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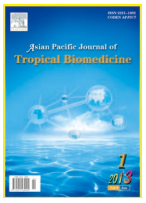

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Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria* spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar

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PEER REVIEW

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Comments

It is apparent from the present study that ingredients in ethylacetate fractions of seaweeds appeared to be the reservoir of antioxidant principles, and seem to play an important role in the free radical scavenging capacity. The present study provides valuable information regarding the potential of these brown seaweeds to develop a viable natural substitute of the existing synthetic antioxidants. The statistical analyses carried out by the authors increased the reliability of the experimental data.
(Details on Page 15)

ABSTRACT

Objective: To evaluate the antioxidant activities and total phenolic contents of brown seaweeds belonging to *Turbinaria* spp. [*Turbinaria conoides* (*T. conoides*) and *Turbinaria ornata* (*T. ornata*) collected from Gulf of Mannar of southeastern coast of India in various *in vitro* systems. **Methods:** The antioxidant activity was evaluated using different *in vitro* systems, *viz.*, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2′-azino-bis-3 ethylbenzothiozoline-6-sulfonic acid diammonium salt (ABTS), H_2O_2/HO radical scavenging, Fe^{2+} ion chelating ability, and reducing potential. Folin-Ciocalteu method was used to determine the total phenolic content of the extracts, and the results were expressed as mg of gallic acid equivalents (GE)/g of the seaweed extracts. Thiobarbituric acid-reactive substances assay was employed to assess the ability of the seaweed extracts to inhibit lipid oxidation. **Results:** Ethyl acetate (EtOAc) fraction of *T. conoides* registered significantly higher phenolic content (105.97 mg GE/g) than that of *T. ornata* (69.63 mg GE/g). Significantly higher antioxidant potential as determined by DPPH (64.14%) radical scavenging activity was registered in EtOAc fraction of *T. ornata*. A higher $ABTS^+$ radical scavenging (IC_{50} 3.16 μg/mL), Fe^{2+} chelating (IC_{50} 0.46 mg/mL), H_2O_2 scavenging (IC_{50} 4.25 mg/mL), lipid peroxidation inhibitory (TBARS, IC_{50} 0.21 mg/mL), and reducing abilities (IC_{50} 52.67 mg/mL) ($P<0.05$) were realized in EtOAc fraction of *T. ornata* than other fractions. **Conclusions:** This study indicated the potential use of *T. conoides* and *T. ornata* as candidate species to be used as food supplements/functional foods to increase shelf-life of food items for human consumption, and nutraceuticals to deter deleterious free radical-induced life-threatening diseases.

KEYWORDS

Brown seaweeds, *Turbinaria conoides*, *Turbinaria ornata*, Antioxidant activity, Total phenolic contents

1. Introduction

Antioxidants are the substances, which can defend serious human diseases including melanoma, cardiac disorders, diabetes mellitus, inflammatory and neurodegenerative diseases[1] that explain their potential use in increasing shelf-life of food and as medicine[2,3]. Free radical-induced oxidation is one of the major reasons in deterioration of nutritional quality, and other physical attributes of food items under storage[4]. In recent years, use of antioxidants of natural origin is considerably enhanced by the concern about the adverse side effects of popularly used synthetic antioxidants *viz.*, butylated hydroxyanisole, butylated hydroxytoluene,

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and other analogues[5].

Very recently, the pharmaceutical and agri-food industries have been at the origin of a great expansion in the demand for seaweeds due to their significant applications as ingredients in functional foods and richness in antioxidant ingredients. It was reported that seaweeds are rich source of bioactive compounds, such as terpenoids, phlor-otanins, fucoidans, sterols and glycolipids, and the extracts or isolated pure components from seaweeds posses a wide range of pharmacological properties such as anticancer, antibacterial, antifungal, anti-viral, anti-inflammatory, anticoagulant, antioxidant, hypoglycaemic, hypolipidemic, antimelanogenic, anti-boneloss, hepatoprotective and neuroprotectiveactivit ies[2,6,7]. More than these, seaweeds are also a wealthy resource of dietary iodine and fibers which can also play an immense part in enhancing the food quality[4]. Earlier reports indicated that the extracts of brown seaweeds belonging to *Turbinaria* spp. were found to have antioxidant and anti-inflammatory activities[8,9]. Additionally, these species registered to have essential nutritional components *viz.*, mineral salts (K, Ca, and Fe), soluble fibers, digestible protein, and polyunsaturated fatty acids, to mention a few[10].

The brown seaweeds contain a large assemblage of species that predominate in the coastal shelf areas of Gulf of Mannar region in southeastern coast of Indian subcontinent. Among various brown seaweeds, *Turbinaria conoides* (J. Agardh) Kuzing (Sargassaceae, Fucales) (*T. conoides*), and *Turbinaria ornata* (Turner) J. Agardh (Sargassaceae, Fucales) (*T. ornata*) are abundantly available in this area throughout different seasons, and therefore these species have been short listed for the present study. Although antioxidant properties of seaweeds were proved by numerous studies from past two decades, there is scanty information regarding the antioxidant potential from this very important species from this particular region. Based on this background, the objectives of the present study were to evaluate the antioxidant activities and total phenolic contents of these seaweed species to understand their beneficial value as human food or as additives. The correlations between total phenolic contents (TPC) and antioxidant capacities of these seaweeds were also evaluated. The results from the present study will be helpful to develop new generation of antioxidants for increasing the shelf-life of food products, as nutraceuticals and/or functional foods, and in combating carcinogenesis and inflammatory diseases.

