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Screening of Microalgae as a Potential Source of Photoprotective Pigments

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ABSTRACT

Absorbance and quantification of microalgal pigments from southeast coast regions (Cuddalore & Parangipettai) was monitored using UV followed by HPLC. Photoprotective pigments of microalgal species such as *Chaetoceros simplex*, *Chlorella marina*, *Nannochloropsis sp*, *Platymonas sp*, *Pavlova lutherii*, *Tetraselmis gracilis*, *Tetraselmis tetrathele*, *Tetraselmis chuii*, and *Synechocystis sp*. were recorded in high light condition. Microalgal species were morphologically identified and isolated using aseptic methods. The strains were cultured under in-vitro condition using Guillard's f/2 & Convey medium. The exponentially grown cells from 2nd to 8th day were subjected to absorption with spectra at 2-day intervals for determining pigments in microalgae. The obtained absorption spectra for each individual strains showed corresponding peaks with the accumulation of different photosynthetic and photo-protective pigments viz. Chlorophyll a, Chlorophyll b, ß-34 carotene and Diatoxanthin etc. Pigments synthesized by all strains were extracted using acetone and were quantified by HPLC-DAD. The present study concludes that the process of acclimation and adaptation of microalgae under in-vitro condition induces many nutraceutical active pigments at a higher concentration which might be due to the pigments present in different phenotypical molecular organization in thylakoids. The results obtained are also helpful to identify bio-marker pigments from different groups of microalgae.

KEYWORDS: Microalgae; Pigments; Spectral absorbance; HPLC;

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INTRODUCTION

In marine environment microalgae frequently experience intensive variations such as high and low light intensity and nutrient concentrations, which is important for their growth and productivity [4]. Light is one of the most prominent sources for photosynthetic organisms like plants, algae and cyanobacteria [7]. They harvest light energy and transformed into chemical energy by the presence of photosynthetic pigments. Light directly affects the photosynthetic rate in the cell. When lead to photo oxidative exceeds damage and affects the growth [11]. To cope with these changes in light regime, microalgae have evolved various adaptive processes of acclimation and adaptation. Here by the evolutionary fitness of species within the environmental constraints are increased [11].

Recently the studies on the spectral composition of light reveal the ability of microalgae to finely balanced light harvesting and photo protective capacity. Moreover, the spectral absorption contributes the information on total in vivo pigment absorption in microalgae [16]. The knowledge of the types and concentration of the pigments provides the mature information regards the photosynthetic process and the algal adaptability at various environments.

Photosynthetic pigments in microalgae appear as highly complex structures whose separation has been proven to be confronting for decades. Further, the diverse molecular structures possess different polarities, such as acidic chlorophylls to the non polar hydrocarbon carotenes. It is to be noted that the species composition of microalgae spatially and temporally affects the quality and quantity of the pigments [13]. So far no single technique or approach has been sufficient for determining the evidence of relevant structure and dynamics of phytoplankton community. High performance liquid chromatography has been serving as a gold standard for measuring pigment concentrations in al biotic sources [14]. Hence our study aimed to evaluate the distribution and abundance of photosynthetic pigments in microalgae of different species. Special attention was given to the culturing technique and pigment determination techniques, in order to determine the diversity of the pigments in micro algal population.

MATERIALS AND METHODS

2.1.Sample collection and maintenance

Sea water samples were collected from different landing centers in the Southeast coastal area of India, *viz.*Cuddalore (11˚42 ’8.6328"N/ 79˚46 ’ 32.4588”E) and Parangipettai (11°29'41.3304"N / 79°46'33.636"E). The samples were collected during pre-monsoon season in the month of June. Plankton nets (diameter -1.5 m) made up of bolting silk cloth (mesh size of 2-20 µm) was employed to collect the samples, which were stored in sterile conditions.

2.2. Identification of micro algae

The aliquots from the mixed species were collected and subjected to compound microscope examination. Microscopic identification of the algal species was performed under 100 x magnifications. The wet mounts of the species were taxonomically determined using identification manual [17].Then the aliquots of mixed species were subjected to serial dilution followed by the quadrant streaking for pure cultures. Stock cultures of pure cultures were maintained in a special room adjacent to the mass culture and maintained in Guillard's f/2 medium and asses the growth potentials at regular spectral course.

2.3. Absorption spectra

Absorption spectra of unialgal species, which is obtained from the aseptic techniques were monitored by regularly/day. Spectral progression was measured using visible wavelength ranges from 400nm-700nm. Thus enables the total pigments in algae with respective wavelength.

2.4. Identification of pigments

The algal cultures (each 10ml) were filtered using GF/F and stored at-20°C until further analysis. The presences of pigments were screened using HPLC analysis coupled with the DAD system to evaluate the pigments existence in the individual species with the help of pigment standards (Beta apo carotenoids).

