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Antioxidant Potentials of Marine Diatom *Skeletonema costatum*

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Abstract

Microalgae contain numerous bioactive compounds that can be commercially used in antioxidant activities of few phenolic compounds in plants are well known and the group of compounds possess antioxidant activity of a biological system of algae. Synthetic antioxidants were used to reduce the harmful effects of free radicals and these synthetic antioxidants may have other harmful effects. Therefore, researching for new antioxidants, especially with natural origin has become a major concern. Samples were extracted by using methanol showed higher antioxidant activity by total antioxidant capacity, DPPH radical scavenging activity and reducing power than hexane extract. One of the results showed that radical scavenging capacity, reducing power and antioxidant capacity is dose dependant. Total phenol content also higher in methanolic extract (0.644 mg gallic acid equivalent /g) than the hexane extract (0.392 mg GAE/g). In the present study, the methanolic extracts of *S. costatum* could be utilized as a good natural source of antioxidants and a possible food supplement.

1. Introduction

Any substance that effectively prevents or delay the adverse effects caused by free radicals and the amount of the antioxidant is less than the substance to the oxidized is known as antioxidant (Halliwell and Gutteridge, 1999). The antioxidant activities of these compounds are mainly involved in scavenging activity against superoxide and hydroxyl radicals chelating ability, triplet oxygen and reducing power (Athukorala *et al.*, 2006). Microalgae produce a wide range of antioxidant compounds including polysaccharides and fatty acids (Chen *et al.*, 2005). Oxidative damage caused by reactive oxygen species induce different kind of serious human diseases and disorder such as cancer, stroke, myocardial infarction, diabetes, septic, muscular dystrophy and neurological disorder, Alzheimer's and Parkinson diseases. They may also cause inadvertent enzyme activation and oxidative damage to cellular system (Wiseman and Halliwall, 1996).

Marine algae are exposed to a combination of light and high oxygen concentrations which led to the formation of free radicals and other strong oxidizing agents and the photosynthetic systems are vulnerable to photodynamic damage because polyunsaturated fatty acids are important structural components of the thylakoid membrane (Sukenik *et al.*, 1993). In recent times, more marine microalgae have been alleged of having strong antioxidant properties, including *Fucus vesiculosus* (Antonio *et al.*, 2001), *Ecklonia cava* (Yasantha *et al.*, 2006), *Petalonia binghamiae* (Takashi *et al.*, 2006) and *Scytosiphonlo mentaria* (Takashi *et al.*, 2004).

Recently, much attention has been focused on the marine microalgae as sources of structurally novel and biologically active metabolites (El-Baky *et al.*, 2008).Microalgae are a promising alternative source of antioxidants (Natrah *et al.*, 2007 and Lee *et al.*, 2010). They produce a wide range of bioactive substances with antimicrobial, enzyme inhibiting, immune stimulant, cytotoxic and antioxidant activities (Venkatesan *et al*., 2007).

Antioxidants involved in oxidation process by scavenging free radicals, chelating catalytic metals and by acting as oxygen scavengers (Buyukokuroglu *et al*., 2001). Microalgae have been developed to some defence system against photo-oxidative damage by oxidative mechanisms to detoxify and eliminate the highly reactive oxygen species. They represent an almost available resource of natural antioxidants due to their vast biodiversity, much more diverse than higher plants. Phenolic compounds are considered as major benefactor to the antioxidant capacity of plants (Asha *et al.,* 2012). These antioxidants also have diverse biological activities such as anti-inflammatory, antimicrobial and anti carcinogenic activities (Chung *et al.,* 1998). Reports on the antioxidant activity of microalgae are limited, especially on the bond between their phenolic contented and antioxidant capability (El-Baky *et al.,* 2008). Reports on the antioxidant activities of microalgae are limited, mainly concerning to the relationship between their phenolic content as well as antioxidant ability (Li *et al.,* 2007). The content and type of antioxidant compounds depends on the microalgae species and growth conditions. Among these, diatoms are widely used in the life science as the source of compounds with diverse structure forms and biological activities. Besides, only limited information on antioxidant activity of microalgae are available (Herrero *et al.,* 2005; Murthy *et al.,* 2005; Tannin Spitz *et al.,* 2005). Although microalgae posses widespread applications in food as well as in pharmaceutical industry.

