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Research Article

GC–MS-BASED PHYTOCHEMICAL PROFILING AND ANTIOXIDANT EVALUATION OF SPATOGLOSSUM SOLIERI

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ABSTRACT

Objectives: The objective of this study was to analyze the phytochemical content of brown alga *Spatoglossum solieri* and evaluate its ability to scavenge free radicals.

Methods: The phytochemical screening of the thallus was performed using different solvent extracts (Aqueous, methanol, ethanol, ethyl acetate, benzene, and petroleum ether). The total phenolic and flavonoid content was estimated, and the phytoconstituents were analyzed using gas chromatography-mass spectrometry (GC-MS). The *in vitro* antioxidant assays, that is, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging, hydroxyl radical scavenging, superoxide radical scavenging, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging, and reducing power were performed.

Results: Phytochemical investigations revealed that methanol and ethanol extracts were rich with a wide range of secondary metabolites such as coumarins, catechins, flavonoids, phenols, saponins, glycosides, tannins, terpenoids, sugars, steroids, and xanthoproteins. The total phenolic and flavonoid content exhibited 62.77 mg GAE/g and 41.74 mg GAE/g, respectively. GC-MS analysis identified 13 unique phytocompounds represented by 15 chromatographic peaks. The major phytocompound includes Neophytadiene, Hexadeconic acid, ethyl ester, Ethyl oleate, Paromomycin, 1-Methyl-4[nitromethy]-4-piperidinol, 8-Ketocopaenal, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4, 3-H-Cyclodeca[b]-furan-2-one, Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl) ethyl ester, and 1,1-Cyclobutanedicarboxylic acid. Methanol extract demonstrated the highest DPPH, hydroxyl, and ABT radical scavenging capacity in terms of *in vitro* antioxidant activity. The extract with the highest superoxide radical scavenging ability was ethanol. Similarly strongest reducing power was demonstrated by the methanol extract.

Conclusion: The high antioxidant activity of *S. solieri* is likely due to its rich phenolic and flavonoid content, as identified through GC-MS and strong performance across multiple *in vitro* radical scavenging assays. In conclusion, there is a great deal of need for additional research into *S. solieri* active principle to pinpoint more of its pharmacological characteristics.

Keywords: *Spatoglossum solieri,* Phytochemicals, Gas chromatography-mass spectrometry, Secondary metabolites, and *in vitro* antioxidant.

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INTRODUCTION

Seaweeds, or marine macroalgae, are ecologically and commercially important resources that serve as a rich reservoir of bioactive compounds [1]. They contain polysaccharides, polyunsaturated fatty acids, phenolics, flavonoids, sterols, terpenoids, and essential vitamins and minerals, many of which have applications in pharmaceuticals, nutraceuticals, and cosmetics [2,3]. Their abundance of secondary metabolites positions seaweeds as promising candidates for the discovery of novel therapeutic agents [4].

Phytochemicals, also known as secondary metabolites, are produced by plants and algae as part of their defense mechanisms against environmental stressors. These compounds play a significant role in promoting health and are beneficial in treating various disorders through their individual effects or through additive and synergistic actions [5,6]. These compounds – including phenolics, flavonoids, alkaloids, tannins, and terpenoids – exhibit diverse biological activities such as antimicrobial, anti-inflammatory, anticancer, and especially antioxidant effects [7-9]. The process of drug discovery often begins with identifying active compounds specifically from marine organisms [10]. In this context, screening plant extracts, mainly seaweeds, has become an effective strategy for uncovering bioactive substances across different plant species [11].

During regular metabolism, reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radicals, and superoxide anions are constantly produced. When ROS levels are high, they induce oxidative stress, which results in lipid peroxidation, DNA damage, protein denaturation, and the development of chronic illnesses such as diabetes, cancer, cardiovascular disease, and neurodegenerative diseases [12]. By scavenging free radicals and preserving redox balance, antioxidants are essential in reducing these negative effects [13].

Seaweeds are especially beneficial in this regard as natural antioxidant sources. Their phytochemical components, particularly flavonoids and phenolics, serve as effective metal chelators and radical scavengers, shielding cells from oxidative stress [14]. Seaweed-derived bioactives are thought to be safer and more environmentally friendly than synthetic antioxidants, which are linked to possible health hazards.

The remarkable quantity of bioactive compounds found in brown algae is known to have a wide range of medicinal and nutritional applications [15-17]. Based on the current understanding of the genus *Spatoglossum*, and specifically *Spatoglossum asperum* [18,19], our study focuses on *Spatoglossum solieri* to determine whether it shares and possibly exceeds these traits, contributing new species-specific information to the genus profile.

Ecological research on the species and reproductive analyses of *S. solieri* has been studied, but not the phytochemical composition and antioxidant potential [20]. Despite known antioxidant activity in *S. asperum*, *S. solieri* remains underexplored, making it a candidate for comparative and novel bioactivity studies. Hence, the present study aimed to (i) characterize the phytochemical composition of *S. solieri*, (ii) identify major metabolites through gas chromatography-mass spectrometry (GC-MS) analysis, and (iii) evaluate its antioxidant potential using multiple radical scavenging assays.

METHODS

Collection and preparation of *S. solieri*

Algal samples were collected from the Rameshwaram shoreline of Tamil Nadu in India (Lat. 09 17.417'N; Long. 079 08.558'E) and were authenticated at the Marine Algal Research Station located at Mandapam in Tamil Nadu. The samples were meticulously cleaned to remove dirt and epiphytes, then chopped and allowed to dry in the shade until they reached a constant weight. The dry material was made into a fine powder using a mechanical grinder and stored in sealed containers until extraction was required.

Seaweed crude extraction

Solvents with increasing polarity were used to extract the powdered seaweed material one step at a time (Aqueous, methanol, ethanol, ethyl acetate, benzene, and petroleum ether). After filtering and condensing each extract under lower pressure, the dried residues were kept for later examination at 4°C.

Phytochemicals screening

The distinct phytochemicals contents found in the various solvent extracts of *S. solieri* were screened out using the methods outlined by Brinda *et al*. [21] and Sathish Kumar *et al*. [9].

Estimation of total phenolics

The Folin-Ciocalteu reagent was used to measure the extract's total phenolic content (TPC), slightly altering the Farasat *et al*. [22] method. To put it briefly, 20 µL of extracts were combined with 100 µL of 1:10 Folin-Ciocalteu reagent, and then 80 µL of 7.5% Na_2CO_3 was added. The test was conducted in a microplate. Following 2 h of incubation in the dark, the absorbance was taken at 600 nm. The standard reference was gallic acid. Gallic acid equivalents (mg GAE/g DW) were used forexpressing TPC.

