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Antioxidant properties of light harvesting pigments of marine diatoms *Chaetoceros simplex* and *Navicula sp.* isolated from Pichavaram, southeast coast of India

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ABSTRACT

In the present study *Navicula* sp. and *Chaetoceros simplex* have been screened for antioxidant properties using total phenolic content, total antioxidant activity, FRAP reducing antioxidant power, DPPH radical scavenging, Hydrogen peroxide radical scavenging and Nitric oxide radical scavenging activity. The diatoms were extracted using different solvents like methanol, acetone and hexane. Antioxidant activities of the extracts varied strongly among the test species and further depended on growth conditions and the solvents used for extraction. The results revealed that the *Chaetoceros simplex* possessed the highest antioxidant capacities and thus it could be a prospective source of natural antioxidants. The results from the different types of extracts clearly indicated that the light harvesting pigments (Chlorophylls, Carotenoids) of *Chaetoceros simplex* exhibited significant antioxidant property.

Introduction

Antioxidants play an important role in inhibiting and scavenging radicals and thereby providing protection against infections and degenerative diseases in humans (Wu *el. al*., 2010). Natural antioxidant compounds exhibit their antioxidant activity by donating or converting the electron free radicals into more stable species and decomposing lipid peroxides into stable final products (Hussain *et. al*., 2008). All photosynthetic organisms must reconcile two requirements: Capturing sufficient number of photons and avoiding the photons' damaging effects. To face the effects of excess energy, the system of photosystem II (PSII) for repairing damage or for scavenging reactive oxygen species (ROS) are activated. Predominantly chlorophylls and carotenoids play fundamental roles in light harvesting and photo protection (Demmig-Adams and Adams, 1996). Light harvesting compounds play vital role in photo protection and regulating the photosystem there by scavenging the free radicals. In the present study an attempt was made to identify the potential microalgae for the purpose of natural antioxidants from Pichavaram coastal area, Tamil Nadu, India. Further, the antioxidant properties of light harvesting pigments and phenolic content have been compared.

Materials and Methods

Sample collection and extraction

The marine diatoms were collected from Pichavaram, southeast coast of India. The collected sample was isolated using different isolation techniques like serial dilution methods and cultured in Guillar's F/2 media. The axenic cultures were maintained at 24 ± 2 ºC of temperature, 4500 ± 500 Lux light intensity and 30 psu salinity. The cells were harvested by centrifuging at 4000 rpm for 10 min and lyophilizing under reduced pressure. A precisely weighed (2 g) amount of ground freeze dried microalgae were extracted for 24 h in 40 ml of methanol, acetone and hexane at room temperature. The extraction was twice repeated and filtered through glass funnel and Whatmann No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using a rotary

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flash evaporator. Finally the dry extracts were lyophilized and stored in refrigerator for further analysis.

Antioxidant activities of microalgae

Total phenolic content

Phenolic contents of micro algal crude extracts were estimated following the method of Taga *et.al*. (1984). Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Phenolic content is expressed as Gallic acid equivalent per gram).

Total pigment content

Total chlorophyll and carotenoid content was measured by spectrophotometer method. Absorbances were measured at 400 to 720nm (Govindjee and Barbara Zilinskas Braun, 1974) and the pigments were extracted by using MN GF 47mm.

Total antioxidant activity

Total antioxidant activity was measured by the method of (Prieto *et. al*., 1999). Absorbance of all sample mixtures was measured at 695 nm. (The Total antioxidant activity is expressed as the number ofequivalents of ascorbic acid).

DPPH radical scavenging assay

The effects of DPPH radical scavenging were determined by the method of Yen and Chen, (1995). Absorbance were measured at 517 nm. The scavenging effect (%) was calculated by using the formulae given by (Duan *et. al*., 2006).

Scavenging effect (%) = (1-(A sample -A sample blank)/A control) × 100

Nitric oxide radical scavenging assay

The nitric oxide radical scavenging was measured by the method of Gulcin (2006). Absorbance was taken at 540 nm. Ascorbic acid was used as positive control. The nitric oxide scavenging activity of the crude extracts was represented as % of scavenging.

Ferric reducing antioxidant power assay

Reducing power of different crude extract samples were determined by the method of Oyaizu (1986).Absorbance of all the solution was measured at 700 nm. (The Ferric reducing antioxidant Power is expressed as the number of equivalents of ascorbic acid).

