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Full Length Research Paper

Evaluation of two different solvent extracts of *Colpomenia sinuosa in vitro* antioxidant activity using standard procedures

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The brown alga, *Colpomenia sinuosa* was subjected to DPPH (1,1- diphenyl-2-picryl hydrazyl), nitric oxide radical, hydrogen peroxide, scavenging of ABTS radical and Total antioxidant capacity (TAC) inhibitory assays to assess its antioxidant property. The dimethyl sulphoxide (DMSO) and methanol extracts showed highest antioxidant activity in DPPH (96.56 and 88.57%), nitric oxide radical (81.2 ± 4.1% and 76.69 ± 3.1 %), hydrogen peroxide (70.7 ± 3.5 and 56.6 ± 2.0%) and ABTS (76.8 ± 3.8% and 74.0 ± 2.8%). The moderate activity was observed in total antioxidant capacity (60.3 ± 4.3 and 57.0 ± 2.0) assay. The activity was higher and comparable to that of commercial antioxidants butylated hydroxy anisole (BHA) (87.38 ± 1.32%) and butylated hydroxy tolune (BHT) (56.05 ± 0.19%) at 2 mg/ml concentration. The significant free radical scavenging activity of *C. sinuosa* indicated that it could be a potential source for natural antioxidant lead molecules.

Key words: ABTS, *Colpomenia sinuosa*, DPPH, hydrogen peroxide, nitric oxide radical.

INTRODUCTION

A free radical is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. The importance of reactive oxygen species and free radicals has attracted increasing attention over the past decade. An over production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free-radical formation (Wong et al., 2000). Reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$) and nitric oxide radical ($NO^{\bullet}$), attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis carcinogenesis (Keli-Chen et al., 2005) and may lead to the development of chronic diseases related to the cardio and cerebrovascular systems (Halliwell and Gutteridge, 1989).

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Free-radical scavengers are antioxidants which can pro-vide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking (Ghosal et al., 1996). The most commonly used synthetic antioxidants presently arebuty-lated hydoxyanisole (BHA), butylatedhydoxytoluene (BHT) Propylgallate (PG) and test butylatedhydroquinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis (Wichi, 1988; Sherwin et al., 1990). Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials to replace synthetic antioxidant.

Marine algae have received special attention as a source of natural antioxidants (Matsukawa et al., 1997). Seaweeds are known source of pharmacological and food additives with potential health effects like antioxi-dative and anticarcinogenic (Lim et al., 2002; Athukurake et al., 2003). Based on the above facts, the brown seaweed *Colpomenia sinuosa* (Derb Et Sol) was studied for its potential antioxidant property. In the present investigation two different solvent extracts of *C. sinuosa* were investigated *in vitro* antioxidant activity using standard procedures.

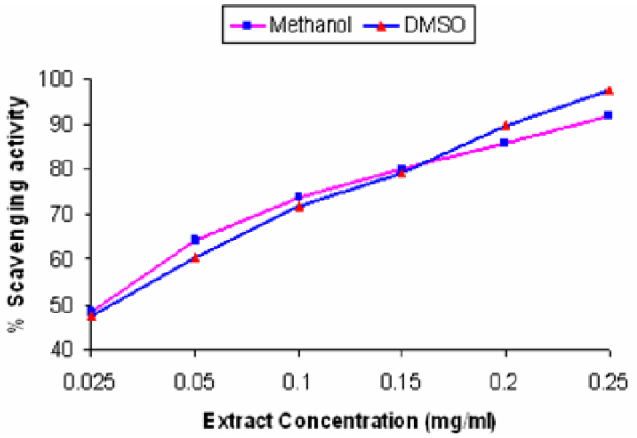

Figure 1. Scavenging activity of *Colpomenia sinuosa* extract on DPPH.