Estimation of flavonoids

A 96-well microplate was utilized to determine flavonoid using the Bouzenad *et al*. [23] approach, with some changes. A solution of 50 µL of the extracted material, 130 µL of MeOH, 10 µL of potassium acetate (CH3COOK), and 10 µL of aluminum nitrate ($Al(NO_3)_2$, $9H_2O$) is added, and the mixture is incubated for 40 min at room temperature. We measured the absorbance at 415 nm. Algal extract (50 µL) and methane (150 µL) were combined, with =quercetin as a reference, to create a blank sample. The values are provided as quercetin (mg QE/g DW) in compliance with the quercetin calibration curve.

GC-MS analysis

GC-MS analysis was carried on ethanolic extracts by the method described by Murugan *et al*. [24] using a Hewlett-Packard 6890/5973 GC-MS running at 1000 eV ionization energy and outfitted with an Agilent 7890A/5975 C GC HP-5. Helium (He, 0.9 mL/min) was employed as the carrier gas in a capillary column (phenylmethyl siloxane, 25 m × 0.25 mm i.d.) with a split ratio of 1:5. The oven's temperature ranged from 80°C (2 min) to 280°C at a rate of 1–40°C/min, the detector's temperature ranged from 250 to 280°C. with a mass scan of 50–600 amu. The relative percentage of each extract constituent was represented as a percentage using peak area normalization, and the GC-MS runs for a total of 50 min.

GC-MS mass spectrum interpretation was performed utilising the Wiley Library and the National Institute of Standards and Technology (NIST) database, having more than 62000 patterns. The mass spectrum of the unknown component was contrasted with the NIST library's collection of recognized components. The components of the test materials were identified by name, molecular weight (MW), and structure.

Antioxidant assays

The antioxidant activity of *S. solieri* extracts (Aqueous, methanol, ethanol, ethyl acetate, benzene, and petroleum ether) was evaluated using five complementary *in vitro* assays to assess their free radical scavenging and reducing potential with concentration ranges such as 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL for all antioxidant assays.

2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant extracts were determined using the DPPH test. As stated by Azizi *et al.* [25], the test was carried out using a 96-well plate. In short, 100 µL of DPPH solution (761 µM DPPH in 80% methanol) was applied to each well of the 96-well plate containing 10 µL of the previously produced sample extract. The combination was allowed to remain at room temperature in a dark environment for 2 h. A microplate spectrophotometer was used to detect the absorbance at 515 nm. The proportion of DPPH free radical scavenging ability was determined by means of the below formula.

$$\text{DPPH scavenging effect (\% inhibition)} = [(A_0 - As)/A_0) \times 100]$$

Where, A_0=absorbance of the DPPH; As=absorbance of the sample.

Hydroxyl radical scavenging

In accordance with Narasimhan *et al*. [26] procedure, 0.6 mL of a 40 mM H_2O_2 solution made in phosphate buffer (7.4) was added to the extract (100 µg/mL). After 10 min of incubation at 37°C, absorbance at 230 nm was measured. For the equivalent blank solutions, phosphate buffer was utilized. The extract and distilled water employed as a control. However, ascorbic acid was employed as a reference. An increase in the scavenging of free radicals was evidenced by a decrease in absorbance. Scavenging activity was calculated as a percentage.

Superoxide radical scavenging

Following Zhang *et al*. [27] method, 10 µL extract along with 10 µL 1,2,3-trihydroxybenzene (THDB) with 3 mmol/L were constantly mixed with 150 µL of Tris-HCl (50 mM, pH = 8.2). To halt the process, Vitamin C (10 µL, 5 mM) was added after 3 min (A0). 150 µL Tris-HCl was mixed constantly to a 10 µL sample that contained Vitamin C and also THDB to create suspension A1. For 3 min, 150 µL of Tris-HCl and 10 µL of THDB were combined to form solution A2. The reaction was then stopped by adding 10 µL of VC and a blank sample solution. 150 µL Tris-HCl was continually mixed with vitamin C, THDB, and a 10 µL blank sample solution to create solution A3.

$$\text{Scavenging (\%)} = [(A0 - A1)/(A2 - A3)] \times 100\%$$

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

ABTS was dissolved in deionized water to a concentration of 7 mM, and potassium persulfate ($K_2S_2O_8$) was added to a concentration of 2.45 mM, as stated by Chakraborty *et al*., [28]. The reaction mixture was not used until it had stood at room temperature for 12–16 h in the dark. After being diluted with MeOH, the resulting brightly colored ABTS had an absorbance of about 0.70 at 734 nm. 5 mL of the aforementioned ABTS solution was combined with 0.1 mL of seaweed extracts to measure the ABTS scavenging activity. The final absorbance was determined using a ultraviolet-Vis spectrophotometer at 743 nm. The formula used to get the percentage of scavenging was ABTS scavenging activity (percent)=((A0−A1)/A0)×100, where A0 is the absorbance, and A1 is the absorbance of the sample.

Reducing power

The methods of Hejna *et al*. [29] were used to measure the ferric iron reduction capability of algal powders. In short, equal parts of the test

sample, 1% potassium ferricyanide, and 2 M phosphate-buffered saline solution were completely combined. The prepared ascorbic acid served as the reference sample. A measurement of the optical density was made at 540 nm. Increased reducing power is correlated with increased absorption. The equivalent of ascorbic acid was used to compute the ferric reducing capacity.

Statistical analysis

All experiments were conducted in triplicate (n=3), data are expressed as mean±standard deviation, and statistical significance was assessed using one-way analysis of variance followed by Tukey's *post hoc* test ($p<0.05$).

RESULTS

Phytochemical screening

Table 1 lists the various types of bioactive chemicals that were found in *S. solieri* extracts derived from various solvents after preliminary screening of phytochemicals. The richest phytochemical profile was found in the methanol and ethanol extracts, testing positive for coumarins, catechins, flavonoids, phenols, glycosides, saponins, terpenoids, tannins, steroids, sugars, and xanthoproteins. Eleven phytoconstituents were detected in the ethyl acetate extract, whereas seven were detected in the aqueous extract. Benzene and petroleum ether extracts displayed relatively less phytochemicals. Since phenolics, flavonoids, tannins, and terpenoids are renowned for their antibacterial, anti-inflammatory, and radical scavenging properties, their preponderance indicates that *S. solieri* may have significant antioxidant and therapeutic potential.

