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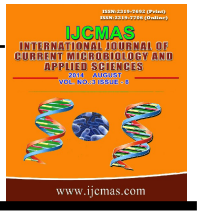

Original Research Article

Evaluation of Antioxidant Properties, Total Phenolic and Carotenoid Content of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

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A B S T R A C T

The search for natural alternatives to replace synthetic antioxidants resulted in extensive study of pigments and phenolic contents and their relation to antioxidant activities. The carotenoid content in microalgae is a specific feature of each species. In order to evaluate the potential of microalgae as new source of safe antioxidants, antioxidant capacity of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana* were evaluated using five antioxidant assays (Total antioxidant activity, DPPH radical scavenging activity, Nitric oxide radical scavenging activity, Hydrogen peroxide radical scavenging activity, Ferric reducing power) in three different solvents methanol, acetone and hexane. Total phenolic content and carotenoid content were also measured. The results showed that the maximum total antioxidant activity was observed in methanol extract of *Isochrysis galbana*. It also shows that the total phenolic and carotenoid content plays a direct vital role in total antioxidant activity. Increase in total phenolic content and carotenoid content shows increase in total antioxidant activity proving that they play a direct vital role in antioxidant properties.

K e y w o r d s

Chaetoceros calcitrans; *Chlorella salina*; *Isochrysis galbana*; Antioxidant assay; Carotenoid content; Total phenolic content

Introduction

In recent years, the use of photosynthetic microorganisms, such as microalgae, in life sciences has received increasing attentions due to their diverse phytometabolic contents with various chemical structures and biological activities (Skulberg 2006). Algal biomass and algae-derived compounds have a very wide range of potential applications, from animal feed and aquaculture to human nutrition and health products (Borowitzka 1988; Soltani et al. 2005; Skulberg 2006).

Polyunsaturated fatty acids, sulfated polysaccharides, phycosterols, heat-induced proteins, phenolic compounds, and pigments including carotenoids are the naturally 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, antihelmintic, 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 production of numerous efficient protective chemicals against oxidative and radical stressors (Tsao and Deng 2004). This scavenger capacity of microalgal contents bring them up as the potential alternative substances against oxidation-associated conditions like chronic diseases, inflammation, skin UV-exposure and also prevention of cardiovascular disorders, of certain ageing related diseases such as Alzheimer and of certain types of cancer (Cao and Prior 1998). Therefore, antioxidants are increasingly being used in food supplements and functional foods (Cuvelier 2001). As synthetic antioxidants are suspected carcinogens (Namiki 1990; Pokorný 1991), there has been a search in recent years to replace these synthetic antioxidants with natural antioxidants. Some algae are considered as rich sources of natural antioxidants (Chkhikvishvili and Ramazanov 2000; Huang and Wang 2004). Unicellular microalgae are a promising alternative source of antioxidants (Li et al. 2007; Natrah et al. 2007; Hajimahmoodi et al. 2010; Rodriguez-Garcia and Guil-guerrero 2008; Chacón-Lee and González-Mariño 2010; Lee et al. 2010). There are a number of reports on the evaluation of antioxidant activity in microalgae and cyanobacteria belonging to the genera Botryococcus (Rao et al. 2006), Chlorella (Wu et al. 2005; Goh et al. 2010), Dunaliella (Herrero et al. 2006), Nostoc (Li et al. 2007), Phaeodactylum (Guzman et al. 2001), Spirulina (Miranda et al. 1998; Jaime et al. 2005; Mendiola et al. 2007), Haematococcus (Cerón et al. 2007) and Chaetoceros (Goh et al. 2010).

An important and well-known class of antioxidants from microalgae is carotenoids. Carotenoids play an important role in quenching reactive oxygen species (ROS) generated during photosynthesis, especially singlet oxygen. Several studies have demonstrated that carotenoids contribute significantly to the total antioxidant capacity of microalgae (Jahnke 1999; Takaichi 2011). It is not clear whether phenolic substances are important antioxidants in microalgae. The fact that the content of phenolic substances in microalgal biomass increases upon exposure to UV-light (Duval et al. 2000; Kovácik et al. 2010) suggests that they indeed play a role in the antioxidative response to this type of stress.