2. Materials and methods

2.1. Seaweed material and description of study area

Seaweeds used in this study were *T. conoides* and *T. ornata*. The seaweeds (2 kg) were collected from Gulf of Mannar of Mandapam region located between 8°48′ N, 78°9′ E and 9°14′ N, 79°14′E on the southwest coast of India. The seaweed samples were shade dried, and powdered after washing thoroughly in fresh water to remove salt and other unwanted materials and stored in airtight containers at room temperature for further work.

2.2. Chemicals and instrumentation

All solvents used for sample preparation were of analytical grade (E-Merck, Darmstadt, Germany). Trichloracetic acid (TCA), 1, 1-dipheny1-2-picrylhydrazyl, 2-thiobarbituric acid, Ferrozine, Folin-Ciocalteu reagent, 2, 2′-azino-bis-(3ethylbenzothiozoline-6-sulfonic acid diammonium salt), Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich Chemical Co. Inc. (St. Louis, MO, USA). All other unlabeled chemicals and reagents were of analytical, spectroscopic or chromatographic reagent grade and were obtained from E-Merck (Darmstadt, Germany).

2.3. Preparation of solvent extracts of the experimental seaweeds Turbinaria spp.

The powdered shade-dried seaweed samples (200 g) were extracted with methanol (500 mL×4) at an elevated temperature (40-45 °C) for 3 h. The samples were filtered with Whatman filter paper No. 1 to obtain the clarified filtrates (1.8 L), which were filtered, through Na_2SO_4 (150 g), and evaporated (40 °C) using rotary evaporator under vacuum to dryness to give a dark green viscous oily mass (100 mL) of methanolic fraction. This dark green viscous oily mass (100 mL) of methanolic crude extract was mixed with an equal volume of distilled water (100 mL), and partitioned successively with *n*-hexane (200 mL×3), dichloromethane (MDC; 200 mL×3), and EtOAc (200 mL×3) to furnish *n*-hexane (600 mL), MDC (600 mL), and EtOAc fractions (600 mL), respectively. The water-free extracts were dried over anhydrous Na_2SO_4 (100 g), and evaporated under reduced pressure using a rotary vacuum evaporator (Buchi, Switzerland) to furnish different solvent fractions of varying polarity.

2.4. Determination of TPC

The amount of total phenolics in samples was determined by established method with suitable modification[11]. All determinations were carried out in triplicate. The TPC was expressed as gallic acid equivalent (GAE) in mg/g sample.

2.5. Quantification of radical scavenging activity

The total radical scavenging activities were determined by already established methods *viz.*, 2, 2′-Azino-bis-3 ethylbenzothiozoline-6-sulfonic acid diammonium salt (ABTS) and 1, 1-diphenyl-2-picryl-hydrazil (DPPH$^{\cdot}$) radical scavenging activity assay with suitable modifications[6]. The results were expressed as % radical scavenging activity.

2.6. Assays for detection of scavenging of short-lived radicals

The ability of the solvent extracts of the *Turbinaria* spp. to scavenge H_2O_2 was determined using established method[12]with suitable modification. The percentage of scavenging of H_2O_2 of seaweed extracts was determined by the following formula: % scavenged (H_2O_2) $=[(A_0-A_1)/A_0]\times 100$, where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of the solvent fractions and standards. The HO$^{\cdot}$ radical scavenging

activity of the crude solvent extracts of the seaweeds was measured using established method[5]. Percentage of HO$^{\bullet}$ radical scavenging activity was determined by comparing the results of the test and standard compounds.

2.7. Thiobarbituric acid-reactive substances formation inhibition assay (TBARS)

This assay was based on the previous method with suitable modification[13]. The model system used for TBARS assay was lyophilized mussel (*Perna viridis* L.) sample as a lipid source. The sample solutions (1 mL, 0.1-0.6 mg/mL) were incubated with the mussel sample (10 mg), AcOH (2 mL, 1.03 g/mL) and an aqueous solution of thiobarbituric acid (TBA, 2 mL, 0.78 g/100 mL) at 95 ℃ for 45 min. The resultant mixture (5 mL) was cooled to room temperature and clarified by centrifugation (8000 r/min, 10 min) to get the supernatant. The absorbance of the supernatant was recorded at 532 nm and the antioxidant capacity was expressed as equivalent mmol/L of malonaldehyde (MDA)/kg of sample. TBARS concentration was calculated using a standard curve based on MDA.

2.8. Total reducing ability

Total reduction capabilities of the crude solvent extracts of the seaweeds were estimated by using the method as described earlier with modifications[9]. The absorbance of the reaction mixture after incubation was measured at 700 nm by using a spectrophotometer (Varian Carry 50 conc UV-visible spectrophotometer). Higher absorbance of the reaction mixture indicated greater reducing power.

2.9. Metal (Fe^{2+}) ion chelating activity

The ferrous ions chelating by the crude extracts and standards were estimated by the earlier method with suitable modification[14]. After the reaction mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm using a spectrophotometer and the results were expressed as in % Fe^{2+} chelating ability. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula: $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

2.10. Statistical analysis

Statistical evaluation was carried out with the Statistical Programme for Social Sciences 13.0 (SPSS Inc, Chicago, USA, Ver. 13.0). Descriptive statistics were calculated for all the studied traits. Analysis were carried out in triplicate, and the means of all parameters were examined for significance (P<0.05) by analysis of variance (ANOVA). Pearson correlation test was used to assess correlations between means. The mean variance in the data set was detected using principal component analysis (PCA). All data were mean centered and scaled to equal unit variance prior to PCA. The selected variables for PCA were the different atioxident assays and phenolic content, as exhibited by different crude extracts of the two brown seaweeds.

