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RESEARCH ARTICLE

Antibacterial and Antioxidant Activity, Total Phenolics and Flavonoid Contents of brown Seaweeds from Southeast Coast of India

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Abstract

Seaweeds are marine macroalgae which are renewable living resources used as food, feed and fertilizer in many parts of the world. Seaweeds are also considered to be a rich source of antioxidants. The present study is aimed to investigate the antibacterial, antioxidant property, total phenolics and flavonoids of aqueous extract of (i) *Spathoglossum asperum* (ii) *Turbinaria conoides* which were obtained from the Southeast Coast of India. DPPH radical scavenging activity and Folin-Ciocalteu reagent were used to determine total antioxidant activity and total phenols of seaweed extracts. Antimicrobial activity was determined by using disc diffusion assay. The aqueous extract of *T. conoides* showed maximum DPPH radical scavenging activity (50.00%) at 30 min compared to *S. asperum* (32.78%) at 25 min. The antibacterial activity of aqueous extract of S. *asperum* and *T.conides* ranged between 9 - 13mm at 30 µl. The results provided evidence that the studied seaweeds might be a potential source as natural antioxidant and antimicrobial agents.

INTRODUCTION

Seaweeds are marine macroalgae which are renewable living resources used as food, feed and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they contain low calorie food, but rich in vitamins, minerals and dietary fibres (Ito and Hori, 1989). In addition to vitamins and minerals, seaweeds are also good sources of proteins, polysaccharides and fibres. Seaweeds provide a source of structurally diverse and biologically active secondary metabolites (Vallinayagam *et al*., 2009).

They are also considered to be a rich source of antioxidants. The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.). Those compounds are widely distributed in seaweeds and are known to exhibit higher antioxidant activities (Cahyana *et al*., 1992).

Algae generally have higher antioxidant activity due to higher contents of nonenzymatic antioxidant components, such as ascorbic acid, reduced glutathione, phenols and flavonoids (Farasat *et al*., 2013). As a result, many marine biosources in the last decades have attracted attention in the search for natural bioactive compounds to develop new drugs and healthy foods. Compounds with antioxidant, antiviral, antifungal, antimicrobial, antitumor and anti-inflammatory activities have been found in brown, red and green algae (Cox *et al*., 2012).

The present study is aimed to investigate the antibacterial, antioxidant property, total phenolics and flavonoid content of aqueous extract of (i) *Spathoglossum asperum* (ii) *Turbinaria conoides* which were obtained from the Southeast Coast of India for future applications in medicine, dietary supplements, cosmetics or food industries.

Materials and Methods

Seaweed collection and processing

Brown seaweeds such as *S.asperum* and *T. conoides* were collected from Mandapam coast in Rameshwaram. The algal samples were handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. It was kept in icebox containing slush ice, transported to the laboratory and washed thoroughly using tap water to remove the salt on the surface of the sample. The water was drained off and the algal material was spread on blotting paper to remove excess water. They were shade dried. The dried seaweeds were finally pulverized in the commercial grinder and the powdered seaweed samples were stored at 4°C and used for further analysis.

Extraction of sample

Each ground sample was weighed and transferred into a beaker. Water was added in the ratio of 1:10 and stirred for 1 h with the aid of a magnetic stirrer. The extraction mixture was left to sediment for at least 1 h before the extract was separated from the residue by filtration through Whatman No. 1 filter paper. The residue was re-extracted twice, and the two extracts were combined. Extracts were produced in duplicates and used to assay the antibacterial and antioxidant activity.

Antimicrobial activity

The aqueous extract of the seaweed was used for antibacterial study (Janarthanan and Sumathi, 2010). Different concentrations (10mg, 20 mg, 30 mg/mL) of the aqueous extract were tested for their antimicrobial activity against pathogenic bacteria strains such as *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherchia coli, and Pseudomonos aeruginosa*. The bacterial cultures were grown in Muller hinton agar and Muller hinton broth (Himedia) (Lopez et al, 2001). Antibacterial activity was measured using the standard method of diffusion disc plates on agar, and then 0.1 mL of each culture of bacteria was spread on agar plate. For antimicrobial assay, all bacterial strains were grown in Muller hinton medium (Himedia) for 24 hours at 37^0C and plated on Muller hinton Agar. For Agar diffusion experiment, Paper disc (6mm in diameter) was placed on the agar medium to load 20µL of the different concentration (10 - 30mg/mL) of aqueous extracts of seaweeds. Inhibition diameters were measured after incubation for 24hrs to 48 hrs at 37^0C.