In this study, antioxidant capacity of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana* were evaluated through five major assays like Total antioxidant activity, DPPH radical scavenging activity, Nitric oxide radical scavenging activity, Hydrogen peroxide radical scavenging activity, Ferric reducing antioxidant power. Further, total phenolic content and carotenoid content were also measured to study their relation to antioxidant capacity.

Materials and Methods

Sample collection and extraction

Chaetoceros calcitrans, *Chlorella salina* and *Isochrysis galbana* strains were obtained from CMFRI (Central Marine Fisheries Research Institute), Mandapam, Tamilnadu, India. Unialgal cultures were developed and maintained in F/2 media. The cultured cells were harvested by centrifugation at 4000 rpm for 10 min. A precisely weighed (2 g) amount of freeze dried microalgae were extracted for 24 h in 40 ml of acetone, methanol and hexane at room temperature. The extraction was repeated twice and filtered through glass

funnel 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 activities of microalgae

Total phenolic content

Phenolic content of crude extracts were estimated by 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 antioxidant activity

Total antioxidant activities were measured by following the method of Prieto et al. (1999). Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

DPPH Radical scavenging assay

The DPPH radical scavenging effect of the extracts was determined by the method of Yen and Chen (1995). The absorbance was measured at 517 nm. The scavenging effect (%) was calculated by using the formulae given by Duan et al. (2006). Synthetic antioxidants, Gallic acid and Ascorbic acid were used as positive controls.

Hydrogen peroxide radical scavenging assay

The ability of microalgae crude extract to scavenge hydrogen peroxide was determined by the standard procedure of Gulçin et al. (2004). The absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide).

Nitric oxide radical scavenging assay

Nitric oxide radicals generated from sodium nitroprusside solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Griess reaction (Gulcin 2006). Absorbance was measured 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 (FRAP)

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

Carotenoid content

Carotenoid content 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 is calculated using the Lichtenthaler equations.

Results and Discussion

In this present study, Total Phenol Content (TPC), and the antioxidant properties such as Total Antioxidant activity, DPPH radical scavenging activity, Nitric oxide radical scavenging activity, Hydrogen peroxide radical scavenging activity, reducing power and carotenoid content of acetone, methanol, and hexane extract of *Chaetoceros*

calcitrans, *Chlorella salina* and *Isochrysis galbana* micro algae were determined.

Total phenol content

The total phenolic content of three different solvent extracts (acetone, methanol, and hexane) of microalgae *C. calcitrans*, *C. salina* and *I. galbana* were determined and the results are presented in **Fig. 1**. The highest phenolic content was found in methanol extract of *I. galbana* (2.57 ± 0.08 mg/g gallic acid equivalent). The lowest phenolic content was recorded in hexane extracts of *C. calcitrans* (0.76 ± 0.02mg /g gallic acid equivalent).

Total antioxidant activity

Total antioxidant activity of different solvent extracts of *C. calcitrans*, *C. salina* and *I. galbana* was showed in Fig. 2. The highest antioxidant activity was observed in methanol extract of *I. galbana* (2.21 ± 0.05 mg/g ascorbic acid equivalent). The lowest activity was noticed in the hexane extract of *C. calcitrans* (0.69 ± 0.06 mg/g ascorbic acid equivalent).

Ferric reducing antioxidant Power (FRAP)

Reducing activity of the microalgae extracts as determined by reducing power assay varied as seen in **Fig. 3**. The reducing power was found to be higher in methanolic extract of *I. galbana* (0.82 ± 0.02 mg/g ascorbic acid equivalent). The lowest reducing power was recorded in hexane extract of *C. calcitrans* (0.27 ± 0.03 mg/g ascorbic acid equivalent).

