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Extraction of phlorotannin-rich polyphenolic compounds from brown macroalgae: Promising antioxidants for functional foods

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Abstract

Highly polymerised phlorotannin-based polyphenolic compounds are ubiquitous yet unique in brown macroalgae. They are potent antioxidants owing to the presence of pluralities of hydroxyl groups enabling them to scavenge numerous free radicals simultaneously and protect cells from oxidative damage. The present study aimed for a comparison of two solvent systems; ethanol:water:acetic acid (E:W:A, 70:29.7:0.3, v/v/v) and acetone:water:acetic acid (A:W:A, 70:29.7:0.3, v/v/v) for polyphenol extraction. The polyphenol extracts of seven brown algae of *Sargassaceae* family (*Turbinaria ornata, T. conoides, T. decurrens, Sargassum ilicifolium, S. tenerrimum, S. wightii* and *S. plagiophyllum*) were analysed for the total phenolic contents (TPC) (76.76-143.56 mg GAE g^{-1}), total phloroglucinol contents (TPhC) (5.01-19.77 mg PGE g^{-1}) and antioxidant activities (IC_{50} < 0.6 mg ml^{-1}). *S. plagiophyllum* displayed the highest phloroglucinol content (19.77 mg PGE g^{-1}) followed by *T. ornata* (13.26 mg PGE g^{-1}) and *T. decurrens* (11.68 mg PGE g^{-1}). *S. plagiophyllum* exhibited the strongest antioxidant activity, with the lowest IC_{50} values in both the DPPH (0.29 mg ml^{-1}) and ABTS (0.09 mg ml^{-1}) assays. These results demonstrate the efficiency of the ethanol solvent system in producing a highly antioxidative, phlorotannin-rich polyphenolic concentrate from macroalgal extracts.

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Introduction

Macroalgae rely on antioxidant polyphenols for their normal growth and protection from environmental oxidative stress, predators, UV light and pathogens ensuring their survival in competitive ecosystem. These inherent polyphenolic compounds are often more stable than terrestrial polyphenols. In brown macroalgae, phlorotannins may account for up to 25% of the algal dry weight; however, their abundance varies considerably among species and is influenced by environmental factors such as salinity, seasonality, habitat, and nutrient availability (Kumar *et al*., 2022; Jang *et al*., 2024). Among the diverse polyphenolic sources, highly polymerised phlorotannins of brown macroalgae are noteworthy for their intricate structure, characterised with multiple hydroxyl groups (Santos *et al.,* 2019). They are complex polymers of phloroglucinol, synthesised *via* the acetate-malonate pathway, linked by biphenyl bonds (fucols), diaryl-ether bonds (phlorethols, hydroxyphlorethols, fuhalols), or both (fucophlorethols) (Zheng *et al.,* 2022). These molecules, ranging from 10 to 100 kDa, exhibit structural variability attributed to different linkage patterns (Menaa *et al.,* 2021). The presence of multiple hydroxyl groups in phlorotannin contributes to their efficacy as antioxidants across diverse oxidation systems, scavenging peroxyl radicals, alkyl peroxyl radicals, superoxide, hydroxyl radicals, nitric oxide, and peroxynitrite in both aqueous and organic environments (El-Beltagi *et al.,* 2022). This activity stems from their capability to donate a hydrogen atom from an aromatic hydroxyl group to a free radical, effectively neutralising them and becoming unreactive phenoxyl radicals, facilitated by the aromatic structure's ability to stabilise an unpaired electron through delocalisation within the π-electron system (Tziveleka *et al.,* 2021). By this action, phlorotannin can mitigate oxidative stress-related damages in cells and thereby maintain normal physiological functions. In addition, they modulate cellular signalling pathways, gene expression, and enzyme activities, contributing to their potential health-promoting effects (Cory *et al.,* 2018).

The human body naturally produces endogenous antioxidants, which can be supplemented with exogenous antioxidants from natural sources or synthetic chemicals such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) to support antioxidant defence mechanisms (Begum *et al.,* 2021). However, the efficacy and safety of antioxidants depend on several factors, including their source, chemical structure, concentration, bioavailability, and intended application. While concerns have been raised regarding the potential adverse effects of certain synthetic antioxidants under specific conditions, naturally derived antioxidants, including marine polyphenols, continue to attract considerable attention owing to their diverse bioactivities and potential health-promoting properties. Previous studies have demonstrated the antioxidant potential of phlorotannins derived from brown algae (Phang *et al.,* 2023). Thus, exploring the antioxidant polyphenolic components of unexplored marine brown macroalgae using green solvents appears to be of great utility for understanding mechanisms that may help combat oxidative stress-related disorders.

