et al. 2018; Rimmer et al. 2021). On the other hand, extracts of brown algae have been known to contain secondary metabolites that are beneficial for health (Perumal et al. 2019; Puspita et al. 2020; Damayanti et al. 2021), including antioxidant activity that can inhibit oxidative stress (Budhiyanti et al. 2012) which can protect against degenerative diseases (Gazali et al. 2018), lowering blood pressure (Kosanić et al. 2019; Park et al. 2023), boosting the immune system (Torres et al. 2019). Some brown algae, such as *Padina*, *Turbinaria*, and *Sargassum*, have been found to have a relatively high content of beneficial compounds (Ponnan et al. 2017; Kumar et al. 2019; Jasmadi et al. 2022).

*Sargassum* spp. thrive in subtidal and intertidal zones, showing ecological dynamics influenced by water temperature, tidal levels, water movement, and substrate type (Afonso et al. 2019; Yip et al. 2020). These microalgae mainly attach to dead corals in shallow waters, highly exposed to ultraviolet radiation, particularly in the coastal areas of Makassar City, where they dominate the marine landscape (Mushlihah et al. 2021). The waters of the Spermonde Archipelago in South Sulawesi are impressively wealthy, hosting 199 identified macroalgae species (Verheij and Prud`homme van Reine 1993). Despite this richness, the *Sargassum* species is still underutilized. Their life cycle was marked by detachment from substrates and subsequent transport by waves to coastal shorelines, accumulating substantial floating biomass (Sissini et al. 2017; Fidai et al. 2020; Robledo et al. 2021; Zhuang et al. 2021; Harb and Chow 2022). Attention to the potential of these species is still very limited, while interest and use of certain species of red and green macroalgae is increasing.

The underutilization of *Sargassum* could be attributed to ecological characteristics, such as exposure to ultraviolet radiation and vulnerability to wave-induced stranding. Meanwhile, red algae, especially species of *Porphyra* and *Gracilaria*, have been extensively studied and cultivated for their valuable agar and carrageenan content (Usov 1998; Bhatia et al. 2015; Liu et al. 2019; Gomes-Dias et al. 2023). Similarly, green algae, such as *Ulva* and *Enteromorpha,* have been used in biofuel production and wastewater treatment (Sode et al. 2013; Suganya et al. 2013; Bikker et al. 2016; Fouda et al. 2022). Commercial success and established cultivation practices of certain macroalgae species may overshadow the potential of underutilized varieties, such as *Sargassum.* The economic feasibility of extensively studied macroalgae may unintentionally lower the interest in exploring the potential of underutilized macroalgae.

Hence, it is crucial to determine the potential of underutilized wild macroalgae by analyzing their nutritional components and bioactive compounds. The aim of this study was to determine the nutritional components and bioactive compounds contained in *Sargassum polycystum* and *Sargassum ilicifolium* collected from the coastal waters of Makassar City, Indonesia, as well as their antioxidant activity.

## MATERIALS AND METHODS

### Study area and materials

Samples of *Sargassum polycystum* C.Agardh, 1824 and *Sargassum ilicifolium* (Turner) C.Agardh, 1820 were collected in April 2023 by hand-picking random sampling at the Gusung Tallang Island (5°7'19.19"S, 119°23'43.91"E), South Sulawesi, Indonesia. After collection, fresh samples were placed into plastic bags filled with ice and transported to the laboratory immediately.

Equipment used in this study includes Spectrophotometer, moisture analyzer, crucibles, a muffle furnace, a distiller, a Kjeldahl apparatus, a Soxhlet extractor, and a Buchner funnel with filter papers, centrifuge, rotary vacuum evaporator, various glassware, and micropipettes.

### Proximate analysis

The sample was cleaned from debris, washed with distilled water to remove impurities and attached organisms, and dried under the sunlight (27-32°C) for approximately 48 hours. The dried sample was cut into pieces and ground into a dry powder using a chopper. Fifty g of dried *Sargassum* powder was placed into an Erlenmeyer flask and macerated with ethanol, ethyl acetate, and hexane. The samples were macerated in each solvent for 2 × 24 hours to extract bioactive compounds in the *Sargassum* powder and then filtered using filter paper. The filtrate was concentrated with an evaporator.

The extract was analyzed for ash, water, carbohydrates, proteins, and fat contents following the AOAC Methods (Latimer 2023).

