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Evaluation of bioprospecting potential of epiphytic *Gracilaria edulis* harvested from seaweed farm in Seriwe Bay, Lombok, Indonesia

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Abstract. *Prasedya ES, Fitriani F, Saraswati PBA, Haqiqi N, Qoriasmadillah W, Hikmaturrohmi H, Nurhidayati SZ, Ariati PEP. 2023. Evaluation of bioprospecting potential of epiphytic Gracilaria edulis harvested from seaweed farm in Seriwe Bay, Lombok, Indonesia. Biodiversitas 24: 5343-5351.* The seaweed industry is strategically important in Indonesia, comprising over 40% of the nation's aquaculture annual production. Despite the industry's promising growth, various challenges and problems remain, including intense epiphyte infestation. Hence, investigating the economic value of these epiphytes could provide new opportunities for potential industrial applications. Epiphytic algae is commonly found growing on commercial seaweeds which causes decreased biomass and increased risk of crop failure. Information regarding these epiphytic algae remains limited. This study investigates the molecular identification of the abundant epiphytic macroalgae found in one of the largest seaweed farms in, Seriwe Bay, Lombok, Indonesia. In addition, the epiphytic bioactive activity is also evaluated for further industrial potential. Molecular identification with the mitochondrial marker *COX1* identifies the epiphytic macroalgae as *Gracilaria edulis* (S.G.Gmel.) P.C.Silva. The Indonesian *G. edulis* is closely related to the *G. edulis* specimens from Malaysia (JQ026083.1), Philippines (KY995636.1 and KY995635.1), and Thailand (JQ026088.1). The outgroup used was the *G. edulis* specimen from India (KP099563.1) because it shows the most distinct relationship to the other specimens. Extracted agar of Seriwe *G. edulis* shows moderate yield (21%) and low gel strength (134 g/cm^2). The phytochemical content analyses show that *G. edulis* agar has a TPC value of 3.65 ± 0.52 mg GAE/g and promising antioxidant activity (DPPH IC_{50} = 797.40 ± 1.50 μg/mL; ABTS IC_{50} = 558.40 ± 1.44 μg/mL). Further phytochemical profiling with GCMS shows various promising major constituents such as tetradecanoic acid, neophyte diene, pentadecanoic acid, and hexadecanoic acid. Therefore, the findings suggest that *G. edulis* displays potential applications in the functional food and cosmetic industry.

Keywords: Agar, antioxidant, epiphytic macroalgae, *Gracilaria edulis*, red macroalgae

INTRODUCTION

Indonesia contributes significantly to the global seaweed production, particularly the commercial carrageenan producing seaweeds from the generas *Kappaphycus* and *Eucheuma*. West Nusa Tenggara (NTB) regency is among the top seaweed producers, along with East Nusa Tenggara, South Sulawesi, and Central Sulawesi (Rimmer et al. 2021). However, currently the Indonesian seaweed industry is also facing various challenges and problems, which have caused a steady decline in recent years. The incidence of diseases and pests could cause significant loss in the biomass of harvested commercial seaweeds affecting the production quantity and also quality (Kambey et al. 2020; Ward et al. 2020). Epiphytic algae are one the most common pests affecting the growth of commercially cultivated seaweeds. Particularly, during rainy season where it could outcompete the growth of commercial seaweeds (Pires et al. 2017). Epiphytic algae could be described as a group of algae that grows attached to the surface of the host seaweed (Arguelles 2019; Jover et al. 2020). This would trigger competition for resources, mainly in light and nutrients, which would further effect the growth of commercial seaweed (Tian et al. 2022).

In recent years, Seriwe Bay as one of the high-producing sites of commercial seaweeds in West Nusa Tenggara (WNT) province, has experienced a decline in seaweed production. One of the potential causing factors is the persistent blooms of non-commercial macroalgae. Excessive nutrients in estuaries and shallow waters could increase the growth of bloom-forming macroalgae such as *Gracilaria* and *Ulva* (Young and Gobler 2016). This condition could result in growth inhibition of commercial macroalgae due to competition with epiphytic macroalgae in nutrition and light sources for photosynthesis (Song et al. 2017). Hence, these algae are called nuisance, pests, or epiphytes, which impacts the productivity of commercial macroalgae (Han et al. 2021). Therefore, increasing research into the bioprocessing of the epiphytic *Gracilaria* is crucial, which is predominantly problematic to the current seaweed industry in NTB.