3. Results

3.1. TPC in the methanolic extracts and solvent fractions of Turbinaria spp..

The methanolic extract was partitioned with *n*-hexane, MDC and EtOAc and the yield obtained was given in Table 1. EtOAc fractions of both *T. conoides* and *T. ornata* registered a significantly higher (P<0.05) TPC (105.97 & 69.63 mg GE/g, respectively), followed by the MDC fractions (51.47 & 12.72 mg GE/g respectively), as compared to other solvent fractions and methanolic extract. The methanolic extract and all solvent fractions of *T. conoides* exhibited significantly higher TPC than corresponding fractions of *T. ornata* (Table 2). The *n*-hexanic extract of *T. ornata* (1.07 GE/g) registered lowest TPC than all other solvent fractions.

Table 1

Yields obtained for methanolic extract (as % w/w of seaweed on dry weight basis) and solvent fractions (as % of total methanolic extract) of *T. ornata* and *T. conoides* (mean±SD).

Seaweed species	methanolic extract (%)	Solvent fractions obtained by partitioning methanolic extract with solvents (%)		
		n-hexane	MDC	EtOAc
T. ornata	8.40±0.36	3.00±1.36	2.50±0.62	1.70±0.39
T. conoides	6.80±0.07	2.50±0.57	2.20±0.44	1.20±0.84

MDC: Dichloromethane; EtOAc: Ethyl acetate.

3.2. Assays for quantification of antioxidant activity by using ABTS

All the seaweed fractions and methanolic extracts displayed antioxidant activities as they were able to scavenge the ABTS$^{\bullet+}$ radical cation. The sequence of antioxidant activity of the different solvent fractions of seaweed *T. ornata* (0.6 μg/mL) as determined by ABTS assay was as follows: *n*-hexanic (30.84 %)>EtOAc (13.91 %)> methanolic (8.04 %)>MDC fraction (6.38 %) (Table 2). MDC fraction of *T. ornata* was realized with higher ABTS radical scavenging ability (17.57 %) than other methanolic extract and other organic fractions. The variation of ABTS radical scavenging activity with concentration (0.1-0.6μg/mL) of the tested extract and fractions were described in Figure 1. It can be observed from the figure that EtOAc fraction of *T. conoides* and methanolic extract of *T. ornata* were more active than other fractions.

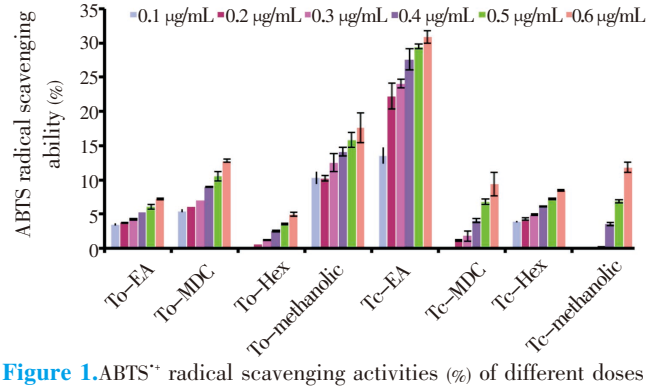

Figure 1. ABTS$^{\bullet+}$ radical scavenging activities (%) of different doses of (0.1, 0.2,0.3,0.4,0.5 and 0.6 μg/mL) methanolic extract and solvent fractions of (EtOAc, MDC and *n*-hexanic) *T. conoides* and *T. ornata*. Values are means of triplicate samples, and expressed as mean±SD. To: *T. ornata*, Tc: *T. conoides*, MDC: Dichloromethanic, EtOAc: Ethyl acetate.

Table 2

Total phenolic content and antioxidant activities of the different crude solvent fractions (methanolic, *n*-hexanic, MDC and EtOAc) of brown seaweeds belonging to *Turbinaria* spp.

TPC and antioxidant activities		Solvent fractions of *Turbinria* spp.			
		Methanolic	*n*-hexanic	MDC	EtOAc
Total reduction capability	*T. conoides*	0.26^{ap}±0.00	0.35^{ap}±0.01	1.07^{ap}±0.02	1.07^{ap}±0.01
(A700 nm)	*T. ornata*	0.79^{bp}±0.01	0.83^{bp}±0.01	1.14^{bp}±0.03	0.28^{bp}±0.00
Total phenolic content	*T. conoides*	16.64^{ap}±0.10	19.26^{ap}±0.68	51.47^{aq}±0.99	105.97^{ar}±1.47
(mg of GAE/g)	*T. ornata*	3.42^{bp}±0.35	1.07^{bp}±0.05	12.72^{bq}±0.62	69.63^{br}±1.50
Scavenging of H_2O_2 (%)	*T. conoides*	5.28^{ap}±0.50	3.49^{ap}±0.30	9.40^{ap}±0.30	18.76^{aq}±0.82
	T. ornata	6.57^{bp}±0.18	3.44^{ap}±0.28	3.57^{bp}±0.24	14.31^{bq}±0.43
Fe^{2+} ion chelating ability (%)	*T. conoides*	58.29^{ap}±3.66	25.46^{aq}±3.22	77.19^{ar}±1.20	25.57^{aq}±0.56
	T. ornata	27.63^{bp}±1.34	8.47^{bq}±0.81	62.44^{br}±2.49	68.67^{bs}±0.56
Hydroxyl radical scavenging activity (%)	*T. conoides*	40.19^{ap}±2.08	5.25^{aq}±0.13	52.03^{ar}±1.28	64.20^{as}±0.92
	T. ornata	30.04^{bp}±1.17	4.45^{bq}±0.31	49.82^{br}±0.40	62.08^{bs}±1.48
Lipid peroxidation inhibitory (TBARS) assay	*T. conoides*	24.23^{ap}±0.34	23.07^{ap}±0.79	10.06^{aq}±0.28	6.03^{aq}±0.52
(mmol/L of MDA equivalent compounds /kg)	*T. ornata*	18.36^{bp}±0.99	18.14^{bp}±0.15	8.91^{bq}±0.45	6.78^{aq}±0.49
ABTS radical scavenging activity (%)	*T. conoides*	12.20^{ap}±0.59	12.94^{ap}±0.56	17.57^{ap}±1.51	13.06^{ap}±0.08
	T. ornata	8.04^{bp}±0.55	30.84^{bq}±0.68	6.38^{bp}±0.35	13.91^{ar}±0.42
DPPH· radical scavenging activity (%)	*T. conoides*	12.22^{bp}±0.17	2.16^{ap}±0.15	34.23^{aq}±1.16	23.32^{ar}±1.41
	T. ornata	ND	2.40^{aq}±0.27	1.68^{bq}±0.08	64.14^{br}±1.66

