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Photosynthetic pigments, lipids and phenolic compounds of three green algae isolated from freshwater ecosystem

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Abstract

Three algal species *Spirogyra neglecta, Pithophora oedogonia* and *Microspora indica* belonging to family chlorophyceae, isolated from freshwater ecosystems were investigated for their photosynthetic pigments, phosphoglycolipids, neutral lipids and phenolic compounds. Results revealed a photosynthetic pigment pattern of chlorophyceae similar to that of higher plants. The main photosynthetic pigments present in the three algae were chlorophyll a, chlorophyll b, carotenoids, xanthophylls, β-carotene and lutein. Chlorophyllide B and a type of xanthophyll were detected only in *Pithophora oedogonia* and *Microspora indica*, and absent in *Spirogyra neglecta*. TLC profile for lipids showed presence of phosphoglycolipids such as monogalactosyldiacylglycerol, digalactosyldiacylglycerol, phosphatidylglycerol, sulfoquinovosyldiacylglycerol and phosphatidylethanolamine in the three algae studied. We also observed presence of neutral lipids such as esters, triglyceride, diglyceride and monoglyceride in three algae studied. Moreover, our study reported different types of phenolic compounds such as coumarin, flavonol, flavone, cinnamic acid and anthocyanin in the three algae studied. The presence of considerable amounts of photosynthetic pigment, lipids and phenolic compounds in all three algae studied may possess possible utilization for nutritional applications.

Keywords: Green algae, lipids, phenolic compounds, photosynthetic pigments.

1. INTRODUCTION

Algae are photosynthetic organisms that are present in most ecosystems, varying from marine and freshwater to desert sands, and from hot boiling springs to snow and ice. It is a very large and diverse group of simple species, usually autotrophic, ranging from unicellular to multicellular. An algae forms the large base upon which the pyramids of food are constructed in ponds and lakes. Algae release oxygen when processing food, increasing the amount dissolved in the water. They absorb more energy from the sun and generate more oxygen than any plant combined. Algae were possibly the first species capable of photosynthesis, and the only photosynthesizers for billions of years before plants appeared on earth. Algae yield an estimated 30 to 50 percent of the global net oxygen. This oxygen is essential for respiration to humans and other terrestrial creatures, and is absorbed when coal, wood, or oil is burned (Srivastava et al., 2020).

Green algae, also known as grass-green algae, are members of the Chlorophyta division, consisting of over 6,000 species. Green algae are eukaryotes wherein their cells are structured into different organelles covered in membranes, including a nucleus and mitochondria. A significant organelle found in eukaryotic algae is the chloroplast, which contains the light-absorbing pigments responsible for capturing energy in sunlight via photosynthesis. Most algae do have secondary pigments, including the brown or yellow carotenoids, and the red or blue phycobilins. Secondary pigments give their vibrant hues to the algae. Their photosynthetic pigments are more complex than plants. Biochemical respiration processes in algae are close to those of other eukaroytes; initial breakdown of carbohydrates, fatty acids, and proteins occurs in the cytoplasm, but ultimate high-energy release steps occur within the mitochondria. Photosynthetic pigments such as chlorophylls a and b, carotene, and xanthophyll are in the same proportions as those of higher plants. The generic green algal cell, which may be motile or non-motile, has a central vacuole, plastid-containing pigments which vary in shape in different species, and a two-layered cellulose and pectin cell wall. Food is contained in the plastides as starch in the pyrenoids as protein cores. All algae contain mainly pigments, proteins, carbohydrates, fat, and nucleic acids. The quantity varies depending on the type of algae (Boyd 2003).

Lipids are esters of fatty acids and alcohols containing a wide number of structurally distinct organic compounds, including fats, waxes, phospholipids, glycolipids etc. Lipids are the most powerful storage energy source and act as insulators of fragile internal organs and hormones, and play an important role as the structural constituents of most cell membranes. There are different types of algae which comprise up to 40 percent of their overall fatty acid mass as these fatty acids can be extracted for different purposes. Algal lipids typically contain ordinary major and minor fatty acids, and are a especially good source of minor polyunsaturated acids (Fleurance 1994).

Algae are considered nutritious owing to their high protein content and high mineral, trace element and vitamin concentrations (Heiba et al., 1993; Naidu et al., 1993). Algae are also added to soils as fertilizer and soil conditioner, as their high potassium and trace element concentrations improve crop quality. They are a critical source of substantially used chemical extracts in the food, pharmaceutical, textile, and cosmetic industries. These can also act as markers of marine ecosystem environmental problems. Since algae grow quickly and are responsive to changing conditions in the environment, they are often among the first species to respond to changes. Algae provide much of the Earth's oxygen, they are the food base for almost all aquatic life, they are an initial source of petroleum products, and provide foods and industrial products for humans.

Algal lipids and pigments are of great commercial interest. Because of its relatively high levels of proteins, essential amino acids, chlorophylls, and polyunsaturated fatty acids (PUFA), the biomass of the one cell green alga, *Chlorella* has been commercially produced and consumed as a dietary supplement (Ramazanov and Ramazanov, 2006). Besides, the species *Ulva* and *Caulerpa* are commonly used in medicines (Ravikumar et al., 2010). At present there is great interest in freshwater algal pigments, lipids, and phenolic compounds. This work was therefore carried out to characterize the pigments, lipids and phenolic compounds present in three selected algae *Spirogyra neglecta, Pithophora oedogonia* and *Microspora indica* belonging to chlorophyceae from aquatic ecosystems of Goa.