The red macroalgae *Gracilaria* is actually well documented to have economic value (Francavilla et al. 2013; Zhang et al. 2019; Pereira et al. 2021) some species are currently cultivated in various countries. The common commercial species for *Gracilaria* are *Gracilarioopsis lemaneiformis* (Bory de Saint-Vincent) E.Y. Dawson, Acleto & Foldvik 1964, *Gracilaria chilensis* C.J. Bird, McLachlan & E.C. Oliveira 1986, *Gracilaria gigas* Harvey 1860, and *Gracilaria longissima* (S.G. Gmelin) Steentoft, L.M. Irvine & Farnham 1995 (Freitas et al. 2021; Caroca-Valencia et al. 2023). In Indonesia, *Gracilaria verrucosa* (Hudson) Papenfuss, nom. Rejic. 1950 is currently the most cultivated species, particularly in the Southern Sulawesi Region (Mulyono et al. 2020). Hence, the red algae *Gracilaria* is emerging as one of the highly valued commercial seaweed globally, along with *Kappaphycus* and *Eucheuma*.

The popular commercial generas *Kappaphycus* and *Eucheuma* are mainly cultivated as carrageenan sources, with a global market size valued at USD 871.66 million in 2022 (Das et al. 2023; Zhang et al. 2023). Although it is still less valued than carrageenan, the agar industry is steadily growing, with a global market size of USD 263.61 million in 2022 (Mendes et al. 2022). However, in the seaweed industry, not only these phycocolloids (polysaccharides derived from seaweeds) are gaining significant attention. Various research also involves the investigation of potential bioactive qualities in seaweeds, such as anticancer capabilities, antiviral, immunomodulatory, antibacterial, and anti-fungal (Sanniyasi et al. 2019; Hentati et al. 2020; Ismail et al. 2020; Lomartire and Gonçalves 2022; Prasedya et al. 2022, 2016). Besides their agar, the red macroalgae *Gracilaria* is also extensively studied as a potential multi-product source for various applications in the food and pharmaceutical industry (Nabil-Adam et al. 2020).

Moreover, for further utilization and bioprospecting, the correct taxonomic status of the epiphytic *Gracilaria* is required. Molecular identification is needed as some *Gracilaria* species tend to have high plasticity which could lead to misidentification. Some *Gracilaria* species are actually poisonous, such as *Gracilaria vermiculophylla* (Ohmi) Papenfuss 1967, have been reported to show harmful effects on metabolism and survival (Martínez-Lüscher and Holmer 2010). Furthermore, misidentification often occurs in macroalgae or seaweeds due to their high plasticity, particularly the *Gracilaria* species (Othman et al. 2018). Until now, there remains limited information on the *Gracilaria* species in Indonesia. In this study, the epiphytic *Gracilaria* in Seriwe Bay is identified and determined based on molecular methods using the mitochondrial gene marker Cytochrome c oxidase 1 (*COX1*) (Lyra et al. 2016; Ng et al. 2017). In addition, various bioactive properties were also evaluated for potential bioprospecting of the epiphyte *Gracilaria*.

MATERIALS AND METHODS

Sample collection

Sampling was conducted in February 2023 in the seaweed farms of Seriwe Bay (08°53.426'S 116°30.701'E), East Lombok District, West Nusa Tenggara Province, Indonesia (Figure 1). All samples were washed with filtered seawater to remove unwanted debris, such as small rocks and sand. The samples were transported in an icebox to maintain humidity (Ramakrishnan et al. 2017). Further cleansing was done in the Lab with sterilized distilled water to remove the remaining contaminants. Cleansed samples were subjected to drying under controlled room temperature (24°C). After three days, the samples were cleansed again with 70% EtOH and 1% Fungicide (Rely+On Virkon, US) to remove bacterial contamination from the thallus surfaces. Further drying was conducted in an oven (40°C) until the sample reached constant weight.