Data are the mean values of triplicate and expressed as mean±SD. Row (p-s) and column values (a-b) with different letters are significantly different ($P<0.05$). MDC: Dichloromethane, EtOAc: Ethyl acetate. The concentration of the crude solvent fractions used were 1 mg/mL for DPPH radical scavenging activity, reducing capacity and H_2O_2 scavenging activity; 0.6 mg/mL for OH radical scavenging activity and Fe^{2+} ion chelating activity; 2 mg/mL for TBARS assay and 0.6 μg/mL for ABTS radical scavenging activity. ND: Non-detectable.

3.3.Determination of scavenging stable radical potential of solvent extracts of seaweeds by 1, 1-diphenyl-2-picryl hydrazyl (DPPH·) method

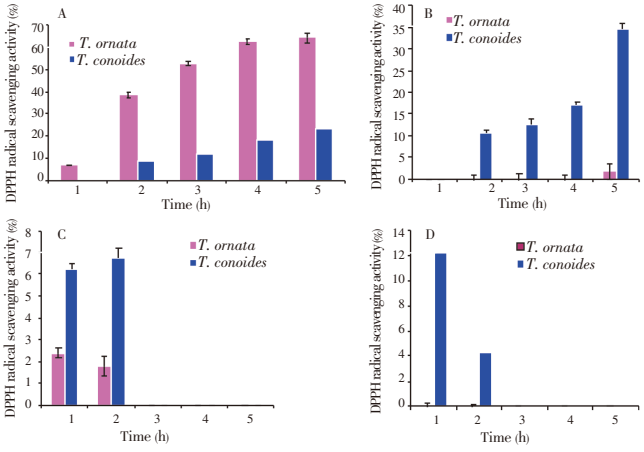

Figure 2. DPPH radical scavenging activities (%) of (A) EtOAc, (B) MDC, (C) *n*-hexanic fractions, and (D) methanolic extracts (0.6 mg/mL) of *T. conoides* and *T. ornata* with respect to various time intervals (0-5 h). Values are means of triplicate samples, and expressed as mean±SD.

DPPH is a useful reagent for investigating the free radical scavenging activities of compounds. EtOAc fractions of both the *Turbinaria* spp. registered significantly higher ($P<0.05$) DPPH radical scavenging activities than methanolic extract and other fractions (Figure 2). DPPH radical scavenging activity of EtOAc fraction of *T. ornata* registered significantly higher ($P<0.05$) (64%) than other solvent extracts (<3%, 1 mg/mL). MDC fraction of *T. conoides* (1 mg/mL) exhibited a significantly higher ($P<0.05$) DPPH radical scavenging activity (34%) followed by EtOAc fraction (23%) at same dose (Table 2).

3.4. Hydroxyl radical scavenging activity

The EtOAc fractions of *T. ornata* (IC_{50} 0.47 mg/mL) and *T. conoides* (IC_{50} 0.44 mg/mL) were found to be highly effective to scavenge HO· radical followed by MDC (IC_{50} 0.59 and 0.58 mg/mL, respectively).The activities were found to be proportionately decreased with concentrations, although EtOAc and MDC fractions of *T. ornata* exhibited significantly higher activities even at lower concentrations (Figure 3). Also, the EtOAc and MDC fractions obtained from *T. conoides* maintained their potential to inhibit formation of HO. radical at a much lower dose.

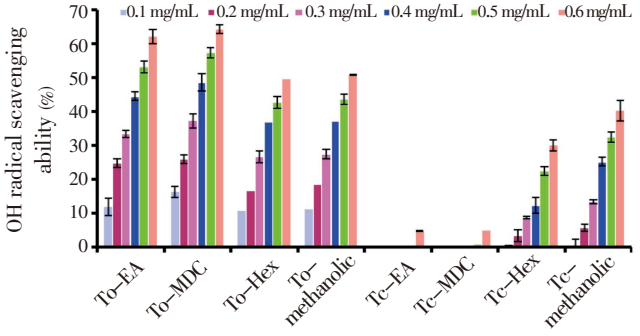

Figure 3. HO· radical scavenging activities (%) of different doses of (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL) methanolic extract and solvent fractions of (EtOAc, MDC and *n*-hexanic) *T. conoides* and *T. ornata*. Values are means of triplicate samples, and expressed as mean±SD. To: *T. ornata*, Tc: *T. conoides*, MDC: Dichloromethanic, EtOAc: Ethyl acetate.

3.5.Hydrogen peroxide scavenging activity

In the present study the EtOAc fraction of *T. conoides* (IC_{50} 1.49 mg/mL) was found to be highly effective to scavenge H_2O_2 followed by MDC (IC_{50} 2.98 mg/mL). The EtOAc fraction

of *T. ornata* realized the lowest IC_{50} value (1.90 mg/mL) followed by methanolic extract (4.25 mg/mL), *n*-hexanic (7.48 mg/mL), and MDC fraction (8 mg/mL), in descending order. The activities of methanolic extract and *n*-hexane fraction were found to be significantly reduced at lower concentrations (0.1-0.3 mg/mL) (Figure 4).