2. MATERIALS AND METHODS

2.1. Materials used

Algal cultures used for the study were *Spirogyra neglecta (*Hassall) Kuetzing*, Pithophora oedogonia* (Montagne) and *Microspora indica* Randhawa. *Spirogyra neglecta* was collected from rice fields of Velha-Canca, Goa. *Pithophora oedogonia*, was collected from aquatic habitats of Zuarinagar, Goa. *Microspora indica* was collected from moist habitation area of Khorlim, Goa. These algae were randomly selected for their easy accessibility.

2.2. Photosynthetic pigment analysis

Quantitative and qualitative analysis of photosynthetic pigments was carried out using different methods such as thin layer chromatography (TLC), UV-Visible spectrophotometer (Schimadzu, UV-2450) and high-pressure liquid chromatography (Waters, HPLC).

2.2.1. Extraction of photosynthetic pigments

Extraction of photosynthetic pigments was carried out according to method described by Sharma and Hall, (1996). 0.5 g of algal tissue were extracted in 2ml of 100% acetone in pestle and mortar at 4^{o}C in dim light followed by centrifugation at 8000 x g for 10 min at 4°C. The supernatant was used for HPLC, spectrophotometric analysis and TLC analysis of pigments.

2.2.2. TLC analysis of pigments

Separation of photosynthetic pigments was carried out using TLC on silica gel plates according to Sankhalkar, (2000). Pigment samples (50 µl) were loaded as discrete spots on the silica plates, 1.5-2 cm from the bottom by means of a syringe. The plates were developed using solvent system. The color spots were identified using their R_f values.

2.2.3. Chlorophyll and carotenoid estimation

Algal tissue was weighed (1g) and extracted in 10 ml of 100 % acetone. The extract was centrifuged for 5 min at 3000 x g and spectral analysis was done from 400-700 nm using spectrophotometer (Schimadzu).

2.2.4. HPLC analysis of pigments

The photosynthetic pigments were separated by HPLC with reverse phase column (Waters Spherisorb ODS 25 µm x 4.6 mm x 250 mm) and a detection programme (Waters 2996 phase diode array detector). 10 µl of the pigment sample was injected into the HPLC column. The gradient for separation was 0-100% ethyl acetate in acetonitrile/ water (9:1) over 25 min with flow rate of 1.2 ml/min and the peaks were detected at 445 nm. The quantity of pigments was calculated from peak area value using β-carotene as external standard. Identification of pigments was carried out using retention time against standards and using spectral profile of individual peaks using PDA detector in the range of 400-700 nm.

2.3. Lipid Analysis

2.3.1. Extraction of total lipids

Total lipids were extracted according to the method described by Turnham and Northcote, (1984). Freshly harvested wet cell pellet (1 g) were homogenized in chloroform: methanol (1:2 v/v) to make the final volume to 15 ml. Lipid extract was centrifuged for 5 min at 3000 x g to get rid of cell debris and to the supernatant, 0.8 ml of double distilled water, 5 ml of chloroform and 5 ml of 0.88% potassium chloride was added in a separating funnel. The mixture was shaken vigorously

for 5 min and total lipids were kept for separation for about 30 min. The lower phase of chloroform contains appreciable amounts of extracted lipids. Total lipids were taken into 10 ml screw capped vials fitted with Teflon lining.

2.3.2. Separation of total lipids by TLC

Separation of total lipids was carried out by thin layer chromatography (TLC) on silica gel H according to Liljenberg and Von Arnold, (1987). Uniform slurry of 100 g of silica gel H was prepared in 100 ml of distilled water. The plates were placed in the drying rack, left at room temperature for an hour and dried at 120°C for 2 h. After cooling the plates, samples (100 µl) were applied as discrete spots, 1.5-2 cm from the bottom of the plate, in chloroform by means of a syringe and the plate was then placed in chromatographic chamber containing eluting solvents. Plates were then air-dried and spots were visualized as bands with iodine vapors in glass chamber, identified by the R_f values (Liljenberg and Kates, 1985).

2.4. Analysis of phenolic compounds

2.4.1. Extraction of phenolic compounds

Algal culture (1 g) was extracted in 5 ml of 80% (v/v) methanolic-HCl using morter and pestle for 5 min at room temperature. This was kept in water bath for 2 h and again homogenized using tissue homogenizer and kept for extraction in dark for 24 h at room temperature. Extracted sample was centrifuged at 6000 x g for 10 min and the supernatant was used for the paper chromatography and spectrophotometric analysis.

2.4.2. Paper chromatography analysis

Ascending paper chromatography was carried out using Whatmann filter paper No. 1 with a solvent system at room temperature. 50 µl of the sample was loaded on the paper and allowed to develop in the solvent mixture. Spots were detected using UV- transilluminator. The compounds were identified based on specific shape and colour of spots.

2.4.3. Spectrophotometric analysis of phenolic compounds

The sample was diluted using 80% methanolic-HCl and the spectral scan was recorded in the range of 190-700 nm on UV-Visible spectrophotometer (Schimadzu 2450) according to Sharma et al., (1998).