2.5. High Performance Liquid Chromatography (HPLC)

2.5.1. Sample preparation for HPLC

The frozen samples were kept outside and thaw for seconds then mix with 90% acetone. (Here we use beta apo carotenoid as an internal standard) and sonicate the algal culture samples about 30 seconds, to break the hard siliceous cell wall. Sonicated samples were centrifuged10,000 rpm for 10 minutes at 40̊ C. The supernatant was filtered through 0.22µm filter with syringe- to avoid the larger particles into the column. Finally samples were injected to the instrument. Mobile phase: method Eluent A: Methanol 50ml: Acetone 30ml: Pyridine 20ml. Eluent B: Methanol 20ml: Acetone 60ml: Acetone 20ml.

RESULTS

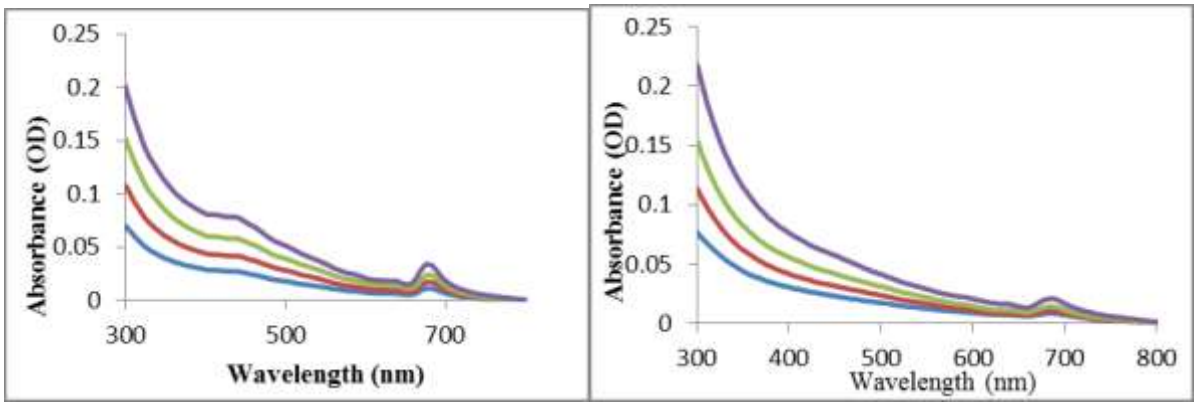
3.1. Identification of Micro algae

Totally 10 species have been isolated from mixed cultures. The species collected, isolated and purified from Estuarine were identified as *Chaetoceros simplex*, *Chlorella marina*,

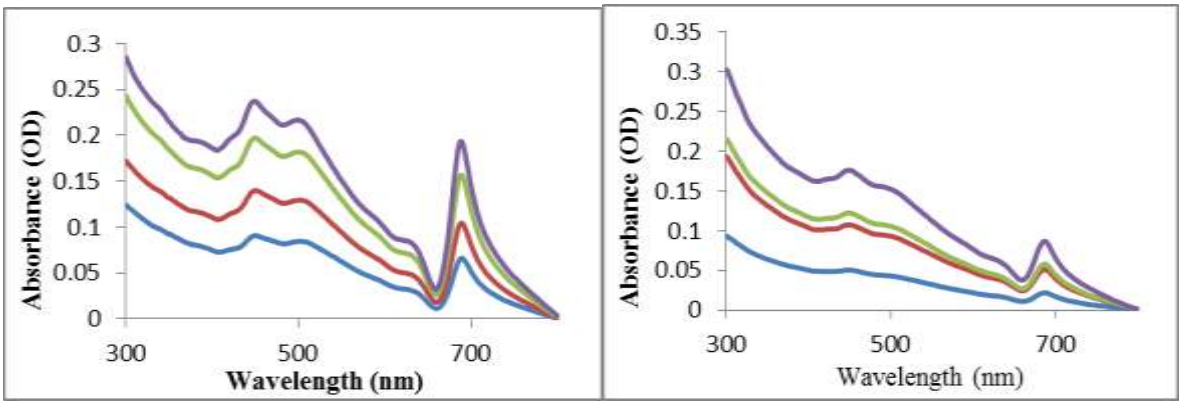
Nannochloropsis sp, Platymonas sp,Pavlova lutherii,Tetraselmis tetrathele, Tetraselmis chuii, Tetraselmis gracilis and Synechocystis sp.

