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Brown and Red Marine Macroalgae as Novel Bioresources of Promising Medicinal Properties

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ABSTRACT

Bioactive compounds from marine macroalgae are gaining immense attention for their application as natural ingredients in various nutraceuticals and food supplements. The present study evaluated the medicinal properties of the organic extracts of four each of brown and red marine macroalgal species, using various *in vitro* assays. Organic extracts of brown algae of Fucophycidan subclass, such as *Sargassum plagiophyllum, Turbinaria decurrens*, and red alga *Hydropuntia edulis*, displayed potential inhibitory properties against antioxidants (IC_{50} 0.2–0.8 mg/mL) and carbolytic enzymes (IC_{50} 0.2–0.9 mg/mL) compared to those exhibited by other studied algae. Noticeably, organic extracts of red alga *H. edulis* and brown alga *T. decurrens* could effectively attenuate pro-inflammatory 5-lipoxygenase (IC_{50} 0.4–0.6 mg/mL), thereby demonstrating their potential application to dissuade inflammatory pathogenesis. This study demonstrated the predominantly available brown and red macroalgae as potential marine bioresources to develop functional food candidates.

KEYWORDS

Marine macroalgae; medicinal properties; carbolytic enzyme inhibition; pro-inflammatory 5-lipoxygenase; spectroscopic fingerprint

Introduction

Benthic marine macroalgae constitutes a unique source of pharmacologically active components and are enriched with natural products that exhibit potential biological properties compared with the terrestrial plants (Deepak et al. 2017). In addition, macroalgal secondary metabolites are gaining significant attention for their utilization in processes like functional foods, medicines, nutraceuticals, and pigments (El Zokm et al. 2021; Ravikumar et al. 2011). Marine macroalgae have been consumed by coastal communities since pre-historic times. For example, different species of brown algae *Sargassum* sp. have been used in traditional Chinese medicine to heal various diseases, such as goiter, for 2000 years (Liu et al. 2012) and have been reported to possess potential anti-inflammatory and anti-hyperglycemic activities (Anusree and Chakraborty 2017a, 2017b). Kombu, wakame, and nori account for greater than one-tenth of the Japanese marine macroalgal diet (Griffin 2015). Recent evidence shows the importance of structurally diverse metabolites isolated from marine macroalgae possessing various biological properties, such as antioxidant (Jacobsen et al. 2019), anti-inflammatory (Paramsivam et al. 2016), antidiabetic (Unnikrishnan et al. 2014), antimicrobial (Thilakan et al. 2016), and cytotoxic activities (Remya et al. 2017). Admittedly, public concern for potential health risk factors regarding synthetic antioxidants has increased, which further supports the utilization of previously reported macroalgae derived natural antioxidants, such as tocopherols, phlorotannins/phenolics, and carotenoids (Jacobsen et al. 2019). Marine macroalgae have been reported to possess antioxidant potential and could augment protection against cellular oxidative damage (El Zokm et al. 2021; Ismail

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et al. 2016). As a result of their antioxidant potential, marine algae could improve immunity, and recent reports have recognized their potential to prevent COVID-19 (Kavitha 2020). Macroalgae derived lipids have demonstrated high positive health impacts, with potential applications in the area of food industries and biopharmaceuticals. Another group of macroalgal compounds are sulfated polysaccharides (fucoidans, carrageenans, and ulvans), which were reported to display numerous bioactivities (Anusree and Chakraborty 2018a; Ismail and Amer 2020; Stranska-Zachariasova et al. 2017). Iota-carrageenan isolated from red macroalgal extract was used as a food thickening agent and could inhibit SARS-CoV-2 infection at 6 μg/mL (Bansal et al. 2020). Sodium oligomannate (SoM), an oligosaccharide isolated from marine macroalga, is the only novel drug approved globally for the treatment of Alzheimer's disease, since 2003. SoM received an approval from the National Medical Products Administration of China in November 2019 for the treatment of mild-to-moderate Alzheimer's disease and to improve cognitive function (Syed 2020).

In 2018, total global marine macroalgal production was greater than 30 million tons (volume-wise fresh weight), valued at $13.3 billion, and more than three times growth was perceived in their global production between 2000 (about 11 million tons) and 2018. Trade of these marine flora increased from $65 million in 1976 to about $1.3 billion in 2018 (FAO 2020). Despite the presence of a large number of marine macroalgae, only a small number are commercially utilized. Out of the global record of more than 200 species of commercially exploited marine macroalgae, nearly 125 were red algae (Rhodophyta) and 64 belonged to brown algae (Ochrophyta, Phaeophyceae) (Mac Monagail et al. 2017; Wade et al. 2020; Zemke-White and Ohno 1999). Previously, researchers assessed the bioactivities of organic extracts of marine macroalgae demonstrating the presence of antioxidants and antimicrobial compounds (Antony and Chakraborty 2020a, 2020b; Deepak et al. 2017). Brown algae, one of the main and relevant taxonomic groups among macroalgae, has been reported to have polyphenols, phlorotannins, flavanoids, sterols, carotenoids, fucoxanthin, alginate, and isoprenoids (Swanson and Druehl 2002). To evaluate the economically important marine macroalgae from the southeastern parts of peninsular India, the current study assessed the pharmacological properties of eight different marine macroalgae, which belong to the subclasses of Fucophycidae, Dictyotophycidae (Phylum Ochrophyta, Class Phaeophyceae) and Rhodymeniophycidae (Rhodophyta), utilizing various *in vitro* models. The ethyl acetate/methanol (EtOAc/MeOH) organic extracts of the studied marine macroalgae were examined for various bioactive properties, such as antioxidant, anti-inflammatory, antihypertensive, antidiabetic, and antimicrobial properties. This study also demonstrated the proton nuclear magnetic resonance (^{1}H NMR)-directed spectroscopic de-convolution of the conspicuous functional group patterns in the organic extracts of the studied marine algae and related their manifestation with the bioactivities (Antony and Chakraborty 2019).