MATERIALS AND METHODS

The brown alga *C. sinuosa* (Derb Et Sol) (Phaeophyta: Phaeophyceae: Dictyosiphonales: Punctariaceae) was collected from Tuticorin coast, Tamil Nadu, India (Lat. 8º45' N; Long. 78º10' E) and identified by the method described by Umamaheshwara Rao (1987). The fresh seaweed was rinsed with fresh water, air dried in shade at 60^{O}C and pulverized into a fine powder. 10 g of seaweed powder was extracted sequentially with 100 ml of diethyl ether (98%) and methanol (99%) in a Soxhlet extractor for six hours and was repeated twice (Lim et al., 2002). The crude extracts were then dried *in vacuum* and the resultant residues were stored in dark at 4^{o}C until further use. Subsequently, the diethyl ether residue was dissolved in Dimethyl sulfoxide (DMSO) and the methanol residue in methanol and subjected to the following assays to assess the antioxidant potential.

DPPH radical scavenging assay

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging was assayed as described by Hwang et al. (2001). 10 µl of the seaweed extract with 0.2 ml of DPPH (100 µM) (Sigma-Aldrich) in methanol solution was incubated at 37ºC for 30 min and the absorbance of the supernatant was measured at 490 nm using ELISA micro plate reader (Bio Rad Laboratories Inc., California, USA, Model 550). Activity calculation was done as follows:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

IC_{50} value concentration of the sample required to scavenge 50% DPPH free radical.

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition was assayed by incubating 2 ml sodium nitroprusside (10 mM), 0.5 ml phosphate buffer saline and 0.5 ml (0.25 mg) of extract solution at 25ºC for 150 min. Then 1 ml of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) was added to 0.5 ml of reaction mixture for 5 min to complete diazotization, subsequently. 1 ml naphthyl ethylene diamine dihydrochloride (NEDD) was added and allowed to stand for 30 min at 25ºC. The absorbances of these solutions were measured at 540 nm (Govindarajan et al., 2003; Badami et al., 2005).

Hydrogen peroxide radical scavenging assay

Ability of the seaweed extracts to scavenge hydrogen peroxide was determined as described by Govindarajan et al. (2003) and Gulcin et al. (2004). One ml (0.25 mg) of the extract was rapidly mixed with 2 ml of 10 mM phosphate buffered (0.1M, pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 min of incubation at 37ºC (Shimazdu, UV- 160A) against a blank (without hydrogen peroxide). The percentage of scavenging of hydrogen peroxide was calculated using the following formula.

$$\text{Percentage scavenged } (H_2O_2) = \frac{A_0 - A_1}{A_0} \times 100$$

(A_0 – Absorbance of control; A_1 – Absorbance of sample)

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging assay

Free radical scavenging activity of the extract was also determined by ABTS (Sigma-Aldrich) radical cation decolourization assay (Re et al., 1999). ABTS radical cation was generated by mixing 20 mM ABTS solution with 70 mM potassium peroxodisulphate and allowing it to stand in the dark at room temperature for 24 h before use. 0.6 ml of extract (0.25 mg) was mixed with 0.45 ml of ABTS reagent and absorbance of these solutions was measured at 734 nm after 10 min.

Total antioxidant activity

Total antioxidant activity was measured by following the method of Mitsuda et al. (1996). 7.45 ml Sulphuric acid (0.6 M solution), 0.9942 g Sodium sulphate (28 mM solution) and 1.2359 g Ammonium molybdate (4 mM) were mixed together in 250 ml with distilled water and labeled as a Total Antioxidant Capacity (TAC). 100 l of extract was dissolved in 1 ml of TAC absorbance was read at 695 nm after 15 min.

RESULTS AND DISCUSSION

Dietary natural antioxidants are reported to help in preventing aging and other diseases. There are some evidences that seaweeds contain compounds with a relatively high antioxidant and antiproliferative activity. Seaweeds are low in fats but contain vitamins and bioactive compounds, like terpenoids, sulfated polysaccharide and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants (Lahaye and Kaffer, 1977).