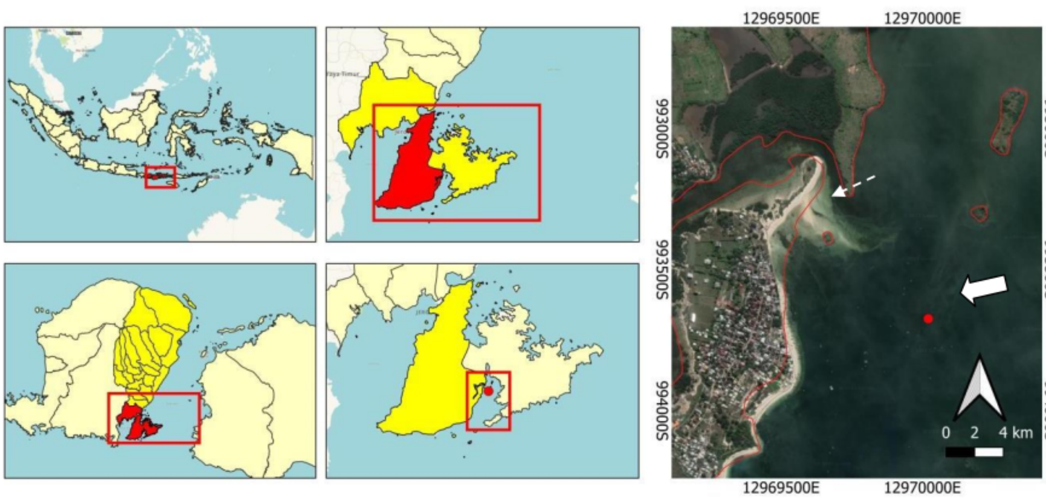

Figure 1. Location of Seriwe Bay in the eastern part of Lombok island, East Lombok District, West Nusa Tenggara Province, Indonesia. The red dot indicates the sampling location. A thick white arrow indicates seaweed farms. Dash white arrows are the estuary

Figure 2 Morphological features of epiphytic *Gracilaria edulis*. Scale bar: 5 cm

Epiphytic *Gracilaria* molecular identification

The red macroalgae *Gracilaria* has a thallus that is commonly cylindrical, compressed, or bladelike. These characteristics make *Gracilaria* macroalgae has a bushy appearance (Figure 2). The dried macroalgae gross samples were deposited to the herbarium of Pusat Unggulan Biosains dan Bioteknologi (PUBB) Universitas Mataram with voucher number PUBBH-1231. Ten small fragments (2-3 cm) from the tips of the thallus were dissected and stored in a ziplock filled with silica gel. Approximately 10 mg of the small fragments were isolated in DNA (Tan et al. 2022). Samples were isolated using the DNAesy Plant Mini Kit (Qiagen, Germany) with modifications, such as adding Proteinase K 10 μL and incubating for 45 minutes at 65°C in a heating block. PCR was performed using PCR Mix Ampliqon Taq DNA Polymerase 2x Master Mix RED (Cat. No. A190303, Germany) with a volume of 50 μL for each reaction. The primers used were *COX1* (upstream COXI43F - 5'TCAACAAATCATAAAGATATTGGWACT3' and downstream COXI1549R - 5'AGGCATTTCTTCAAA NGTATGATA3'). The reaction mix consisted of 25 μL PCR mix, 20 μL RNase-free water, 1 μL forward and reverse primers (0.4 μM concentration), and 3 μL DNA volume. The PCR product was subjected to Sanger sequencing. The Basic Local Alignment Search Tool (BLAST) in NCBI was used to determine the molecular ID. The *Gracilaria edulis COX*1 sequence has been deposited to NCBI genbank under the accession number OR682794. Further phylogenetic analyses of the sequencing data were carried out using various applications such as BioEdit (version 7.2), ClustalX (version 2.2), and Chromas (version 2.6.6) (Ng et al. 2017).

Epiphytic *Gracilaria* agar content analyses

The agar extraction was carried out based on the method described by Li et al. and Vuai with minor modifications (Li et al. 2008; Vuai 2022). The dried powder of *Gracilaria* (1 g) was boiled in 100 mL of distilled water for 60 minutes. The solution was filtered with nylon cloth while hot using a vacuum filter funnel. The extracted solution was kept at room temperature (24°C) until a gel was formed. The gels were kept in deep freeze (-20°C) for 48 h. The frozen gel was thawed at room temperature and dried in an oven at 60°C temperature for 48 h. The extracted agar was determined with Fourier Transformed Infrared Spectra (FTIR). Approximately two grams of dried agar powder was mixed with potassium bromide. The FTIR spectra were documented in transmission mode from 4000-500 cm^{-1} range with 2 cm^{-1} resolution (Belattmania et al. 2021).