Materials and Methods

Collection of marine macroalgae and initial processing

Depending on the availability of macroalgae during the November-February season, fresh samples of four brown, including *Lobophora variegata* (J. V. Lamouroux) Womersley ex E. C. Oliveira (1 kg), *Stoechospermum polypodioides* (J.V. Lamouroux) J. Agardh (1 kg) (belonging to subclass Dictyotophycidae), *Turbinaria decurrens* Bory (20 kg), and *Sargassum plagiophyllum* C. Agardh (20 kg) (belonging to subclass Fucophycidae), and four red algae, namely *Gracilaria corticata* (J. Agardh) J. Agardh (1 kg), *Portieria hornemannii* (Lynbye) P. C. Silva (3 kg), *Acanthophora spicifera* (M. Vahl) Borgesen (1 kg), and *Hydropuntia edulis* (S. G. Gmelin) Gurgel & Fredericq (20 kg) belonging to the subclass Rhodymeniophycidae were brought from the Mandapam coast of the Gulf of Mannar (8° 48′ N, 78° 9′ E and 9° 14′ N, 79°14′ E) of Ramanathapuram district, Tamil Nadu (Figure 1). The macroalgae were identified by Dr. Chellaiah Periaswamy, Aquaculture Foundation of India. Samples

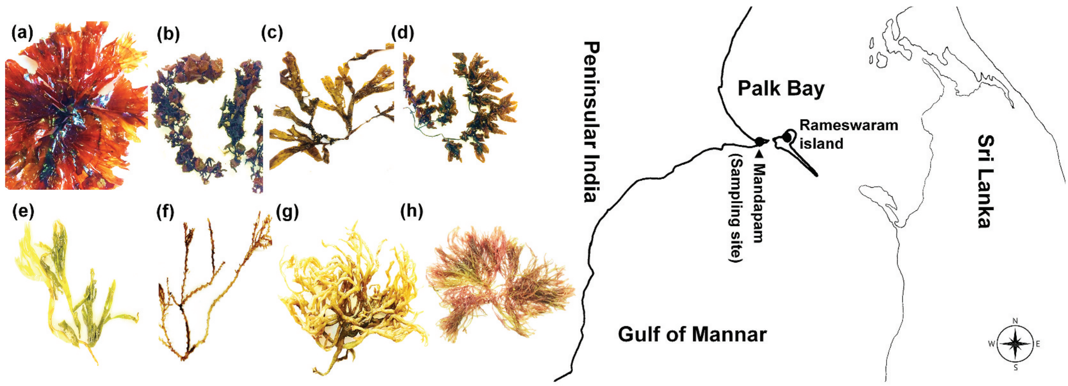

Figure 1. Illustrative photographs of the marine macroalgae (a) *L. variegata*, (b) *T. decurrens*, (c) *S. polypodioides*, (d) *S. plagiophyllum*, (e) *G. corticata*, (f) *P. hornemanii*, (g) *A. spicifera*, and (h) *H. edulis*, and geographical location showing the sampling site of the marine macroalgae at the Mandapam region of Gulf of Mannar.

were dried and carried to the laboratory, followed by repeated washing with distilled water to eliminate sand and other particles. Further, samples were cleaned using distilled water to eliminate salt before shade-drying. The shade-dried materials were thereafter ground before being kept in air-tight packages.

Chemicals and instrumentations

The chemicals and solvents used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA), E-Merck (Darmstadt, Germany), HiMedia (West Chester, PA, USA) and Sisco Research Laboratories (Mumbai, India). Buffers and molar solutions required for assays were freshly prepared, as reported earlier. The UV spectra were acquired using an ultraviolet-visible (UV-VIS) spectrophotometer (Agilent Cary® 50 UV–Vis spectrophotometer, Santa Clara, CA, USA). IR spectra were documented with a Perkin-Elmer Fourier transform infrared (FTIR) spectrophotometer (Perkin-Elmer FTIR2000, Waltham, MA, USA). ^{1}H NMR spectral data were recorded in an NMR spectrometer (Bruker Avance AV 500, 500 MHz Karlsruhe, Germany) in deuteriated chloroform ($CDCl_3$) and tetramethylsilane (TMS $C_4H_{12}Si$, δ_H 0 ppm) as an internal standard. The NMR results (chemical shifts, δ_H) were expressed in ppm (parts per million) and were analyzed using MestReNova (version 7.1.1–9649, Mestrelab Research S.L) software.

Preparation of organic extract of macroalgae

Organic extracts of the studied macroalgae (500 g each) were prepared by soaking initially with hexane (2 × 1 L) for 3 h at room temperature, followed by hot extraction (~80°C) in EtOAc/MeOH (1:1, v/v) (3 × 1 L) for 7–8 h. The extract was filtered using a through anhydrous Na_2SO_4 loaded on a filter paper (Whatman No. 1). The clarified filtrate was concentrated using a rotary evaporator (Heidolph, Germany) at 50°C to yield the crude extract, which was kept at 4°C for further analyses.

Quantitative profiling of total phenolic content

Total phenolic contents for the algal organic extracts were analyzed by Folin-Ciocalteu method (Wojdylo et al. 2007). Gallic acid was used as the standard, and based on the standard curve, the results were articulated in milligram of gallic acid equivalents (mg GAE/g) of the algal extracts.

Screening the biological activities of the crude extract

Antioxidant activity

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activities of the organic extracts derived from the test marine macroalgae were analyzed by adapting an earlier method (Chakraborty et al. 2017). Concisely, different concentrations (0.1–2 mg/mL) of the algal crude extracts were dissolved in methanol followed by addition of 0.1 mM methanolic solution of DPPH and incubated at room temperature (~28°C) under dark. UV absorbance of control (DPPH in MeOH) and samples at 514 nm was measured against a blank (MeOH) at regular intervals using a spectrophotometer.

2, 2′-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) diammonium salt radical scavenging activity was measured by following a previously described procedure (Chakraborty et al. 2017). In short, ABTS free radical was formulated by the addition of 2.4 mM $K_2S_2O_3$ to 7 mM ABTS in deionized water and was let to react by keeping it in dark for overnight at room temperature. This intensely colored reagent was diluted using MeOH until it could attain an absorbance of 0.71 ± 0.01. Thereafter, the algal extracts (0.1 to 2 mg/mL) were treated with ABTS solution before recording the absorbance at 734 nm.

DPPH and ABTS activities were also expressed by AEAC ascorbic acid equivalent capacity (AEAC) in comparison with ascorbic acid (IC_{50} 0.42 mg/mL), an antioxidant agent, and was expressed as AEAC (mgAA/100 g) = IC_{50} (ascorbate)/IC_{50} (sample) × 10^5. A lesser IC_{50} value signified greater AEAC, which is directly proportional to the antioxidant activities.

Hydrogen peroxide scavenging and ferrous ion chelating assays were determined by an established method (Antony and Chakraborty 2019), and all the antioxidant activities were recorded as percent inhibition, expressed as {(absorbance of control-absorbance of sample)/absorbance of control} multiplied by 100, and the IC_{50} values were calculated.

Thiobarbituric acid-reactive species (TBARS) formation inhibitory activity

The potential of the crude algal extracts to inhibit lipid peroxidation was evaluated by thiobarbituric acid reactive species assay as described by Chakraborty et al. (2017). The results were shown as mM of malondialdehyde (MDA) equivalent compounds formed per kg sample (MDAEQ/kg sample).