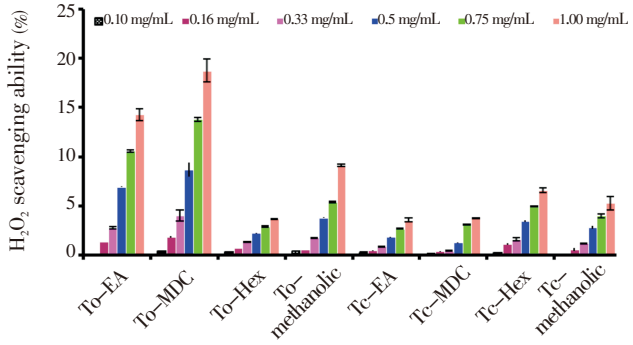

Figure 4. H_2O_2 radical scavenging activities (%)of different doses of (0.10, 0.16, 0.33, 0.50, 0.75 and 1.00 mg/mL) methanolic extract and solvent fractions of (EtOAc, MDC and *n*-hexanic) *T. conoides* and *T. ornata*. Values are means of triplicate samples, and expressed as mean±SD. To: *T. ornata*, Tc: *T. conoides*, MDC: Dichloromethanic, EtOAc: Ethyl acetate.

3.6. Lipid peroxidation inhibitory activities -TBARS

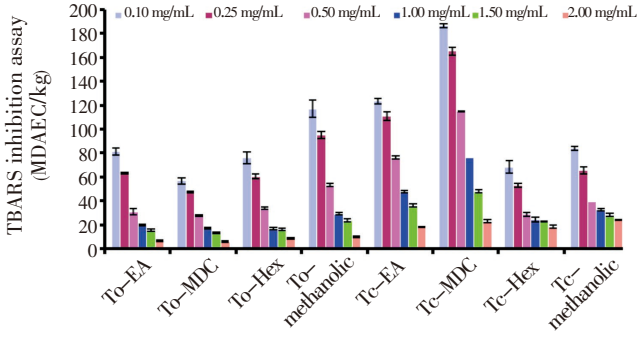

Figure 5. Lipid peroxidation inhibitory assay (%) of different doses of methanolic extract and solvent fractions of (EtOAc, MDC and *n*-hexanic) *T. conoides* and *T. ornata*. Values are means of triplicate samples, and expressed as mean±SD. To: *T. ornata*, Tc: *T. conoides*, MDC: Dichloromethanic, EtOAc: Ethyl acetate.

The EtOAc and MDC fractions of *T. ornata* registered significantly higher TBARS inhibition ability (6.78 and 8.91 MDAEC/kg, respectively) ($P< 0.05$) at 2 mg/mL with respect to inhibit lipid peroxidation (Table 2) than methanolic and *n*-hexanic fractions (18.36 and 18.14 mmol/L MDAEC/kg, respectively), which were not significantly different ($P>0.05$). It is evident from Figure 5 that the TBARS inhibition ability is dose dependent and were found to be proportionately decreased with concentrations. The IC_{50} value of the methanolic extract and different organic solvent fractions revealed the order of activity as: EtOAc fraction (0.21 mg/mL)>*n*-hexane fraction (0.24 mg/mL)>methanolic extract (0.26 mg/mL)>MDC fraction (0.43 mg/mL). The lipid peroxidation inhibitory capacities of EtOAc and MDC fractions of *T. conoides* (6.03 and 10.06 MDAEC/kg, respectively at 2 mg/mL) were significantly higher ($P<0.05$) than that recorded for methanolic extract and *n*-hexanic fraction (24.23 and 23.07 mmol/L MDAEC/kg, respectively) (Table 2). The results obtained from TBARS assay indicate the effectiveness of different *Turbinaria* spp. to prevent lipid oxidation *in vitro*.

3.7. Evaluation of total reduction capability

The IC_{50} value of methanolic extract and different solvent fractions of *T. conoides* revealed the order of activity as: EtOAc (60.59 mg/mL)>MDC (67.35 mg/mL)>*n*-hexane (236.46 mg/mL)>methanolic (543.98 mg/mL). The same trend was apparent in lower concentrations (0.33-0.75 mg/mL). In *T. ornata* the IC_{50} value of reducing activities of different organic solvent fractions and methanolic extract revealed the order of activity as: EtOAc = MDC (52.67 mg/mL)>methanolic extract (71.22 mg/mL)>*n*-hexanic (79.70 mg/mL). A dose dependency was observed in all the solvent fractions (Figure 6).

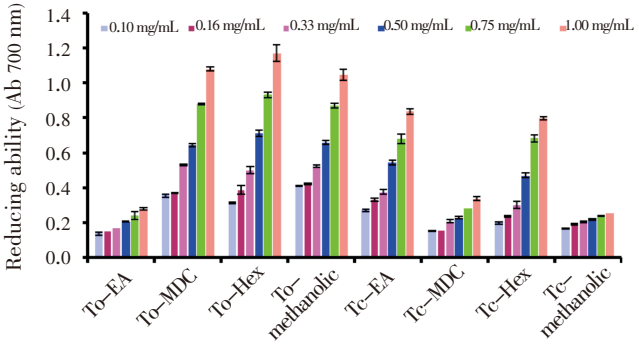

Figure 6. Reducing capacities (A700 nm) of different doses of (0.10, 0.16, 0.33, 0.50, 0.75 and 1.00 mg/mL) methanolic extract and solvent fractions of (EtOAc, MDC and *n*-hexanic) *T. conoides* and *T. ornata*. Values are means of triplicate samples, and expressed as mean±SD. To: *T. ornata*, Tc: *T. conoides*, MDC: dichloromethanic, EtOAc: ethyl acetate.