3.2. Phytoplankton spectral measurements

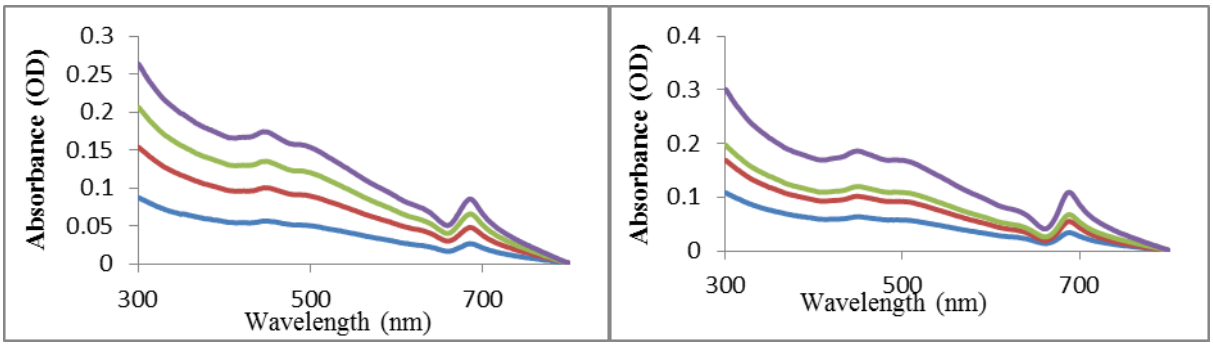
3.2.1. Visible spectroscopy



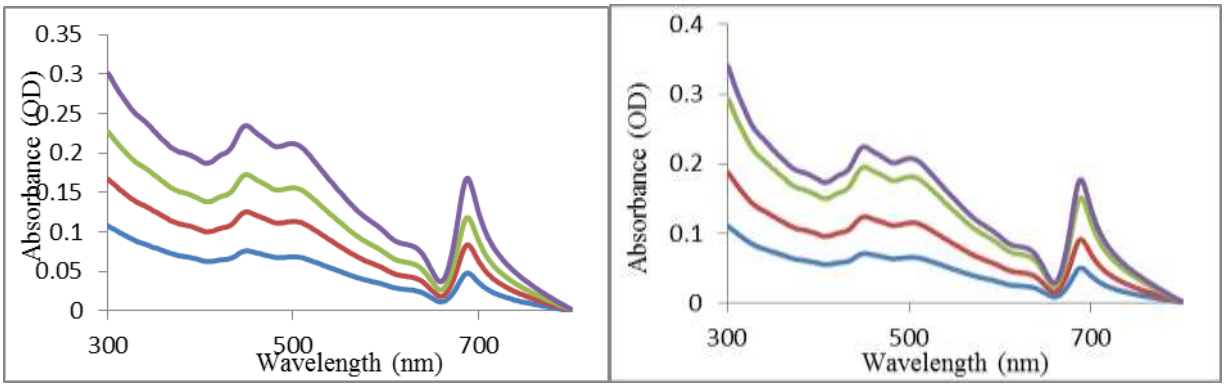
1) *Chaetoceros simplex* 2) *Pavlova lutherii*



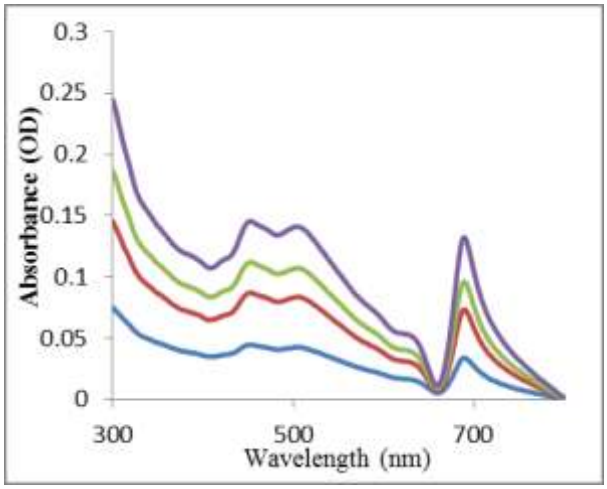
3) *Chlorella marina* 4) *Tetraselmis gracilis*



5) *Tetraselmis tetrathele* 6) *Tetraselmis chuii*



7) *Synechocystis sp* 8) *Platymonas sp*



9) *Nannochloropsis sp*

Fig. 1-9 The spectral images of the different microalgae (at different day intervals)