Despite growing interest in marine-derived antioxidants, comparative studies on the polyphenolic composition and antioxidant potential of several economically important Sargassaceae species from the Gulf of Mannar remain limited. In particular, studies evaluating phlorotannin-rich extracts obtained using environmentally sustainable solvent systems are scarce. Addressing these gaps is essential for identifying promising macroalgal resources and unlocking their potential for functional food and nutraceutical applications. The present study evaluates the antioxidant potential of polyphenolic extracts from seven economically significant marine macroalgae of the Sargassaceae family, namely *Turbinaria ornata, T. conoides, T. decurrens, Sargassum ilicifolium, S. tenerrimum, S. wightii* and *S. plagiophyllum*, from the Gulf of Mannar region in peninsular India. Polyphenolic extracts from each species were prepared using two different solvent mixtures and subsequently characterised for their phytochemical composition, including total phenolic content (TPC) and total phloroglucinol content (TPhC). The antioxidant activities were assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) radical scavenging assays using *in vitro* chemical models.

Materials and methods

Chemicals and instrumentation

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were sourced from Sigma-Aldrich Chemicals Co. Inc. (St. Louis, MO, USA). Dimethyl sulfoxide and 2,4-dimethoxy benzaldehyde were sourced from E-Merck (Darmstadt, Germany). Sulphuric acid, phloroglucinol, Folin-Ciocalteu (FC) reagent, glacial acetic acid, chloroform, and petroleum ether were obtained from MRECK Specialties Private Limited (Worli, Mumbai). Sodium carbonate was sourced from S.D fine chemicals limited (Boisar, Maharashtra). Analytical-grade solvents such as acetone, ethanol, methanol, and ethyl acetate were from E-Merck (Darmstadt, Germany), redistilled in an all-glass system. Double-distilled water was utilised throughout the study. All solvents and reagents used were of analytical grade. UV absorbance was measured using a Varian Cary 50 UV-Vis spectrophotometer (Walnut Creek, USA). Concentration of the crude extracts was performed using a rotary vacuum evaporator (Heidolph, Germany).

Sample collection and preparation of polyphenol extracts from selected brown algae

Seven brown macroalgae species (20 kg each of *T. conoides, T. ornata, T. decurrens, S. ilicifolium, S. tenerrimum, S. plagiophyllum* and *S. wightii*) were harvested during December from the Gulf of Mannar coastline of peninsular India (Mandapam, 8º 48'N, 78º 9'E and 9º 14'N, 79º 14'E). The macroalgae were then thoroughly washed, shade-dried and subsequently pulverised. The powdered shade dried algal materials (100 g) were extracted with two different sets of solvent (400 ml each) of acetone: water: acetic acid (A:W:A) (70:29.7:0.3, v/v/v) and ethanol: water: acetic acid (E:W:A) (70:29.7:0.3, v/v/v), at 60°C for 12 h in water bath. These solvent systems were selected based on their reported efficacy in extracting polyphenolic compounds from brown macroalgae and were employed to comparatively assess their ability to recover phlorotannin-rich polyphenolic extracts through environmentally sustainable extraction approaches. The extracts were filtered, passed via anhydrous sodium sulphate and concentrated using a rotary evaporator under partial vacuum to afford crude polyphenolic extracts of seven macroalgal species. The crude extract (20 g) of each macroalgae was suspended in 150 ml of distilled water and subjected to partitioning with 100 ml of n-hexane (4 times) to eliminate pigments. The n-hexane phases were pooled and evaporated. Subsequently, the aqueous phase underwent partitioning with ethyl acetate four times (100 ml each) and was concentrated to obtain polyphenolic extracts. (Fig.1).