DPPH activity

DPPH radical scavenging activities (%) of different extracts of three microalgae are presented in Fig. 4. All these microalgae extracts possessed the ability to scaveng DPPH at various degrees, the DPPH radical scavenging activity was found to be higher in methanolic extract of *I. galbana* (34.18%) The hexane extract of *C. calcitrans* showed the minimum DPPH radical scavenging activity of 9.31%.

Hydrogen peroxide radical scavenging

Hydrogen peroxide radical scavenging activity of different solvent extracts of *C. calcitrans*, *C. salina* and *I. galbana* were shown in Fig. 5. Hydrogen peroxide radical scavenging activity was found to be maximum in methanol extract of *I. galbana* (67.33%), whereas minimum in hexane extract of *C. calcitrans* (11.45%).

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging assay of different solvent extracts of three microalgae were shown in **Fig. 6**. Results indicated that the highest scavenging activity was observed in methanol extract of *I. galbana* (37.33%), whereas minimum in acetone extract of *C. calcitrans* (15.46%).

Carotenoid content

Carotenoid content of acetone, methanol and hexane extracts of *C. calcitrans*, *C. salina* and *I. galbana* were shown in **Fig. 7**. Results shows that the highest carotenoid content was observed in methanol extract of *I. galbana* (7.63±0.08 $mg.g^{-1}$), whereas minimum in acetone extract of *C. calcitrans* (1.74±0.04 $mg.g^{-1}$).

It is well-known that carotenoids are important contributors to antioxidant activity in microalgal biomass (Kobayashi et al. 1997; Herrero et al. 2006). Carotenoid

content showed a significant contribution to the evaluated antioxidant assays such as Total Antioxidant activity, DPPH radical scavenging activity, Nitric oxide radical scavenging activity, Hydrogen peroxide radical scavenging activity, Ferric reducing antioxidant power.

Phenolic content as measured in this study was comparable to the previous studies of Geetha et al. (2010), Hajimahmoodi et al. (2010) and Li et al. (2007), although Cha et al. (2010) detected higher levels in extracts from C. vulgaris obtained by pressurised liquid extraction at elevated temperatures using 90% ethanol as extractant. In our study, the highest phenolic content was found to be higher in methanol extract of (2.57 ± 0.08 mg/g) were observed in *I. galbana* and lowest phenolic content was recorded in hexane extracts of *C. calcitrans* (0.76 ± 0.02). According to Manivannan et al. (2012) methanol extract of *Chlorella marina* exhibited higher activity which was followed by diethyl ether and hexane extract. This may be due to the differences in the polarity of the solvents used.

The highest antioxidant activity was observed in methanol extract of *I. galbana,* (2.21 ± 0.05 mg/g) and the lowest activity was noticed from the hexane extract of *C. calcitrans* (0.69 ± 0.06 mg/g). Similarly, Sivakumar and Rajagopal (2011) reported that highest antioxidant activity was observed in methanol extract from eight green algal species. Uma et al. (2011) observed that the methanolic extracts displayed greater potential in all antioxidant assays when compared to ethanolic and acetone extract of green microalgae *Desmococcus olivaceous* and *Chlorococcum humicola*.

Maximum DPPH radical scavenging activity was found in methanol extract of *I. galbana* (34.18%). The hexane extract of *C. calcitrans* showed the minimum DPPH radical scavenging activity of 9.31%. Similarly both methanolic and acetone extracts of *D. olivaceous* and *C. humicola* showed a significant dose dependent reduction of DPPH radicals which was recorded by Uma et al. (2011). Lee et al. (2010) reported that 80% methanol extract and organic solvent fractions of microalgae showed notable activities indicating the higher efficacy for scavenging of free radicals. The reducing power was found to be higher in methanolic extract of *I. galbana* (0.82 ± 0.02 mg/g). The lowest reducing power was recorded in lowest concentration of hexane extract of *C. calcitrans* (0.27 ± 0.03 mg/g). Similarly, Kuda et al. (2005) reported that the highest amount of reducing power was observed in the highly polar water extract of *S. lomentaria* and the minimum reducing power was observed in ethanol extract and crude fucoidan, these were dose-dependent.