The agar yield was calculated using the formula below:

Agar yield (%) = [(Dry weight of agar (g)/ Dry weight of macroalgae (g))] x 100

The gel strength (gcm^{-2}) was measured by taking a 1.5% *w/v* solution of *Gracilaria* agar and autoclaved at 120°C for 30 min. The agar was kept at room temperature to form a gel and further stabilized at 5°C overnight in a refrigerator. The agar gel strength was then measured with Brookfield CT3 Texture Analyzer at 20°C (Brookfield Engineering Labs. Inc) using a cylindrical probe (TA10 Cylinder 12.7 mm diameter, 35 mm long) (Belattmania et al. 2021).

Epiphytic *Gracilaria* extract preparation

The red epiphytic macroalgae *Gracilaria* was subjected to extraction with ethanol. The extract preparation was conducted based on the method in previous work (Prasedya et al. 2022). The dried macroalgae sample for phytochemical analyses was ground into a powder with a food mill grinder. Next, 100 g of sample powder was extracted with ethanol solvent at 1:10 (*w/v*). The mixture was incubated in an ultrasonicator for 60 minutes at 50°C and 50 kHz. The solid residue was discarded, and the supernatant was subjected to rotary evaporation for 2 hours at 60°C temperature. The resulting extract paste was subjected to further analyses.

Epiphytic *Gracilaria* extracts total phenolic content

The total phenolic content (TPC) of epiphytic *Gracilaria* extract was estimated with the Folin-Ciocalteu method (Prasedya et al. 2021). In absolute ethanol, polyphenol gallic acid (GAE) was standard (1 mg/mL). The extract (100 μL) was combined and mixed with 0.75 mL of the Folin-Ciocalteu reagent (diluted tenfold in dH_2O before use). The mixture was incubated at room temperature for 5 min before adding 750 μL sodium carbonate (Na_2CO_3). After 90 min, the absorbance of the mixture was measured at 725 nm with a UV-Vis spectrophotometer (Multiskan-Go, Thermo Scientific). The TPC value of samples was revealed as Gallic acid equivalents in milligrams per gram of the extract (mg GAE/g).

Epiphytic *Gracilaria* extract antioxidant activity

The antioxidant activity of *Gracilaria* extract was determined by the two most common methods, the DPPH and ABTS assay (Azeem et al. 2022). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed on samples at a concentration of 0.1% in absolute ethanol. A volume of 100 μL of extract was mixed with 100 μL DPPH reagent. An additional 200 μL ethanol solvent was used as blank. Absorbance was measured at 517 nm using a spectrophotometer. The IC50 value represents the percentage inhibition value. The DPPH measurement was calculated using the following equation:

% inhibition = [(Control Abs - Blank Abs) - (Sample Abs - Blank Abs) / (Control Abs - Blank Abs)] x 100%.

Where: Abs refers to absorbance.

The ABTS method describes the ability of the extract to scavenge the radical ABTS (2, 2 azino bis (3-etheylbenzothiazoline-6-sulphonicacid) diammonium salt). ABTS solution was prepared by mixing 5 ml of 7 mM ABTS stock solution with 88 μL of 140 mM potassium persulfate solution and incubating the mixture for 16 hours. A volume of 125 μL ABTS was transferred to another dark container, and 10 mL absolute ethanol was added until absorbance was 0.7±0.02 at 734 nm. 0.1 mL of *Gracilaria* extract solution (1, 3, 10, 30, 100, 300, 1000 μg/mL) was mixed with 0.1 mL of ABTS solution. Absorbance was measured at a wavelength of 734 nm using a UV-visible spectrophotometer after incubation in the dark for 6 minutes. Measurements were made three times with vitamin C for comparison. The IC50 value represents the percentage inhibition value.

GC-MS analyses

The *Gracilaria* extract was characterized by gas chromatography (GC-MS) method according to Bharathithasan et al. (2021) with minor modifications. The GC-MS was coupled with a quadrupole mass spectrometer QP2010 Ultra (Shimadzu) system. This system was fitted with a RTX5 capillary column (30 m × 0.25 mm of internal diameter, × 0.25 mm of film thickness and maximum temperature of 37°C (El Wahidi et al. 2015). The injection, transfer line, and ion source were all heated up to 280°C temperature. The oven temperature was set to increase from 80°C (hold for 2 minutes) to 280°C at a rate of 3°C/min. The crude extracts were diluted with a suitable solvent (1/100, v/v) and filtered. A syringe was used to extract the diluted crude extract (1 L) free of particulates, which was then injected into an injector at a split ratio of 10:1. The full-scan mass spectral scan range of 40-550 amu was gathered as data for this investigation. The proportion of peak area will establish the crude extract compounds percentage composition. The interpretation of mass spectrum analyses was conducted using the National Institute Standard and Technology (NIST) database.