### Phytochemical screening

The *Sargassum* spp. extracts underwent qualitative analysis for active compounds, including alkaloids, flavonoids, hydroquinone phenols, steroids, triterpenoids, saponins, and tannins based on the Harborne Method (Harborne 1998).

### Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) analysis

The quantitative analysis for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in *Sargassum* spp. extracts were determined by the spectrophotometric method (Saeed et al. 2012). Gallic acid was used as the standard, with concentrations ranging from 0.1 to 1.0 mg/mL), then mixed with Folin-Ciocalteu reagent for TPC analysis. Following a dark incubation period of 30 minutes, sodium carbonate solution was added, and the reaction mixture was incubated for an additional 30 minutes at 25°C. Absorbance was measured at 765 nm, and the resulting data were used to construct a calibration curve and then to calculate TPC in extracts, expressed as mg Gallic Acid Equivalents (GAE) per gram extract. For TFC determination, rutin standards were similarly prepared and mixed with an aluminum chloride solution (2% to 5%), followed by a dark incubation period of 40 minutes. Absorbance was measured at 415 nm, and a calibration curve using rutin standards was used to calculate TFC in extracts, expressed as mg Rutin Equivalents (RE) per gram. The analysis was carried out in triplicate.

### DPPH assay for antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay for antioxidant activity follows the method by Brand-Williams et al. (1995). A fresh DPPH solution was prepared in ethanol. If necessary, the test sample or extract at concentrations ranging from 0.1 to 1.0 mg/mL was dissolved in an appropriate solvent. The DPPH solution and the sample were mixed in a 1:1 ratio and incubated in the dark for 30 to 60 minutes at room temperature. After incubation, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. A higher percentage of DPPH radical scavenging activity indicated greater antioxidant activity. Ethanol as a solvent was used as a blank or negative control. For validation, a positive control containing quercetin was included in the assay. This positive control helped confirm the assay's sensitivity to detect antioxidant properties and provided a reliable reference for comparing the antioxidant activity of the test sample.

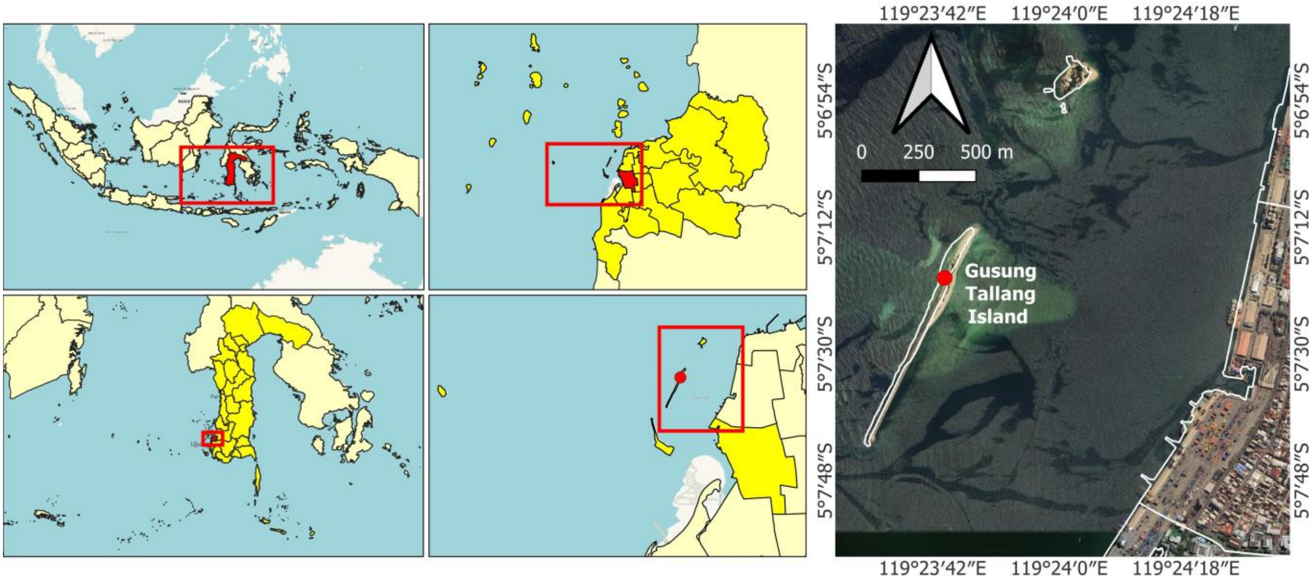


**Figure 1.** The Sampling site is coastal water near Makassar City, South Sulawesi, Indonesia. The red dot indicates the sampling site at Gusung Tallang Island (5° 7'19.19"S, 119°23'43.91"E)