Table 1.Different pigments and their wavelength of 9 algal species

S.No.	Name of the species	Pigments	Wavelength (nm)
1	*Chaetoceros simplex*	Pra, Dd, Dt, Chl-a, Pheo-a, β- Car.	420,430,443,670,447
2	*Chlorella marina*	Chlorophyllide-b, Vx, Ax, Dd, Zx, Leu, Cx, Divinyl Chl-a, Chl-a, Pheo-a, β-car.	470,477,490,453,475,467,433,430,665,447
3	*Pavlova lutherii*	Pra, Vx, Dd, Dt, Zx, Leu, Chl-a, Chl-b, Pheo-a, β- Car.	4420,477,443,453,475,433,430,665,447
4	*Platymonas sp*	Vx, Zx, Leu, Chl-a, Pheo-a, β- Car.	477,443,453,475,433,430,665,447
5	*Synechocystis sp*	Vx, Ax, Zx, Leu, Chl-a, Pheo-a, β-car.	477,490,453,475,433,430,665,447
6	*Tetraselmis chuii*	Dt, Chl a, Pheo-a, β- Car.	443,430,480,665,447
7	*Tetraselmis gracils*	Dt, Cx, Chl-a, Pheo-a, β-car.	443,467,430,665,447
8	*Tetraselmis tetrathele*	Vx, Zx, Leu, Chl-a, Pheo-a.	477,490,453,430,665
9	*Nannochloropsis sp.*	Ax, Dd, Zx, Leu, Divinyl Chl-a, Chl a Pheo-a, β- Car.	490,477,453,475,433,430,665,447

3.3. HPLC chromatogram of different algal species and its pigments patterns

The pigment composition of individual species was analyzed by HPLC-DAD system and the results showed the presence of prominent pigments such as Chlorophyll-a,Chlorophyllide-b, Astaxanthin, Canthaxanthin, Violaxanthin, Diadinoxanthin, Diatoxanthin Pheophytin-a, Divinyl chlorophyll-a, Leutin, Zeaxanthin and β-Carotene.

Table 2.Maximum and minimum concentrations of pigments present in the microalgal species.

S.no	Name of the species	Name of the pigments	Concentration mg/µl	
			Maximum	Minimum
1	*Chaetoceros simplex*	Pra, Dd, Dt, Chl-a, Pheo-a, β- Car.	18.74639 (Dt)	1.42371 (β- Car)
2	*Chlorella marina*	Chlo-b, Vx, Ax, Dd, Zx, Leu, Cx, Divinyl Chl-a, Chl-a, Pheo-a, β-car.	4.61957 (Ax)	1.17361 (Dd)
3	*Pavlova lutherii*	Pra, Vx, Dd, Dt, Zx, Leu, Chl-a, Chl-b, Pheo-a, β- Car.	61.77803 (Dt)	1.15467 (β-C)
4	*Platymonas sp*	Vx, Zx, Leu, Chl-a, Pheo-a, β-Car.	6.97312 (Zx)	1.36175 (Pheo-a)
5	*Synechocystis sp*	Vx, Ax, Zx, Leu, Chl-a, Pheo-a, β-car.	7.07779 (Vx)	1.09016 (Zx)
6	*Tetraselmis chuii*	Dt, Chl a, Pheo-a, β- Car.	5.83544 (Dt)	1.04839 (β-C)
7	*Tetraselmis gracilis*	Dt, Cx, Chl-a, Pheo-a, β-car.	6.40342 (Dt)	1.03806 (β– C)
8	*Tetraselmis tetrathele*	Vx, Zx, Leu, Chl-a, Pheo-a.	7.11095(chl-a)	1.31048 (Leu)
9	*Nannochloropsis sp.*	Ax, Dd, Zx, Leu, Div- Chla, Chl-a Pheo-a, β- Car.	7.27167 (Dd)	1.29847 (Ax)

(Chl a - Chlorophyll a, Chl b - Chlorophyll b, Pra – Prasinoxanthin, β-C – Beta carotene, An - Antheraxanthin, Zx – Zeaxanthin, Dt - Diatoxanthin, Chl b - Chlorophyll b, Per - Peridinin, Ax – Astaxanthin, Cx - Canthaxanthin, Leu –Leutin, Div-chla – Divinylchlorophyll a, Dd – Diadinoxanthin and Chlo-b – Chlorophyllide b, Pheo-a- Pheophytin a, Vx- violaxanthin)

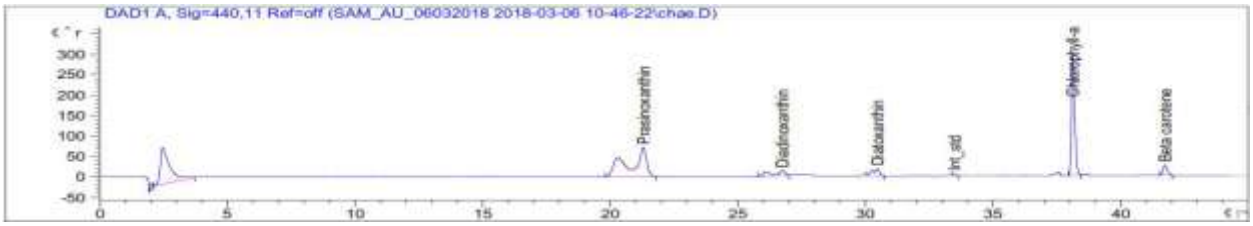


Fig.10. HPLC spectrum of *Chaetoceros simplex*. showing the pigments of Prasinoxanthin, Diadinoxanthin, Diatoxanthin, Chl-a, Pheophytin-a and β-Carotene. Comparatively Diatoxanthin (18.74639 ng/µl) was found predominant, which is species specific in nature.