DPPH radical scavenging assay

The increase in scavenging activity of *C. sinuosa* extracts on DPPH radicals was concentration dependent (Figure 1). The DMSO and methanol extract exhibited a strong scavenging activity on DPPH (96.56 and 88.57%) at 0.25 mg concentration (Figure 2). Inhibition above 50% was

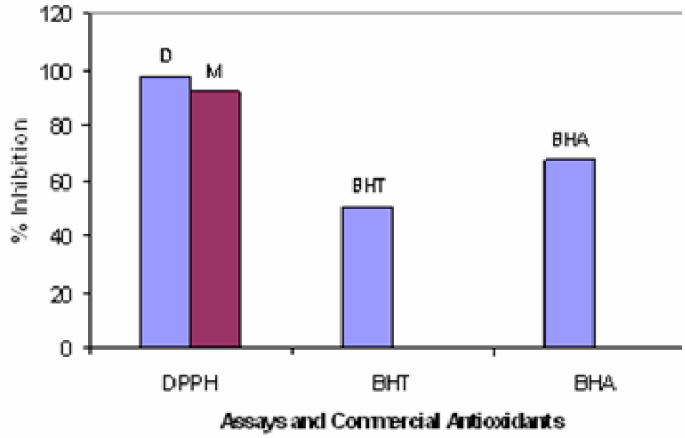

Figure 2. DPPH radical scavenging activity of methanol and DMSO dissolved diethyl ther extracts (0.25 mg/ml) compared with commercial antioxidants (2 mg/ml). DPPH - DPPH radical scavenging assay; BHT- Butylated Hydroxy Tolune; BHA-Butylated Hydroxy Anisole; D-DMSO dissolved diethyl ether extract; M-Methanol extract.

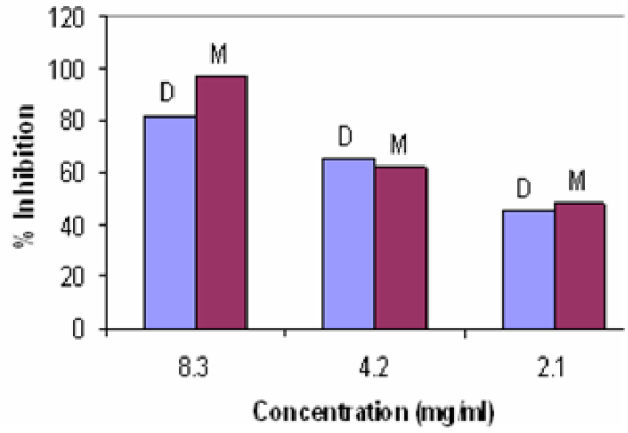

Figure 3. Nitric oxide radical inhibiting activity of *Colpomenia sinuosa.*D-DMSO dissolved diethyl ether extract; M-Methano.

observed for both DMSO dissolved and methanol extracts at a concentration of 0.05 mg. The IC_{50} values for DMSO dissolved and methanol extracts were 0.03 and 0.0275 mg/ml, respectively (Figure 7). The scavenging activity was greater than that of commercial antioxidant BHA and BHT at 2 mg/ml concentration (Badami et al., 2005; Heo et al., 2005). The IC_{50} values in the present study are comparable to that of organic and aqueous fractions of brown alga *Ecklonia cava*. It was also found that the IC_{50} value of *C. sinuosa* was higher than that of BHT but less than that of –tocopherol (Senevirathane et al., 2006).

The study of Yuan et al. (2005) revealed that the IC_{50} values of *Palmaria palmate* extract was lower than that of *C. sinuosa* extract. However, the extractions of active ingredients depend upon various polarity nature of the solvent system and the quantum of the active principles in the particular resources. It was also found that DPPH scavenging activity of BHA and BHT (Gulcin, 2005) are comparable to the activity of *C. sinuosa* extract. The IC_{50} activity of DMSO dissolved and methanol extract of *C. sinuosa* was comparatively stronger than ethanolic extract of Nori, Kombu, Wakame, Hijiki and the known antioxidant Vitamin E (Ismail and Hang, 2002).