RESULTS AND DISCUSSION

Epiphytic *Gracilaria* molecular identification

The bloom-forming *Gracilaria* is seen to be abundant in estuarine environments (Mendes et al. 2022). This type of nuisance algae is widely distributed, and its growth is associated with agriculture and domestic waste locations (Joniver et al. 2021). These characteristics match the seaweed farm location in Seriwe Bay (Figure 1). However, the taxonomical status of these nuisance algae remains poorly understood.

The molecular identification of epiphytic *Gracilaria* is determined with the mitochondrial cytochrome c oxidase subunit I (*COX1*). The amplified *COX1* region of epiphytic *Gracilaria* shows above 99% coverage of *G. edulis* (S.G. Gmelin) P.C. Silva 1952 (Table 1) based on BLAST results. Hence, the epiphytic Gracilaria sequence was compared to other *COX*1 *Gracilaria edulis* sequences from the NCBI genebank database for further downstream bioinformatic analyses. The reference sequence from India (KP099563.1) is used as the outgroup, because it is the most distantly related compared to other *G. edulis* sequences (Figure 3). The Indonesian *G. edulis* (SRW032) show close relationship to the reference sequences from Malaysia (J1026083.1), Philippines (KY995636.1 and KY995635.1), and Thailand (JQ026088.1). It has been reported that the Southeast Asia region share similar seaweed diversity (Hehre and Meeuwig 2016). Interestingly, some sequences from the Philippines (KY995676.1 and KY9956761) show close similarity to the sequences from China (JQ026081.1). In addition, the Philippine reference sequences (KY995675.1 and KY995641.1) share similar sequences to *G. edulis* found in Japan (KF214693.1). This shows that there is a need to add more sequences in the future for *G. edulis* from various locations in Indonesia. Due to the economic value of *G. edulis*, many sequences have been submitted to the NCBI database which originated from Philippines, China and India. However, up to now, there are no *G. edulis* sequences from Indonesia based on the mitochondrial-encoded *COX1* region.

Epiphytic *Gracilaria* agar content analyses

The chemical characteristics of the agar extracted from Seriwe Bay *G. edulis* (SRW032) were compared to *G. edulis* species from other origins (Table 1). Based on previous work, the agar yield from *G. edulis* could vary. This is influenced by growth conditions, seasonal, and also extraction methods. The agar extracted from *G. edulis* by Kalimuthu and Ramalingan (1996) resulted in a significantly higher yield (43%) than Meena (11%). Another study extracted agar from *G. edulis* origin in Tanzania, showing a similar yield at 17%. Our study shows a similar agar yield obtained from *G. edulis* at 21%. Compared to other *G. edulis* studies, our current study showed considerable gel strength of the agar extracted. However, to reach the high agar quality requirement for the market, a gel strength greater than 750 g/cm^{-2} is needed (Wang et al. 2017). Hence, modifications and optimizations of the agar extraction method are needed to explore further the potential of agar from *G. edulis* (Xiao et al. 2023). However, certain industries also utilize agar with low gel strength, which is used as a gelling agent for spreading foods, cosmetics, and soft-texture confectionery (Gu et al. 2017).

The chemical characteristics of the extracted agar from *G. edulis* were determined using the infrared (IR) spectrophotometry method (Figure 4). The absorbance at

1250 cm^{-1} confirms the presence of sulfate esters. The absence of IR-bands at 705,805, and 1070 cm^{-1} suggests the presence of sulfate groups in the extracted agar. The bonds at 1434 and 1256 cm^{-1} are common to all spectra of polysaccharides and are associated with the stretching of CH_3/CH_2 groups. The absorbance at the wavelength of 925-935 cm^{-1} detected in the sample agar is potentially due to the presence of 3,6-anhydrogalactose, and it was observed that the presence of a band at 930 cm^{-1} suggest the presence of 3, 6-anhydrogalactose bridges, which confirms the chemical composition of extracted agar (Vuai 2022). The vibrational band at 1600 cm^{-1} presented the CO and NH groups responsible for forming conjugated peptide bonds (Barros et al. 2013). It was concluded that the analysis of sample IR-spectra confirms the substance extracted from *G. edulis* represents the chemical characteristics of the hydrocolloid agar.