Antioxidant activity

DPPH radical scavenging activity

Qualitative Analysis

100 µL of aqueous extracts were taken in the microtitre plate. 100 µL of 0.1% methanolic DPPH was added over the samples, incubated for 30 minutes in dark. The samples were then observed for discolouration. The purple colour indicates the absence of antioxidant activity. The yellow colour indicates the presence of strong antioxidant activity and pale color was an indication of weak activity respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitaive Analysis

Aqueous extract of 100 µL from qualitative assay were mixed with 2.7mL of methanol. Then 200 µL of 0.1% methanolic DPPH was added.The suspension was incubated for 30min in dark condition. Subequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of BHT. Sample blank and control samples were performed according to the method. Scavenging effect of DPPH radical was calculated using the following equation

Antioxidant activity (%) =Absorbance of control - Absorbance of sample/ Absorbance of Control *100

Total phenolic content

The folin - ciocalteau reagent has been used for estimation of total phenolic extracts according to Lister and Wilson 2001, with slight modification. Five concentrations of all crude extracts of the plants have been prepared and then 100µL have been taken from each concentration. Acid mixed with with 0.5mL of F.C reagent (1/10) dilution and 1.5ml Na_2co_3 (2% w/v). The blend was incubated in the dark @R.T for 15 minutes. The absorbance of blue coloured solution of all samples was measured at 765nm using UV-Vi spectrophotometer. The result was expressed in mg of gallic acid equivalent (GAE) per g dry weight of Seaweed powder.

Estimation of Total Flavanoid Content

Aluminium Chloride Calorimetric method

The aluminium chloride calorimetric method was modified from the procedure reported by woisky and salatino. Quercetine was used to make the calibration curve. 10mg of Quercetin was dissolved in 80% ethanol and then diluted to 25, 50 &100µg/mL. The diluted standard solution (0.5mL) were separately mixed with 1.5mL of 95% ethanol, 0.1mL of 10%Aluminium chloride, 0.1mL of 1M Potassium acetate and 2.8mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415nm with

spectrophotometer. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank.

Results

The results of antioxidant and antibacterial activity of aqueous extract of *S. asperum* and *T. conoides* were shown in the Table 2 & Table 3 respectively. The total phenol and flavonoid content were presented in Table 4.

Table 1: Qualitative Analysis of Antioxidant activity

Samples	**Response**
Control (methanol)	Negative
Control (BHT)	Positive
S. asperum	Negative
T.conides	Positive

Table 2: Quantitative Analysis of Antioxidant activity

Samples	**DPPH Assay (%)**						
	0 min	**5 min**	**10 min**	**15min**	**20 min**	**25min**	**30min**
S. asperum	25.41	26.23	29.51	30.32	31.97	32.78	32.78
T.conides	38.52	41.80	45.08	47.54	48.36	48.36	50

The aqueous extract of *T. conoides* showed maximum DPPH radical scavenging activity (50.00%) at 30 min compared to *S. asperum* (32.78%) at 25 min (Table 2).