3. RESULTS

3.1. Analysis of photosynthetic pigments

The photosynthetic pigments were studied using TLC and HPLC as well as spectrophotometric measurements. Photosynthetic pigments such as chlorophyll, carotenoids and xanthophylls were observed in three algae studied (Fig. 1, 2 & 3; Table no. 1, 2 & 3).

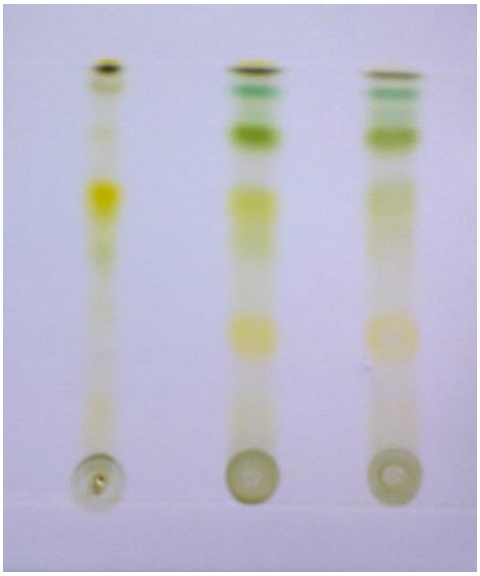


Fig. 1 Thin layer chromatogram of photosynthetic pigments extracted from three algae studied. A-*Spirogyra neglecta,* B-*Pithophora oedogonia,* C-*Microspora indica.*

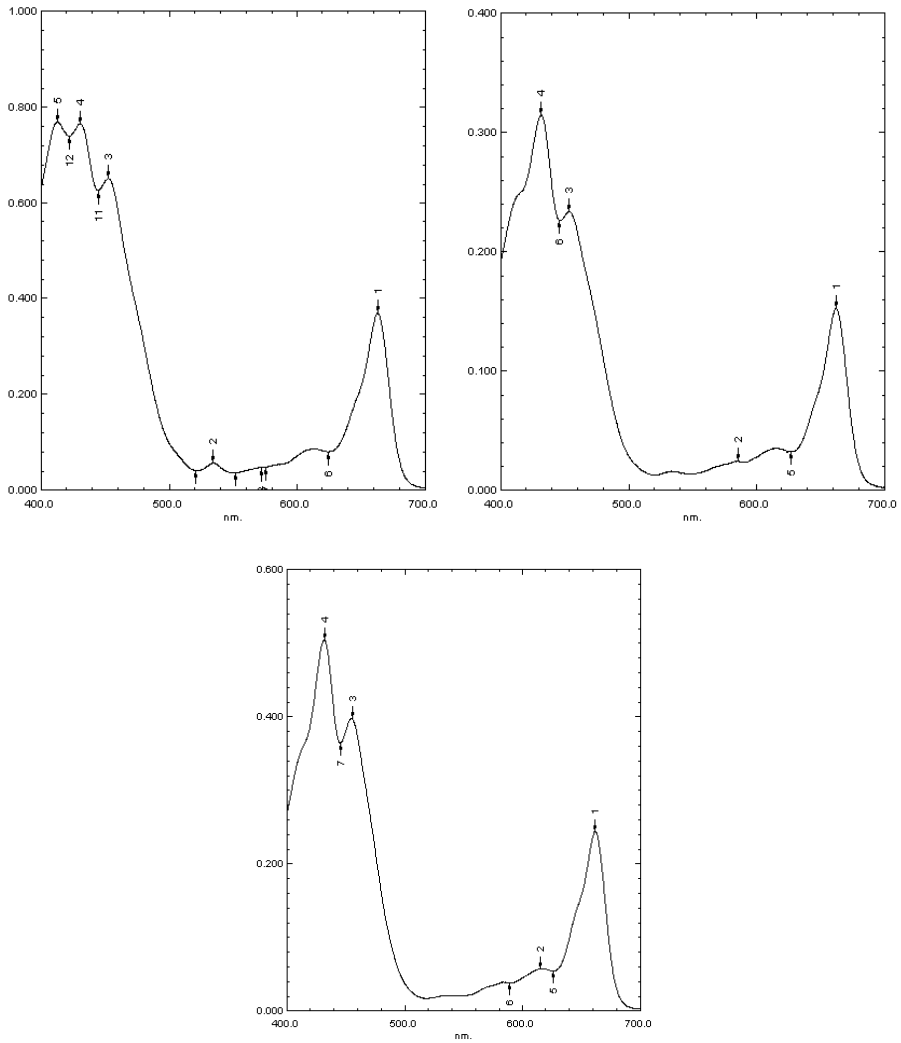


Fig. 2 Spectral analysis of photosynthetic pigments of three algae studied. A-*Spirogyra neglecta,* B- *Pithophora oedogonia,* C-*Microspora indica.*