Statistical analysis

To analyze the statistical proposition, the data were subjected to two way and one way ANOVA using statistics software (SPSS, ver.16).

Results and Discussion

Total phenolic content

The total phenolic content of acetone, methanol and hexane extract of microalgae was evaluated (Fig. 1). The radical scavenging power of the compound was determined by the presence of aromatic rings and hydroxyl groups in phenolic compounds and its derivatives like flavonoids, lignins, phenols, tannins and phenylproponoids (Dziedziz and Hudson, 1983). Many of the phenolic compounds such as pheophytin from green microalgae (Nishibori and Nanniki, 1988) and phlorotannins from brown microalgae (Nagayana *et al.,* 1988) were reported as antioxidants. But it is not the major contributor of antioxidant capacities in microalgae (Li *et al*., 2007). Here the highest phenolic content was found in methanol extract of *Chaetoceros simplex* 0.58±0.07 mg/g.In contrast the hexane extract of *Chaetocerous sp.* compared to *Nannochloropsis sp.* showed the highest phenolic content and negative effect on methanolic extract. Interestingly it showed higher antioxidant activity on other assays. Thus clearly elucidate there is no correlation between antioxidant capacity and phenolic content (Nagayama *et al*., 2003). All of the solvent extracts the lowest phenolic content was showed in hexane extract of *Navicula sp.* 0.22±0.051(mg/g gallic acid equivalent) than *Chaetoceros simplex*.

Pigment Content

The total pigment content was observed at visible region from 400 nm to 720 nm (Govindjee and Barbara Zilinskas Braun, 1974). Use spectrophotometer (Fig. 2). The absorbance value showed the abounded pigments in *Navicula sp.* and *Chaetoceros simplex* at 645 nm and 650 nm of Chl c (Allen *et al.,* 1969) and Chl b (Thomas 1971) respectively and 495 nm of Carotenoids (Cho and Govindjee, 1970) were measured in the same species, whereas in *Navicula sp.* maximum peak pick value was found at 485 nm and 540 nm of Carotenoids (Cho and Govindjee, 1970) and chlorophylls at 702 nm (Litvin *et al.,* 1969), whereas the methanolic extract of *Chaetoceros simplex* (3.75±0.01 mg/g) showed higher yield as well as antioxidant