Hydrogen peroxide radical scavenging activity (%) was found to be maximum in methanol extract of *I. galbana* (67.33%), whereas minimum in hexane extract (11.45%) of *C. calcitrans*. Similar report was proposed by Uma et al. (2011) stating that methanolic extract of *D. olivaceous* exhibited 39% scavenging activity and the acetone extract of *C. humicola* exhibited 15% scavenging activity.

In this study, the highest scavenging activity was observed in methanol extract of *I. galbana* (37.33%), whereas minimum in acetone extract of *C. calcitrans* (15.46%). This is lined with the finding of Lee et al. (2010), who found that the ethyl acetate of *H. porphyrae* (30.1%) and the 80% of methanol extract of *O. unicellularis* (49.3%) exhibited significantly higher nitric oxide radical scavenging effects than those of the commercial antioxidants.

Fig.1 Total phenolic content of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

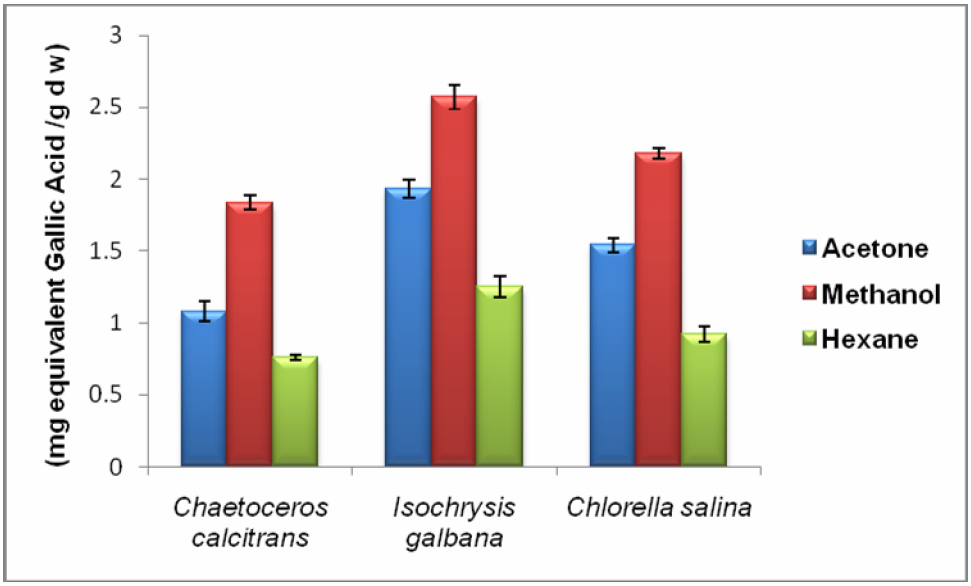


Fig.2 Total antioxidant activity of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