In vitro antioxidant assays

The free radical scavenging activity of the polyphenolic extracts were examined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) scavenging assay (Wojdylo *et al*., 2007). α-Tocopherol was used as the standard antioxidant. Briefly, for the DPPH assay, different concentrations (0.1–2.0 mg mL^{-1}) of the algal crude extracts were prepared in methanol and mixed with 0.1 mM methanolic DPPH solution. The reaction mixtures were incubated in the dark at room temperature (~28°C) for 10 min. Thereafter, the absorbance of the control (DPPH solution in methanol), α-tocopherol standard, and sample extracts was measured at 517 nm against a methanol blank using a spectrophotometer.

For the ABTS assay, 7 mM ABTS dissolved in double-distilled water was mixed with 2.45 mM potassium persulphate and incubated in the dark for 16 h. The resulting solution was diluted with methanol to an absorbance of 0.700 at 734 nm. The algal extracts were serially diluted to concentrations ranging from 125 to 2000 ppm. Subsequently, 1 ml of each extract was mixed with 2 ml of the $ABTS^+$ working solution, and the absorbance of the control, α-tocopherol standard, and samples was measured at 734 nm.

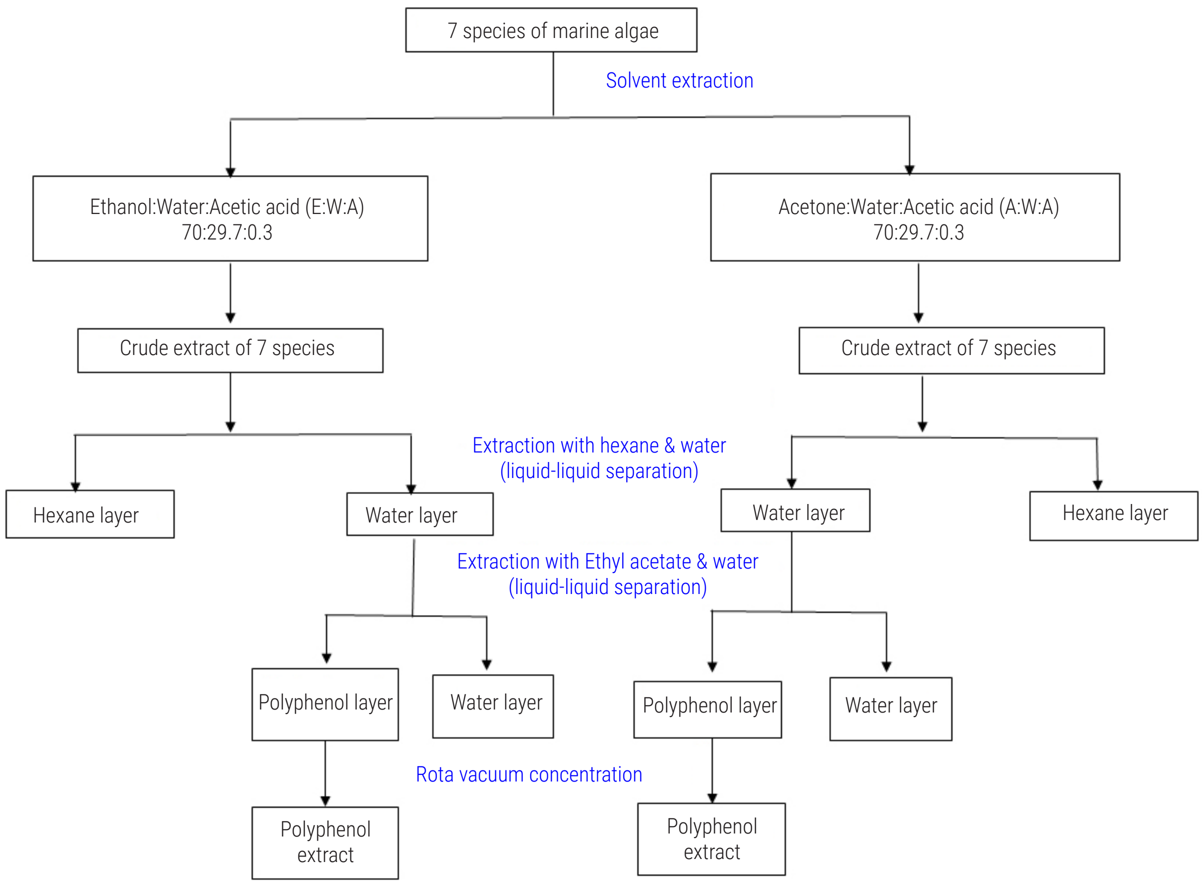

Fig. 1. Schematic representation of isolation of polyphenol from seven selected brown macroalgae species