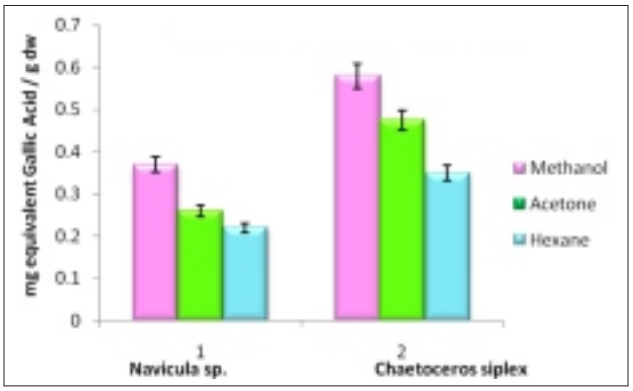

Fig. 1. *Total phenolic content of the microalgae*

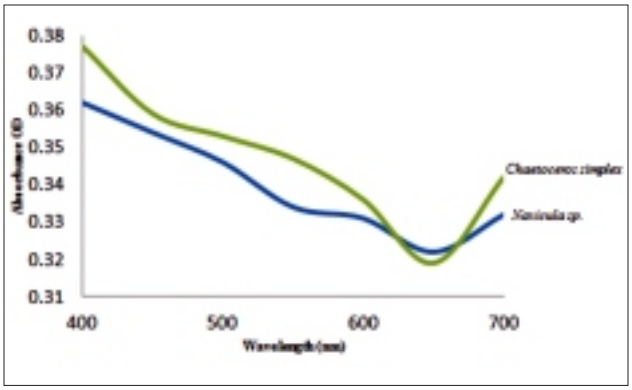

Fig. 2. *Spectral absorption of microalgae*

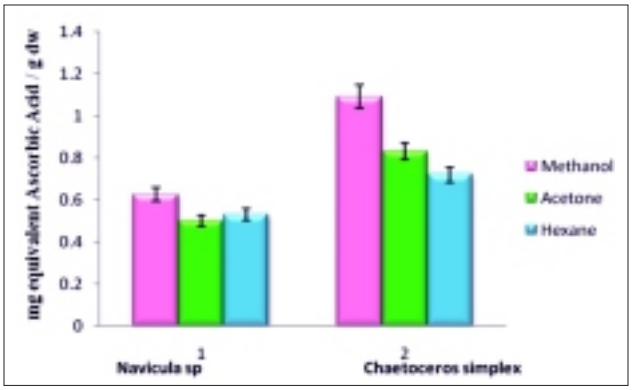

Fig. 4. *Total antioxidant Activity*

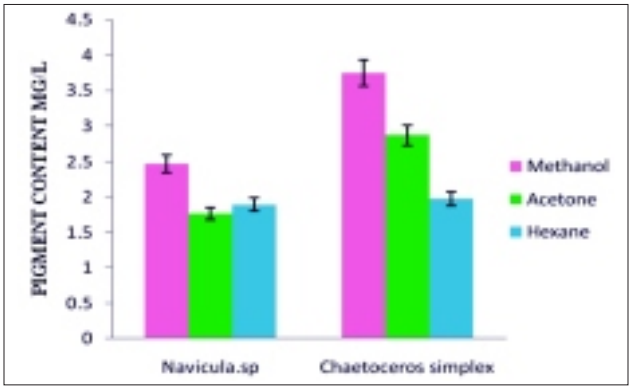

Fig. 3. *Pigment content of the microalga*

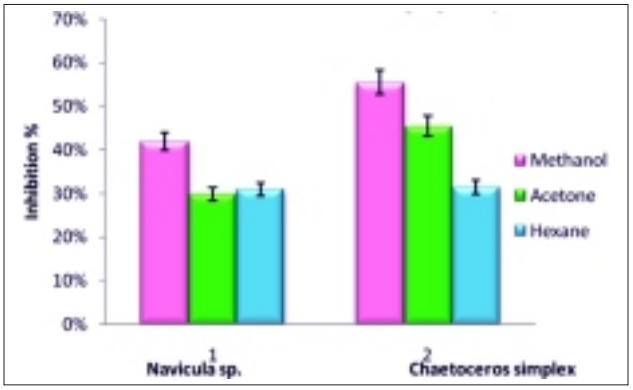

Fig. 5. *DPPH radical scavenging activity*

capacity overthe phenolic content (1.76±0.03mg/g) in hexane extract of the same species (Fig. 3).The pigments proportion was found to be maximum when compared to the phenolic content of *C. simplex* light harvesting pigment may responsible for antioxidant activity in *C. simplex*.

Total antioxidant activity

The total antioxidant activity of different species of methanol, acetone and hexane extracts are shown in Fig. 4.Tthat the highest antioxidant activity was found at methanol extract of *Chaetoceros simplex* (1.09±0.032 mg/g) intently the methanolic extract of *Chaetoceros calcitrans* (C. Saranya *et.al.,* 2014) and the lowest activity was found in acetone extract of *Navicula sp.*0.5± 0.028 mg/g gallic acid equivalent. Similarly, Uma *et al.* 2011) observed the greater potentials of antioxidant activity at methanol extract over other solvents.

DPPH radical scavenging activity

DPPH radical scavenging activity is given in Fig 5. The higher activity was noted in methanol extract of *Chaetoceros simplex* (56%) among the extracts. Comparably methanol extract of *Chaetoceros calcitrans* showed 14% of reducing power than green micro algae (Saranya *et al.,* 2014). Similarly Lee *et al.* (2010) reported that 80% of the methanol extract and organic solvent fractions of microalgae showed notable activities, indicating the highest efficiency for scavenging of free radicals, whereas the acetone extract of *Navicula sp*. showed 30% lower activity. Likewise Kuda *et al.* (2005) reported that the highest and the lowest effect of reducing power were dose dependent.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity is shown at Fig. 6. The maximum hydrogen peroxide radical scavenging was found in acetone extract of *Navicula sp*.(12%) likewise hexane extract of the diatom *Chaetocerous calcitrans* (11.45%) over green micro algae (Saranya *et al.,* 2014) whereas the methanol extract of *Chaetoceros simplex* (5%) showed lowest radical scavenging activity than *Navicula sp.*

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured in different solvent extracts of methanol acetone and hexane is displayed in Fig. 7. The maximum activity was found in methanol extract of *Chaetoceros simplex* (35%) and the minimum was noted in acetone extract of *Navicula sp.* (13%).

