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Original article

Evaluation of *Gelidiella acerosa*, the red algae inhabiting South Indian coastal area for antioxidant and metal chelating potential

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ABSTRACT

In vitro antioxidant potential and metal chelating activity of various solvent fractions of *Gelidiella acerosa* were evaluated by different antioxidant assays, like ferric reducing antioxidative power (FRAP) assay and scavenging activities for hydrogen peroxide, hydroxyl radical and nitric oxide. Among all the fractions, benzene showed the highest 1,1-diphenyl,2-picrylhydrazyl (DPPH) radical, nitric oxide and hydrogen peroxide scavenging activity when compared to standard BHT with IC_{50} values of 306.50 ± 3.04, 328.79 ± 14.25 and 275.43 ± 72.99 μg/mL, respectively. Total antioxidative capacity and reducing power was found to be significantly higher in the dichloromethane fraction. Highest ferrous ion chelating activity was observed in ethyl acetate and benzene fractions. Dimethylsulfoxide fraction exerted the highest hydroxyl radical scavenging effect. Moreover, benzene and dichloromethane fraction showed the highest total polyphenolic content of 18.86 ± 0.27 and 17.69 ± 0.353 μg/mg of dry extract, respectively. Preliminary cytotoxic studies suggest that benzene and dichloromethane fraction has no cytotoxic effect, hence, they can be used as effective antioxidant for treating reactive oxygen species (ROS) mediated diseases.

1. Introduction

Reactive oxygen species (ROS), such as hydroxyl radicals, superoxide, nitric oxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative damage that in turn leads to geriatric degenerative conditions, like cancer and a wide range of other human diseases [1]. Antioxidants are free radical scavengers, which postpone the oxidation and block the chain initiated by high-energy molecules thereby, protecting the body against oxidative damage [2]. Although many synthetic antioxidants are promising for various human ailments, their pro-oxidant or cytotoxic nature at higher concentration prevents them from long-term use. These findings, together with the consumers' interests in natural food, have reinforced the efforts for the development of alternative antioxidants of natural origin [3]. Numerous studies have been focused on natural antioxidants in terrestrial plants and their application in food systems to prevent oxidation. Nowadays, aquatic plants are also gaining interest as a potential source of antioxidants. Over the past decades, seaweeds or their extracts have been shown to produce a variety of compounds and some of them have been reported to possess biological activity of potential medicinal value [4,5]. Therefore, new interest has been developed to search natural and safe antioxidative agents from marine sources. In folk medicine, seaweeds have been used for a variety of remedial purposes, e.g. for the treatment of eczema, gallstone, gout, crofula, cooling agent for fever, menstrual trouble, renal problems and scabies [6]. More recent reports revealed that marine algae possess rich sources of antioxidant compounds with potential free radical scavenging activity as in *Halimeda tuna* [7] and *Acanthophora spicifera* [8]. Some active antioxidant compounds from marine algae were also identified as phylopheophylin in *Eisenia bicyclis* [9], phlorotannins in *Sargassum kjellamanianum* [10] and fucoxanthin in *Hijikia fusiformis* [11]. In India, seaweeds are exploited mainly for the industrial production of phycocolloids, such as agar-agar, alginate, carrageenan and not for health aspects. As reports regarding the antioxidant properties of seaweeds are very limited, we attempted to screen for the antioxidant activity of the commonly available seaweeds from Southern coast of India. Preliminary screening for antioxidant activity of seaweeds inhabiting Gulf of Mannar has shown that methanolic extract of *Gelidiella acerosa* exhibited excellent antioxidant activity [12].

G. acerosa is a perennial red algae (Rhodophyceae) widely distributed throughout the year along the South coastal region of India i.e., Gulf of Mannar. *G. acerosa* is widely used in agar production and in the treatment of gastrointestinal disorders [13]. S-ACT-1, a sulfono glycolipid of *G. acerosa* showed potent sperm motility stimulating activity under in vitro condition [14]. S-PC-1, a sphingosine derivative was found to act as non-steroidal anti progesterone contraceptives [15]. Reports regarding the pharmacological application of *G. acerosa* are still at its infancy. Since the preliminary work

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was promising, the present study focus on the evaluation of metal chelating property and antioxidative activity of different solvent extracts of *G. acerosa*.

2. Methods

2.1. Sample collection

Seaweed *G. acerosa*, which was washed off the shore was collected along the south Indian coastal area, Tamil Nadu and the species were identified according to the reference Oza and Zaidu [16] and Krishnamurthy and Joshi [17].