Total phenolic and flavonoid content

The TPC was calculated as mg GAE/g DW, whereas the total flavonoid content was calculated as mg QE/g DW, GAE and QE are gallic acid and quercetin, respectively. The methanol extract of *S. solieri* contained 41.74 mg QE/g total flavonoids and 62.72 mg GAE/g total phenolics, according to quantitative analysis. The results unambiguously demonstrate that the methanol extract of *S. solieri* has a significant quantity of total phenolic and flavonoid content.

GC–MS analysis

The ethanol extract of S. solieri was subjected to GC-MS analysis, which revealed the presence of 15 distinct peaks corresponding to 13 different phytocompounds (Table 2 and Fig. 1). These compounds were recognized on the basis of their retention times, MWs, molecular formula, and comparison with the NIST database. Among the detected metabolites, neophytadiene was the most abundant (40.87%), followed by hexadecanoic acid ethyl ester (12.16%), hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester (6.74%), ethyl oleate (6.36%), and 8-ketocopaenal (5.57%). Other notable compounds included paromomycin, 1-methyl-4-(nitromethyl) piperidin-4-ol, 1-dodecen-3-ol, bosartol-F, dichloroacetic acid tetradecyl ester, and 4,8,12,16-tetramethylheptadecan-4-olide.

Antioxidant activity

DPPH

DPPH radical scavenging activity was dose-dependent in all solvent extracts of *S. solieri* (Table 3 and Fig. 2). The greatest inhibition was exhibited by benzene extract, which reached 93.16% at 800 µg/mL. Petroleum ether and ethyl acetate came in second and third, respectively, with 84.36% and 80.24%. Extracts of methanol and ethanol were moderately active, and at the maximum concentration, all three of the most active extracts outperformed the reference (ascorbic acid). As promising natural radical scavengers, these results highlight the significant antioxidant potential of *S. solieri* extracts in benzene, petroleum ether, and ethyl acetate.

Hydroxyl radical scavenging

The hydroxyl radical scavenging ability of *S. solieri* was demonstrated by all solvent extracts (Table 4 and Fig. 3). Petroleum ether (84.34%) and ethyl acetate (78.16%) were the next two substances that showed the largest effect, with the benzene extract achieving 98.13% inhibition at 800 µg/mL. Extracts of both methanol as well as ethanol showed considerable inhibition (53.16% and 61.22%), and at the highest test dose, all three of the best extracts performed better than ascorbic acid (69.33%). According to these findings, *S. solieri* extracts in benzene, petroleum ether, and ethyl acetate exhibit strong hydroxyl radical scavenging properties and show promise as natural antioxidant candidates.

Superoxide radical scavenging

All the solvent extracts of *S. solieri* showed the dose-dependent superoxide radical scavenging activity (Table 5, Fig. 4). Benzene and petroleum ether extracts were the most effective with 94.31% and 90.31% inhibition at 800 µg/mL. Ethyl acetate also showed substantial activity with 84.16% at the higher concentration. Methanol and ethanol extracts were moderately active. Results show that benzene and petroleum ether extracts of *S. solieri* have potent superoxide scavenging properties.

ABTS

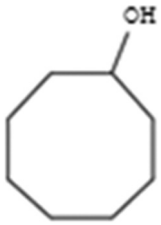
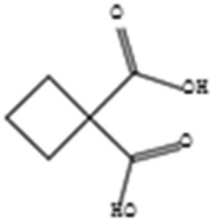
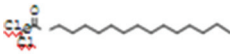
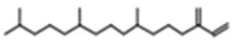
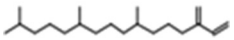
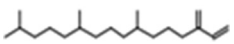
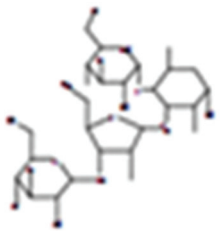
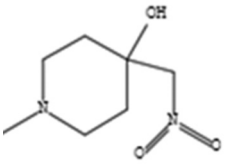
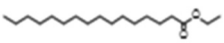
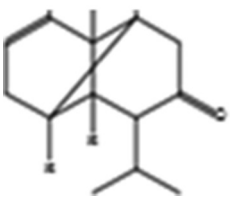
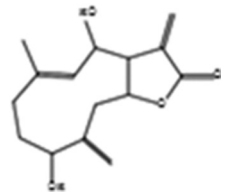
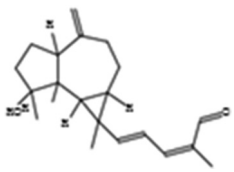
All *S. solieri* extracts showed dose-dependent ABTS scavenging activity (Table 6 and Fig. 5). Benzene (93.26%) and petroleum ether (89.31%) extracts demonstrated the highest inhibition at 800 µg/mL concentration. Ethyl acetate extract also showed considerable activity (76.22%), while methanol and ethanol extracts revealed modest scavenging effects (66.31% and 60.22%). The standard antioxidant, ascorbic acid, achieved 70.16% inhibition. These findings confirm that *S. solieri* possesses excellent ABTS scavenging potential.

Table 1: Preliminary phytochemical screening of *Spatoglossum solieri*+present; – absent

Bioactive compounds	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	Aqueous
Alkaloids	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–
Catechins	+	–	+	+	+	+
Coumarins	–	+	+	+	+	–
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	–
Phenols	+	+	+	+	+	+
Quinones	–	–	–	–	–	–
Saponins	+	+	+	+	+	+
Steroids	–	–	–	+	+	–
Sugars	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	–	+	+	+	+	–
Xanthoproteins	+	+	+	+	+	+

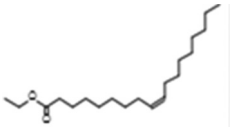
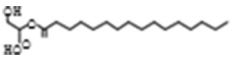
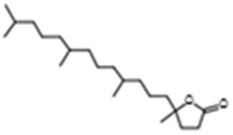
(+): Indicates presence, (–): Indicates absence

Table 2: Phytochemicals identified using gas chromatography – mass spectrometry chromatogram of ethanol extract of *Spatoglossum solieri*

Peak No	Peak retention time (min)	Peak area %	Compound name	Molecular formula	Molecular weight	Structure
1	16.54	3.30	1-Dodecen-3-o	$C_8H_{16}O$	128	
2	18.05	3.70	1,1-Cyclobutanedicarboxylic acid	$C_6H_8O_4$	144	
3	18.92	3.13	Chloroacetic acid, 2-tetradecyl ester	$C_{16}H_{30}C_{l2}O_2$	324	
4	20.51	17.3	Neophytadiene	$C_{20}H_{38}$	278	
5	20.78	10.36	Neophytadiene	$C_{20}H_{38}$	278	
6	20.99	13.44	Neophytadiene	$C_{20}H_{38}$	278	
7	20.66	3.03	Paromomycin	$C_{23}H_{45}N_5O_{14}$	615	
8	20.68	4.52	1-Methyl-4-(nitromethyl) *piperidin*-4-ol	$C_7H_{14}N_2O_3$	174	
9	22.21	12.16	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284	
10	22.30	5.57	8-Ketocopaenal	$C_{15}H_{20}O_2$	232	
11	23.13	4.14	3H-Cyclodeca[b] furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a, 4	$C_{15}H_{20}O_4$	264	
12	23.72	2.94	Bosartol-F	$C_{20}H_{28}O_2$	300	