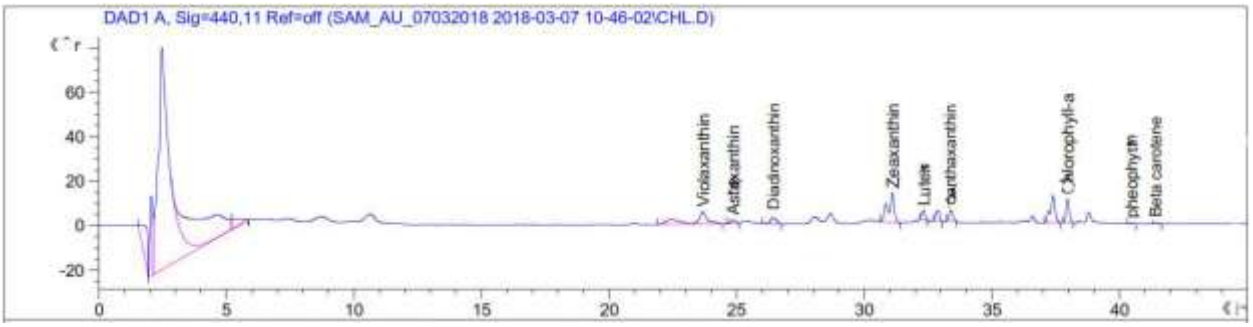


Fig.11. HPLC spectrum of *Chlorella marina* showing the pigments of Chlorophyllide b, violaxanthhin, Astaxanthin, Diadinoxanthin, Zeaxanthin, Leutin, Canthaxanthin, Chlorophyll-a, Divinyl chlorophyll a, Pheophytin-a and β-Carotene. Here the signature pigments of chlorophyll a (116.38128ng/µl) and b (3.67731ng/µl) were noted in Chlorophyceae and the accessory pigments especially Astaxanthin (4.61957 ng/µl) was observed as photoptotective pigment.

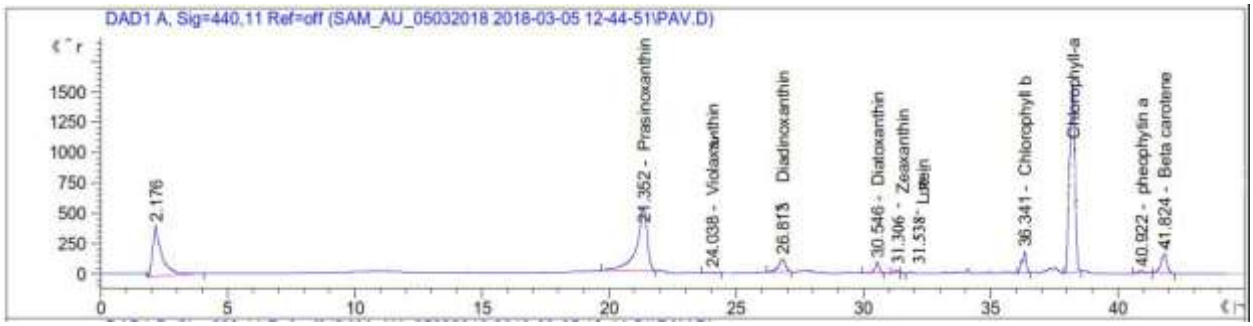


Fig.12. HPLC spectrum of *Pavlova sp.* shows the pigments of Pheophorbide a, Prasinoxanthin, Violaxanthin, Diadinoxanthin, Diatoxanthin, Zeaxanthin,Leutin,Chl b, Chl a, Pheophytin a and β-carotene. Here the highest pigment concentration was found in Chlorophyll a (34.69956 ng/µl) of Peridinin and the lowest were found in β-carotene (1.15467 ng/µl).