Other bioactivities

Anti-inflammatory activities of the crude algal extracts were assessed by using pro-inflammatory enzymes 5-lipoxygenase (5-LOX) and cyclooxygenases (COX-1, COX-2) (Baylac and Racine 2003; Larsen et al. 1996). Inhibitory properties of the macroalgal extracts against α-amylase, α-glucosidase, and dipeptidyl peptidase-IV (DPP-IV), which are crucial in the degradation of incretins and glucose metabolism, were utilized for evaluating their anti-diabetic potential (Hamdan and Afifi 2004). The α-glucosidase and α-amylase inhibitory activities of the crude extracts were analyzed according to the procedure used by Dangkulwanich et al. (2018). Antihypertensive activity of the crude extracts was evaluated using a commercial angiotensin-I converting enzyme (ACE-I from rabbit lung, 20 μL, 20 mu) (Udenigwe et al. 2009), and the decrease in absorbance was determined at 345 nm against the reagent blank (distilled water). Anti-hypercholesterolemic assays were performed by measuring the inhibition activity of hydroxymethylglutaryl coenzyme-A reductase enzyme (Krishnan and Chakraborty, 2019). The results were recorded as percentage inhibition of the enzymes, $(A_{CT}-A_{SP})/A_{CT} \times 100$, A_{CT} = Absorbance of control, A_{SP} = Absorbance of sample, and the results were calculated as IC_{50} (mg mL^{-1}).

Antimicrobial activity

The crude extracts of macroalgae were dissolved in MeOH at two different concentrations (Thilakan et al. 2016). The pathogenic microorganisms referred in the study were *Escherichia coli, Vibrio parahemolyticus, Aeromonas caviae*, and methicillin-resistant *Staphylococcus aureus* (MRSA).

Antimicrobial properties of the samples were tested by agar well-diffusion method (El-Masry et al. 2000) and determined by recording the diameters of the zone of inhibition (mm) of crude extracts relating to the inhibition zone of standard drug (ampicillin) (Kizhakkekalam et al. 2020). The wells were made on Muller Hinton Agar plates using a sterile cork borer, which was previously inoculated with the pathogenic bacterial cultures. The wells were filled with crude algal extract (50 µL; 1 mg, 5 mg), whereas DMSO was used as the negative and chloramphenicol as the positive control. The plates were incubated at 37°C for 24 h. A negative control (10% DMSO) was run simultaneously along with the extract suspension to note the effects of the solvent. Antimicrobial activities were expressed as activity index, IZ_S/IZ_0, where IZ_0 = inhibition zone (mm) of a standard drug, IZ_S = inhibition zone (mm) of the test sample.

Screening of crude extracts by spectroscopic methods

FTIR spectra of the algal extracts were recorded on a FTIR spectrophotometer with a scan range of 4000 and 400 cm^{-1}. Protons at the characterized regions in the ^1H NMR spectra of the eight crude marine macroalgal extracts were integrated according to the specific splitting patterns in the corresponding areas. NMR spectra of the samples were analyzed on the basis of characteristic regions such as δ_H 0.5–2 (saturated hydrocarbons/non-oxygenated aliphatic groups/aliphatic acetoxy groups), δ_H 2–2.5 (alkyl alkanoates/acetyl groups/aromatic acetoxy groups), δ_H 2.5–3.5 (methoxy, halogenated aliphatic groups, aliphatic alcohols), δ_H 4.5–6.5 (olefins, cyclic benzylic, alkanoates), and δ_H 6.5–8.5 (aromatic protons).

Statistical analysis

Statistical Program for Social Sciences (SPSS Inc., Chicago, IL, USA; ver. 13.0) was used for the one way analysis of variance (ANOVA) to perceive the significant difference between the means. Data were expressed in means as triplicate determinations ± standard deviation, and significant differences were noted as $P < .05$. The mean variance in the data set was identified using principal component analysis (PCA), and the bioactivities of the algal organic extracts were selected as the variables for analysis. Pearson correlation was performed between the variables.

Results and discussion

The present study evaluated the medicinal activities, such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and antihypertensive potentials of the organic extracts (EtOAc/MeOH, 1:1, v/v) of eight different marine macroalgae belonging to the subclasses of Fucophycidae, Dictyotophycidae (Phaeophyceae), and Rhodymeniophycidae (Rhodophyta), using different *in vitro* models to demonstrate their antioxidant, anti-inflammatory, antihypertensive, antidiabetic, and antimicrobial potential (Table 1), and the activities were statistically correlated.

Bioactive potential of the organic extracts of selected marine macroalgae

Antioxidant activities

Evaluation of the antioxidant properties of the studied macroalgal extracts was carried out by DPPH, ABTS, H_2O_2, and ferrous ion chelating assays. Higher DPPH scavenging activity was observed for brown alga *T. decurrens* (IC_{50} 0.27 mg/mL) followed by those displayed by the organic extracts of *S. plagiophyllum* (0.58 mg/mL), *L. variegata* (0.66 mg/mL), and *H. edulis* (0.82 mg/mL) (Table 1). ABTS radical quenching potential of the crude extracts also showed a similar trend as DPPH radical scavenging activities (Table 1, Fig. S1). Significantly greater AEAC values of the macroalgae belonging to the subclass Fucophycidae (*T. decurrens* and *S. plagiophyllum*) with regard to DPPH radical scavenging activities (>500 mg AA/100 g) further corroborated their significance as functional food

Table 1. Percentage yield, total phenolic contents and bioactivities of the organic extracts of marine macroalgae.