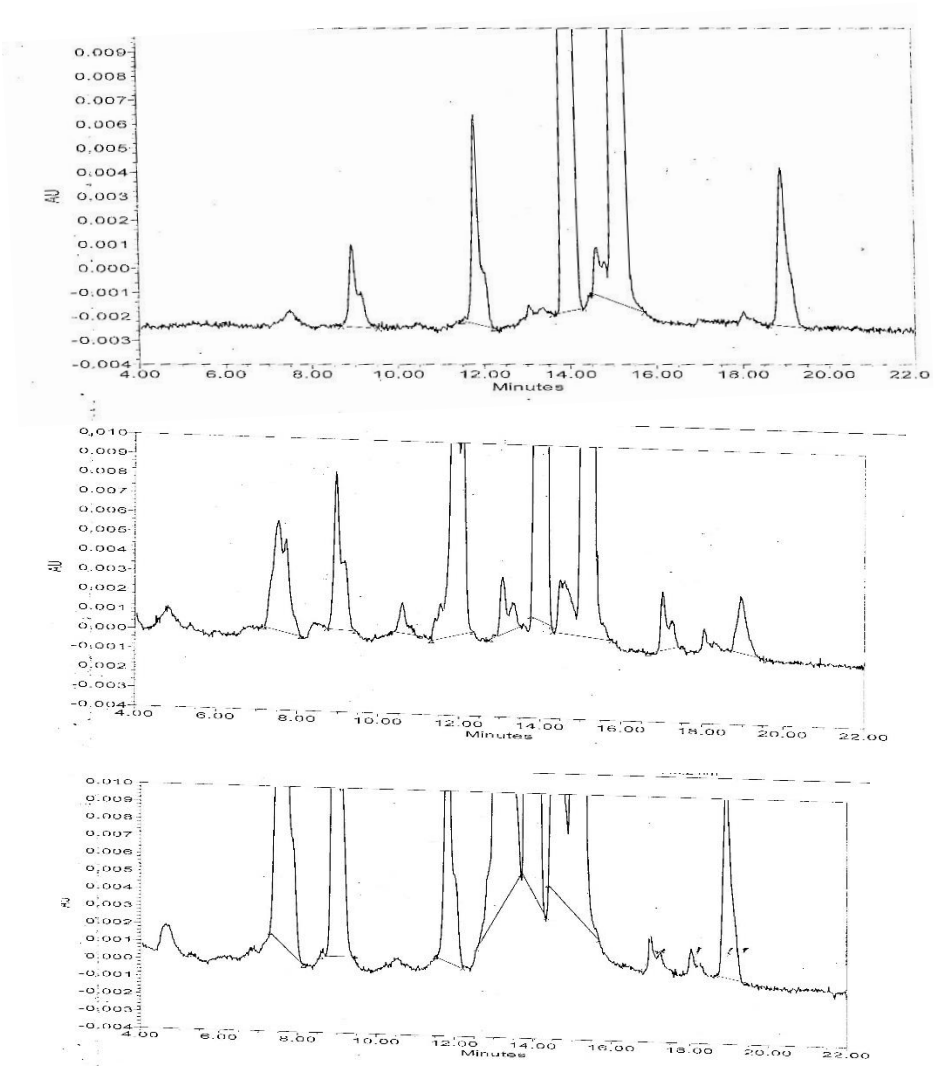


Fig. 3 HPLC analysis of photosynthetic pigments of three algae studied. A-*Spirogyra neglecta,* B- *Pithophora oedogonia,* C-*Microspora indica.*

Table No. 1 Thin layer chromatography profile of photosynthetic pigments of three green algae studied.

Sr. No.	Rf x100	Colour of the spot	*Spirogyra neglecta*	*Pithophora oedogonia*	*Microspora indica*
1	100	Orange	β -carotene	β-carotene	β-carotene
2	97	Grey	Lutein	Lutein	Lutein
3	95	Green	Chlorophyll a	Chlorophyll a	Chlorophyll a
4	80	Light green	-	Chlorophyll	Chlorophyll
5	70	Green	Chlorophyll b	Chlorophyll b	Chlorophyll b
6	55	Yellow	Xanthophylls	Xanthophylls	Xanthophylls
7	30	Yellow	Xanthophylls	Xanthophylls	Xanthophylls
8	21	Yellow	-	Xanthophylls	Xanthophylls

Table No. 2 Spectral analysis of photosynthetic pigments of three green algae studied.

Sr. no.	Peaks	*Spirogyra neglecta*	*Pithophora oedogonia*	*Microspora indica*
1	660	Chlorophyll a	Chlorophyll a	Chlorophyll a
2	615	Chlorophyll	Chlorophyll	Chlorophyll
3	530	Chlorophyll	Chlorophyll	Chlorophyll
4	470	β- carotene	β- carotene	β- carotene
5	440	Carotenoids	Carotenoids	Carotenoids
6	410	Carotenoids	Carotenoids	Carotenoids

Table 3 HPLC analysis of photosynthetic pigments (μg g^{-1} f. w.) in three green algae studied.