3.8. Ferrous ion (Fe^{2+}) chelating activity

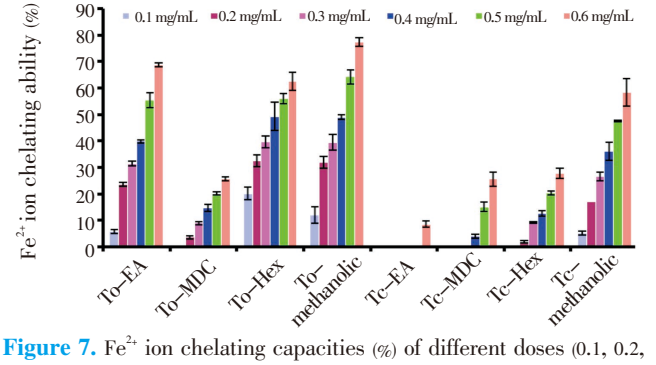

Figure 7. Fe^{2+} ion chelating capacities (%) of different doses (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL) of methanolic extract and solvent fractions of (EtOAc, MDC and *n*-hexanic) *T. conoides* and *T. ornata*. Values are means of triplicate samples, and expressed as mean±SD. To: *T. ornata*, Tc: *T. conoides*, MDC: Dichloromethanic, EtOAc: Ethyl acetate.

In present study different fractions of *Turbinaria* spp. demonstrated a marked capacity for Fe^{2+} ion binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity. The EtOAc and MDC fractions of *T. ornata* (0.6 mg/mL) exhibited significantly higher Fe^{2+} chelating abilities and accordingly, the IC_{50} values exhibited by different solvent fractions of this seaweed followed the order: MDC (0.43 mg/mL)>EtOAc (0.46 mg/mL)> methanolic (1.03 mg/mL)>*n*-hexanic (4.37 mg/mL) fractions. The *n*-hexanic fraction was found to be ineffective towards Fe^{2+} chelating abilities (<10%, 0.6 mg/mL) (Table 2). At lower concentrations (0.3-0.5 mg/mL), EtOAc and MDC

fractions of *T. ornata* realized significantly ($P<0.05$) higher Fe^{2+} chelating activity than methanolic extract. However, it is interesting to note that the Fe^{2+} chelating abilities of MDC fraction of *T. conoides* (0.6 mg/mL) as observed in this study, registered significantly higher ($P<0.05$) (IC_{50} 0.39 mg/mL) than methanolic extract and other solvent fractions. No significant differences are apparent with respect to Fe^{2+} chelating abilities between EtOAc (25.57%), and *n*-hexanic fraction (25.46%) (Table 2). The scavenging activity exhibited a dose dependent behavior. Accordingly, lowest IC_{50} was registered by MDC fraction of *T. conoides* (0.39 mg/mL) towards Fe^{2+} chelating abilities followed by the methanolic fraction (0.53 mg/mL). It can be observed from the figure that (Figure 7) the methanolic extract and solvent fractions of *T. ornata* showed higher ability to chelate Fe^{2+} ions than that exhibited by the tested samples from *T. conoides*.

3.9. Correlations between TPC in Turbinaria spp. and antioxidant activity assays

TPC assay showed significant correlation with $HO^{\cdot}$ radical and H_2O_2 scavenging activity ($n=8$, $R=0.738$, $P<0.05$ and $n=8$, $R=0.957$, $P< 0.01$, respectively). From this observation it may be inferred that phenolic compounds present in the algal extracts are responsible for its radical scavenging ability. A negative correlation ($n=8$, $R=-0.717$, $P<0.05$) was realized between TPC and TBARS assay with different solvent fractions and antioxidant assays. Likewise, the TPC and Fe^{2+} ion chelating activity exhibited negative correlation ($n=8$, $R=0.217$, $P>0.05$). TPC also showed no significant correlation with DPPH ($n=8$, $R=-0.089$, $P>0.05$) and ABTS radical scavenging activities ($n=8$, $R=-0.054$, $P>0.05$) among different solvent fractions and antioxidant assays.

4. Discussion

No simple relationship has been universally accepted between antioxidant capacity of food products vis-à-vis their health benefits. The "antioxidant activity" measured by an individual assay reflects the chemical reactivity under the specific conditions applied in that assay. It is, therefore, necessary to assay the methanolic extracts and the solvent fractions from *Turbinaria* spp. using various methods, which can give an overall idea about their antioxidant activity. The assays were principally based on quantification of phenolic components and reactive oxygen species scavenging activity by different mechanisms *viz.*, hydrogen transfer, electron transfer, and hydroxyl radical scavenging ability peroxide radical scavenging activity. Evaluation of the ability to inhibit lipid oxidation in model systems, reducing ability and metal ion chelating ability were also considered. The validation of the antioxidant potential of the seaweed extracts using different dimensions enable us to get a better understanding of the mechanism of antioxidative properties operating with the experimental seaweed extracts.

Phenolic compounds are considered to exhibit radical scavenging properties[15]. Several studies demonstrated a significant correlation between the phenolic content and the antioxidant activity in seaweed extracts[16]. It is evident from the present observations that a higher percentage of TPC was observed in the polar solvent fractions (EtOAc and MDC) of *Turbinaria* spp. indicate their high antioxidant potential. Earlier reports indicated the presence of phenolic compounds viz. catechin and epigallocatechin in EtOAc fraction of brown seaweeds, particularly *Turbinaria* spp.[17,18]. It was also reported that the aqueous fraction of *T. conoides* is endowed with a higher phenolic content (49.16 mg GE/g) in[18].