The percentage of antioxidant scavenging activity of the sample was computed using the following equation:

% Inhibition = $((A_0-A_1)/A_0)*100$

Where, A_0 is the absorbance of control and A_1 is the absorbance of the sample. The percentage inhibition is plotted against different concentrations, and IC_{50} values were obtained from the graph. The results were expressed as $IC_{50.}$

Total polyphenol content (TPC) and total phloroglucinol (TPhC) content

The Folin-Ciocalteu (FC) method was employed to assess the total phenolic content of the crude macroalgal extracts of seven studied species (Farasat *et al.*, 2014). In brief, in triplicate vials, a known amount of the sample was dissolved in MeOH (5 mg ml^{-1}). This solution was then combined with 2.5 ml of FC reagent and 2 ml of sodium carbonate (0.7 N in distilled water). The resulting reaction mixture was incubated for 2 h at room temperature in dark, and the absorbance was measured at 760 nm. The total phenolic content was calculated based on the gallic acid equivalents (GAE) calibration curve (Hatami *et al.*, 2014), expressed in milligrams per gram of the sample. The gallic acid calibration curve was prepared using concentrations ranging from 10 to 100 ppm (R^2=0.9909).

Quantitative determination of total phloroglucinol content (TPhC) was done with 2,4-dimethoxy benzaldehyde (Catarino *et al.*, 2019). In brief, equal volumes of a stock solution containing 2,4-dimethoxybenzaldehyde (2 g per100 ml glacial acetic acid) and HCl (16 ml concentrated HCl per 100 ml glacial acetic acid) were mixed just before use to create the working reagent. An aqueous solution of the dry crude extract (10 µl of a 20 mg ml^{-1}solution in methanol) was combined with 2.5 ml of the working reagent and 10 µl of DMF. The mixture underwent incubation for 60 min at a temperature between 30 and 33°C, followed by measuring the absorbance at 515 nm against the blank. The phloroglucinol calibration curve was prepared using concentrations ranging from 156 to 10,000 ppm (R^2=0.993).

Statistical analysis

Statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences, version 21.0; SPSS Inc., CA, USA). Data were expressed as mean±standard deviation (Mean±SD, n=3). Differences among groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Pearson's correlation analysis was performed to assess the relationships among TPC, TPhC, DPPH IC_{50}, and ABTS IC_{50} values. Statistical significance was considered at $p<0.05$.

Results and discussion

Polyphenolic extracts derived from brown macroalgae offer a natural and bioactive resource with potential applications in promoting health. As we continue to explore the multifaceted benefits of these, it is evident that they are rich in bioactive phytochemicals with significant antioxidant properties, and thereby novel candidates for nutritional supplements and pharmaceutical applications (Pereira and Cotas, 2023). The extraction of polyphenols from brown macroalgae poses several challenges, primarily due to the complex structure of the algal cell walls and the diverse solubility characteristics of polyphenolic compounds (Cotas *et al.,* 2020). Efficient extraction methods are essential to maximise yield and maintain the bioactivity of these compounds. Traditional extraction methods often involve the use of organic solvents, which can be harmful to the environment and pose health risks. Therefore, there is a growing interest in developing green extraction technologies that align with the principles of green chemistry, which emphasise the use of environmentally friendly solvents and processes.

Percent yield of polyphenol extract

The study compared the efficiency of two solvent systems, ethanol:water:acetic acid (E:W:A) and acetone:water:acetic acid (A:W:A), for extracting polyphenols from algal species. The E:W:A system exhibited a higher mean percentage yield of crude polyphenolic extracts (11.38%) compared with the A:W:A system (8.42%) (Table 1). The choice of solvent is a critical factor in polyphenol extraction, as it directly impacts the yield of phenolic compounds. Previous literature (Li *et al*., 2017) emphasised the use of polar solvents, such as aqueous solutions of methanol, ethanol, acetone, or distilled water, for the effective extraction of phenolic compounds. Among these solvents, ethanol has gained considerable attention owing to its low toxicity, biodegradability, safety for human consumption, and compatibility with green extraction approaches (Chaabani *et al*., 2023). Furthermore, ethanol-water mixtures have been reported to improve extraction efficiency by enhancing mass transfer and increasing the permeability of the biological matrix, thereby facilitating the release of phenolic compounds (Chaabani *et al*., 2023). Consistent with these observations, Sharifi-Rad *et al.* (2025) demonstrated that ethanol-based extraction yielded higher concentrations of phytochemicals and superior antioxidant activity compared with other extraction systems. Therefore, the higher extraction yield observed for the E:W:A system in the present study may be associated with the favourable extraction characteristics of ethanol-based solvent mixtures. The present findings are also consistent with previous reports indicating that ethanol extracts generally exhibit higher phenolic concentrations than water extracts, which tend to show lower phenolic recovery (Sanchez-Camargo *et al*., 2016).