2.2. Preparation of crude extracts

The seaweeds were washed with alcohol, water and dried under shade. The dried seaweeds were stored in an airtight container, which will be stable for at least 1 year. The air dried seaweeds were powdered and successively extracted with different solvents, like petroleum ether (PE), benzene (BE), dichloromethane (DM), chloroform ($CHCl_3$), ethyl acetate (EtOAc), acetone (AC), methanol (MeOH) and dimethysulfoxide (DMSO) in a Soxhlet apparatus. The extracts were dried under reduced pressure

under vacuum dessicator until dryness and the percentage of yield was calculated. The dried extract was dissolved in distilled water containing less than 0.02% of methanol or Tween 20 as solvents and used for further analysis. The extraction procedures were done at temperature less than 40 °C to avoid thermal degradation of the compounds. The yield of the extract was calculated as below:

$$\text{Yield of the extract} = \frac{\text{Weight of the beaker with extract} - \text{Weight of the empty beaker}}{\text{Weight of the sample in grams}} \times 100$$

2.3. Chemicals

Nicotiamide adenine dinucleotide (NADH), thiobarbituric acid (TBA), 1,1-diphenyl, 2-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-S-triazine (TPTZ) were purchased from Sigma–Aldrich, USA. RPMI 1640 and lymphocyte separation medium were purchased from Himedia. All the other chemicals were of highest purity grade commercially available

2.4. In vitro antioxidant assay

2.4.1. Total antioxidative power

Total antioxidative power of *G. acerosa* was assessed by ferric reducing antioxidant power (FRAP) assay according to the method of Benzie et al. [18]. FRAP assay depends upon the ability of the sample to reduce ferric tripyridyltriazine (Fe(III)–TPTZ) complex at low pH to intensive blue colored ferrous tripyridyltriazine (Fe (II)–TPTZ), which is read at 593 nm. First, 1.5 mL of freshly prepared FRAP reagent (25 mL of 300 mM/L of acetate buffer pH 3.6, 2.5 mL of 10 mM/L TPTZ in 40 mM/L of HCl, 20 mM/L of ferric chloride solution) were mixed with 50 μL of various concentration of seaweed (100–500 μg/mL) extracted successively in different solvents and 150 μL of distilled water. Absorbance was monitored for 4 min (every 10 s) at 593 nm. Aqueous solution of known $FeSO_4 \times 7H_2O$ (100–1000 μg/mL) was used as standard for calibration. The relative activity of the sample was compared with standard ascorbic acid (100–500 μg/mL).

2.4.2. Free radical scavenging assay

The free radical scavenging activity of *G. acerosa* was measured by DPPH• according to Shimada et al. [19]. DPPH (333 μL of 0.1 mM) in methanol was added to 1 mL of different doses of *G. acerosa* extracts (100–500 μg/mL) in water. The mixture was shaken vigorously, allowed to stand at room temperature for 30 min and the absorbance was read at 517 nm in a UV–visible spectrophotometer (U-2800 model, Hitachi, Japan). The lower the absorbance of the reaction mixture indicated higher free radical scavenging activity. BHT (100–500 μg/mL) was used as positive control. The percent DPPH• scavenging effect was calculated from the following equation:

$$\text{DPPH}^{\bullet}\ \text{Scavenging effect}\,(\%) = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100$$

where A_{cont} was the absorbance of the control reaction and A_{test} was the absorbance in the presence of the seaweed extract.

2.4.3. Hydroxyl radical scavenging activity

The ability of seaweed to scavenge OH• was assessed using the classic deoxyribose degradation assay described by Halliwell et al. [20]. Different concentrations of various solvent fractions of *G. acerosa* (100–500 μg) in distilled water were treated with l mL of reaction buffer (containing 1 mM EDTA, 10 mM $FeCl_3$, 10 mM deoxyribose, 10 mM H_2O_2, 1 mM ascorbic acid and 50 mM phosphate buffer pH 7.4). The mixture was incubated at 37 °C for 1 h. An amount of 1.0 mL of the incubated mixture was mixed with 1 mL of 10% TCA and 1 mL of 0.4% TBA (in glacial acetic acid pH 3.5) to develop the pink chromogen measured at 532 nm. BHT (100–500 μg/mL) was used as positive control. The hydroxyl radical scavenging activity of the extract was reported as percentage inhibition of deoxyribose degradation and was calculated as above.

2.4.4. Hydrogen peroxide (H_2O_2) scavenging activity

The ability of the *G. acerosa* to scavenge H_2O_2 was determined according to the method of Gulcin et al. [21]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorption at 230 nm. Different doses of *G. acerosa* (100–500 μg/mL) in distilled water were added to H_2O_2 solution (0.6 mL, 40 mM). Ascorbic acid (100–500 μg/mL) was used as positive control. Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 of *G. acerosa* and standard compound was calculated as above.

2.4.5. Nitric oxide radical (NO•) scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the Griess reaction [22]. The reaction mixture (1 mL) containing sodium nitroprusside (10 mM, 0.5 mL), phosphate buffer saline (0.25 mL) and various concentration of *G. acerosa* (100–500 μg/mL) were incubated at 25 °C for 150 min. After incubation, 0.25 mL of the reaction mixture was mixed with 0.5 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 0.5 mL of naphthyl ethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min at 25 °C. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. BHT (100–500 μg/mL) was used as positive control. The NO• scavenging activity of *G. acerosa* extract was reported as percentage inhibition and was calculated as above.