The antioxidant activities of many different types of microalgae in the South Indian coastal area are still unknown. Microalgae have enormous biodiversity, even much more diverse than available higher plants, representing an almost unexploited resource of natural antioxidants. However, all the groups of microalgae cannot be used as natural sources of antioxidants, due to their broadly varied contents of target products, growth rate or yields, and other factors (Li *et al.,* 2007). The main objective of the present study is to assess the *in vitro* antioxidant activity using methanol and hexane extract of *S.costatum* isolated from Vellar estuary, Parangipettai, Southeast Coast (Bay of Bengal), India

2. Materials and Methods

2.1. Micro algae culture

The marine phytoplankton, *Skeletonema costatum* were cultured in F/2 Guilard (1975) media in the laboratory condition. Morphological identification was followed by standard protocol of Venkataraman (1939).

2.2. Extraction

The harvested biomass (2 g) was extracted using methanol and hexane separately at room temperature. The extraction was repeated thrice and filtered through glass funnel and Whatmann No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator. Finally the dry extracts were lyophilized and stored in refrigerator for further analysis (Lim *et al.,* 2002).

2.4. Antioxidant assays of diatom

2.4.1. Determination of total phenolic content

Phenolic contents of crude extracts were estimated by the method of Taga *et al*. (1984). 100 µl of sample aliquot was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min. at room temperature. After incubation, 100 µl of 50% Folin Ciocalteau's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand at room temperature for 30 min. in the dark. Absorbance of all the sample solutions was measured at 720 nm using Spectrophotometer (Phenolic content is expressed as Gallic acid equivalent (GAE) per gram).

2.4.2. Total antioxidant activity

The total antioxidant activity was determined by phosphomolybdinum and Seedevi *et al*. (2014). 2.0 ml of sample at various concentrations (50-250 µg/ml) was mixed with 1.0 ml reagent solution (28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min. under water bath. After the mixture had been cooled to room temperature, the absorbance of each solution was measured at 695 nm using Spectrophotometer against blank. The L-ascorbic acid was used as a standard and the total antioxidant activity was expressed as ascorbic acid equivalent.

2.4.3. Scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl radicals (DPPH)

The scavenging ability on DPPH radicals was determined according to the method of Shimada *et al*. (1992). Each sample (0.1–10 mg/ml) in 2g/l acetic acid solution (4 ml) was mixed with 1ml of methanolic solution containing DPPH radicals, which resulted a final concentration of 10 mmol/l DPPH. The mixture was shaken vigorously and left to stand for 30 minutes in the dark and the absorbance was then measured at 517 nm against blank. Ascorbic acid was used for comparison. The scavenging ability was calculated by

$$\text{Scavenging ability (\%)} = \frac{(\Delta A517 \text{ of control} - \Delta A517 \text{ of sample})}{\Delta A517 \text{ of control}} \times 100$$

2.4.4. Reducing power

The reducing power was determined as described by Seedevi *et al*. (2014). Briefly, 1 ml of algal extract (0.1-2 mg/ml) in phosphate buffer (0.2 M pH 6.6) was mixed with 1 ml of potassium ferricyanide (1%, w/v) and incubated at 50ºC for 20 min. Afterwards, 2.0 ml of TCA (10% w/v) was added to the mixture to terminate the reaction. The solution was mixed with 1.25 ml of ferric chloride (0.1% w/v) and the absorbance was measured at 700 nm. The L-ascorbic acid was used as a standard.