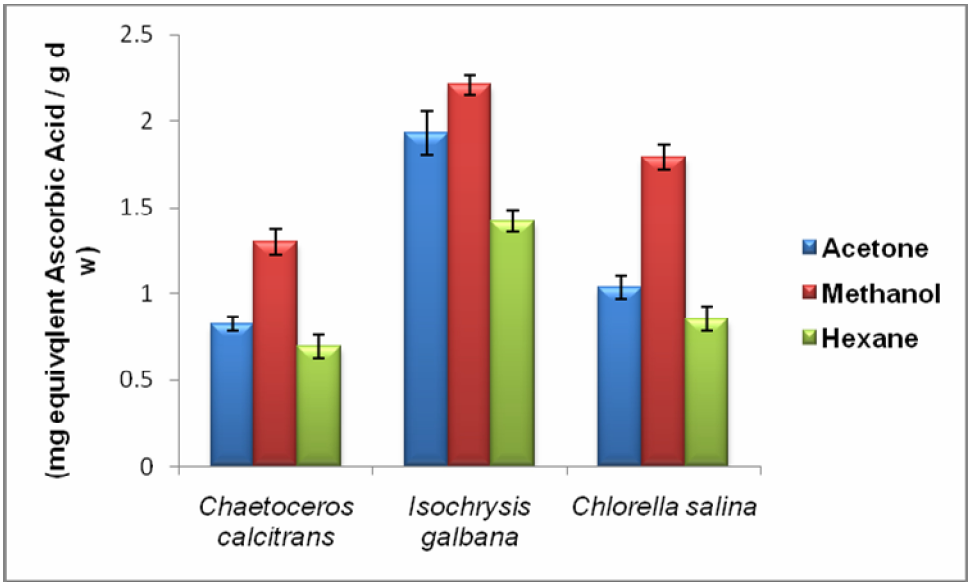


Fig.3 Ferric reducing antioxidant power (FRAP) of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

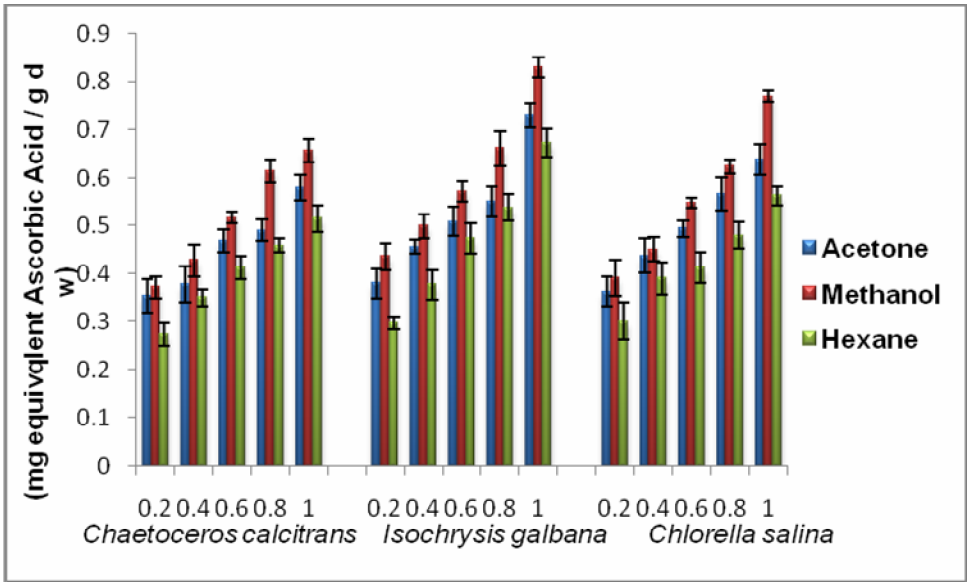


Fig.4 DPPH activity of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

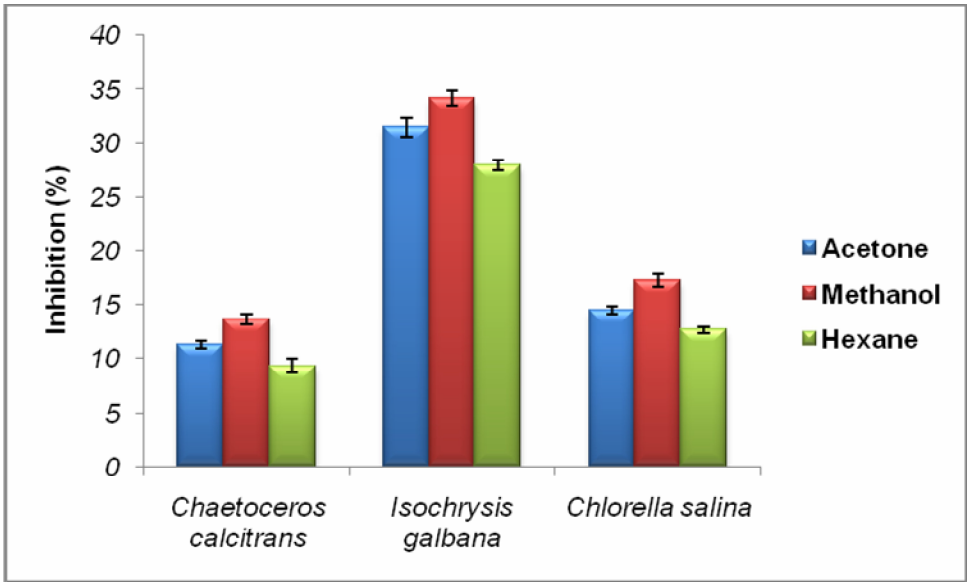


Fig.5 Hydrogen peroxide radical scavenging assay of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