		L. variegata	*T. decurrens*	*S. polypodioides*	*S. plagiophyllum*	*G. corticata*	*P. hornemannii*	*A. spicifera*	*H. edulis*
†Yield		9.8 ± 0.08	8.0 ± 0.02	3.2 ± 0.03	8.6 ± 0.18	2.0 ± 0.20	6.1 ± 0.16	5.2 ± 0.25	8.2 ± 0.69
‡Total phenolic content		39.4^{d} ± 0.21	55.7^{a} ± 0.52	35.2^{e} ± 0.62	54.7^{b} ± 0.13	34.6^{f} ± 0.01	52.2^{c} ± 0.57	23.5^{g} ± 0.74	55.8^{a} ± 0.25
€TBARS activity		7.9^{c} ± 0.01	6.9^{d} ± 0.00	8.99^{a} ± 0.03	8.2^{b} ± 0.02	4.7^{f} ± 0.19	2.5^{h} ± 0.59	3.5^{g} ± 0.13	5.7^{e} ± 0.07
Antioxidant activity (AOA)									
DPPH scavenging	§IC_{50}	0.66^{f} ± 0.21	0.27^{h} ± 0.62	0.97^{d} ± 0.38	0.58^{g} ± 0.99	1.9^{a} ± 0.07	1.3^{c} ± 0.14	1.5^{b} ± 0.02	0.82^{e} ± 0.18
	¥AEAC	454.5^{c} ± 0.42	1111.1^{a} ± 0.16	309.3^{e} ± 0.29	517.2^{b} ± 0.26	157.9^{h} ± 0.39	230.8^{f} ± 0.85	200.0^{g} ± 0.57	365.85^{d} ± 0.02
ABTS scavenging	§IC_{50}	0.4^{d} ± 0.35	0.2^{f} ± 0.22	0.43^{d} ± 0.02	0.3^{e} ± 0.05	0.7^{b} ± 0.06	0.5^{c} ± 0.03	1.0^{a} ± 0.09	0.44^{d} ± 0.18
	AEAC	750^{d} ± 0.05	1500^{b} ± 0.18	1000^{c} ± 0.09	697.7^{e} ± 0.16	4714.3^{a} ± 0.06	600^{g} ± 0.03	300^{h} ± 0.63	681.8^{f} ± 0.27
H_2O_2 scavenging		0.31^{e} ± 0.87	0.22^{f} ± 0.12	0.45^{d} ± 0.18	0.25^{f} ± 0.06	0.51^{c} ± 0.78	0.72^{b} ± 0.32	0.98^{a} ± 0.19	0.32^{e} ± 0.22
Fe^{2+} radical scavenging		0.33^{c} ± 0.35	0.25^{d} ± 0.22	0.38^{c} ± 0.02	0.26^{d} ± 0.05	0.55^{b} ± 0.06	0.59^{b} ± 0.03	0.71^{a} ± 0.09	0.38^{c} ± 0.18
§Anti-inflammatory activity (IC_{50})									
5-LOX		1.52^{b} ± 0.09	0.52^{g} ± 0.06	1.45^{c} ± 0.21	0.92^{e} ± 0.36	0.65^{f} ± 0.01	1.03^{d} ± 0.85	1.98^{a} ± 0.97	0.40^{h} ± 0.73
COX-1		1.61^{c} ± 0.26	0.93^{e} ± 0.69	1.75^{b} ± 0.03	0.91^{e} ± 0.08	0.73^{f} ± 0.96	1.51^{d} ± 0.21	2.01^{a} ± 0.88	0.51^{g} ± 0.22
COX-2		1.58^{b} ± 0.07	0.65^{f} ± 0.73	1.58^{b} ± 0.74	0.82^{d} ± 0.05	0.71^{e} ± 0.11	1.48^{c} ± 0.15	1.96^{a} ± 0.05	0.49^{g} ± 0.02
COX-1/COX-2		1.02	1.43	1.11	1.11	1.03	1.02	1.03	1.04
§Antidiabetic activity (IC_{50})									
α-amylase		5.0^{a} ± 0.56	0.52^{f} ± 0.78	1.9^{c} ± 0.25	0.82^{d} ± 0.07	0.62^{e} ± 0.23	2.5^{b} ± 0.52	1.9^{c} ± 0.02	0.45^{g} ± 0.02
α-glucosidase		2.5^{b} ± 0.07	0.53^{f} ± 0.37	1.7^{d} ± 0.17	0.41^{g} ± 0.54	0.78^{e} ± 0.52	5.9^{a} ± 0.84	2.1^{c} ± 0.89	0.42^{g} ± 0.03
DPP-IV		0.61^{b} ± 0.05	0.31^{c} ± 0.26	0.78^{a} ± 0.17	0.55^{b} ± 0.40	0.34^{c} ± 0.03	0.81^{a} ± 0.02	0.59^{b} ± 0.62	0.22^{d} ± 0.11
#Antimicrobial activity									
Escherichia coli		1.0	2.4	1.13	0.80	0.80	1.66	0.80	1.20
Vibrio parahemolyticus		1.4	0.73	2.53	0.66	2.66	0.66	2.53	0.66
Aeromonas caviae		1.13	0.93	0.93	0.66	0.86	0.66	0.86	0.80
MRSA		0.93	1.6	1.13	0.80	1.26	1.93	1.66	1.06
§Antihypertensive activity (IC_{50})									
ACE-I inhibition		1.01^{e} ± 0.03	0.98^{f} ± 0.26	1.53^{c} ± 0.98	0.81^{g} ± 0.07	1.11^{d} ± 0.89	1.87^{b} ± 0.78	2.18^{a} ± 0.06	0.51^{h} ± 0.08
§Anti-hypercholesterolemic activity (IC_{50})									
HMGCR inhibition		1.21^{d} ± 0.88	1.62^{c} ± 0.05	2.56^{b} ± 0.96	1.18^{e} ± 0.76	0.99^{g} ± 0.18	2.55^{b} ± 0.87	2.97^{a} ± 0.19	1.05^{f} ± 0.22

The samples were analyzed in triplicate (n = 3), and expressed as mean ± standard deviation. †Yield of EtOAc/MeOH extract is represented as % w/w of seaweed on dry weight basis. ‡Total phenolic contents were represented as mg of gallic acid equivalence mg GAE/g.

€TBARS activity was represented as mM MDAEQ kg^{-1}.

§IC_{50} values were represented as mg/mL. ¥AEAC was represented in mgAA/100 g.

#Antimicrobial activity was expressed as activity index (inhibition zone of the test sample divided by inhibition zone of a standard drug).

agents (Table 1). The present findings were consistent with earlier reports, which elucidated the isolation of sargachromanol D, E, K (Lee and Seo 2011), (+)-epiloliolide (Peng et al. 2018), and cholest-5-en-3-ol (Jenifer et al. 2017) with antioxidant properties from the brown marine macroalgae *Sargassum siliquastrum*, *Sargassum naozhouense* (Phaeophyceae) and *Gracilaria foliifera* (Rhodophyceae), respectively. Likewise, the DPPH scavenging activity of methanolic extract of red macroalga *L. variegata* (IC_{50} 0.66 mg/mL) was similar to that exhibited by the EtOAc/MeOH extract of an earlier report (IC_{50} 0.66 mg/mL) (Sathyaseelan et al. 2015).