Nitric oxide radical inhibition assay

Nitric oxide is generated from amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells in the brain (Moncada et al., 1991). The toxicity and damage caused by NO• and O_2• is multiplied as they react to produce reactive peroxynitrite (ONOO), which lead to serious toxic reactions in the biomolecules, like proteins, lipids and nucleic acids (Moncada et al., 1991; Yermilo et al., 1995). Scavengers of nitric oxide compete with oxygen and lead to the production of nitric oxide (Marcocci et al., 1994). The present investigation on *C. sinuosa* extract (DMSO and methanol) shows better activity (Figure 3). *C. sinuosa* extract at 8.3 mg/ml concentration showed an inhibition of 81.2 ± 4.1 (DMSO dissolved) and 76.69 ± 3.1 (methanol). The IC_{50} values for both the extracts were 2.6 and 2.7 mg/ml, respectively (Figure 7). These IC_{50} values of nitric oxide radical assay was comparable to the IC_{50} values of *Cassia fistula* (*Linn*) bark extract as reported by Raju Ilavarasan et al. (2002). Badami et al. (2005) reported that, the IC_{50} value of ethyl acetate, petroleum ether and methanol extracts of *Aporosa lindleyana* root inhibit the nitric oxide generation. The suppression of NO• release may be partially attributed to direct scavenging by both extracts of *C. sinuosa,* which decrease the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro* (Senevirathene et al., 2006).

Hydrogen peroxide radical scavenging assay

Many species of seaweed possess scavenging ability of hydrogen peroxide (Athukurake et al., 2003; Siriwardhana et al., 2003). The measurement of H_2O_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2 (Czochra and Widensk, 2002). It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells (Gulcin, 2006).

The inhibitive effect of seaweed extract was subjected to hydrogen peroxide scavenging assay and was found to be moderate (Figure 4) when compared to other assays. The IC_{50} values were 2.7 and 4.2 mg/ml for DMSO dissolved and methanol extracts, respectively (Figure 7).

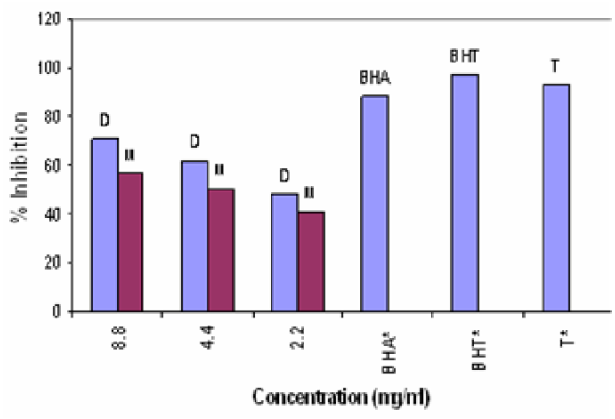

Figure 4. Hydrogen peroxide radical scavenging activity of *Colpomenia sinuosa.* BHT- Butylated Hydroxy Tolune; BHA-Butylated Hydroxy Anisole; T-α-Tocopherol; D-DMSO dissolved diethyl ether extract; M-Methanol extract; *BHA, BHT, T (α-Tocopherol) - 75 mg/ml.

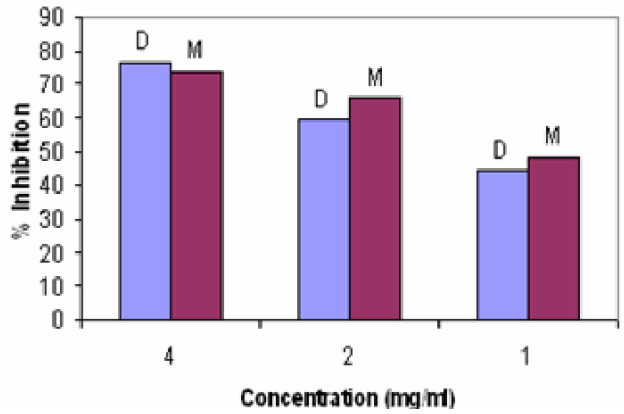

Figure 5. ABTS radical cation scavenging activity of *Colpomenia sinuosa.* D-DMSO dissolved diethyl ether extract; M-Methanol extract.

The inhibition of 70.7 ± 3.5% and 56.6 ± 2.0% observed for DMSO dissolved and methanol extracts at 8.8 mg/ml concentration were lower than the activity of commercial antioxidants of BHA and BHT and – Tocopherol (Heo et al., 2005). These results indicate the antioxidant molecular leads dissociate in different proportions with different solvent system. Nevertheless *C. sinuosa* proved to possess the vital antioxidant lead molecules.