Pigments	Retention time (min)	*Spirogyra neglecta*	*Pithophora oedogonia*	*Microspora indica*
Carotenoids	**7.56**	0.050126	0.143298	0.430413
Xanthophylls	**8.94**	0.102724	0.114029	0.364675
Xanthophylls	**11.86**	-	0.455881	0.238034
Chlorophyll b	**13.06**	0.656250	0.043427	0.665319
Chlorophyllide B	**13.88**	-	0.910747	3.679461
Chlorophyll a	**15.03**	0.773889	0.883530	1.723225
Chlorophyll	**16.04**	-	0.040155	-
β-carotene	**18.92**	0.102093	0.049010	0.169162

3.1.1. Photosynthetic pigments (TLC)

The photosynthetic pigments were studied using TLC in three different algae (Fig 1, table no. 1). In *Spirogyra neglecta,* the main photosynthetic pigments observed were identified as β–carotene, chlorophyll a, chlorophyll b, carotenoids and xanthophylls (Fig. 1, table no. 1). The spot no. 1 with Rf value 100 showed orange colour, was identified as β–carotene. Spot no. 2 with Rf value 97 showed grey colour and identified as lutein, spot no. 3 with Rf value 95 was green in colour and identified as chlorophyll a and spot no. 4 with Rf value 70, which was green in colour was identified as chlorophyll b. Spot no. 5 and 6 showed yellow colour and both identified as xanthophylls.

In *Pithophora oedogonia*, the pigments identified were β–carotene, chlorophyll a, Chlorophyll b, chlorophyll, carotenoids and xanthophylls (Fig. 1, table no. 1). In *Pithophora*, 8 different spots were observed. Spot number 1 with Rf value 100 showed orange colour which was identified as β–carotene. Spot number 2 with Rf value 97 showed grey colour and identified as lutein. Spot number 3 with Rf value 95 was chlorophyll a, showed dark green colour. Spot number 4 with Rf value 80 showed light green colour and identified as a type of chlorophyll. Spot number 5 with Rf value 70 showed green colour which was identified as chlorophyll b. Spot number 6, 7 and 8 with Rf value 55, 30 and 21 showed yellow colour which are different types of xanthophylls.

The main pigments identified were β–carotene, chlorophyll a, chlorophyll b, carotenoids and xanthophylls in *Microspora indica* (Fig. 1, table no. 1). In *Microspora* also, 8 similar spots were observed. Rf value 100 showed orange colour which was identified as β–carotene. Spot number 2 with Rf value 97 showed grey colour and identified as lutein. Spot number 3 with Rf value 95 was chlorophyll a, showed dark green colour. Spot number 4 with Rf value 80 showed light green colour and identified as a type of chlorophyll. Spot number 5 with Rf value 70 showed green colour which was identified as chlorophyll b. Spot number 6, 7 and 8 with Rf value 55, 30 and 21 showed yellow colour which were identified as different types of xanthophylls.

3.1.2. Spectrophotometric analysis of photosynthetic pigments

Spectrophotometric analysis of photosynthetic pigments of three algae were studied using spectrophotometer (Fig. 2, table no.2). The absorption scan of *Spirogyra neglecta* showed 6 peaks in the visual region. The peaks observed in the visual region were 410 nm, 440 nm, 470, 530, 615 and 660 nm, which were identified as carotenoids, β–carotene, chlorophyll b and chlorophyll a (Fig. 2 and table no. 2).

It was observed that absorption scan of *Pithophora oedogonia* also showed 6 peaks in the visual region. The peaks observed in the visual region were 410 nm, 440 nm, 470, 530, 615 and 660 nm, which were identified as carotenoids, β–carotene, chlorophyll b and chlorophyll a (Fig. 2 and table no. 2).

Absorption spectra of *Microspora indica* showed similar 6 peaks each in the visual region. The peaks observed in the visual region were 410 nm, 440 nm, 470, 530, 615 and 660 nm, which were identified as carotenoids, β–carotene, chlorophyll b and chlorophyll a (Fig. 2, table no. 2).

3.1.3. HPLC analysis of photosynthetic pigments

HPLC data showed different photosynthetic pigments in three algae studied (Fig. 3 and table no. 3). In *Spirogyra neglecta*, the different photosynthetic pigments were carotenoids, xanthophylls, chlorophyll b, chlorophyll a and β-carotene. In *Pithophora oedogonia*, the different photosynthetic pigments were carotenoids, xanthophylls, chlorophyll b, chlorophyllide B, chlorophyll a, chlorophyll and β-carotene. The different photosynthetic pigments present in *Microspora indica* were same as that of *Pithophora* i.e. carotenoids, xanthophylls, chlorophyll b, chlorophyllide B, chlorophyll a, chlorophyll and β-carotene.

3.2. Analysis of lipids

3.2.1. Analysis of phosphoglycolipids

Phosphoglycolipids separation was carried out by thin layer chromatography in three algae studied (Fig. 4, table no. 4). Identification of these spots was done according to Rf values compared with standards. In *Spirogyra neglecta,* 4 different phospho-glycolipids spots were observed with Rf values 100, 98, 68 and 22 which were identified as pigments, monogalactosyldiglyceride, Phosphatidylethanolamine and digalactosyldiglyceride.

Fig. 4 Thin layer chromatogram of phosphoglycolipids extracted from three algae studied. A-*Spirogyra neglecta,* B- *Pithophora oedogonia,* C-*Microspora indica.*

Table 4 Thin layer chromatography profile of phosphoglycolipids of three green algae studied.