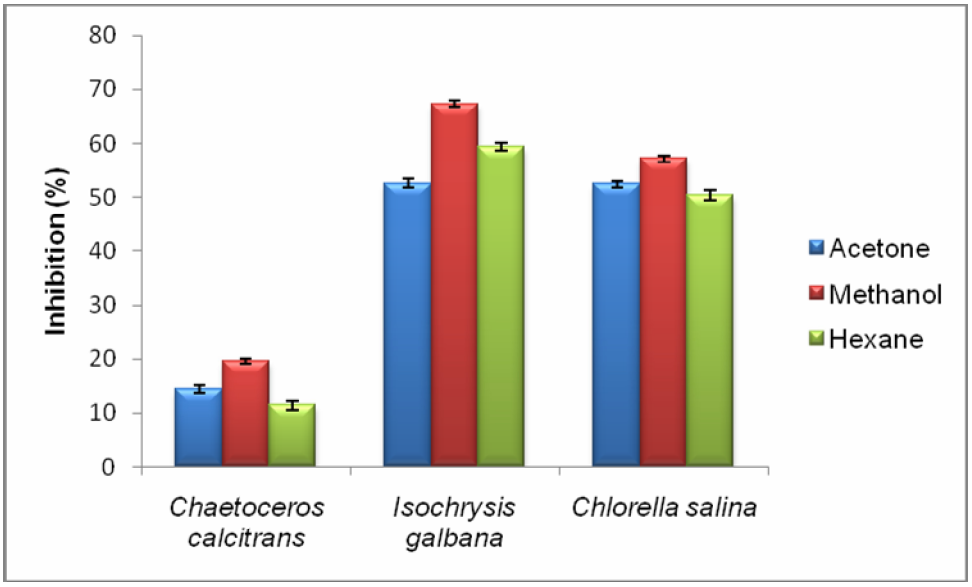


Fig.6 Nitric oxide radical scavenging assay of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*