Table 1. Percent yield of polyphenolic extracts in ethanol: water: acetic acid (E:W:A) and acetone: water: acetic acid (A:W:A) of seven selected macroalgae species

Seaweed species	Solvent systems	
	E:W:A	A:W:A
T. ornata	$13.12^{c} \pm 0.40$	$12.57^{a} \pm 0.67$
S. plagiophyllum	$16.39^{a} \pm 0.50$	$10.27^{c} \pm 0.56$
T. decurrens	$12.76^{d} \pm 0.24$	$10.56^{b} \pm 0.40$
S. ilicifolium	$14.00^{b} \pm 0.30$	$7.56^{e} \pm 0.30$
S. tenerrimum	$9.22^{e} \pm 0.20$	$9.70^{d} \pm 0.30$
T. conoides	$6.17^{g} \pm 0.40$	$4.11^{g} \pm 0.60$
S. wightii	$8.04^{f} \pm 0.70$	$4.18^{f} \pm 0.70$

Samples were analysed in triplicate (n=3) and expressed as mean±SD. Means followed by different superscripts (a-g) within the same column indicate significant differences (p<0.05)

The findings highlight the importance of solvent selection in maximising polyphenol recovery, with the E:W:A system showing greater potential for producing higher yields of bioactive compounds while also offering environmental advantages associated with green extraction approaches. Ethanol has been recognised as a green solvent owing to its low toxicity, biodegradability, renewability, and suitability for applications involving food and pharmaceutical products. These attributes support the growing shift towards sustainable extraction strategies that minimise environmental and health impacts. Among the studied algal species extracted using E:W:A, *S. plagiophyllum* exhibited the highest percent yield (16.39%), while *T. conoides* showed the lowest yield (6.17%). This variability in extraction yield emphasises the importance of considering species-specific characteristics during the extraction process.

Total phenolic content (TPC) and total phloroglucinol contents of polyphenol extracts

In brown algae, the predominant polyphenolic compounds are primarily composed of phlorotannins, which are polymers consisting of phloroglucinol (1,3,5-trihydroxybenzene) (Maheswari and Babu, 2022). The total phenolic contents of polyphenolic extracts were significantly different ($p<0.05$) with greater phenolic contents recorded for *S. plagiophyllum* (143.56 mg GAE g^{-1}) and (121.36 mg GAE g^{-1}) in ethanol: water: acetic acid and acetone: water: acetic acid, respectively (Fig. 2a), followed by *T. ornata* in E:W:A extract (101.66 mg GAE g^{-1}) and *T. decurrens* (100.01 mg GAE g^{-1}) in E:W:A extract.

Polyphenolic extracts of *S. plagiophyllum* exhibited significantly greater total phloroglucinol contents (19.77 mg PGE g^{-1} in E:W:A and 16.04 mg PGE g^{-1} in A:W:A), followed by those displayed by the polyphenolic extracts of *T. ornata* (13.26 mg PGE per g in E:W:A) and *T. decurrens* (11.68 mg PGE per g in E:W:A extract) (Fig. 2b). Among the studied species, lowest phloroglucinol contents were observed in *S. tenerrimum* (6.71 and 5.01 mg PGE per g) in both the solvent systems (E:W:A and A:W:A).