### Data analysis

Descriptive data analysis was done using Microsoft Excel. Results are presented as means with standard deviations. This comprehensive approach helped us thoroughly interpret and present both qualitative and quantitative results from phytochemical screening and the DPPH assay. Additionally, Pearson correlation analysis and Two-way ANOVA, using PAST 4.07b, were employed to determine correlations and differences between Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and $IC_{50}$ values for antioxidant activity.

## RESULTS AND DISCUSSION

### Proximate analysis

The nutritional composition of two *Sargassum* species, i.e., *S. polycystum* and *S. ilicifolium*, is presented in Table 1. *S. ilicifolium* has a slightly higher water content (9.43%) than *S. polycystum* (8.80%), while *S. polycystum* has a higher fiber content (16.20%) than *S. ilicifolium* (12.80%). However, both species have 1.30% fat content. Water content in both species is below 10%, indicating optimal stability and a reduced risk of microbial growth. Monitoring water content is crucial for assessing self-life and determining the best storage methods to prevent microorganism growth, such as bacteria, small insects, and molds (Mannheim et al. 1994; Tun Norbrillinda et al. 2016; Obluchinskaya and Daurtseva 2020).

*Sargassum polycystum* has a protein content of 9.51%, while *S. ilicifolium* has 8.55%, indicating a slight difference in protein levels. Protein content in food significantly determines the quality of the food material (Friedman 1996). The crude fiber content of *S. polycystum* reached 16.20%, while *S. ilicifolium* was 12.80%. Based on the dietary guidelines of 28 g/day for adult women and 34 g/day for adult men, a daily energy intake of 2000 kcal and 2400 kcal, respectively (US Department of Agriculture (USDA) and US Department of Health and Human Services 2020), the crude fiber content of the studied macroalgae were fall within the recommended range. Dietary fiber positively affects digestion, heart disease risk, and blood sugar regulation (Nurjanah et al. 2016; Hitoe and Shimoda 2017; He et al. 2022). These results emphasize the beneficial role of crude fiber in promoting digestive health and overall well-being. The use of crude fiber should consider overall diet and individual health conditions.

### Bioactive compounds

The qualitative analysis of bioactive compounds in *S. polycystum* and *S. ilicifolium* was based on color changes or precipitates formed after adding reagents (Rengasamy et al. 2020). These bioactive substances include alkaloids, flavonoids, saponins, tannins, hydroquinone phenols, and steroids/triterpenoids (Table 2). Understanding the composition of these bioactive substances is valuable for predicting their potential benefits for humans.

The *S. polycystum* contains flavonoids, hydroquinone phenol, and saponins. On the other hand, *S. ilicifolium* contains flavonoids, phenol hydroquinone, tannins, and steroids (Table 2). There are some differences in the bioactive substances in both species, even though they were collected from the same location and time. Besides species differences, the variation may be due to the age of the macroalgae and the depth of its habitat. According to previous studies, the chemical composition of macroalgae is influenced not only by species but also by factors such as maturity, reproductive type, environment, and seasons (Kumar et al. 2021; Miguel et al. 2023). In our case, the sampling was done close to the shoreline, about two meters in depth, and the macroalgae, including its roots, were collected. In particular, the leaves of *S. polycystum* (from our samples) were not dense, and the stems were soft, indicating it might be in the early growth stage.

Moreover, *S. ilicifolium* grows more densely, with broader and thicker leaves than *S. polycystum*. Light exposure and nutrients significantly influence each species' growth and chemical composition (Cronin and Hay 1996), leading to differences in bioactive substances. Even within the same species, variations in the content of bioactive compounds may occur. These variations can be due to differences in the species' age during harvest, growth conditions, and varying nutrient absorption.