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging assay

The ABTS radical reactions involve electron transfer and the process take place faster rate when compared to DPPH radicals. Re et al. (1999) reported that the decolorization of the $ABTS^{\bullet+}$ radical also reflects the capacity

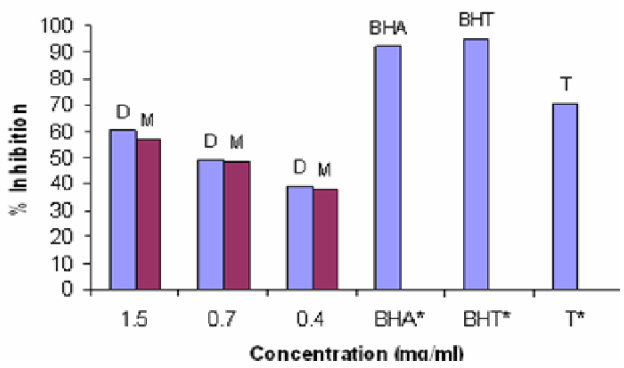

Figure 6. Total antioxidant activity of *Colpomenia sinuosa.* BHT- Butylated Hydroxy Tolune; BHA-Butylated Hydroxy Anisole; T-α-Tocopherol; D-DMSO dissolved diethyl ether extract; M-Methanol extract; *BHA, BHT, T (α-Tocopherol) - 75 µg/ml.

of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. In the ABTS radical cation scavenging activity, the seaweed *C. sinuosa* extract showed concentration dependent scavenging activity (Figure 5). The DMSO dissolved extract showed activity of 76.8 ± 3.8% and methanol extract showed activity of 74.0 ± 2.8% for the concentration of 4 mg/ml. The IC_{50} values were 1.4 and 1.1 mg/ml for DMSO dissolved and methanol extracts, respectively (Figure 7). Katalinic et al. (2006) found that, in this assay, ABTS radical cation was generated directly in stable form using potassium persulfate. Generation of radical before the antioxidants added prevents interference of compounds, which affect radical formation. This modification makes the assay less susceptible capacity. The extracts of *C. sinuosa* showed higher scavenging activity compared to grape seed extract (Keli-Chen et al., 2005). ABTS was used to reconfirm the antioxidant property of the seaweed extract *C. sinuosa*.

Total antioxidant activity

The total antioxidant activity of extract was calculated based on inhibition percentage of 60.3 ± 4.3 and 57.0 ± 2.0, respectively (Figure 6). The antioxidant activity increases with increasing concentration. The IC_{50} values were 0.8 and 0.9 mg/ml for DMSO dissolved and methanol extracts (Figure 7). However, the *C. sinuosa* extracts showed lesser activity than the standards of BHA, BHT and -tocophorol.

Conclusion

All these results denote that *C. sinuosa* could be an important source of antioxidant molecules.Further studies are required to elucidate the compound showing antioxi

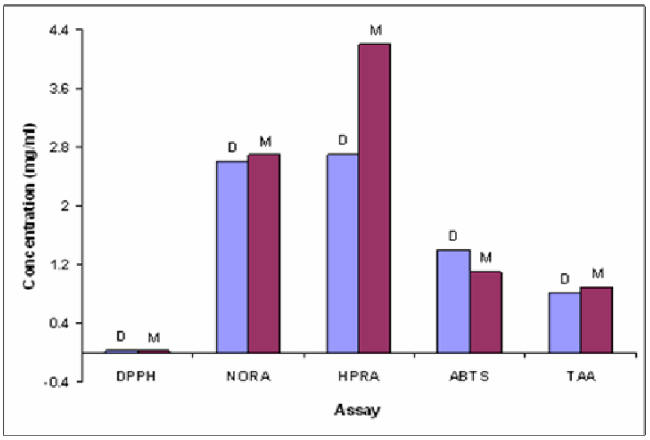

Figure 7. IC_{50} values for *Colpomenia sinuosa* in different assays.
DPPH - DPPH radical scavenging assay; NORA-Nitric oxide radical scavenging assay; HPRA-Hydrogen peroxide radical scavenging assay; TAA-Total antioxidant assay; D-DMSO dissolved diethyl ether extract; M-Methanol extract.

dant property *in vivo* and the compound could evolve as an anticancer drug in near future.

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