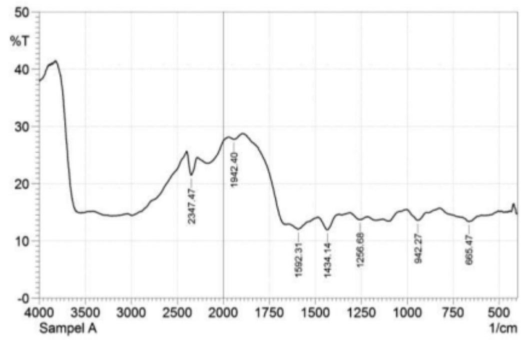

Figure 4. FT-IR spectra of *Gracilaria edulis* extracted agar

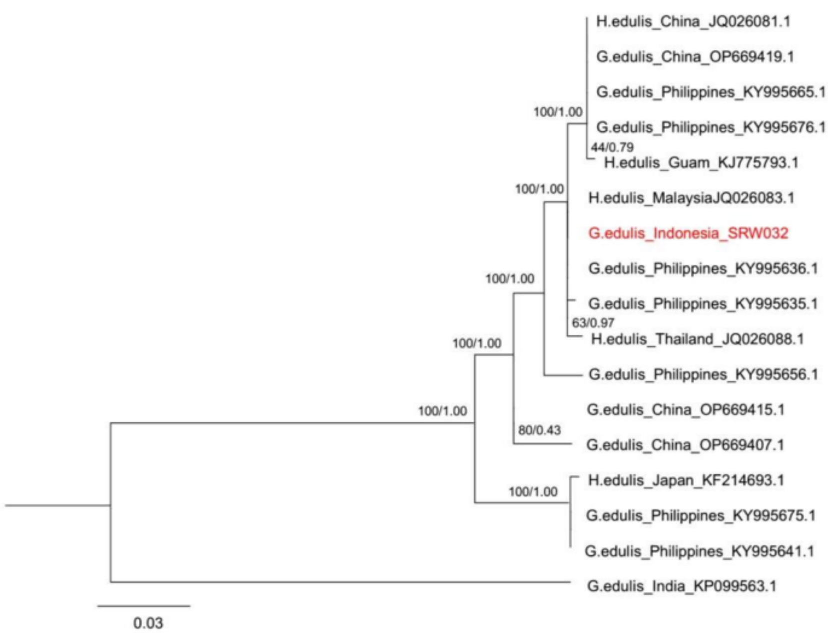

Figure 3. Simplified phylogenetic tree showing current relationships between *Gracilaria edulis* from various locations based on the mitochondrial *COXI* region. Nodal values denote the maximum Likelihood of bootstrap support. Sequence ID in red refers to the sequence retrieved from the specimen used in the current study

Table 1. Other studies reported the yield and gel strength of the *Gracilaria edulis* species

Species	Origin	Yield (%)	Gel strength (g/cm^{-2})	Reference
G. edulis	Indonesia	21	134	Current study
G. edulis	India	43	120	(Kalimuthu and Ramalingan 1996)
G. edulis	India	11	490	(Meena et al. 2006)
G. edulis	Tanzania	17	110	(Vuai 2022)

Epiphytic *Gracilaria* bioactive potential

The phytochemical and bioactive potential of epiphytic *Gracilaria* was evaluated based on total phenolic content (TPC) and antioxidant activity. The total phenolic content (TPC) obtained from *G. edulis* extract was 3.65±0.52 mg GAE/g. This is higher compared to a study done by Gunathilaka et al. (2019), which shows *G. edulis* extract demonstrates a relatively low TPC, ranging from 0.5-2 mg GAE/g in various solvents. Previous studies of different *Gracilaria* species, *Gracilaria salicornia* (C.Agardh) E.Y.Dawson and *Gracilaria corticata* (J.Agardh) J.Agardh shows higher TPC 12-13 mg GAE/g (Ghannadi et al. 2016). However, this is still significantly low compared to brown macroalgae such as *Sargassum* (TPC ≥50 mg GAE/g) (Sunarwidhi et al. 2022). Compared to red macroalgae, the group of brown macroalgae possesses more phenolic compounds, particularly phlorotannins (Mekinić et al. 2019). However, the Folin-Ciocalteu method also has limitations, which sometimes gives misinterpretation. Hence, the Folin-Ciolcateu method could not be applied indiscriminately since different phytochemical constituents may impair the assay's accuracy (Martins et al. 2021).