(*Contd...*)

Table 2: (*Continued*)

Peak No	Peak retention time (min)	Peak area %	Compound name	Molecular formula	Molecular weight	Structure
13	23.93	6.36	Ethyl oleate	$C_{20}H_{38}O_2$	310	
14	24.18	6.74	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	
15	25.72	3.02	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	

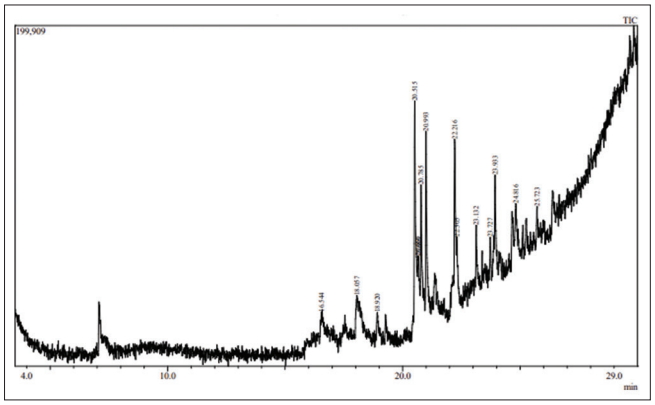


Fig. 1: Gas chromatography-mass spectrometry chromatogram of ethanol extract of *Spatoglossum solieri*

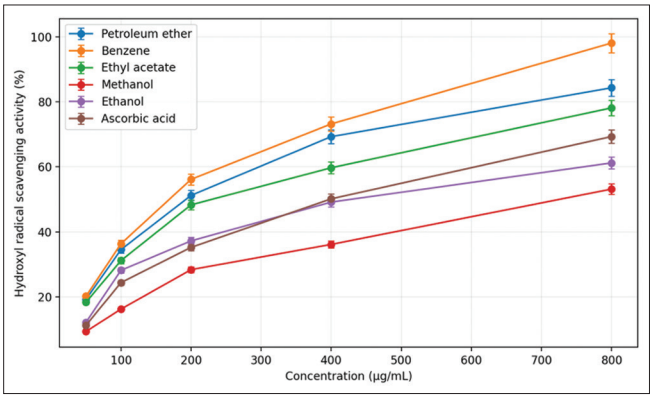


Fig. 3: Hydroxyl radical scavenging activity of *Spatoglossum solieri* extracts

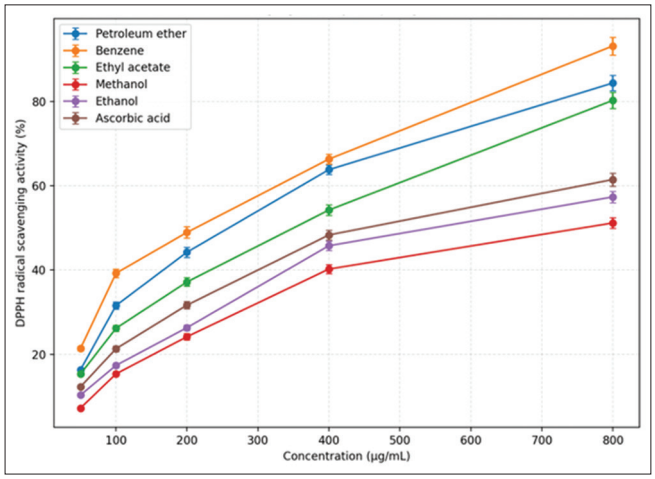


Fig. 2: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of *Spatoglossum solieri* extracts

Reducing power

All solvent systems under evaluation showed an increase in the reducing power activity of *S. solieri* extracts that was concentration-dependent (Table 7 and Fig. 6). Methanol outperformed the standard at 800 µg/mL, demonstrating the highest reducing power among the extracts. Benzene and ethyl acetate had the most activity, followed by ethanol extract. For lowering activity, petroleum ether extract was the least effective. These findings imply both methanol as well as ethanol *S. solieri* extracts have a considerable ability to donate electrons.

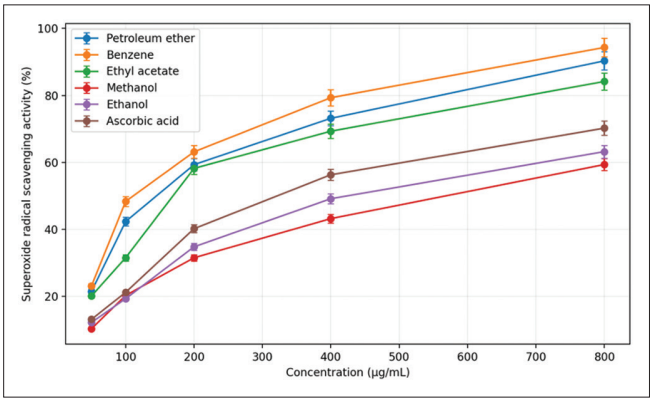


Fig. 4: Superoxide radical scavenging activity of *Spatoglossum solieri* extracts

DISCUSSION

The current study is the first to conduct a complete examination of the phytochemical content, GC-MS characterization, and antioxidant activity of the brown algae *S. solieri*. The findings revealed that methanol and ethanol extracts contained a diverse range of secondary metabolites, including flavonoids, phenolics, tannins, terpenoids, and steroids, all of which are renowned for significant antioxidant activity contributions by donating hydrogen to a free radical to scavenge potential damage, among other pharmacological activities.

