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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.
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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