Alkaloids were detected in *S. polycystum*. Alkaloids have activity as antibiotics (Chuah et al. 2016) and anti-inflammatory agents (de Souza et al. 2009), reducing pain, improving blood circulation, restoring stamina after childbirth, and preventing infections in the uterus (Zheng et al. 2017; Poole et al. 2018; Zhu et al. 2020; Sellami et al. 2022). Phenolic compounds were detected in both species. Phenolics possess anti-inflammatory properties, act as antioxidants, and contribute to carcinogen detoxification and anti-cholesterol (Michalak and Chojnacka 2015; Ravichanthiran et al. 2018; Torres et al. 2019). Saponin was detected only in *S. polycystum*. It has the potential to be an anti-inflammatory and help heal wounds. Previous studies have demonstrated that *Sargassum* spp. have antimicrobial activity (Susilowati et al. 2015; El Shafay et al. 2016; Telles et al. 2018; Abu-Khudir et al. 2021) to prevent pathogenic bacterial infection and reduce inflammation.

**Antioxidant activity**

Antioxidants prevent free radical autoxidation reactions in lipid oxidation (Pangestuti and Kim 2015). Antioxidant activity could be evaluated using free radical DPPH to measure radical scavenging ability by donating electrons to DPPH radicals (Table 3). The secondary metabolite analysis of both *Sargassum* species showed the presence of phenolic compounds, alkaloids, phenol hydroquinone, saponin, flavonoids, and tannins (Table 2). Phenolic compounds, distinguished by O-H bonds, showed increased antioxidant activity due to their lower dissociation bond than other groups, facilitating efficient hydrogen release and donation (Bendary et al. 2013).

The results of the antioxidant activity showed that the $IC_{50}$ of *S. polycystum* and *S. ilicifolium* were 51.34 and 51.25 ppm, respectively. These results suggest that both *Sargassum* species have the potential to serve as potential antioxidants (less than 100 ppm) (Table 3). Antioxidant activity was categorized based on the $IC_{50}$ values obtained through the DPPH assay. A sample with an $IC_{50}$ value below 100 ppm is considered a potent antioxidant (Nurhasnawati et al. 2019). The $IC_{50}$ value reflects the concentration required to scavenge 50% DPPH radicals. The result of this study was consistent with previous research on antioxidants in *Sargassum* sp. The $IC_{50}$ values of *Sargassum* spp. from the coastal area of Gunung Kidul were 65.28 to 69.27 ppm (Lailatussifa et al. 2017; Sedjati et al. 2018). $IC_{50}$ of *Sargassum* from the coastal area of West Aceh was 68.89 ppm (Gazali et al. 2018). The results showed that *Sargassum* spp. may have the potential to be an antioxidant.

**Table 1.** Nutritional components of *Sargassum polycystum* and *Sargassum ilicifolium* from coastal water of Makassar City, South Sulawesi, Indonesia

| Parameters | *S. polycystum* | *S. ilicifolium* |
|---|---|---|
| Water | 8.80±0.63 | 9.43±0.29 |
| Ash | 28.07±0.22 | 38.07±0.22 |
| Protein | 9.51±0.06 | 8.55±0.04 |
| Fat | 1.30±0.05 | 1.30±0.05 |
| Crude fiber | 16.20±0.52 | 12.80±0.06 |
| Carbohydrates | 52.31±0.85 | 42.64±0.13 |

**Table 2.** Phytochemical assay of *Sargassum polycystum* and *Sargassum ilicifolium* from coastal water of Makassar City, South Sulawesi, Indonesia

| Secondary metabolite | *S. polycystum* | *S. ilicifolium* | Positive test result |
|---|---|---|---|
| Alkaloid | | | |
| Mayer | - | - | White precipitate |
| Wagner | - | - | Brown precipitate |
| Dragendroff | + | - | Orange precipitate |
| Flavonoids | + | + | Yellowish |
| Phenol hydroquinone | + | + | Yellowish brown |
| Saponin | + | - | Stable foam |
| Tanin | - | + | White precipitate |
| Steroids | - | + | Yellowish brown |
| Triterpenoids | - | - | Yellowish brown |

**Table 3.** Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and $IC_{50}$ value of *Sargassum* spp. from various locations

| Species | Sampling | TPC (mg GAE/g) | TFC (mg RE/g) | IC50 (ppm) | Sources |
|---|---|---|---|---|---|
| *Sargassum* sp. | Gunung Kidul | 57.97 | - | 69.27 | (Sedjati et al. 2018) |
| *Sargassum* sp. | West Aceh | 563.22 | - | 68.89 | (Gazali et al. 2018) |
| *Sargassum hystrix* J.Agardh 1847 | Gunung Kidul | 114.26 | - | 65.28 | (Lailatussifa et al. 2017) |
| *Sargassum polycystum* | Pohuwato waters | 713.00 | - | 77.38 | (Manteu et al. 2018) |
| *Sargassum muticum* (Yendo) Fensholt 1955 | Bangladesh | 66.52 | 68.11 | 40.74 | (Afrin et al. 2023) |
| *Sargassum polycystum* | Makassar | 365.96 | 93.75 | 51.34 | *This study* |
| *Sargassum ilicifolium* | Makassar | 382.94 | 92.99 | 51.25 | *This study* |