On a comparable note, the organic extract of the studied marine macroalgae belonging to the subclass Fucophycidae (*T. decurrens* and *S. plagiophyllum*) registered higher hydrogen peroxide radical scavenging and metal-binding activities (IC_{50} < 0.3 mg/mL) than those exhibited by other species. Metal chelating abilities of the macroalgal extracts might be attributed to the occurrence of polyphenolic compounds as reported previously (Antony and Chakraborty 2019; Anusree et al. 2016). It is of note that among the studied macroalgae, total phenolic content was considerably greater for the organic extracts derived from *T. decurrens* (55.7 mgGAE/g) and *S. plagiophyllum* (54.7 mgGAE/g). A comparison of the total phenolic contents of the other macroalgae showed total phenolic content ranging from 55 to 20 mgGAE/g (Table 1). Earlier studies reported the presence of considerable phenolic contents of the organic extracts of marine algae *T. decurrens* and *P. hornemannii* (Chakraborty et al. 2013; Fatima et al. 2016). The present study showed considerably lower phenolic contents of the EtOAc-MeOH extract of *A. spicifera* (23.5 mgGAE/g), which could be corroborated with the significantly reduced antioxidant activities ($IC_{50} \geq 0.7$ mg/mL). Polyphenolic compounds were found to be UV protective in macroalgae and were reported to function as a chemical defense mechanism (Luder and Clayton 2004). The results were similar to the chloroform extract (40.58 mgGAE/g) of *A. spicifera* collected from Malaysia (Zakaria et al. 2011). It is apparent that marine macroalgae are enriched with compounds having polyhydroxylated groups, electron-rich centers, and unsaturations, which could donate electrons to quench free radicals. It was demonstrated in the present study that brown macroalgae (phylum Ochrophyta, class Pheaophyceae) exhibited considerably greater antioxidant activity compared to those displayed by the red marine algae (phylum Rhodophyta), as also documented by a previous report (Indu and Seenivasan 2013). Comparative antioxidant activities of the organic extracts of the studied marine macroalgae are represented in Figure 2.

Thiobarbituric acid reactive species inhibitory activity

Lipid oxidation in food items has a key influence on daily life as it results in distasteful flavors and disagreeable odors through the conversion of triacylglycerols and fatty acids to form oxidation products causing rancidity in foods. TBARS assay makes use of one of the dominant aldehydes, malondialdehyde, to react with thiobarbituric acid (TBA) to form a colored compound that can be measured spectrophotometrically (Zeb and Ullah 2016). Marine macroalgal extracts were found to be efficient in inhibiting MDA formation as those contain antioxidants, which interrupt the development of oxidation products in food matrices. Lipid oxidation inhibitory activity of the studied organic extracts of *S. polypodiodes* (~9 mM MDAEQ/kg) was significantly greater ($p < .05$) when compared to other species (Table 1), which could be corroborated with its potential antioxidant activities and considerable presence of phenolic compounds. Conspicuously, TBARS inhibitory activities of the studied brown algae (8.99–6.9 mM MDAEQ/kg) were considerably higher than those exhibited by the red algae (5.7–2.5 mM MDAEQ/kg), which did not demonstrate potential antioxidant properties in comparison with the former.

Anti-inflammatory activity

Among the marine macroalgae belonging to the subclass Fucophycidae, organic extracts of *T. decurrens* and *S. plagiophyllum* displayed potential attenuation properties against 5-LOX (IC_{50} 0.52 and 0.92 mg/mL, respectively) and COX-2 isoform (IC_{50} 0.65 and 0.82 mg/mL, respectively). Notably, *H. edulis* showed considerably higher activity for 5-LOX inhibition (IC_{50} 0.40 mg/mL),

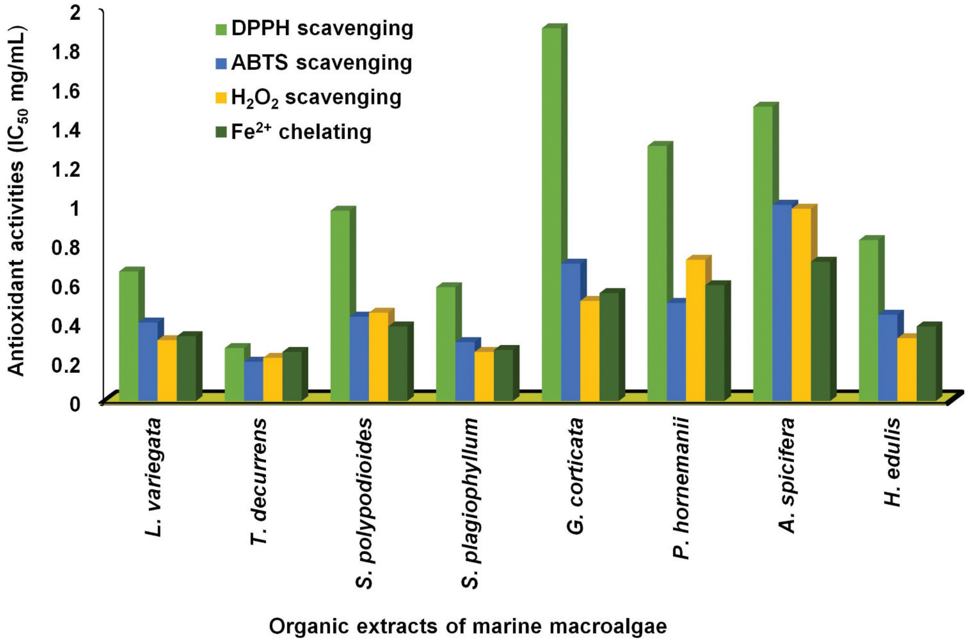

Figure 2. Graphical representation of antioxidant activities of the organic extracts of marine macroalgae.

followed by that of *G. corticata* (IC_{50} 0.65 mg/mL), among the marine macroalgae belonging to subclass Rhodymeniophycidae. The studied macroalgal species of Dictyotophycidae origin did not show prospective attenuation potential against these constitutive pro-inflammatory enzymes. 5-LOX is an enzyme involved in lipid peroxidation, a key step in the biosynthesis of leukotrienes that initiates allergic and inflammation reactions. Therefore, leukotriene inhibitors are of great interest since they can control osteoporosis, cardiovascular, and other inflammatory diseases (Gür et al. 2018). Consequently, the abilities of the organic extracts of the marine algae belonging to subclass Fucophycidae and Rhodymeniophycidae to attenuate 5-LOX enzyme involved in the biosynthesis of leukotrienes could result in recognizing potential leads to develop naturally-originated anti-inflammatory agents. Previous studies on pharmacological properties of macroalgae belonging to the phyla Ochrophyta and Rhodophyta (Antony and Chakraborty 2019; Anusree and Chakraborty 2018b) reported similar results. Preliminary examination of the aqueous extracts of *T. conoides* isolated from the Gulf of Thailand has established its anti-inflammatory activity with reference to standards phenylebutazol and acetylsalicylic acid (Boonchum et al. 2011). Notably, the inhibition ratio of COX-1 to COX-2 depicts the selective attenuation towards the inducible enzyme COX-2 than the constitutive COX-1. Conspicuously, greater attenuation (with lesser IC_{50} value) against COX-2 than COX-1 recognized the greater selectivity profile of the organic extracts of marine algae. Among various marine macroalgal extracts, *T. decurrens* exhibited considerably greater anti-inflammatory selectivity ratio (~1.4). Noticeably, the selectivity ratio for all the studied algal extracts was greater than 1, which signified that the organic extracts of marine macroalgae could be ideal candidates to develop promising pharmacophore agents.