This is probably why in some studies, total phenolic activity positively correlates with antioxidant activity, and other studies negatively correlate with total phenolic activity (Kim and Lee 2020; Muflihah et al. 2021). Our current study shows that the TPC negatively correlates with antioxidant activity. The antioxidant activity of *G. edulis* extract from Seriwe showed promising activity based on DPPH (IC_{50} = 797.40 ± 1.50 μg/mL) and ABTS (IC_{50} = 558.40 ± 1.44 μg/mL) assay (Figure 5). The IC_{50} value is a measurement which shows the antioxidant activity of the tested samples (Olugbami et al. 2014). The higher the IC_{50} value, the weaker the antioxidant activity of the tested sample (Paudel et al. 2014). Our study shows that *G. edulis* shows significantly stronger antioxidant activity than *Gracilaria gracilis, Gracilaria* sp., and *Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva (Goutzourelas et al. 2023). Another previous report also shows lower antioxidant activity in *Gracilaria lemaneiformis* (Bory) Weber-van Bosse in both DPPH (IC_{50} = 9.62 ± 0.35 mg/mL) and ABTS (IC_{50} = 23.85 ± 1.78 mg/mL) assays (Long et al. 2022). These antioxidant values are much lower compared to brown seaweeds such as *Fucus vesiculosus* L. with DPPH IC_{50} of 614 μg/mL (Corsetto et al. 2020).

Various factors could induce different antioxidant activities in seaweeds. The antioxidant activity of certain macroalgae is highly dependent on environmental factors (Michalak et al. 2022). Another factor that must be considered is the extraction solvent. However, an indication of the potential antioxidant activity could be a good lead for further exploration and utilization. The antioxidant potential of *G. edulis* is similar to that of tropical plants (Mustafa et al. 2010). Further optimizing extraction conditions may produce different antioxidant activities (Reboleira et al. 2020).

GCMS analyses

Based on the GCMS assay, the evaluation of the phytochemical constituents in Seriwe *G. edulis* revealed some promising bioactive compounds (Figure 6). The major constituents detected in the *G. edulis* extract were Pentadecanoic acid RT 10.006 (2.98%), Neophytadiene RT 10.006 (2.98%), Tetradecaonoic acid RT 9.568 (2.88%), Hexadecanoic acid RT 10.621 (32.96 %), Octadenol RT 11.534 (7.41 %), Oleic acid RT 11.699 (8.07%), cholest-5-en-3-ol RT 18.463 (21.38%). There were various compounds detected in *G. edulis* extract that could be utilized in various industries, such as Tetradecanoic acid, which was present in the *G. edulis* extract. This compound, also known as myristic acid, can potentially be used as soap and cosmetic ingredients (Becker et al. 2010). Another interesting compound was neophytadiene, which is known for its potential anti-inflammatory and antibacterial activities (Kaur et al. 2023; Singh et al. 2023; Toh et al. 2023). The *Gracilaria* species, *G. edulis* is an economically important commercial seaweed in Philippine, China, and India. In addition to antioxidant activity, hypoglycemic potential is also mentioned, which could lead to the development of metabolic drugs. This is potentially due to the use of pentadecanoic acid. This compound is an essential fatty acid that supports healthy metabolic processes (Venn-Watson and Butterworth 2022). A major constituent of hexadecenoic acid was detected at RT of 10.621. This compound, known as palmitic acid, is commonly used in cosmetic applications (Čižinauskas et al. 2017). The active compounds pentadecanoic acid and hexadecenoic acid are also found in other studies showing phytochemical profile of *Gracilaria* extract (Guo et al. 2017; Kasanah et al. 2019). The occurrence of cholest-5-en-3 ol has also been reported in previous reports from red macroalgae *Gratuloupia turuturu* Yamada and *Laurencia papillosa* (C.Agardh) Grev. (Plouguerné et al. 2006; Kavita et al. 2014).

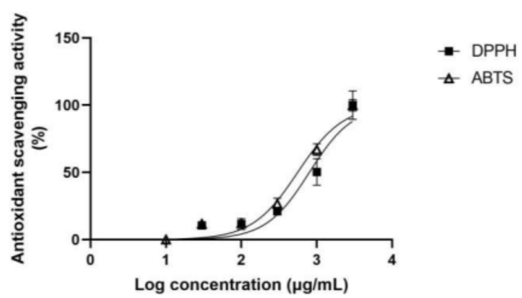

Figure 5. Antioxidant activity of Seriwe *G. edulis* extracts determined by DPPH and ABTS. Experiments were done in triplicates; values are expressed as means ± SEM