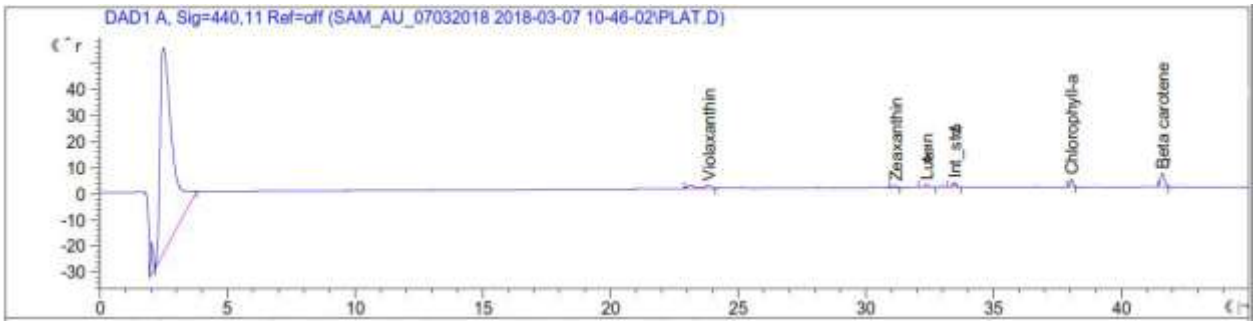


Fig.13. HPLC spectrum of *Platymonas sp*. showing the pigments namely Violaxanthin, Zeaxanthin, Leutin, Chlorophyll-a, Pheophytin a and β-carotene. The highest pigment concentration was noticed in Zeaxanthin (6.97312 ng/µl) and lowest concentration was observed in Pheophytin-a (1.36175 ng/µl)

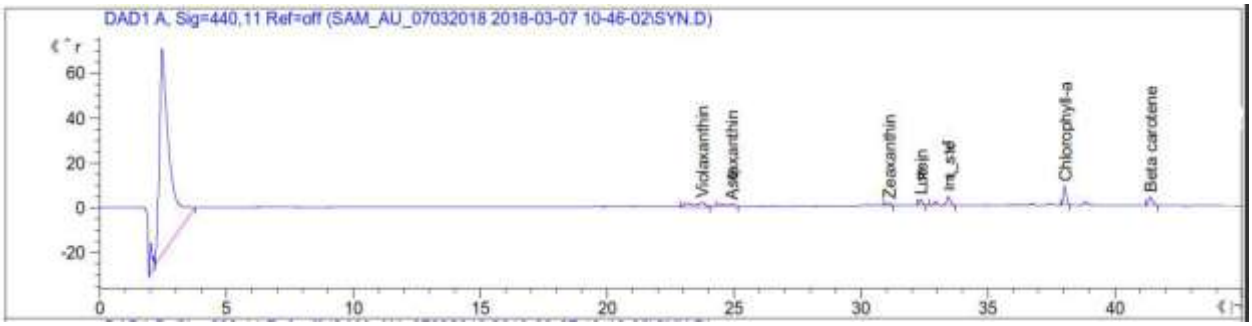


Fig.14. HPLC spectrum of pigments present in *Synechocystis sp.* of Violaxanthin, Astaxanthin, Zeaxanthin, Leutin, Chl-a, Pheophytin-a and β-Carotene. The highest pigment concentration was found in Violaxanthin (7.07779 ng/µl) and the lowest concentration in Zeaxanthin (1.09016ng/µl)

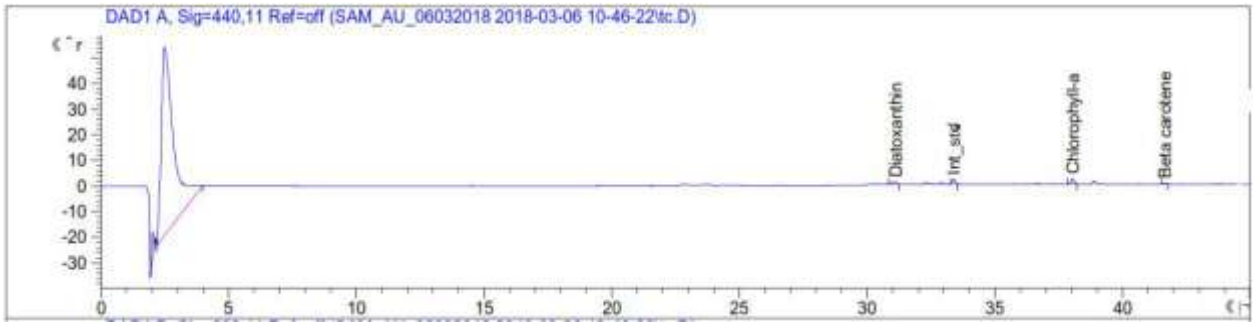


Fig.15. HPLC spectrum of *Tetraselmis chuii* pigments of Diatoxanthin, Chlorophyll a, Pheophytin a and β-carotene. The highest pigment concentration of Tetraselmis chuii was found in Diatoxanthin (5.83544ng/µl) whereas β-carotene was found in lowest concentration of (1.04839 ng/µl)