Pearson's correlation analysis revealed a strong positive correlation between TPC and TPhC ($r=0.967$, $p<0.001$), underscoring the intrinsic relationship between the overall polyphenolic content and the specific presence of phloroglucinol-based compounds, particularly phlorotannins, in the studied macroalgae. The higher TPC values observed in *S. plagiophyllum*, *T. ornata*, and *T. decurrens* corresponded with elevated TPhC values, indicating that these species are rich sources of phloroglucinol-containing polyphenols. The phytochemical evaluation is essential for optimising extraction processes and accurately evaluating the antioxidant potential of algal extracts. Notably, *S. plagiophyllum* exhibits significant variations in phenolic content (143.56 to 121.36 mg GAE per g) and phloroglucinol content (19.77 to 16.04 mg PGE per g) depending on the choice of solvent. The variance in TPhC among various algal species may be attributed to genetic factors, environmental conditions, or different extraction methods employed in previous studies (Aminina *et al.,* 2020). Genetic factors influence the biosynthesis of secondary metabolites, including polyphenols, leading to species-specific variations in their content (Zhan *et al.,* 2022). Earlier studies reported the presence of considerable phenolic contents of the organic extracts of brown algae species *T. ornata* and *T. decurrens* (Thambi and Chakraborty, 2022). However, there are only limited reports of evaluation of polyphenol and phloroglucinol contents of *S plagiophyllum*, which showed the highest TPC and TPhC in the present study.

Antioxidant activities of crude polyphenol extracts

Evaluation of the antioxidant properties of the studied polyphenol extracts was carried out by DPPH and ABTS scavenging assays. In the present study, it was observed that the E:W:A solvent derived crude extracts demonstrated superior antioxidant potential compared to those obtained from the A:W:A solvent system (IC_{50} E:W:A<IC_{50} A:W:A). This may be attributed to higher polarity and ability of ethanol to form stronger hydrogen bonds with phlorotannins, enhancing both the extraction and stability of antioxidants. The maximum activity was observed in case of *S. plagiophyllum* for both DPPH (IC_{50} 0.29 mg ml^{-1}) and ABTS assay (IC_{50} 0.09 mg ml^{-1}) (Fig. 2c-d). The lowest activity was recorded in *S. ilicifolium* (IC_{50} 0.59 mg ml^{-1}) for DPPH assay and *T. conoides* (IC_{50} 0.42 mg ml^{-1}) for ABTS assay. The significantly greater radical scavenging activity of *S. plagiophyllum* analysed *via* DPPH and ABTS assay were found to be positively correlated and such consistency is valuable in the field of antioxidant research, as it enhances the reliability of these assays. The study also established a strong positive correlation between DPPH and ABTS IC_{50} values ($r=0.807$, $p<0.001$), indicating a high degree of agreement between the two antioxidant assays in evaluating the radical scavenging potential of the studied macroalgal extracts. Previous literature reports that the antioxidant effect is closely associated with polyphenols and particularly the phlorotannins, the oligomers or polymers of phloroglucinol (Heffernan *et al.,* 2015; Generalić *et al.,* 2019). Extracts with high total phenolic content demonstrated the greatest antioxidant activity, with *S. plagiophyllum* exhibiting the highest TPC, TPhC, and potent radical scavenging potential among the species investigated. Given the limited information available regarding the antioxidant properties and phlorotannin composition of *S. plagiophyllum*, the present findings provide novel insights into its phytochemical richness and highlight its potential as a promising natural source of bioactive antioxidants for future nutraceutical and pharmaceutical applications.

Free radicals and reactive oxygen species (ROS), including superoxide radical (O_2), hydroxyl radical (OH), peroxyl radical (ROO), and hydrogen peroxide (H_2O_2), emerge as byproducts in diverse metabolic pathways within our body leading to cellular damage, DNA mutations, inflammation, and a range of health consequences