Phytochemicals and antioxidant relevance

Phenolics are essential chemicals because of their scavenging characteristics caused by their hydroxyl groups. Phenolic and

Table 3. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (%) of *Spatoglossum solieri*

Extract	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL
Petroleum ether	16.31±0.42^{c}	31.56±0.88^{c}	44.22±1.21^{c}	63.81±1.09^{b}	84.36±1.84^{b}
Benzene	21.36±0.51^{b}	39.22±1.04^{b}	48.92±1.33^{b}	66.31±1.16^{b}	93.16±2.11^{a}
Ethyl acetate	15.31±0.39^{c}	26.16±0.71^{c}	37.16±1.02^{c}	54.23±1.24^{c}	80.24±1.93^{b}
Methanol	7.22±0.18^{f}	15.34±0.44^{f}	24.16±0.78^{f}	40.22±1.06^{e}	51.16±1.21^{e}
Ethanol	10.31±0.26^{e}	17.36±0.53^{e}	26.31±0.69^{e}	45.76±1.11^{d}	57.33±1.36^{d}
Ascorbic acid	12.27±0.31^{d}	21.31±0.64^{d}	31.66±0.88^{d}	48.31±1.14^{d}	61.46±1.52^{c}

Values are expressed as Mean±standard deviation, n=3, as an analysis of variance test p<0.05% level. Means in each column with superscripts (s) are significant different (p<0.05)

Table 4: Hydroxyl radical scavenging activity (%) of *Spatoglossum solieri*

Extract	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL
Petroleum ether	19.22±0.58^{b}	34.54±1.04^{b}	51.22±1.54^{b}	69.31±2.08^{b}	84.34±2.53^{b}
Benzene	20.16±0.61^{a}	36.34±1.09^{a}	56.16±1.68^{a}	73.22±2.20^{a}	98.13±2.94^{a}
Ethyl acetate	18.31±0.55^{c}	31.16±0.93^{c}	48.31±1.45^{c}	59.73±1.79^{c}	78.16±2.34^{c}
Methanol	9.31±0.28^{f}	16.22±0.49^{f}	28.36±0.85^{f}	36.13±1.08^{f}	53.16±1.59^{f}
Ethanol	12.16±0.36^{e}	28.16±0.84^{e}	37.22±1.12^{e}	49.16±1.47^{e}	61.22±1.84^{e}
Ascorbic acid	11.22±0.34^{d}	24.38±0.73^{d}	35.22±1.06^{d}	50.16±1.50^{d}	69.33±2.08^{d}

Values are expressed as Mean±standard deviation, n=3, as an analysis of variance test p<0.05% level. Means in each column with superscripts (s) are significant different (p<0.05)

Table 5: Superoxide radical scavenging activity (%) of *Spatoglossum solieri*

Extract	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL
Petroleum ether	21.51±0.65^{b}	42.36±1.27^{b}	59.33±1.78^{b}	73.16±2.19^{b}	90.31±2.71^{b}
Benzene	23.16±0.69^{a}	48.36±1.45^{a}	63.16±1.89^{a}	79.31±2.38^{a}	94.31±2.83^{a}
Ethyl acetate	20.13±0.60^{c}	31.56±0.95^{c}	58.22±1.75^{c}	69.31±2.08^{c}	84.16±2.52^{c}
Methanol	10.36±0.31^{f}	20.30±0.61^{f}	31.54±0.95^{f}	43.22±1.30^{f}	59.36±1.78^{f}
Ethanol	12.24±0.37^{e}	19.33±0.58^{e}	34.81±1.04^{e}	49.16±1.47^{e}	63.22±1.90^{e}
Ascorbic acid	13.16±0.39^{d}	21.22±0.64^{d}	40.22±1.21^{d}	56.31±1.69^{d}	70.22±2.11^{d}

Values are expressed as Mean±standard deviation, n=3, as an analysis of variance test p<0.05% level. Means in each column with superscripts (s) are significant different (p<0.05)

Table 6: 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (%) of *Spatoglossum solieri*

Extract	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL
Petroleum ether	18.22±0.55^{b}	31.34±0.94^{b}	46.27±1.39^{b}	58.54±1.76^{b}	89.31±2.68^{b}
Benzene	21.16±0.63^{a}	33.16±0.99^{a}	49.31±1.48^{a}	63.27±1.90^{a}	93.26±2.80^{a}
Ethyl acetate	15.22±0.46^{c}	30.26±0.91^{c}	44.16±1.32^{c}	53.19±1.60^{c}	76.22±2.29^{c}
Methanol	11.86±0.36^{e}	22.36±0.67^{e}	31.56±0.95^{e}	45.22±1.36^{e}	66.31±1.99^{e}
Ethanol	10.31±0.31^{f}	19.96±0.60^{f}	26.13±0.78^{f}	42.31±1.27^{f}	60.22±1.81^{f}
Ascorbic acid	12.22±0.37^{d}	24.31±0.73^{d}	36.11±1.08^{d}	51.84±1.56^{d}	70.16±2.10^{d}

Values are expressed as Mean±standard deviation, n=3, as an analysis of variance test p<0.05% level. Means in each column with superscripts (s) are significant different (p<0.05)

Table 7: Reducing power of *Spatoglossum solieri*

Extract	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	800 µg/mL
Petroleum ether	0.224±0.007^{f}	0.248±0.007^{f}	0.284±0.009^{f}	0.312±0.009^{f}	0.341±0.010^{f}
Benzene	0.264±0.008^{c}	0.289±0.009^{c}	0.331±0.010^{c}	0.354±0.011^{c}	0.383±0.011^{c}
Ethyl acetate	0.224±0.007^{d}	0.273±0.008^{d}	0.318±0.010^{d}	0.351±0.011^{d}	0.388±0.012^{d}
Methanol	0.305±0.009^{a}	0.343±0.010^{a}	0.381±0.011^{a}	0.421±0.013^{a}	0.468±0.014^{a}
Ethanol	0.284±0.009^{b}	0.345±0.010^{b}	0.395±0.012^{b}	0.418±0.013^{b}	0.441±0.013^{b}
Ascorbic acid	0.281±0.008^{e}	0.324±0.010^{e}	0.359±0.011^{e}	0.388±0.012^{e}	0.429±0.013^{e}

Values are expressed as Mean±standard deviation, n=3, as an analysis of variance test p<0.05% level. Means in each column with superscripts (s) are significant different (p<0.05)

flavonoid molecules are good natural antioxidants for their capacity to donate hydrogen atoms or electrons, bind metal ions, and inhibit lipid peroxidation [30]. It is reported that the polyphenols can reduce free radicals by activating antioxidant enzymes thereby eliminating α-tocopherol radicals, and inhibit oxidases [31]. Compared to previous studies, *S. solieri* has higher phenolic (62.72 mg GAE/g) and flavonoid (41.74 mg QE/g) content than *Turbinaria ornata* (14.14 mg GAE/g; 9.98 mg QE/g) [32] and *Colpomenia sinuosa* (56.45 mg GAE/g; 12.13 mg QE/g) [33]. These findings indicate that *S. solieri* may be a richer natural source of phenolic antioxidants than many other brown algae. Given

