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Antioxidant properties and carotenoid content of *Coscinodiscus centralis* and *Chaetoceros calcitrans* isolated from Vellar estuary, southeast coast of India

V. THANAPPAN*, K. SUBA SANKARI, M. SURESH AND P. ANANTHARAMAN
Centre of advanced studies in Marine Biology, Faculty of Marine Sciences
Annamalai University, Parangipettai - 608 502, Tamil Nadu, India
**E-mail : vthanappan57@gmail.com*

ABSTRACT

In the past decades, food scientists have been searching for natural alternatives to replace synthetic antioxidants. In order to evaluate the potential of microalgae as new source of safe antioxidants, the biomass of *Coscinodiscus centralis* and *Chaetoceros calcitrans* have been compared for their antioxidant property using total antioxidant and total phenolic content assays. In addition the study has assessed the carotenoid content of the test microalgae. The microalgae were extracted with ethanol/water and alternatively a three-step fractionation procedure using successively hexane, ethyl acetate and water. Antioxidant activity of the extracts varied strongly among species and further depended on growth conditions and the solvent used for extraction. From the presents study, it was confirmed that laboratory cultivated samples of *C. calcitrans* exhibited highest antioxidant capacities compared with *C. centralis* and thus it could be a potential new source of natural antioxidants. The results from the different types of test extracts clearly indicated that in addition to the well-studied carotenoids, the phenolic compounds showed its significant antioxidant capacity.

Introduction

The marine environments have different phytometabolic content with rich source chemical and biological diversity and marine organisms are highly potent and commercializing interesting compounds for applications and also food industry, cosmetic industry, neutraceuticals, pharmaceutical industry and other industries important compounds (Ali *et al*., 2014). Algae contains biochemical products (carotenoids, vitamins, phycobiliproteins and polyunsaturated fatty acids, including the omega-3 and omega-6 fatty acids) and pigments including this all are natural origin, functional ingredients which have positive effects on the health of man and animals (Pulz and Gross 2004).

A large number of studies on the microalgal bioactive compounds have oriented to the anti-inflammatory, antiviral, antimicrobial, anthelmintic, cytotoxic, immunological and enzyme inhibition properties (Dufosse *et al.,* 2005; De la Noue and De Pauw 1988; Singh *et al.,* 2005), Moreover, because of phototropic life of microalgae and their permanent exposure to high oxygen and radical stresses, they have a high capability for the production of numerous efficient protective chemicals against oxidation and radical stressors (Tsao and Deng, 2004). This scavenger capacity of micro algal contents brings them up as the potential alternative substances against in oxidation-associated conditions like chronic diseases, inflammation, aging or skin UV-exposure. There are many studies on the antioxidant activity of some species belonging to the genera of *Botryococcus* (Rao *et al.,* 2006), *Chlorella* (Wu *et al.,* 2005), *Dunaliella* (Herrero *et al*., 2006), *Nostoc* (Li *et al.,* 2007), *Phaeodactylum* (Guzman *et al.,* 2001), *Polysiphonia* (Duan *et al.,* 2006), *Scytosiphon* (Kuda *et al*., 2005) and *Spirulina* (Jaime *et al.,* 2005; *Miranda et al*., 1998). The aim of the present study was hence, to identify new sources of safe and inexpensive antioxidants from microalgae using *Coscinodiscus centralis* and *Chaetoceros calcitrans*. The strains were collected from Vellar estuary, Parangipettai, Tamilnadu, India and cultured in

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laboratory conditions by measuring the antioxidant activity, total phenolic content, Deoxy ribose radical scavenging activity and reducing power in various extracts.

Materials and Methods

Sample collection and extraction

The two microalgae *Chaetoceros calcitrans* and *Coscinodiscus centralis* were collected from Vellar estuary, Parangipettai, Tamilnadu. Unialgal cultures were developed and maintained in F/2 media. The cultured cells were harvested by centrifugation at 3500 rpm 10 min.A precisely weighed two grams of each freeze dried microalgal species were extracted for 24 h in 40 ml of acetone, methanol and hexane at room temperature (25±1°C). The extraction was repeated twice and filtered through glassfunnel with 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.

Antioxidant activitie of microalgae

Total phenolic content

he total phenolic content of the crude extracts was estimated by he method of Taga *et al.* (1984). One hundred microlitres of aliquot sample were added to 2.0 ml of 2% Na2 CO and allowed to stand for 2 min at rom temperature. After incubation, 100µ of 50% Folin Ciocalteau's phenol reagent was added and mixed thoroughly. After 30 min of incubation at room temperature in the dark, the absorbace of all the samples was measured at 720 nm using spectrophotometer. The results were expressed as Gallic acid equivalent (GAE) /g dry weight of microalgae and calculated as mean value ± SD (n = 3).

Total antioxidant activity

Total antioxidant activity of crude extracts were measured by according to the method of (Prieto *et al.,* 1999). TAC (Total Antioxidant Capacity) reagent consisting of 7.45 ml sulphuric acid (0.6 Mm solution), Sodium sulphate (28 Mm solution) 0.9942 g and ammonium molybdate (4 Mm solution) were mixed together in 250 ml distilled water. 300 µl of extract was dissolved in 3ml of TAC reagent. TAC reagent was replaced by distilled water for the blank. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Determination of carotenoid content

The carotenoid content of each algal speices was estimated spectrophotometrically according to the method of Lichtenthaler and Buschmann (2001). Aliquots of the extracts were diluted 15-300 times with 90% (v/v) methanol in water and absorbances were measured at 470, 652 and 665 nm and carotenoid content was calculated using the Lichtenthaler equations.