Antidiabetic activity

Maintenance of blood glucose level has become a discerning issue in the treatment of diabetes mellitus. One of the methods to reduce glucose levels or to control the release of glucose from foods is the inhibition of carbolytic enzymes. Among the studied macroalgae, organic extracts of brown alga

T. decurrens (subclass Fucophycidae) and red alga *H. edulis* (subclass Rhodymeniophycidae) demonstrated considerably greater α-amylase and α-glucosidase attenuation potential ($IC_{50} \leq 0.6$ mg/mL) compared to other algal species as well as the synthetic inhibitor acarbose (IC_{50} 0.8 mg/mL), which has a limited efficacy due to gastrointestinal side effects. Notably, these above stated organic extracts of macroalgae also showed considerable higher attenuation activity against serine exopeptidase DPP-4 (IC_{50} 0.2–0.4 mg/mL). The antidiabetic potential of brown macroalgae was supported by a previous report (Pirian et al. 2017), even though there were insufficient descriptions on antidiabetic activities of red algae. An earlier report demonstrated that the solvent extracts of *Padina tetrastromatica* (Phaeophyceae) and *Gracilaria salicornia* (Rhodophyta) exhibited potential DPP-4 inhibition activity (Chakraborty and Antony 2019). Chakraborty and Antony (2019) reported *abeo*-oleanenes as inhibitors of starch digestive enzymes from *G. salicornia*. While mannitol derived from marine macroalgae has been used as a sweetener in food for people with diabetes, blood glucose level would increase to a lesser extent compared to sucrose, thus resulting in a relatively lower glycemic index (Qin 2018). Reports of Ali et al. (2017) put forward the antidiabetic potential of plastoquinones isolated from *Sargassum serratifolium*. Both α-amylase and α-glucosidase inhibition was found to increase with increasing concentration of algal extracts, as cited by the earlier reports of Chiasson and Rabasa-Lhoret (2004) and Pirian et al. (2017). Even though Teixeira et al. (2007) reported the antidiabetic activity of the acetone extract of *L. variegata* (IC_{50} 20 mg/mL), organic EtOAc-MeOH extract of the same demonstrated comparatively greater activity and also exhibited the presence of polyphenolic constituents in the extract. Markedly, polyphenols with multiple hydroxylated moieities could bind with these enzymes related to the glucose metabolism, thereby blocking the enzyme from binding with carbohydrates, and this might be the reason for their high antidiabetic activities. Kim et al. (2008) showed that supplementation of marine macroalgae could control blood sugar and might be effective in improving antioxidant enzyme activities and lowering blood lipids, thus reducing risk factors for cardiovascular disease in diabetic patients.

Antimicrobial activity

Among the brown marine macroalgae, *T. decurrens* (antimicrobial activity index, A.I. 2.4) showed significantly greater activity index, followed by *S. polypodioides* (A.I. 1.1), *S. plagiophyllum* (A.I. 0.8), and *L. variegata* (A.I. 1.0), in descending order against the pathogen *Escherichia coli* (Fig. S2). *P. hornemannii* registered significantly higher activity (A.I. 1.6) among red algae, followed by *H. edulis* (A.I. 1.2), *G. corticata*, and *A. spicifera* (A.I. 0.80). These results agreed with the studies of Sethi (2014) and Fatima et al. (2016), which demonstrated the antimicrobial activities of *T. conoides* and *P. hornemannii* against *Escherichia coli* and *Vibrio parahaemolyticus*, respectively. Paramsivam et al. (2016) illustrated the antimicrobial potential of aqueous extract of *G. Salicornia*, which displayed prominent activity against human pathogens. Glombitza and Große-Damhues (1985) reported the antibiotic properties of the compounds derived from macroalgal extracts, which further ascertained the potential antimicrobial activity of these marine flora. Introduction of *Sargassum wightii*, as a functional food ingredient, imparted ready-to-eat tuna jerky with improved antimicrobial quality (Hanjabam et al. 2017). Marinomed Biotech AG, Austria developed an over-the-counter drug called Carragelose® from a red algae for use against a host of respiratory infection, and it is available as nasal spray and lozenge in more than 40 countries.

Antihypertensive and antihypercholesterolemic activities

In the search for ACE-inhibitors from natural resources, marine macroalgae has shown growing potential in the field of nutraceuticals and functional food industries (Anusree and Chakraborty 2018b; Makkar and Chakraborty 2018). Angiotensin-I converting enzyme inhibition activity was notably greater for the organic extract of *H. edulis* (IC_{50} 0.51 mg/mL) than those exhibited by other algal extracts. Among brown algae, *S. plagiophyllum* (IC_{50} 0.81 mg/mL) displayed a greater attenuation potential against ACE-I. HMGCR inhibitory activities of the crude extracts of *H. edulis* (IC_{50} 1.05 mg/mL) and *S. plagiophyllum* (IC_{50} 1.18 mg/

mL) were noticeably higher than those exhibited by other marine macroalgal species (Table 1). A previous study showed that the crude extracts of *G. salicornia* displayed ACE-I inhibitory property (Antony and Chakraborty 2019). Macroalgal protein hydrolysates were reported to exhibit prospective ACE-inhibitory activity and could be used in developing functional food ingredients, which could regulate hypertension and oxidative stress (Paiva et al. 2017).

Correlation analysis

Strong positive correlation was observed among antioxidant, antidiabetic, lipid inhibition activities, and total phenolic content of organic extracts of the studied macroalgae ($R^2 = 0.884$). ABTS and DPPH scavenging activities were also positively correlated with the total phenolic content ($R^2 > 0.8$), as demonstrated by El Zokm et al. (2021) and Ismail et al. (2016). The correlation studies between polyphenolic constituents and bioactivities of the organic extracts of macroalgae were analyzed statistically using PCA. The first (PC1) and second principle components (PC2) accounted for 69.65% and 30.34% of the variance, respectively (Figure 3). The positive correlation with TPC and antioxidant, anti-diabetic, and lipid inhibition activities demonstrated that higher amount of polyphenols could result in higher bioactivities.