Sr. no.	Rf x100	*Spirogyra neglecta*	*Pithophora oedogonia*	*Microspora indica*
1	100	Pigments	Pigments	Pigments
2	98	Monogalactosyldiglyceride	Monogalactosyldiglyceride	Monogalactosyldiglyceride
3	94	-	Monogalactoosyldiglyceride	Monogalactoosyldiglyceride
4	90	-	Monogalactosyldiglyceride	-
5	82	-	Sulfoquinosylglycerol	Sulfoquinosylglycerol
6	68	Phosphatidylethanolamine	Phosphatidylethanolamine	Phosphatidylethanolamine
7	41	-	Phosphotidylglycerol	Phosphotidylglycerol
8	22	Digalactosyldiglyceride	Digalactosyldiglyceride	Digalactosyldiglyceride

In *Pithophora oedogonia,* 8 different phospho-glycolipids spots were observed. Spot no. 1 with Rf value 100 was identified as pigments, Spot number 2, 3, 4 with Rf value 97, 94, 90 was identified as monogalactosyldiglyceride, Spot number 5 with Rf value 82 was identified as sulfoquinosylglycerol, spot no. 6 with Rf value 68 as phosphatidylethanolamine. Spot number 7 with Rf value 41 was identified as phosphotidylglycerol, Spot number 8 with Rf value 22 was identified as digalactosyldiglyceride.

In *Microspora indica,* 7 different phospho-glycolipids spots were observed which were identified as Spot no. 1 with Rf value 100 as pigments, Spot number 2, 3, 4 with Rf value 97, 94 was identified as monogalactosyldiglyceride, Spot number 4 with Rf value 82 was identified as sulfoquinosylglycerol, spot no. 5 with Rf value 68 as phosphatidylethanolamine. Spot number 6 with Rf value 41 was identified as phosphotidylglycerol, Spot number 7 with Rf value 22 was identified as digalactosyldiglyceride.

3.2.2. Analysis of Neutral lipids

Neutral lipids separation was carried out by thin layer chromatography in three algae studied (Fig.5 and table no. 5). In *Spirogyra neglecta*, 6 different spots were observed which were identified as esters with Rf value 100, two spots of diglyceride with Rf values 85 and 70, three spots of monoglyceride with Rf values 46, 26 and 15 respectively.

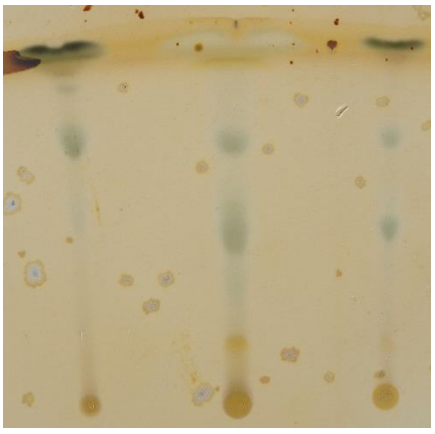


Fig. 5 Thin layer chromatogram of neutral lipids extracted from three algae studied. A-*Spirogyra neglecta,* B- *Pithophora oedogonia,* C-*Microspora indica.*

Table 5 Thin layer chromatography profile of neutral lipids of three green algae studied.

Sr. no.	Rf x100	*Spirogyra neglecta*	*Pithophora oedogonia*	*Microspora indica*
1	100	Esters	Esters	Esters
2	96	-	Triglyceride	Triglyceride
3	85	Diglyceride	Diglyceride	-
4	70	Diglyceride	Diglyceride	-
5	63	-	-	Diglyceride
6	46	Monoglyceride	Monoglyceride	Monoglyceride
7	26	Monoglyceride	Monoglyceride	Monoglyceride
8	15	Monoglyceride	Monoglyceride	Monoglyceride

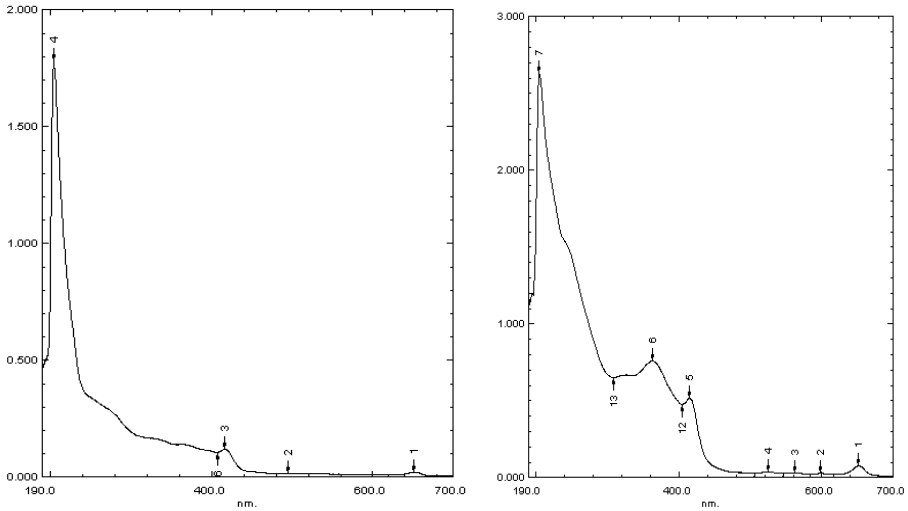
In *Pithophora oedogonia,* 7 different spots were identified as esters with Rf value 100, triglyceride with Rf value 96, two spots of diglyceride with Rf values 85 and 70, and three spots of monoglyceride with Rf values 46, 26 and 15 respectively.