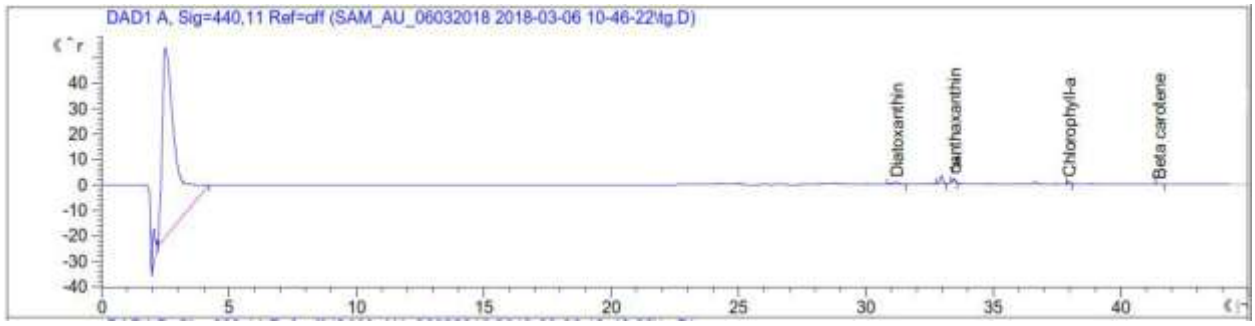


Fig.16. HPLC spectrum of *Tetraselmis gracilis* pigments are Diatoxanthin, Canthaxanthin, Chla, Pheophytin-a and β-carotene. Here the highest concentration was found in diatoxanthin (6.40342 ng/µl) and the lowest was noted on β-carotene (1.03806 ng/µl)

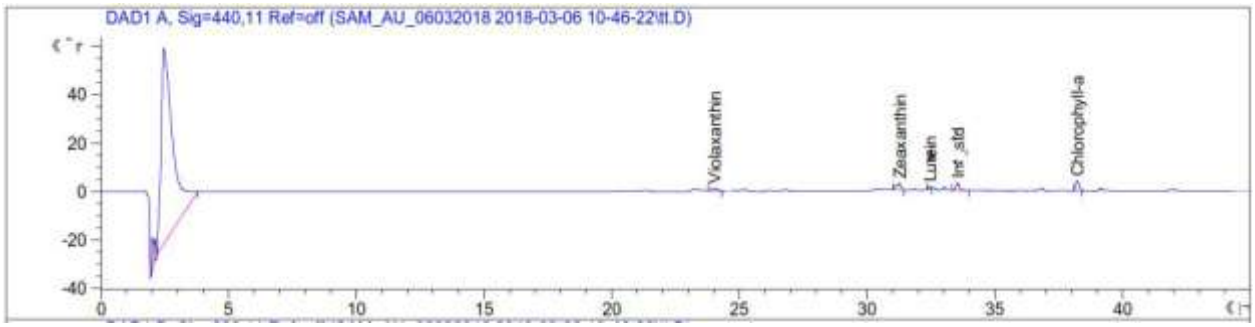


Fig.17. HPLC spectrum of *Tetraselmis tetrathele* pigments are Violaxanthin, Zeaxanthin, Leutin, Chlorophyll-a and Pheophytin-a. Here the maximum pigment concentration was found in Chlorophyll-a (7.11095 ng/µl) and the minimum was noted in Leutin (1.31048 ng/µl).

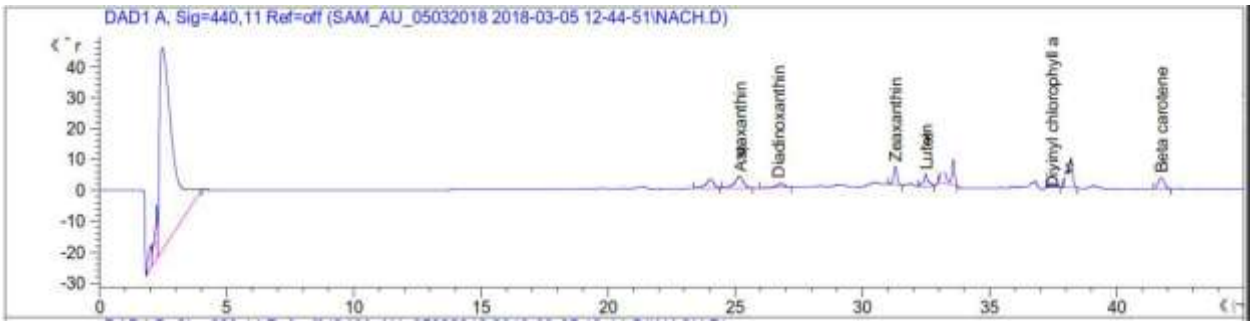


Fig.18. HPLC spectrum of *Nannochloropsis sp.* pigments are Aataxanthin, Diadinoxanthin, Zeaxanthin, Leutin,Divinyl chl-a, chl-a, pheophytin-a and β-carotene.here the maximum pigment concentration was observed in Diadinoxanthin (7.27167 ng/µl) and the minimum was noted in (1.29847 ng/µl)