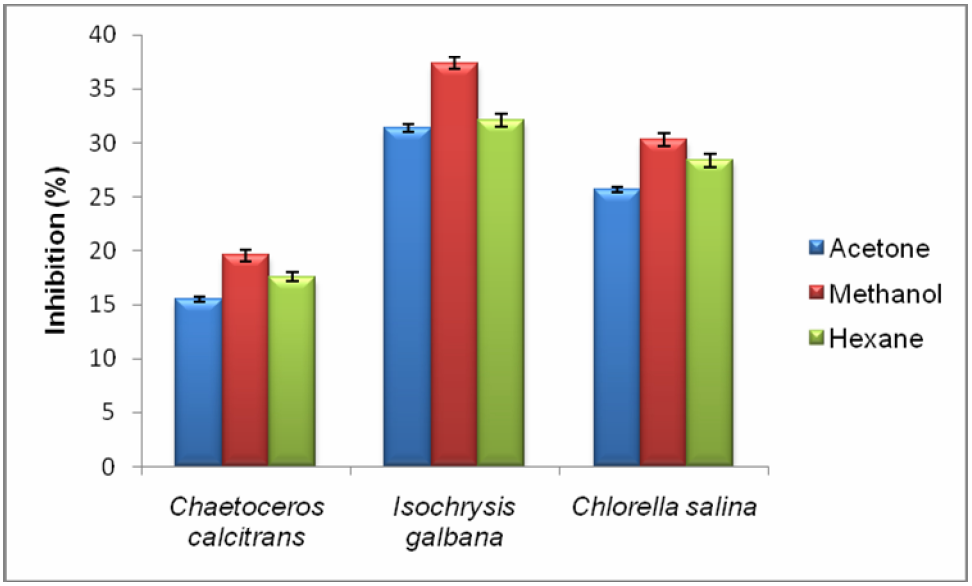
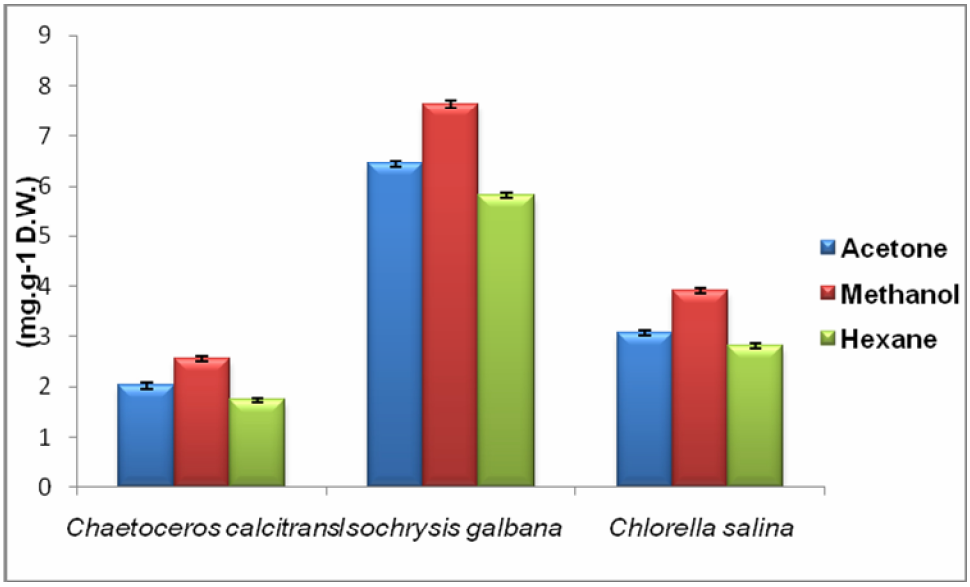


Fig.7 Carotenoid content of acetone, methanol and hexane extracts of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*



Highest carotenoid content was observed in methanol extract of *I. galbana* (7.63 ± 0.08 mg.g^{-1}), whereas minimum in acetone extract of *C. calcitrans* (1.74±0.04mg.g^{-1}). Similarly (Goiris et al. 2012) reported that the highest carotenoid content was observed in the ethanol/water extracts 7.8 mg g-1 biomass of *Isochrysis sp., Phaeodactylum sp., Chlorella sp.* and *T. suecica*.

The results suggest that when using microalgae biomass as a source of natural antioxidants, not only carotenoids but also phenolic compounds should be considered. Although the content of phenolic substances in microalgae is in the lower end of the range reported for terrestrial plants (Cai et al. 2004), it was comparable to the carotenoid content in the studied samples. Further identification of phenolic substances from microalgae is required to evaluate whether microalgae may contain novel phenolic compounds that are not known from terrestrial plants. Further, it should be noted that carotenoid content in some microalgae can be increased up to 140 mg g-1 biomass by manipulating environmental conditions (Spolaore et al. 2006) and that the same might be true for phenolic compounds. In addition, phenolic compounds appear to be responsible for the antioxidant activity of microalgae extracts.

However, in the present study, both carotenoid content and total phenolic content contributed significantly to antioxidant capacity. Though the results shows that both carotenoid and total phenolic content plays a vital role in total antioxidant capacity, it is still unclear whether they individually affect the antioxidant activity of microalgae. So, there is a need for vast experimental analysis to strongly prove the individual role of carotenoid and phenolic content in antioxidant capacity. Moreover, on the basis of the results obtained the antioxidant properties of microalgae

shows that it can be used for a variety of beneficial chemo-preventive effects.

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