In *Microspora indica,* 6 different spots were observed which were identified as esters with Rf value 100, triglyceride with Rf value 96, diglyceride with Rf value 63, three spots of monoglyceride with Rf values 46, 26 and 15 respectively.

3.3. Analysis of phenolic compounds

3.3.1.Spectrophotometric analysis of phenolic compounds

Spectrophotometric analysis of phenolic compounds of all three algae was done using spectrophotometer and the spectral scan was carried out from 190-700 nm (UV and visual range) (Fig. 6 and table no. 6). Identification of these peaks was based on spectral profile.



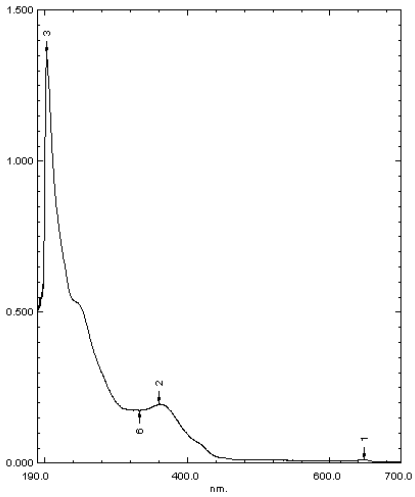


Fig. 6 Spectral analysis of phenolic compounds of three algae studied. A-*Spirogyra neglecta,* B- *Pithophora oedogonia,* C- *Microspora indica.*

Table 6 Spectral analysis of phenolic compounds of three green algae studied.

Sr. no.	Peaks	*Spirogyra neglecta*	*Pithophora oedogonia*	*Microspora indica*
1	215	Coumarin	Coumarin	Coumarin
2	230	-	Flavonol	Flavonol
3	270	-	Flavonol	Flavonol
4	350	-	Flavone	Flavone
5	410	Anthocyanin	Anthocyanin	-
6	520	Anthocyanin	-	-
7	540	-	Anthocyanin	-
8	600	-	Anthocyanin	-
9	660	Anthocyanin	Anthocyanin	Anthocyanin

In *Spirogyra neglecta*, the absorption spectra of phenolic compounds showed one peak in the UV region at 215 nm which was identified as coumarin and 3 peaks in visible region at 410, 520 & 660 nm, between 400-700 nm, which were identified as anthocyanin.

It was observed that absorption scan of *Pithophora oedogonia* showed 8 peaks in both UV and visible range. Four peaks were observed in the UV range at 215 nm, 230 nm, 270 nm and 350 nm which were identified as coumarin, 2 flavonol and flavone respectively. Peaks in the visible range were at 410 nm, 540 nm, 600 nm and 660 nm, all were identified as anthocyanins.

It was observed that absorption scan of *Microspora indica* showed 5 peaks in both UV and visible range. Four peaks were observed in the UV range and only one peak in the visible range. Peaks in the UV range were at 215 nm, 230 nm, 270 nm and 350 nm which were identified as coumarin, 2 flavonol, flavone respectively. Absorption spectra in the visible range were at 660 nm, which was identified as anthocyanin.

3.3.2. Paper chromatography analysis of phenolic compounds

The phenolic compounds of three algae were also studied using paper chromatography (Fig. 7 and table no. 7).

Individual spots were identified using Rf value & compared with standards. Tentative nature of these phenolic compounds is given in Table no. 7. Paper chromatogram for phenolic compounds of *Spirogyra neglecta* also showed 5 different spots with Rf value 97 (flavonol), Rf value 78 (coumarin), Rf value 67 (unidentified), Rf value 60 (cinnamic acid) and Rf value 25 (anthocyanin). In *Pithophora oedogonia*, 5 different spots were observed on the paper chromatogram with Rf values 97, 75, 54, 32 and 16 which were identified as flavonol, coumarin, cinnamic acid, flavone and anthocyanin respectively. In *Microspora indica* also, 5 different spots were identified with Rf values 97, 72, 54, 34 and 15 which were identified as flavonol, coumarin, cinnamic acid, flavone and anthocyanin respectively.

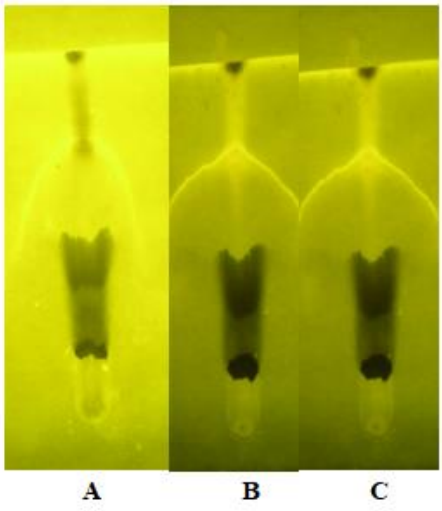


Fig. 7 Paper chromatogram of phenolic compounds extracted from three algae studied. A-*Spirogyra neglecta,* B- *Pithophora oedogonia,* C-*Microspora indica.*

Table 7a. Paper chromatography profile of phenolic compounds of *Spirogyra neglecta*.