The present study reveals that the solvent fractions especially the EtOAc and MDC fractions of *Turbinaria* spp. contain secondary metabolites of antioxidant potential attributed from its DPPH radical scavenging ability. It was reported that active compounds from brown seaweeds with antioxidative properties are phlorotannins and fucoxanthin[15]. The antioxidant property exhibited in present study may mainly be due to the presence of such compounds or any other potential antioxidants with centre/s of unsaturation present in them.

The assay applied in this study was according to the improved technique for the generation of $ABTS^{\cdot-}$, which involves the direct production of the blue/green $ABTS^{\cdot-}$ chromophore though the reaction between ABTS and $K_2S_2O_8$[6]. The higher ABTS radical scavenging ability exhibited by the *n*-hexanic fraction may be explained due to the presence of carotenes/other pigments with long hydrocarbon chain[4]. There are reports which showed that also reported that hexane, chloroform and methanol extracts of *Porphyra yezoensis* exhibited antioxidant activities[19] attributed to the presence of β-carotene, chlorophyll analogues (pheophytin) and amino compounds (leucine, phenylalanine and mycosporine-like amino acid, usujirene). There are also other reports claiming that seaweeds contain antioxidant compounds which include some pigments such as fucoxanthin and astaxanthin, polyphenols such as phlorotannins, chlorophyll related compounds, phospholipids, flavonoids, bromophenols and polysaccharides[4,15].

Hydroxyl radical scavenging activity assay was employed to understand the scavenging potential of methanolic extract and different solvent fractions from seaweeds against short-lived radicals, *viz.*, $HO^{\cdot}$ radicals. $HO^{\cdot}$ radicals were reported to abstract H- atoms from lipid membranes, and thus bring about peroxide reactions of lipids. The $HO^{\cdot}$ scavenging activities of brown seaweeds were reported to be due to polyphenolic compounds such as phlorotannins which can act as electron traps and are responsible for the multifunctional antioxidant properties such as scavenging of hydroxyl radicals, peroxy radicals or superoxides[4]. Ascorbic acid was also reported to be the principle component responsible for $HO^{\cdot}$ scavenging activities recorded in brown seaweeds[20]. There are also other reports which showed that seaweed extracts are potential $HO^{\cdot}$ scavengers[5]. In the present study EtOAc fractions of *Turbinaria* spp. realized higher activities thereby signifying the importance of using EtOAc to isolate potential antioxidant molecules.

H_2O_2 is a non radical compound, and is of potential biological significance because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells (singlet oxygen and $HO^{\cdot}$ radicals)[21]. Thus, removal of H_2O_2 is very essential to protect the biological system in general, and food components, in particular. Earlier studies showed that seaweeds contained polyphenolic compounds such as phlorotannins which can act as electron traps and are responsible for the multifunctional antioxidant properties such as scavenging of hydroxyl radicals, peroxy radicals or superoxides[4]. It was reported that extracts of some brown seaweeds registered more than 90% H_2O_2 scavenging activity[22], thereby supporting the very fact that brown seaweeds are rich source of natural antioxidant compounds, which can scavenge H_2O_2 radical. Many other species of seaweeds were also reported in literature to possess potential H_2O_2 scavenging activity[23].

As a result of oxidation, unsaturated fatty acid (with $\geqq 2$ olefinic double bonds) were reported to break down into low molecular weight aldehydes causing off-flavors (rancid flavor) in oils, and can react with the free amino groups of phospholipid, proteins, and nucleic acids, leading to structural modifications, which induce dysfunction of immune systems[24]. The fatty acid breakdown products essentially contain malondialdehyde (MDA), which was measured through their reaction with thiobarbituric acid (TBA)[25]. The lower values in mille moles of MDA equivalent compounds formed/kg (MDAEC/kg) indicate a higher lipid peroxidation inhibitory effect. The results obtained from TBARS assay indicate the effectiveness of both *Turbinaria* spp. to prevent lipid oxidation *in vitro*. Earlier studies revealed that EtOAc and MDC fraction are the major fractions of seaweeds harboring the principle antioxidative components[8]. The inhibition in lipid peroxidation may be due to the presence of polyphenolic antioxidants that were reported to disrupt free-radical chain reaction by donating proton to fatty acid radicals to terminate chain reactions, may have roles to inhibit lipid peroxidation[4]. There are other reports which suggests that extracts of brown seaweeds belonging to *Turbinaria* spp. are anticipated to be very got inhibitors of lipid peroxidation[9].

The reducing abilities of chemical extracts and/or compounds generally depends on the presence of reductones, which have been shown to impart antioxidant action by breaking the free radical chain by donating a hydrogen atom[26]. The presence of reductants (*i.e.* antioxidants) in the solvent fractions apparently reduces the Fe^{3+}/ferricyanide complex to its Fe^{2+} form, which can be monitored by measuring the formation of Perl's Prussian blue at 700 nm[26]. Results obtained in the present study are in accordance with the earlier reports suggesting that brown seaweeds collected from different regions were found to be endowed with potential reducing abilities and antioxidant properties[5,16]. It was also reported that reducing power exhibited by solvent extracts of seaweeds belonging to *Turbinaria* spp. was comparatively higher than α-tocopherol[18].