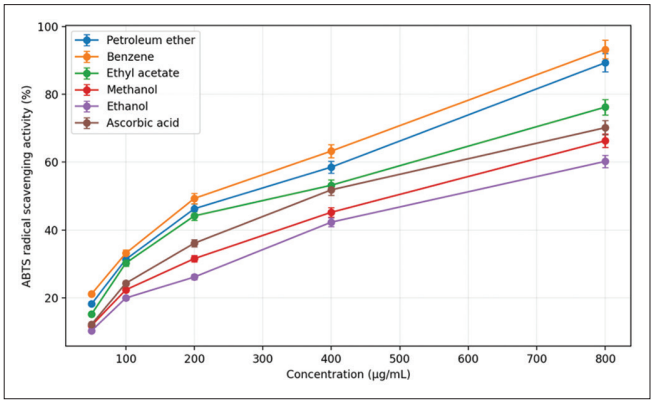


Fig. 5: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity of *Spatoglossum solieri* extracts

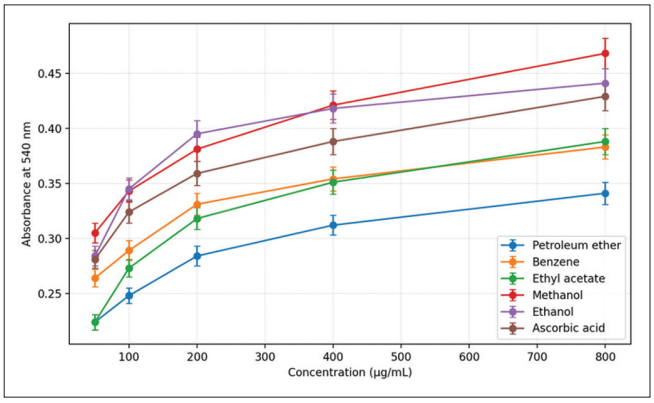


Fig. 6: Reducing power activity of *Spatoglossum solieri* extracts

its superior phenolic content in comparison to other brown algae like *T. ornata*, *S. solieri* could be marketed as a high-potency component for anti-aging [34].

GC-MS identified metabolites

The GC-MS study revealed 15 bioactive chemicals, of which neophytadiene was dominant. Neophytadiene is reported to have anti-inflammatory, antibacterial, and antioxidant effects [35]. Similarly, hexadecanoic acid ethyl ester is well-known for its anticancer, hepatoprotective, and anti-inflammatory properties [36]. Ethyl oleate has been reported antibacterial and antioxidant properties [37]. Other substances, such as 8-ketocopaenal and paromomycin, provide antifungal and antibacterial properties [38,39]. The pharmacological diversity of these metabolites highlights *S. solieri* therapeutic potential and shows that extracts may work synergistically through numerous bioactive pathways.

Antioxidant assays

The antioxidant capabilities of *S. solieri* extracts were thoroughly investigated using DPPH, ABTS, superoxide, hydroxyl radical scavenging, and reducing power assays. In reducing power assays, the reducing ability is commonly related with the presence of reductones, which donate a hydrogen atom to break the free radical chain. A compound's reducing capacity can be a good indication of its antioxidant potential [40]. In all studies, solvent extracts demonstrated strong dose-dependent effects, with benzene, petroleum ether, and ethyl acetate often demonstrating the highest inhibition rates, frequently outperforming the reference ascorbic acid at maximal doses. Methanol and ethanol extracts both displayed moderate action, with methanol showing particularly strong results in decreasing power tests. The total phenolics and flavonoids concentrations are consistent with the antioxidant activity of *S. solieri* extracts. Many of the radical scavenging methods studied *in vitro* are supported by phenolic and flavonoids compound, which have excellent capacity to donate electrons and neutralize ROS. The benzene, petroleum ether, and ethyl acetate extracts of *S. solieri* demonstrated superior radical inhibition, indicating that these bioactives were successfully extracted. Based on these quantitative results, *S. solieri* is in the upper range for brown macroalgae (62.72 mg GAE/g phenolics and 41.74 mg QE/g flavonoids), which is consistent with previous research that found a strong correlation between increased antioxidant properties and higher phenolic and flavonoid concentrations [41,42]. This validates the observed hierarchy in extract activity and supports the mechanistic interpretation of the chemical assay data. The current findings support *S. solieri* high antioxidant potency, establishing its nonpolar extracts as extraordinary radical scavengers capable of competing with traditional seafood "functional meals" and well-known nutraceuticals. Although methanol extracts showed higher phenolic content, nonpolar solvents such as benzene and petroleum ether may preferentially extract lipophilic antioxidants, including terpenoids, fatty acid esters, and hydrocarbons, which are effective against specific radical systems. These compounds likely contribute to the enhanced scavenging observed in nonpolar extracts. This is consistent with previous research demonstrating that sulfated polysaccharides, carotenoids, and macroalgal phlorotannins not only have a high scavenging ability but also aid in the prevention of oxidative stress-related illnesses. Despite their strong antioxidant activity, the use of benzene and petroleum ether raises toxicity concerns. Therefore, future studies should focus on replacing these solvents with food-grade alternatives such as ethanol or ethyl acetate while maintaining extraction efficiency. According to current advances in algae drug development, future research should focus on extraction optimization utilising safe solvents, extensive chemical identification, and efficacy validation in cellular and animal models for pragmatic and regulatory reasons [41-43]. *S. solieri* has higher antioxidant activity than *T. ornata* [44], *Sargassum* and *Polycladia* [42,45], and *S. asperum* [15], according to prior studies. *S. solieri* extract demonstrated a higher DPPH scavenging activity than *Hypnea musciformis* [46] and *Sarconema scinaioides* [32].

These findings provide significant evidence for *S. solieri* potential as a viable natural antioxidant candidate. Natural alternatives produced from marine resources are of great interest due to widespread concern about the safety of synthetic antioxidants [12]. *S. solieri* phytochemical richness, wide spectrum of pharmacological metabolites, and significant antioxidant activity indicate its prospective for their usage in the food, pharmaceutical, and nutraceutical productions.

CONCLUSION

This study is the first to report GC-MS-based phytochemical profiling and multi-assay antioxidant evaluation of *S. solieri* demonstrates remarkable antioxidant activity. This study reveals its potential as a source of natural antioxidants. This implies that there are a lot of strong nonpolar antioxidant compounds in the algae. Although the impressive results of petroleum ether and benzene extracts in various radical scavenging tests are noteworthy, they also draw attention to a significant problem because of toxicity issues. Characterizing the active ingredients, maximizing extraction using safe, food-grade solvents, and confirming bioactivity in cellular or organismal models should thus be the main goals of future research. Thus, future work should focus on isolating active compounds using food-grade solvents and validating bioactivity *in vivo*.