Results and Discussion

In the present study, the total phenolic contents of *Skeletonema costatum* were determined and expressed as mg/ gallic acid equivalent (GAE) in order to make a comparison between two extract of marine diatom and identified as a natural source for phenolic compounds. The total phenolic content (TPC) of *S.costatum* along with standard gallic acid is shown in Figure 2. Methanol extract showed high phenolic content of 0.644 mg GAE/g whereas the minimum (0.392 mg GAE/g) phenolic content was noted in hexane extract. The variation in phenol content in different solvent extract may be due to the differences in the polarity of the solvents used and thereby the different phenolic components which may be differentially eluted (Uma *et al.*, 2011).

Similar result was reported by Manivannan *et al.* (2012) in *Chlorella marina* that methanol extract showed high total phenolic content of 0.647±0.052 mg GAE/g and minimum activity was in diethyl ether extract of 0.368± 0.126 mg GAE/g. Hemalatha *et al.* (2013) observed the highest phenolic content was found to be in methanol and acetone extract of 0.78 ± 0.032 and 0.63 ± 0.031 mg GAE /g in *Chlorella marina* and hexane extract of *Nitzchia clavata* was 0.34 ± 0.028 mg GAE /g. Karthikeyan *et al.* (2013) in *Odontella mobiliensis* showed higher amount of phenol content (0.75 ± 0.006) was recorded.

The total antioxidant activity of *S.castatum*, showed maximum radical scavenging activity was absorbed in methanol extract (59%) and the minimum was absorbed in hexane (46%). Ascorbic acid was used as a standard and all the activities were relatively lower than that of standard. In methanol extract, maximum antioxidant activity was showed at concentration of 3.2 mg/mL. While minimum was at 0.1 mg/mL concentration in hexane extract. Lekamera *et al.* (2008) also reported similar result in *Colpomenia sinuosa* that antioxidant activity increases with increasing concentration and extract showed lesser activity than standard. Recently, Saranya *et al.* (2014) have further reported that methanol extract showed higher antioxidant capacity than hexane extract.

Reducing power of various concentrations of *S.costatum* extracts behaved in a quantity dependent manner (0.15 to 0.75 mg/mL). Similar to the total antioxidant activity (TAA), methanol extract showed better reducing power than hexane. The reducing capacity of various concentrations of *S.costatum* extracts behaved in a dose dependent manner (0.2 to1.0 mg/mL). The reducing power increased with increasing concentration in all samples analysed. Similar trend was also achieved by Chandini *et al.* (2008) and Ganesan *et al.* (2008). Among the two extracts, methanolic extract exhibited higher radical scavenging activity when compared to hexane (Manivannan *et al.*, 2012; Kumar *et al.*, 2008).

In DPPH radical scavenging activity, methanol extract showed higher activity than hexane extract and this assay has been extensively used for screening antioxidant activity such as polyphenols and anthocyanins, from marine algae (Sanchez-Moreno, 2002; Duan *et al.*, 2006; Chandini *et al.*, 2008). The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation (Ganesan *et al.*, 2011). The increase the scavenging activity of *S.costatum* extracts on DPPH radicals was concentration dependent similar to the finding of Lekamera *et al.* (2008). In DPPH radical scavenging activity is found to have maximum at 10 mg/mL concentration in methanol extract and minimum at 1mg/mL in hexane extract **(Fig. 5)**. Ganesan *et al.* (2011) also found maximum activity in methanol extract. This assay revealed that the extracts might prevent reactive radical species from damaging biomolecules such as lipoprotein, DNA, aminoacids, sugar, proteins and PUFA in biological and food systems (Uma *et al.*, 2011).

Conclusion

The present finding was concluded that the methanolic extracts of *S.costatum* is capable of scavenging a wide range of synthetic and naturally occurring free radicals. It is also evident from the present study that methanolic extracts of *S.costatum* could be utilized as a good natural source of antioxidants and possible food supplement. The data may contribute to a rational basis for the use of antioxidant rich marine algal extracts in the therapy of diseases related to oxidative stress. In addition, the results also indicate that phenolic compounds might be not major contributors to the antioxidant activities of *S.costatum*. The finding of the current report appears to be useful for further research aiming to isolate, identify and characterize the specific antioxidant compounds in *S.costatum* for its industrial and pharmaceutical applications.

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