Spectroscopy-guided functional group fingerprinting

Spectral fingerprint analysis of characteristic functional groups in the organic extracts of the marine macroalgae were analyzed by FTIR and ^{1}H NMR spectroscopic techniques (Table 2). These analyses demonstrated the occurrence of variable functional groups that could result in potential biological activities of these macroalgal extracts (Figure 4). The FTIR spectrum of *L. variegata, S. polypodioides, S. plagiophyllum, G. corticata, P. hornemannii*, and *A. spicifera* showed broad bands around the region 3200–3600 cm^{-1} designating hydrogen bond – OH stretching or primary N-H stretching, which further attributed the presence of polyphenols or amides. The bands between 2500 and 2800 cm^{-1} of *S. polypodioides* could be attributed to the = C-H alkenic stretching vibrations corresponding to the

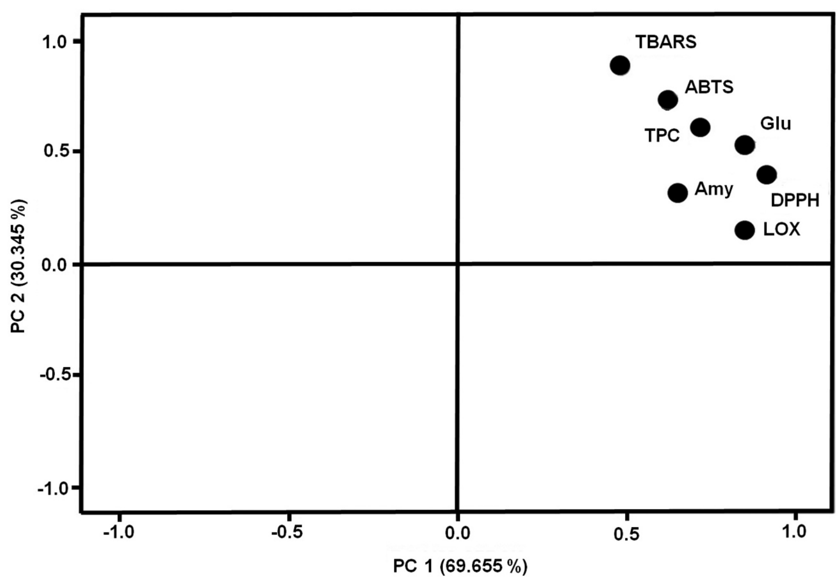

Figure 3. Correlation plot diagram (PCA 1 and PCA 2 in rotated space) representing relationships between various bioactivities (antioxidant, anti-inflammatory, antidiabetic) *vis-a-vis* total phenolic content (TPC) of solvent extracts of the studied marine macroalgal species.

Table 2. Proton integral of organic extracts of marine macroalgae.

	Proton integral (ΣH) at specified chemical shift (δ_H)					
Macroalgae	δ_H0.50–2.00[a]	δ_H2.00–2.50[b]	δ_H2.50–3.50[c]	δ_H3.50–4.50[d]	δ_H4.50–6.50[e]	δ_H6.50–8.50[f]
L. variegata	52.11	4.23	17.7	2.22	4.39	10.0
T. decurrens	138.28	18.30	24.16	2.38	19.63	62.1
S. polypodioides	11.59	3.21	7.41	1.00	4.02	33.6
S. plagiophyllum	115.47	7.35	5.00	1.49	17.4	57.0
G. corticata	23.14	2.74	13.4	1.64	4.24	11.0
P. hornemannii	40.22	2.42	6.00	1.55	9.17	52.1
A. spicifera	27.51	2.22	3.00	1.06	1.83	49.0
H. edulis	154.72	8.26	17.6	0.92	15.00	148.38

[a]Saturated hydrocarbons/non-oxygenated aliphatic groups/aliphatic acetoxy groups
[b]Alkyl alkanoates/allylic/acetyl groups ($RCH_2C(=O)OR_1$)/(CH_2 = CH-CH_3)/(RC(=O)CH_3)/aromatic acetoxy groups
[c]OCH_3/RCH_2-X/RCH_2OH
[d]Anomeric protons for polysaccharides/aliphatic region
[e]Alkanoates/olefinic ($RCH_2C(=O)$-OCH_3)/RCH = CHR_1/cyclic benzylic groups
[f]Aromatic protons (Ar-H)

signal of double bonds or electron-rich centers. Similarly, an intense band along the region 1600–1800 cm^{-1} connoted the presence of C = O groups. The specific band at 1100 cm^{-1} for the studied species could correspond to C-O stretching, and the characteristic fingerprint regions were recognized by the presence of = C-H bending (675–1000 cm^{-1}) vibrations. FTIR fingerprint analysis of the macroalgal extracts could reasonably infer the presence of a greater number of polar functional groups.

Characteristic protons occurring in the organic extracts of the studied macroalgae were scrutinized and specifically assigned using deconvoluted ^{1}H NMR spectral data in conformity with the chemical shift values and proton integrals (Figure 5, Table 2). ^{1}H NMR spectral method of deconvolution could rapidly lead to an inference of the number and types of protons that could be related to the bioactive constituents in the studied algal organic extracts. Among the eight experimental species, organic extract of *T. decurrens* displayed a higher number of integrated protons in the region between δ_H 2–2.5 (ΣH = 18.30), indicating the possible presence of alkyl alkanoates/acetyl groups compared to those observed in *S. plagiophyllum* (ΣH = 7.35), *L. variegata* (ΣH = 4.23), and *S. polypodioides* (ΣH = 3.21). Singlet peaks occurring between δ_H 2.5–3.5 might attribute to methoxy and aliphatic alcohols or halogens, which were found to be greater for *T. decurrens* (ΣH = 24.16) than those exhibited by *L. variegata* (ΣH = 17.7), *S. marginatum* (ΣH = 7.41), and *S. plagiophyllum* (ΣH = 5.00), whereas only *H. edulis* (ΣH = 17.6) registered a significantly higher proton among red algae, along this region.