DISCUSSION

Among the groups of microalgae as described such as Chlorella *marina*, *Nannochloropsis sp*., *Pavlova lutherii*, *Platymonas sp*., *Tetraselmis gracilis*, *T. tetrathele*, *T. chuii* and *Synechocystis sp*. shows vast diversity of pigment profiles as documented earlier. Marine microalgae have great biodiversity for photoacclimation and photoprotection in photosynthesis [6]. The thylakoid membranes are embedded with pigments which are discrete light complex in phytoplankton,The light harvesting complexes may differ from phytoplankton classes and the way it is embedded in thylakoid membrane [8]. PSI : PSII membrane regions are seen stacked and unstacked in thylakoids, mostly in green algae [1]. In contrast lateral segregation of PSI : PSII were recorded in phyla of *Bacillariophyceae*, *Eustigmatophyceae* and *Haptophyta* [8]. *Chlorophyceae*, *Eustigmatophyceae* and *cyanobacteria* species are sensitive to high light intensity upon exposure emission of some photo protective pigments such as Canathaxanthin and Astaxanthin, Whereas *Pavlova lutherii* is exceptional [8]. Prasinoxanthin, Violoxanthin and Fucoxanthin are light sensitive pigments.With reference to the previous reports in our study we recorded Prasinoxanthin shows maximum concentration of (14.13660 ng/µl) present in *Pavlova lutherii* when compared to that of *Chaetoceros simplex* (2.99542 ng/µl) and Astaxanthin were observed maximum in *Synechocystis sp* (5.45758 ng/µl),*Chlorella marina* (4.61957 ng/µl) and least concentration in *Nannochloropsis sp* (1.29847 ng/µl) and elevated level of Canthaxanthin was observed maximum in *Chlorophytes* of *Chlorella marina* (4.25886 ng/µl) and minimum of *Tetraselmis gracilis*(1.50772 ng/µl) may due to optical signature or intra cellular self-shading of chloroplast [10]. Whereas violaxanthin was found highest concentration in *Synechocystis sp* (7.07779 ng/µl) and lowest concentration in *Pavlova lutherii*

(1.25671 ng/µl). In this respect, photoprotective pigments in thylakoids might be responsible for mutualistic association of nutrient present in estuary water than in open ocean against various biotic and abiotic stresses.moreover algal strains isolated from estuaries shows higher (2.5 to 5 times) and faster qE than strains isolated from the open ocean or from coastal ecosystems [12]. Few papers have investigated the xanthophyll cycle in microalgae other than diatoms [2, 5, 9]. On the whole the results of the pigment level while photo acclimation were studied, which is related to the fact that the in-vitro environment is highly light encouraged.

CONCLUSION:

The quantification of Microalgal pigment profiles were evaluated using analytical methods of spectrophotometer followed by HPLC. Hence the results clearly explain the signature pigments of different phylum of micro algae (Chlorophyta, Eustigmataceae, Haptophyta, Cyanobacteria, Baillariophyta and Ochrophyta) which were show absorption maxima at visible wavelength from 400-700nm. Based on the results, *Pavlova lutherii*and *Chaetoceros simplex* showed the prominent pigments of diatoxanthin concentration at 61.77803ng/µl & 18.74639 ng/µl respectively. However the phyla of Eustigmophyta, Cyanobacteria, Chlorophyta with respective species of *Nannochloropsis sp* (1.29847 ng/µl), *Synechoystis sp* (5.45758 ng/µl) and *Chlorella marina* (4.61957 ng/µl) has dominant pigments of Astaxanthin respectively observedvia HPLC as well as spectral absorbance at 490nm.Whereas in chlorophyta especially the species of *Chlorella marina* (4.25886 ng/µl) and *Tetraselmis gracilis* (1.50772 ng/µl) has Canthaxanthin maximum at 467nm. And moreover prasinoxanthin predominantly present in bacillariophyta and haptophyta of species respectively *Chaetoceros simplex* (2.99542 ng/µl) and *Pavlova lutherii* (14.13660 ng/µl). Whereas β – Carotene present minimum concentration in all microbial species. These kinds of studies will be helpful to identify bio-marker pigments from different group of phytoplankton in coastal waters and to identify their spectral absorbance peaks and otherwise their reflectance signals in linking with satellite sensor based observations and to link with hyper spectral remote sensing.

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