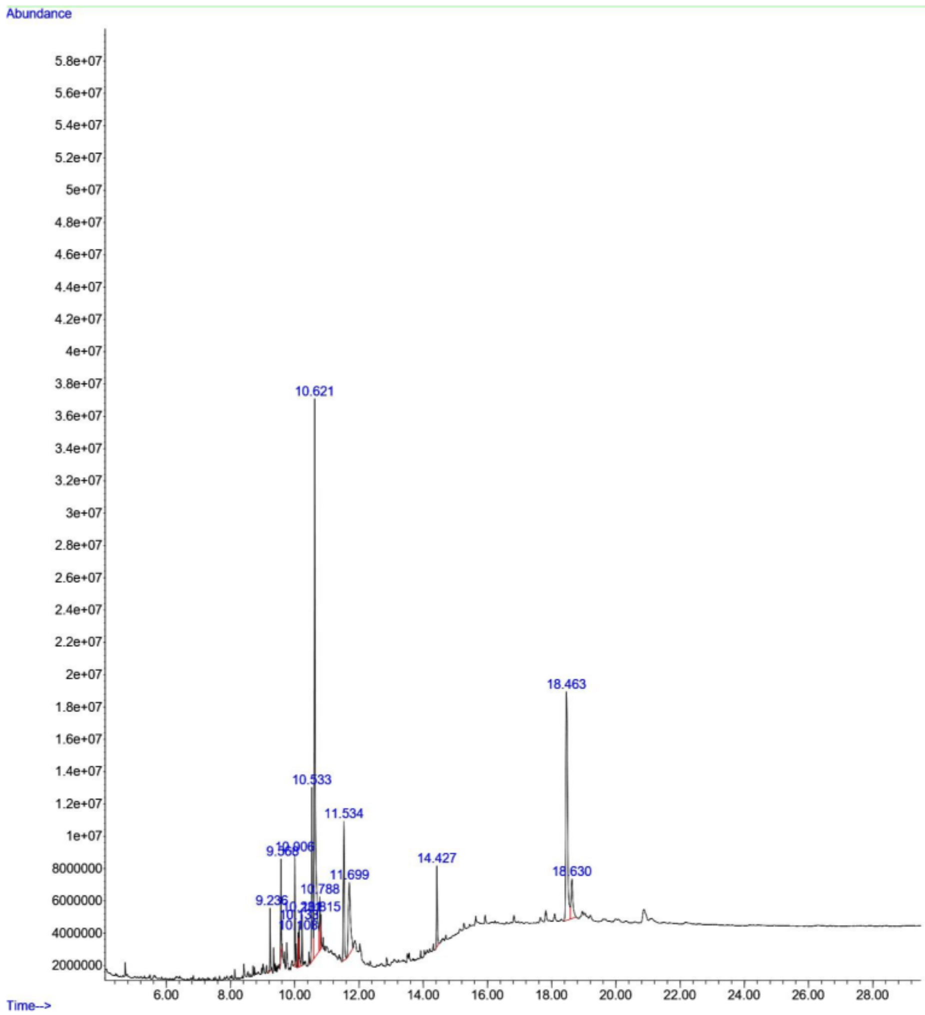

Figure 6. GCMS profile of Seriwe *Gracilaria edulis* ethanol extract

In conclusion, the epiphytic red macroalgae, abundant in the seaweed farms of Seriwe Bay, Lombok, Indonesia, were identified as *Gracilaria edulis*. This *Gracilaria* species is more closely related to the specimens found in the Philippines, Malaysia, and Thailand. Which are distinct from the *G. edulis* species found in China, Japan, and India. The agar content, phytochemical profile, and antioxidant activity of epiphytic macroalgae carry potential assets as they may have a strong industrial value. Additionally, more studies are needed on the potential of existing epiphytes in seaweed farms. The utilization of these epiphytic macroalgae may contribute to increasing the production of the commercial seaweeds *Kappaphycus* and *Eucheuma* in various seaweed farms.

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Proper Nouns You may need to use a capital letter for this proper noun.

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Possessive

Missing "," Review the rules for using punctuation marks.

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Confused You have used either an imprecise word or an incorrect word.

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Wrong Article You may have used the wrong article or pronoun. Proofread the sentence to make sure that the article or pronoun agrees with the word it describes.

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P/V You have used the passive voice in this sentence. You may want to revise it using the active voice.

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Proofread This part of the sentence contains an error or misspelling that makes your meaning unclear.

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