Statistical analysis

Three replicates of each sample were used for statistical analysis and the values were presented as mean ± SD. Pearsons correlation and One-way ANOVA the statistical significance were performed using SPSS, version 16.0 software to study the relationship between antioxidant activities and carotenoid contents.

Results and Discussion

In total, two diatoms *Chaetoceros calcitrans* and *Coscinodiscus centralis* were observed and isolated. The isolated diatoms were cultured in Guillard F/2 medium. The harvested dried biomass were extracted with acetone, methanol and hexane and the crude extact obtained were tested for antioxidant activity. In the present study, total phenol content (TPC) and the antioxidant properties such as Total Antioxident activity and carotenoid content of acetone, methanol and hexane extract of *Chaetoceros calcitrancs* and *Cosinodiscus centralis*.

Total phenolic contents

The total phenolic content of acetone, methanol and hexane extracts of *Coscinodiscus centralis* and *Chaetoceros calcitrans* was analyzed (Fig. 1). According to the obtained results, the highet amount of phenolic content was found in methanol extract of *C. calcitrans* (0.72 ± 0.011 mg/g gallic acid equivalent) while, the lowest phenolic content was observed in hexane extracts of *C. centralis* (0.40 ± 0.05 mg/g gallic acid equivalent). Accoding to the oneway ANOVA, the carotenoid content of isolated diatoms was singnificant.

Total antioxidant activity

The total antioxidant activity of acetone, methanol and hexane extracts of *Coscinodiscus centralis* and *Chaetoceros calcitrans* is shown in Fig. 2. The higher activity (0.99 ± 0.017 ascorbic acid equivalent/g) was observed in methanolic extract of *C. calcitrans* followed by the lowest activity in the hexane extract of *C. centralis* (0.31 ± 0.020 mg/g ascorbic acid equivalent). Accoding to the oneway ANOVA, the

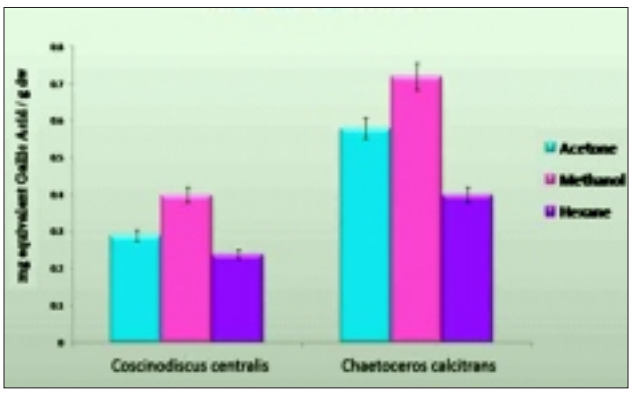

Fig. 1. *Total Phenolic Content*

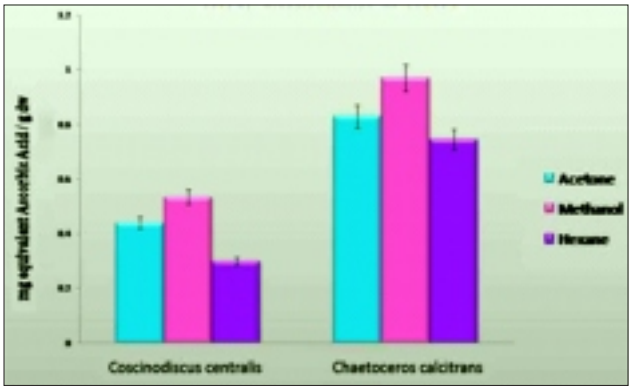

Fig. 2. *Total Antioxidant Activity*

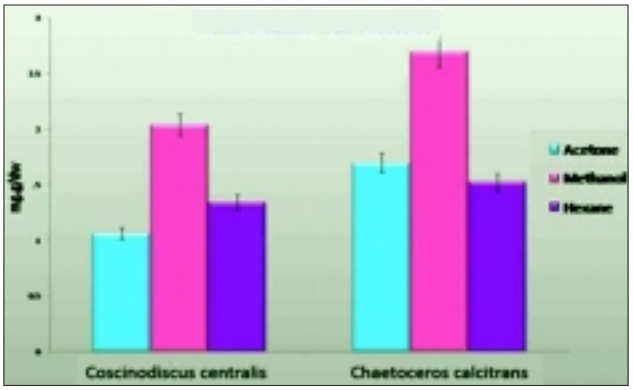

Fig. 3. *Total Carotenoid Content*

carotenoid content of isolated diatoms was singnificant.

Carotenoid content

Carotenoid content of acetone, methanol and hexane extracts of *Coscinodiscus centralis* and *Chaetoceros calcitrans* is given in Fig. 3. The methanolic extract of *C. calcitrans* exhibited highest carotenoid content (2.55 ± 0.075mg.g^{-1} Dry weight), whereas the lowest carotenoid content (1.1 ± 0.065mg.g^{-1} Dry weight) was observed in the acetone extract of *C.centralis.* Accoding to the oneway ANOVA, the carotenoid content of isolated diatoms was singnificant. It is well known that, carotenoids play important role in scavenge several active oxygen species (ROS) such as $1O^2$, O^{2-}, H_2O_2, hydroxyl radicals (HO-) and peroxy radicals both *in vitro* and *in vivo.* Several studies have demonstrated that carotenoids contribute significantly to the total antioxidant capacity of microalgae (Jahnke 1999; Takaichi 2011).

In conclusion, the antioxidant capacity *Chaetoceros calcitrans* was higher than that of *Coscinodiscus centralis.* This may be due to the presence of higher amount of carotenoids and further detailed studies are needed. Hence, in future *Chaetoceros calcitransI* may find important and wide application in the food and pharmaceuticals industries due to its high antioxidant activities.

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