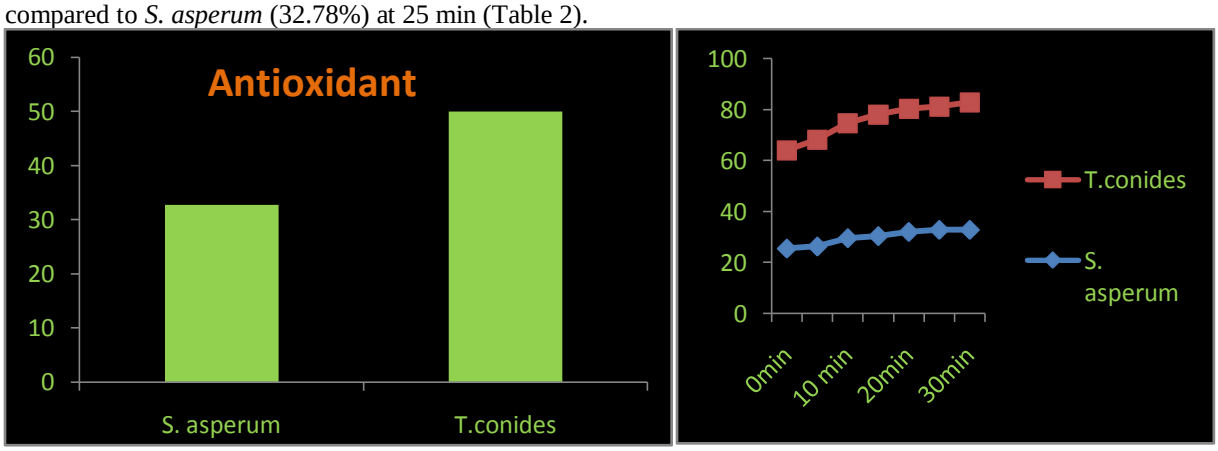

Fig.1: Antioxidant activity of *T.conides* and *S.asperum*

The antibacterial activity of aqueous extract of *S. asperum* and *T.conides* ranged between 9 - 13mm at 30 µl. The *S. asperum* showed maximum activity against *B. subtilis* (13mm) and minimum activity against *E.coli* (9mm). The *T.conides* showed maximum activity against *B. subtilis* and S.*aureus* (13mm) and minimum activity against *B. cereus* (9mm). Whereas no activity was seen against *Pseudomonas* sp.

The quantitative phytochemical estimation of total phenols and flavonoids were estimated in *S. asperum* and *T.conides*. In *S. asperum*, the total phenol and flavonoid were 2.33 mg GAE/g and 7.50mg/g respectively. In *T.conides* the total phenol and flavonoid were 2.50 mg GAE/g and 3.75mg/g respectively.

Table 3: Antimicrobial activity of *S. asperum* and *T. conides*

Bacterial Strains	Seaweeds	Zone of Inhibition (mm)		
		10µl	20 µl	30 µl
Bacillus cereus	***S. asperum***	-	8	10
	T.conides	-	-	9
Bacillus subtilis	***S. asperum***	-	12	13
	T.conides	-	10	13
Pseudomonas	***S. asperum***	-	-	-
	T.conides	-	-	-
E.coli	***S. asperum***	-	9	9
	T.conides	-	-	11
S. aureus	***S. asperum***	-	10	12
	T.conides	10	11	13

Table 3: Total Phenol and Flavanoid of *S.asperum* and *T. conides*

Samples	Total Phenol Content (mg GAE/g)	Total Flavanoid Content (mg/g)
S. asperum	2.33	7.50
T.conides	2.50	3.75

Discussion

In the current study, the DPPH radical scavenging method was used to evaluate the antioxidant capacity of the seaweed extracts, because the use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers (Nickavar *et al.*, 2007).

The antioxidant activity of seaweeds was in accordance with their amount of phenolic and flavonoid contents. Several reports have indicated a close relationship between total phenolic content and high antioxidant activity and many researchers demonstrated that phenolic compounds were one of the most effective antioxidants in brown algae (Namjooyan *et al*., 2007).

The antioxidant activity is strongly dependent on the collection seasons, phenol and flavonoid content and types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential (Kumar et al.,2008).

The majority of the compounds isolated from marine algae are responsible for the antimicrobial activity. The maximum antimicrobial activity shown by brown algae in the present work confirms to the earlier work (Reichelt and Borowitzka, 1984). Disc diffusion methods are extensively used to investigate the antimicrobial activity of natural substances. In the present work, the aqueous extracts of two different seaweeds were evaluated for antimicrobial activity against pathogenic bacteria. *S. asperum* and *T.conides* showed maximum activity against *Bacillus subtilis*.

This study showed that agar diffusion methods using different test microorganisms are valuable tool for the antimicrobial activity of seaweeds. There have been many reports on the screening of seaweeds for antimicrobial activity. This capability of the seaweeds can be attributed to synthesis of bioactive secondary metabolites. The complexity of antimicrobial properties in seaweeds is due to their multiple inhibitory properties. It may be due to

both long term defense as well as rapid activation induced by environmental conditions. The intra specific variability in the production of secondary metabolites in seaweeds is generally related to seasonal variation; differences in the extraction protocols to recover the active metabolites and assay methods that would result in different susceptibilities of the target strains (Kayalvizhi., 2012).

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