The Total Phenolic Content (TPC) of *S. polycystum* and *S. ilicifolium* were 365.96 and 382.94 mg GAE/g extract, respectively. Phenolic compounds, including subclasses flavonoids and phenol hydroquinone, are known for their antioxidant capabilities. These compounds play a pivotal role in neutralizing free radicals, contributing significantly to the antioxidant potential of the sample. The increased total phenol content showed an increased antioxidant capacity, in line with the $IC_{50}$ values through the DPPH assay. The Total Flavonoid Content (TFC) of *S. polycystum* was 93.75 mg RE/g, and *S. ilicifolium* was 92.99 mg RE/g for *S. ilicifolium*. Based on the results of Table S1, TFC has a higher correlation with the $IC_{50}$ value than TPC. Table S2. showed that the TPC, TFS, and IC50 values of *S. polycystum* and *S. ilicifolium* were not significantly different.

**Table S1.** The Pearson Correlation (Linear) matrix and plot of TPC, TCF, and IC50 value.

| | A (TPC) | B (TFC) | C (IC50) |
|---|---|---|---|
| A(TPC) | | 0.046963 | 0.035079 |
| B (TFC) | 0.99728 | | 0.011883 |
| C (IC50) | 0.99848 | 0.99983 | |

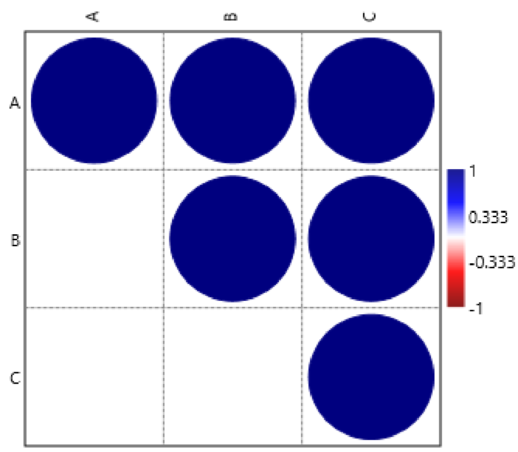


**Table S2.A.** Two-way ANOVA (without replication) analysis between Total Phenolic Content (TPC) and $IC_{50}$ value

| | Sum of squares | df | Mean square | F | p (same) |
|---|---|---|---|---|---|
| Rows (between samples) | 162647 | 5 | 32529.5 | 1.044 | **0.4816** |
| Columns | 274198 | 1 | 274198 | 8.802 | 0.03127 |
| Error | 155755 | 5 | 31150.9 | | |
| Total | 592600 | 11 | | | |

**Table S2.B.** Two-way ANOVA (without replication) analysis between Total Flavonoid Content (TFC) and $IC_{50}$ value

| | Sum of squares | df | Mean square | F | p (same) |
|---|---|---|---|---|---|
| Rows (between samples) | 0.180625 | 1 | 0.180625 | 1.609 | **0.425** |
| Columns | 1770.31 | 1 | 1770.31 | 1.58E+04 | 0.005069 |
| Error: | 0.112225 | 1 | 0.112225 | | |
| Total: | 1770.6 | 3 | | | |

In summary, *S. polycystum* contains alkaloids, flavonoids, phenol hydroquinone, and saponins, while *S. ilicifolium* contains flavonoids, phenol hydroquinone, tannins, and steroids. The $IC_{50}$ for antioxidant activity of *S. polycystum* and *S. ilicifolium* was found to be 51.34 ppm and 51.25 ppm, respectively. Further investigations using several methods to assess antioxidant activity are suggested for a comprehensive understanding of the antioxidant potential of these underutilized brown algae. The findings provide a promising starting point, and future studies should consider a broader spectrum of assays to establish a more comprehensive assessment of the antioxidant capabilities of *Sargassum* spp.

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