The reduced form of iron (Fe^{2+}) can stimulate and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[27-30]. As a result chelators of Fe^{2+} ion can be considered as potential inhibitors of lipid peroxidation. It was reported that low-molecular compounds in the dried brown seaweed Scytosiphon lomentaria with Fe^{2+} iron chelating activity[17]. There are other reports that the phlorotanins which are usually present in the polar solvent fractions of brown seaweeds are strong chelators of heavy metals[4,28]. The Fe^{2+} chelating abilities of the seaweed fractions were also reported to be due to the presence of non phenolic compounds like different types of polysaccharides present in the seaweed extracts[32]. A negative correlation observed between TPC and Fe^{2+} chelating abilities proves that in this study chelating ability of algae could be due to the presence of compounds other than phenolics and these seaweeds could be potential rich sources of natural antioxidants. Molecules with hydroxyl, sulfhydryl, carbonyl, and phosphate groups were reported to possess favourable structure-function configuration resulting in Fe^{2+} chelating abilities, and apparently compounds including phenolic acids, flavonoid quercetin, andphenolic glycosides are noted to chelate transition metal ions like Fe^{2+} iron. These active compounds might have a synergistic effect, playing an important role in antioxidant activity by the inhibition of oxidation and chelating effects.

The positive correlation observed between TPC and radical scavenging activities of seaweed extracts is in agreement with the earlier literature data[33]. Negative correlation realized between TPC and TBARS assay apparently indicate that antioxidant activity did not depend only on total phenol content, but also on other factors as there may be some active metabolites other than phenolics such as polysaccharides capable of inhibiting the TBA-MDA adduct formation. Likewise, the total phenolic content and Fe^{2+} ion chelating activity exhibited negative correlation thus suggesting the presence of some compounds other than phenolics capable of chelating transition metals. Earlier studies conducted by other researchers also showed that polysaccharides (*e.g.* alginates and fucoidan) and/or phytochelatins were more effective than phlorotannins for the detoxification and resistance to copper accumulation in Ascophyllum nodosum[31]. In addition, some peptides as well as proteins found in seaweed extracts have also been reported to possess the abilities to chelate metal ions[31]. The results lead to the conclusion that algal polyphenols are probably not strong chelators of transition metals. However, further study is needed to elucidate the mechanism of antioxidant action of different compounds in the seaweed extracts. No significant correlation between phenolic contents and DPPH and ABTS radical scavenging activities in the seaweed extracts also indicated the presence of compounds other than phenolics (small molecular weight polysaccharides, pigments, proteins or peptides), to be involved in the antioxidant activity.

There are other studies which are in agreement with our present observation that some seaweed extracts exhibited a lower correlation between TPC and antioxidant activity[35]. The present study propose a path to further research on these solvent extracts, particularly of ethyl acetate fraction from *Turbinaria* spp. as starting materials for bioassay guided purification, and characterization of the compounds accountable for their antioxidant activity, for application in food, pharmaceutical, and cosmetic industry.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Reactive oxygen species formed during aerobic metabolism in human tissue cells result in extensive oxidative damage leading to several life-threatening human diseases. Therefore, consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these undesirable events. Since there is an increased interest in the antioxidants of natural origin in recent times in place of synthetic ones due to concern about their potential toxic effects, it is rational to explore natural alternatives to isolate antioxidant principles for use as food supplements and/or nutraceutical supplements. There are limited efforts to unearth the vast marine sources to understand their antioxidative potential. Recent studies indicated that antioxidants were found in some marine organisms including seaweeds.

Research frontiers

Although antioxidant properties of seaweeds were proved by numerous studies from past two decades very few of them have been studied in detail from this very important delta region. The present study provide us with valuable information regarding the antioxidative defense mechanism of the brown seaweeds belonging to *Turbinaria* spp.. The present study realized the potential antioxidant properties of brown seaweeeds *T. conoides* and *T. ornata* abundantly available alongwith the south-east coast of Indian subcontinent. The statistical analysis carried out by the authors increased the reliability of the experimental data.

Related reports

Brown seaweeds constitute potential sources of natural antioxidants, including phenolics and isoprenoids (Swanson and Druehl, 2002). Extensive studies have been conducted on the antioxidant activities belonging to brown seaweeds, and were found to be endowed with potential antioxidant properties (Heo *et al*., 2005). It was reported that the varius extracts obtained from brown seaweeds exhibited nutraceutical value as potent antioxidants via alleviations of radical-induced toxicities (Kim *et al*., 2010), anti-obesity and antioxidant properties (Matanjun *et al*., 2010), DPPH radical scavenging ability, reducing power and metal-chelating activity (Prabhasankar *et al*., 2009).

Innovations and breakthroughs

In this study the polar ethylacetate fractions of *Turbinaria* spp. was found to be potential reservoir of phenolic content and high $DPPH^{\cdot}$, $HO^{\cdot}$ scavenging ability, reduction capability, and Fe^{2+} chelating activity thereby signifying the potential use of this seaweed as potential reservoir of antioxidative principles. These results indicated a significant correlation of total phenolic content in ethylacetate fractions of seaweeds with ABTS, DPPH, hydroxyl radical scavenging activities, and Fe^{2+} chelating ability, thereby indicating that polyphenols present in algal fractions, particularly in ethylacetate fraction are responsible for its radical scavenging ability, lipid peroxidation ability and chelating ability.

Applications

This study indicated the potential use of *T. conoides* and *T. ornata* as valuable sources of natural antioxidants to be used as food supplement for increasing the shelf-life of food industry and as candidate nutraceuticals in combating free-radical induced diseases.

Peer review

It is apparent from the present study that ingredients in ethylacetate fractions of seaweeds appeared to be the reservoir of antioxidant principles, and seem to play an important role in the free radical scavenging capacity. The present study provides valuable information regarding the potential of these brown seaweeds to develop a viable natural substitute of the existing synthetic antioxidants. The statistical analyses carried out by the authors increased the reliability of the experimental data.

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