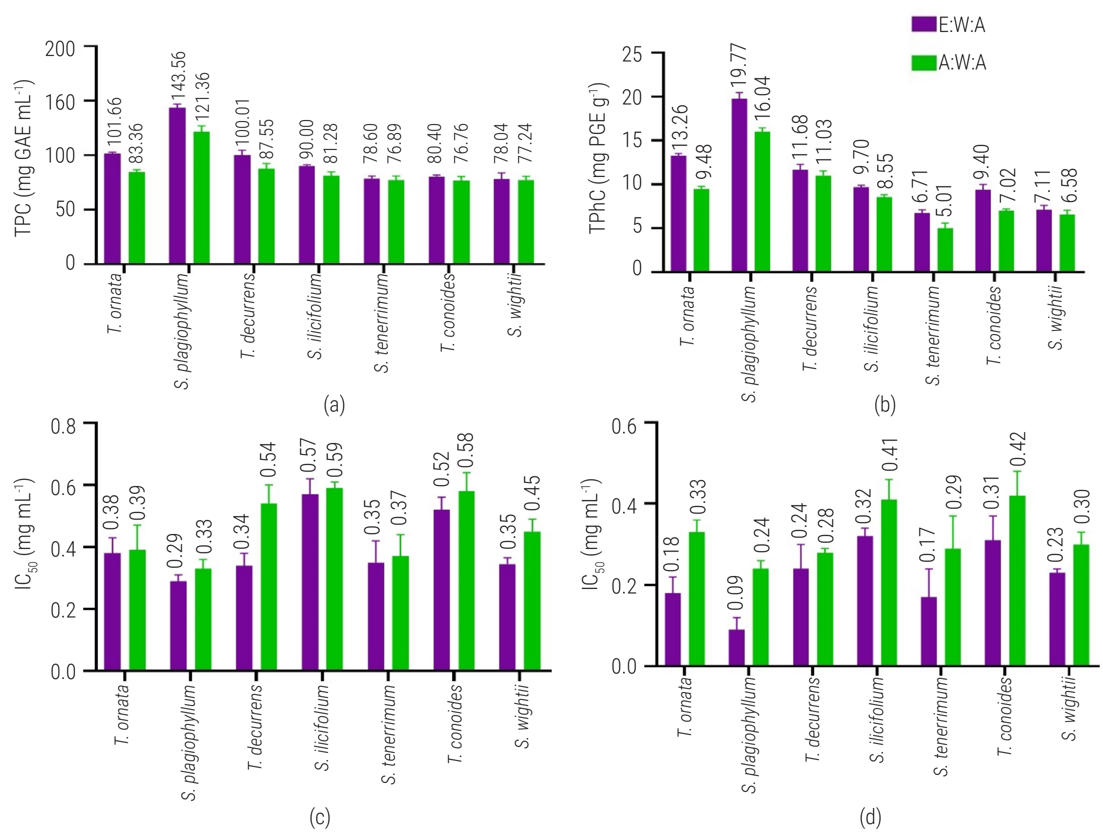

Fig. 2. Total phenolic content (2a) total phloroglucinol content; (2b) antioxidant activities; (2c) DPPH assay and (2d) ABTS assay) of polyphenolic extracts of seven selected macroalgal species. Data are presented as mean ± standard deviation (n=3). α-Tocopherol was used as the standard antioxidant, with IC_{50} values of 0.21±0.01 mg $ml^{-1}$and 0.16±0.03 mg $ml^{-1}$for the DPPH and ABTS assays, respectively

(Seo *et al.*, 2022). The antioxidant activity of polyphenol extracts from brown algae, especially phlorotannins, is due to their chemical and structural features. With multiple phenolic rings, polyhydroxylated groups, and electron-rich centers, these compounds neutralise free radicals, disrupt radical formation, and chelate metal ions (Begum *et al*., 2021). Their effectiveness is linked to their phenolic structure, aromatic ring substitutions, hydroxyl group positions, and side chains (Mathew *et al*., 2015). Thus, the diverse polyphenolic compounds in brown algae provide strong antioxidant protection against oxidative stress.

Polyphenolic extracts from brown macroalgae are rich in bioactive phytochemicals with significant antioxidant properties, making them valuable candidates for nutritional supplements and pharmaceutical applications. Efficient extraction is crucial due to the complex structure of algal cell walls. The study compared the efficiency of two solvent systems, ethanol:water:acetic acid (E:W:A) and acetone:water:acetic acid (A:W:A), for extracting phlorotannin-rich polyphenolic compounds from brown macroalgae. The E:W:A solvent system was more effective, resulting in higher extraction yields and greater antioxidant activity. Pearson's correlation analysis revealed a strong positive relationship between total phenolic content and total phloroglucinol content, as well as between the DPPH and ABTS assays, highlighting the consistency of the phytochemical and antioxidant assessments employed in this study. Among the brown algae investigated, *S. plagiophyllum* exhibited the highest extraction yield (16.39%), potent antioxidant properties, and the highest total phloroglucinol content. The study highlights the potential of brown seaweed-derived polyphenols, particularly those from *S. plagiophyllum*, as promising natural antioxidants for health-promoting applications.

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