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AUTHORS' CONTRIBUTION

Aishwarya S: Conceptualization, Methodology, Experimental, Data curation, Formal analysis, Software, Visualization, Validation, and

Writing – Original Draft. Sridhar S: Initiated the framework of the research idea, Methodology, Experimental, Data curation, Supervision, Writing – Original Draft, Visualization, Validation, Review and Editing critically.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Hernández-Herrera RM, González-González MF, Velasco-Ramírez AP, Velasco-Ramírez SF, Santacruz-Ruvalcaba F, Zamora-Natera JF. Seaweed extract components are correlated with the seeds germination and growth of tomato seedlings. Seeds. 2023;2(4):436-48. doi: 10.3390/seeds2040033
2. Sathish Kumar B, Murugesan S. Evaluation of fatty acid, mineral and vitamin compositions of *Laurencia papillosa* from the South East Coast of India. Indo Am J Pharm Sci. 2018;5(4):2681-7.
3. Sathish Kumar B, Murugesan S. Proximate and amino acid compositions of marine red alga *Laurencia papillosa* (C. Agardh) Greville from South East Coast of India. J Glob Trends Pharm Sci. 2018;9(1):4987-95.
4. Nedumaran T, Arulbalachandran D. Seaweeds: A promising source for sustainable development. In: Thangavel P, Sridevi G, editors. Environmental Sustainability. New Delhi: Springer India; 2015. p. 65-88. doi: 10.1007/978-81-322-2056-5_4
5. Mahomoodally MF. Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. Evid Based Complement Alternat Med. 2013;2013:617459. doi: 10.1155/2013/617459, PMID 24367388
6. Ramu C, Gayathri N, Priya G, Sathish Kumar B, Sekar T. Integrated phytochemical analysis of *Strychnosnux-vomica* using molecular spectroscopy (ultraviolet-vis and Fourier transform infrared) and hyphenated chromatography (gas chromatography-mass spectrometry). Asian J Pharm Clin Res. 2025;18(8):124-31. doi: 10.22159/ajpcr.2025v18i8.54684
7. Vinoth Kumar R, Murugesan S, Bhuvaneswari S. Phytochemical analysis of red alga *Champia parvula* (C. Agardh) collected from Mandapam coast of Tamil Nadu, India. Int J Adv Pharm. 2015;4(3):15-20.
8. Jacobsen C, Sørensen AM, Holdt SL, Akoh CC, Hermund DB. Source, extraction, characterization, and applications of novel antioxidants from seaweed. Annu Rev Food Sci Technol. 2019;10:541-68. doi: 10.1146/annurev-food-032818-121401, PMID 30673506
9. Sathish Kumar B, Vishnu Kiran M, Vinoth Kumar R, Murugesan S. Phytochemical and physicochemical evaluation of marine red algae *Laurencia papillosa* (C. Agardh) Greville. J Pharm Med Res. 2022;7(1):133-5. doi: 10.30799/jpmr.059.22070102
10. Arulvasu C, Sekaran GD, Prabhakaran B, Kalaiselvi V, Kathirvel N, Lakshmanan V. Cytotoxic effect of crude venom isolated from sea anemone *Calliactis tricolor* on human cancer cell lines. Indian J Geo Mar Sci. 2020;49:601-9.
11. Starlin T, Prabha PS, Thayakumar BK, Gopalakrishnan VK. Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. Bioinformation. 2019;15(6):425-9. doi: 10.6026/97320630015425, PMID 31312080
12. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, *et al*. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694. doi: 10.3389/fphys.2020.00694, PMID 32714204
13. Sathyaseelan T, Murugesan S, Sivamurugan V. Structural identification and antioxidant properties of methanolic extract of brown algae *Lobophora variegata* (JVF Lamouroux) Womersley ex EC Oliveira. Int J Innov Pharm Biosci Res Technol. 2015;2:165-78.
14. Vinayagamurthy K, Kumar DP, Yalavarthi K, Ramudu KN, Ravichandran J, Stephen NM, *et al*. Biological significance of polyphenols as functional molecules in biomaterial preparations. In: Verma C, editor. Science and Engineering of Polyphenols. United States: John Wiley & Sons, Limited; 2024. p. 548-83. doi: 10.1002/9781394203932.ch23
15. Pandithurai M, Murugesan S. Free radical scavenging activity of methanolic extracts of brown alga *Spatoglossum asperum*. J Chem Pharm Res. 2014;6(7):128-32.
16. Thennarasan S, Murugesan S. Biochemical composition of marine brown alga *Lobophora variegata* from Mandapam in the South East Coast of Tamil Nadu. Int J Pharm Pharm Sci. 2015;5:25-9.
17. Pandithurai M, Murugesan S, Bhuvaneswari S, Thennarasan S. *In vitro* α-amylase and α-glucosidase inhibition activity of methanolic extract of marine brown alga *Spatoglossum asperum*. Int J Adv Pharm. 2015;4(5):83-7.
18. Ara J, Sultana V, Qasim R, Ehteshamul-Haque S, Ahmad VU. Biological activity of *Spatoglossum asperum*: A brown alga. Phytother Res. 2005;19(7):618-23. doi: 10.1002/ptr.1699, PMID 16161033
19. Tanna B, Choudhary B, Mishra A, Chauhan OP, Patel MK, Shokralla S, *et al*. Antioxidant, scavenging, reducing, and anti-proliferative activities of selected tropical brown seaweeds confirm the nutraceutical potential of *Spatoglossum asperum*. Foods. 2021;10(10):2482. doi: 10.3390/foods10102482, PMID 34681531
20. Lluch JR, Ribera A, Gómez Garreta A. New data on the reproductive structures of *Spatoglossum solieri* (Chauvin ex Montagne) Kützing (Dictyotales, Phaeophyceae). Botanica Marina. 2004 M;47(4):281-6. doi: 10.1515/BOT.2004.033
21. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on Merugan kizhangu. Bull Med Ethnobot Res. 1981;3:84-6.
22. Farasat M, Khavari-Nejad RA, Nabavi SM, Namjooyan F. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. Iran J Pharm Res. 2014 Winter;13(1):163-70. PMID 24734068, PMCID PMC3985267
23. Bouzenad N, Ammouchi N, Chaib N, Messaoudi M, Bousabaa W, Bensouici C, *et al*. Exploring bioactive components and assessing antioxidant and antibacterial activities in five seaweed extracts from the Northeastern Coast of Algeria. Mar Drugs. 2024;22(6):273. doi: 10.3390/md22060273
24. Murugan S, Natarajan S, Soundararajan S, Sathish Kumar B, Subbiah M. Comparative insights into ultraviolet-B radiation-induced biochemical modulations in *Senna auriculata*: A gas chromatography-mass spectrometry profiling study. Asian J Pharm Clin Res. 2025;18(2):82-9.
25. Azizi MN, Loh TC, Foo HL, Akit H, Izuddin WI, Yohanna D. Brown and green seaweed antioxidant properties and effects on blood plasma antioxidant enzyme activities, hepatic antioxidant genes expression, blood plasma lipid profile, and meat quality in broiler chickens. Animals (Basel). 2023;13(10):1582. doi: 10.3390/ani13101582, PMID 37238013
26. Narasimhan MK, Pavithra SK, Krishnan V, Chandrasekaran M. *In vitro* analysis of antioxidant, antimicrobial and antiproliferative activity of Enteromorpha antenna, *Enteromorpha linza* and *Gracilaria corticata* extracts. Jundishapur J Nat Pharm Prod. 2013 Nov;8(4):151-9. doi: 10.17795/jjnpp-11277. PMID 24624206, PMCID PMC3941891
27. Zhang Y, Fang H, Xie Q, Sun J, Liu R, Hong Z, *et al*. Comparative evaluation of the radical-scavenging activities of fucoxanthin and its stereoisomers. Molecules. 2014;19(2):2100-13. doi: 10.3390/molecules19022100
28. Chakraborty K, Joseph D, Praveen NK. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. J Food Sci Technol. 2015 Apr;52(4):1924-35. doi: 10.1007/s13197-013-1189-2 PMID 25829573, PMCID PMC4375198
29. Hejna M, Dell'Anno M, Liu Y, Rossi L, Aksmann A, Pogorzelski G, *et al*. Assessment of the antibacterial and antioxidant activities of seaweed-derived extracts. Sci Rep. 2024;14(1):21044. doi: 10.1038/s41598-024-71961-8, PMID 39251803
30. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules. 2023;28(4):1845. doi: 10.3390/molecules28041845, PMID 36838831
31. Aulifa DL, Wibowo DP, Safitri N, Budiman A. Formulation of effervescent granules from red ginger (*Zingiberis officinale* roscoe var. Rubrum) extract and its antioxidant activity. Int J Appl Pharm. 2022 Jan; 7;14(1):112-5.
32. Farghl AA, Al-Hasawi ZM, El-Sheekh MM. Assessment of antioxidant capacity and phytochemical composition of brown and red seaweeds samples of Red Sea Coast. Appl Sci. 2021;11(23):11079. doi: 10.3390/app112311079
33. Manam VK, Subhaiah M. Phytochemical, amino acid, fatty acid and vitamins investigation of marine seaweeds *Colpomenia sinuosa* and Halmeriaporphyroides collected along southeast coast of Tamil Nadu, India. World J Pharm Res. 2020;9:1088-102.
34. Zhu F, Ma XH, Qin C, Tao L, Liu X, Shi Z, *et al*. Drug discovery prospect from untapped species: Indications from approved natural product drugs. PLoS One. 2012;7(7):e39782. doi: 10.1371/journal.pone.0039782, PMID 22808057
35. Bhardwaj M, Sali VK, Mani S, Vasanthi HR. Neophytadiene from *Turbinaria ornata* suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and Sprague Dawley rats. Inflammation. 2020;43(3):937-50. doi: 10.1007/s10753-020-01179-z,