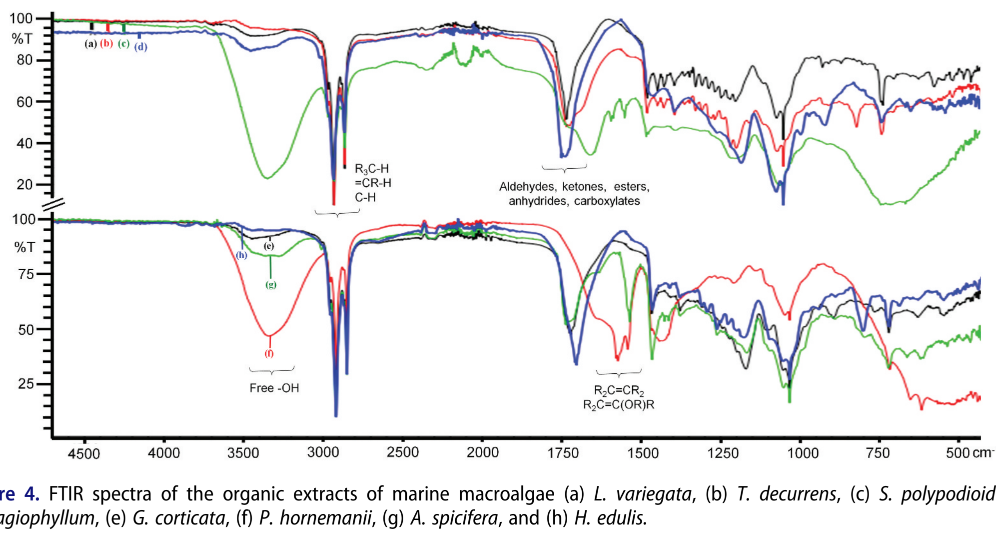

Figure 4. FTIR spectra of the organic extracts of marine macroalgae (a) *L. variegata*, (b) *T. decurrens*, (c) *S. polypodioides*, (d) *S. plagiophyllum*, (e) *G. corticata*, (f) *P. hornemanii*, (g) *A. spicifera*, and (h) *H. edulis*.

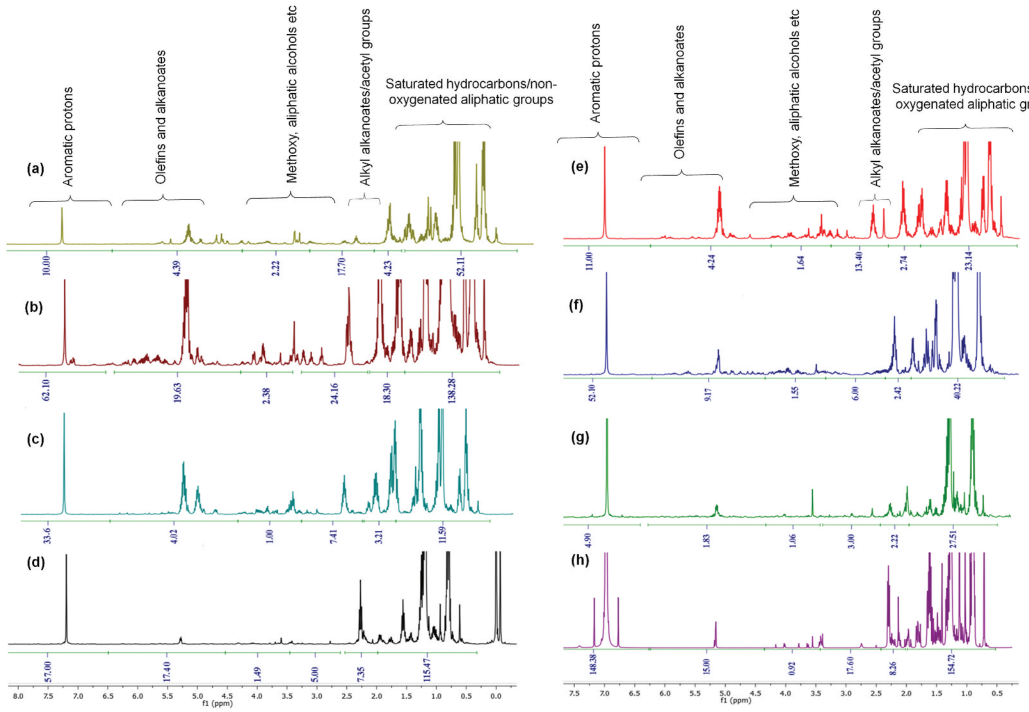

Figure 5. ^{1}H NMR spectral data representing organic extracts of marine macroalgae (a) *L. variegata*, (b) *T. decurrens*, (c) *S. polypodioides*, (d) *S. plagiophyllum*, (e) *G. corticata*, (f) *P. hornemanii*, (g) *A. spicifera*, and (h) *H. edulis*. The proton peaks at the characteristic regions were integrated, and sum of the associated protons at these regions were calculated.

Organic extract of *H. edulis* displayed ^{1}H NMR peaks at δ_H 6.0–8.5 (ΣH = 148.4), which could be related to aromatic compounds. Notably, the ^{1}H-NMR spectra of the crude extracts of *T. decurrens* and *H. edulis* acknowledged the presence of significantly higher proton integrals at δ_H 4.5–6.5 (ΣH = 19.6 and 15, respectively) that might be attributable to the presence of protons coupled with the hydride of alkyl alkanoates and olefins. Intense signals exhibited by the organic extract of *T. decurrens* along the downfield regions were ascribed to highly electronegative functionalities, which could be responsible for imparting its greater bioactivities. The proton integrals in the downfield section of the ^{1}H NMR spectra (δ_H 3–7) were feeble for *P. hornemannii, L. variegata*, and *A. spicifera*, wherein comparatively lesser biological activities of the organic extracts of these marine algae suggest that these functional groups could be responsible for the studied medicinal properties (Figure 5). On the other hand, the organic extracts of *T. decurrens* displayed greater proton integrals in the deshielded region of the ^{1}H NMR spectrum, which might be associated with the higher bioactivities of the organic extract of this species. Presence of electronegative groups in the organic extracts of *T. decurrens, H. edulis*, and *S. plagiophyllum* might recognize the prominent medicinal properties as also substantiated by *in vitro* bioactivity assessments. Considerable co-linearity was perceived among the electronegative groups positioned in the downfield regions of ^{1}H NMR spectra *vis-a-vis* pharmacological properties of the organic extracts of the studied macroalgae.

Conclusions

Marine macroalgae are every so often considered as wonder biota of the ocean. They are unique marine living resources with wide-ranging ecological connotation and economic significance and offer an enormous prospect for the blue economy. A total of eight marine algae belonging to the subclasses of Fucophycidae, Dictyotophycidae, and Rhodymeniophycidae were assessed for *in vitro* antioxidant, anti-inflammatory, antidiabetic, anti-hypercholesterolemic, antihypertensive, and antimicrobial activities. Among the studied species, those belonging to the subclasses Fucophycidae and Rhodymeniophycidae exhibited noticeably greater pharmacological potential. Particularly, the organic extracts of *T. decurrens, H. edulis*, and *S. plagiophyllum* disclosed significantly greater medicinal properties than those originated from other studied marine algae. The present study further demonstrated that spectral fingerprinting could rapidly comprehend the probable structural classes implicated in the organic extracts of the studied species and their correlation to depict bioactive potential. Substantial positive correlation between the phenolic content with antioxidant properties recognized that phenolic compounds were responsible for potential bioactivities. The present study acknowledged the utilities of marine macroalgae *T. decurrens, H. edulis*, and *S. plagiophyllum* as promising biological resources to develop pharmaceutical agents and functional foods.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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