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power assay are directly proportional to the antioxidant capacity. Interestingly most of the ferric reducing agent possessed antioxidant properties (Su-Hua Goh *et al.,* 2010). Fig. 8 shows the ferric reducing

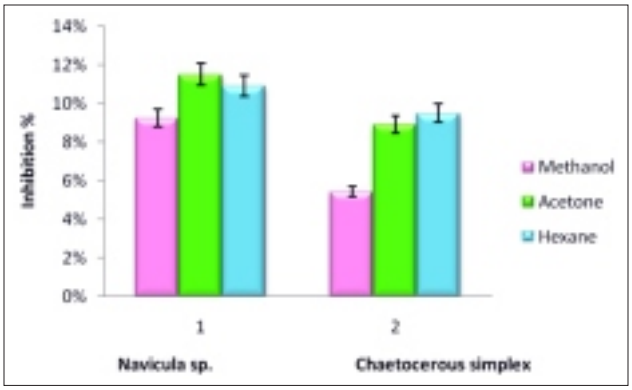

Fig.6. *Hydrogen peroxide radical scavenging activity*

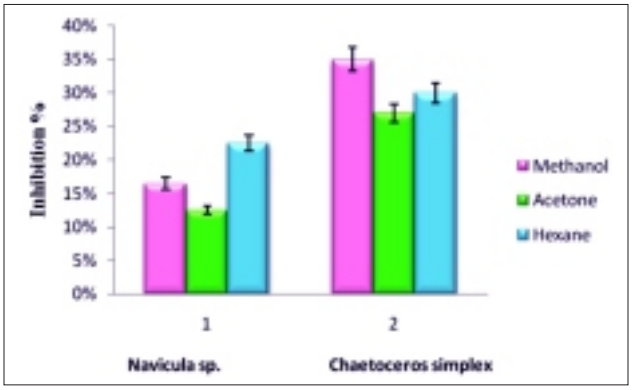

Fig. 7. *Nitric oxide radical scavenging activity*

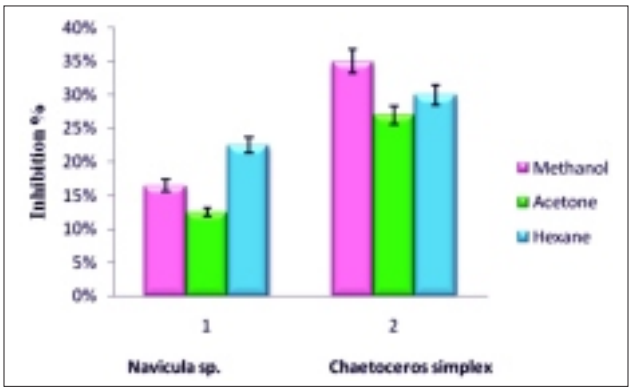

Fig. 8. *Ferric reducing antioxidant power assay*

antioxidant power of different solvent extracts of methanol, acetone and hexane. The reducing activity of microalgae were found to be highest in methanol extract of 0.72±0.057mg/g (ascorbic acid equivalent) in *Chaetoceros simplex* at 1ml concentration and the lowest reducing activity were observed in acetone extract of 0.405±0.067mg/g (ascorbic acid equivalent) in *Navicula sp.*

In this present study the antioxidant properties of light harvesting pigments of two diatoms *Chaetoceros* simplex and *Navicula sp.* were determined. When compared to the total phenolic content of the above mentioned species showed highest antioxidant properties in terms of reducing and radical scavenging in methanolic extract of *Chaetoceros simplex* compared to *Navicula sp.* indicates that phenolic compound might not be the major source of antioxidant properties in these two diatoms Further, the species was evaluated for the phytoconstituents. Light harvesting pigments (chlorophylls and carotenoids) and phenolic content were analysed. Based on the results, it is concluded that light harvesting pigments may be ascribed to the highest antioxidant potential of the *C. simplex*. Hence, the pigments of *C. simplex* could be used as a natural antioxidant supplement instead of synthetic pigments.

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