PMID 31981060

36. Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC MS. Int Sch Res Notices. 2014;2014:1-13.
37. Saravanakumar K, Chelliah R, Ramakrishnan SR, Kathiresan K, Oh DH, Wang MH. Antibacterial, and antioxidant potentials of non-cytotoxic extract of *Trichoderma atroviride*. Microb Pathog. 2018;115:338-42. doi: 10.1016/j.micpath.2017.12.081, PMID 29307755
38. Abd Algaffar SO, Seegers S, Satyal P, Setzer WN, Schmidt TJ, Khalid SA. Sandalwood oils of different origins are active *in vitro* against *Madurella mycetomatis*, the major fungal pathogen responsible for eumycetoma. Molecules. 2024;29(8):1846. doi: 10.3390/molecules29081846, PMID 38675665
39. Olajuyigbe OO, Onibudo TE, Coopoosamy RM, Ashafa AO, Afolayan AJ. Bioactive compounds and *in vitro* antimicrobial activities of ethanol stem bark extract of *Trilepisium madagascariense* DC. Int J Pharmacol. 2018;14(7):901-12. doi: 10.3923/ijp.2018.901.912
40. Injeti N, Gubbiyappa KS. GC-MS analysis and *in silico* approaches of *Indigofera prostrata* and *Lantana camara* constituents for anti-Alzheimer studies. Int J Appl Pharm. 2024;16(4):100-7.
41. Monroy-García IN, Torres-Romero S, Castro-Ochoa LD, Mendoza-Acosta A, Viveros-Valdez E, Ayala-Zavala F. Bioactive compounds from marine macroalgae: A natural defense against oxidative stress-related diseases. Stresses. 2025;5(1):22. doi: 10.3390/stresses5010022
42. Tziveleka LA, Tammam MA, Tzakou O, Roussis V, Ioannou E. Metabolites with antioxidant activity from marine macroalgae. Antioxidants (Basel). 2021;10(9):1431. doi: 10.3390/antiox10091431, PMID 34573063
43. Gomez-Zavaglia A, Prieto Lage MA, Jimenez-Lopez C, Mejuto JC, Simal-Gandara J. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. Antioxidants (Basel). 2019;8(9):406. doi: 10.3390/antiox8090406, PMID 31533320
44. Chia YY, Kanthimathi MS, Khoo KS, Rajarajeswaran J, Cheng HM, Yap WS. Antioxidant and cytotoxic activities of three species of tropical seaweeds. BMC Complement Altern Med. 2015;15:339. doi: 10.1186/s12906-015-0867-1, PMID 26415532
45. Boi VN, Cuong DX, Khanh Vinh PT. Effects of extraction conditions over the phlorotannin content and antioxidant activity of extract from brown algae *Sargassum serratum* (Nguyen Huu Dai 2004). Free Radic Antioxid. 2016;7(1):115-22. doi: 10.5530/fra.2017.1.17
46. Honey O, Nihad SA, Rahman MA, Rahman MM, Islam M, Chowdhury MZ. Exploring the antioxidant and antimicrobial potential of three common seaweeds of Saint Martin's Island of Bangladesh. Heliyon. 2024;10(4):e26096. doi: 10.1016/j.heliyon.2024.e26096, PMID 38404817