Rf value	Colour of the spot	Colour of spot under UV	Components
97	Brownish green	Brown	Flavonol
78	Dark yellow	Yellowish brown	Coumarin
67	Grey	Black	-
60	Light yellow	Yellow	Cinnamic acid
25	Dark yellow	Black	Anthocyanin

Table 7b. brown **Paper chromatography profile of phenolic compounds of *Pithophora oedogonia*.**

Rf value	Colour of the spot	Colour of the spot under UV	Components
97	Brownish green	Brown	Flavonol
75	Light	Yellow	Coumarin
54	Light brown	Black	Cinnamic acid
32	Yellow	Black	Flavone
16	Dark yellow	Black	Anthocyanin

Table 7c. Paper chromatography profile of phenolic compounds of *Microspora indica.*

Rf value	Colour of the spot	Colour of the spot under UV	Components
97	Brownish green	Brown	Flavonol
72	Yellowish brown	Yellow	Coumarin
54	Light brown	Black	Cinnamic acid
33	Yellow	Black	Flavone
15	Dark yellow	Black	Anthocyanin

4. DISCUSSION

In the present investigation, photosynthetic pigments were isolated from fresh water algae viz. *Spirogyra neglecta, Pithophora oedogonia* and *Microspora indica* (Fig. 1, 2, 3 & Table no. 1, 2, 3). Results revealed key photosynthetic pigments as β–carotene, carotenoids, xanthophylls, chlorophyll a and chlorophyll b. Bhandari et al., (2012) observed pigments of four algal species, *Caulerpa sertulariodies, Chaetomorpha media, Enteromorpha intestinalis* and *Ulva fasciata* isolated from marine environments belonging to the chlorophyceae family. Earlier results showed similar pigment pattern to that of higher plants and thus corroborates our findings. Apart from the main pigments characteristic of higher plants, some class-specific pigments such as prasinoxanthin or siphonoxanthin or Loroxanthin in some orders of prasinophyceae and ulvophyceae have been reported in previous studies of chlorophyta members (Schagerl et al., 2003; Yoshii et al., 2004), which were not detected in this research. Secondary carotenoids such as ketocarotenoids, canthaxanthin or astaxanthin (Lorquin 1997; Fabregas 1998) have also not been encountered in the present investigation. Synthesis of secondary carotenoid is evidently induced by a variety of factors, such as depletion of nitrogen, excessive light supply and high salinity (Schagerl et al., 2003; Fabregas, 1998).

Compared to other algal groups, members of the order Siphonales (*Caulerpa*) exhibit peculiar pigment characteristics. Siphonoxanthin and siphonein are indicative of this family as is the relative abundance of α-carotene compared to β- carotene (Benson and Cobb 1981; Hegazi et al., 1998). In *Caulerpa filiformis,* the absence of siphonoxanthin and siphonein suggests that it is not a definitive character (Strain 1965). In the study conducted by Hegazi et al., (1998) chlorophyll b, micronone, microxanthin, neoxanthin, siphonein and siphonoxanthin were found to be the main characteristic pigments of *Caulerpa prolifera*. Bhandari et al., (2012) observed that the pigment composition of *Caulerpa sertulariodes* showed the occurrence of neoxanthin, lutein, violaxanthin, antheraxanthin, chlorophyll b, chlorophyll a, chlorophyllide-B and α-carotene.

Lutein and loroxanthin are the main carotenoids present in *Chaetomorpha okamurae* along with 9'-cis neoxanthin, violaxanthin, antheraxanthin and β-carotene (Yoshi et al., 2004). Shie et al., (2005) first reported the presence of zeaxanthin in *Chaetomorpha basiretorsa*. Lutein, neoxanthin, violaxanthin, antheraxanthin, chlorophyll b, chlorophyll a, chlorophyllide-B and β-carotene were the pigments detected in the sample of *Chaetomorpha media*, while loroxanthin and 9'-cis neoxanthin were absent (Bhandari et al., 2012).

Enteromorpha intestinalis showed pigments characteristic of higher plants namely, neoxanthin, violaxanthin, antheraxanthin, lutein, chlorophyll a, chlorophyll b, chlorophyllide-B, phaeophytin B, phaeophorbide B and β-carotene (Bhandari et al., 2012). Takaichi and Mimuro (1998) detected the presence of violaxanthin, lutein, chlorophyll a, chlorophyll b and β-carotene in *Ulva* and *Spirogyra*. The present study confirmed the presence of carotenoids, xanthophylls, chlorophyll b, chlorophyll a and β-carotene (Benson and Cobb 1981; Bhandari et al., 2012).

In previous studies, (Dere et al., 1998) it was observed that there were changes at the pigment level of algal species that live in an environment where light stratification is seen. The presently investigated freshwater algae (*Spirogyra, Pithophora* and *Microspora*) (Fig. 1, 2, 3 & Table no. 1, 2, 3) as a result of their characteristic habitat is

subjected to wide daily and seasonal variation in light quality and quantity. Pigment spectrum outlined above may therefore be adapted for optimum light harvesting in this environment. It is quite possible that some of the pigment such as chlorophyllide B reported could also be due to epoxidation product as a result of handling the tissue or tissue experiencing oxidative stress in nature.

Phosphoglycolipids and neutral lipids in the three green algae namely *Spirogyra neglecta, Pithophora oedogonia* and *Microspora indica* were studied (Fig. 4, 5 & Table no. 4, 5). Our study showed presence of phosphoglycolipids such as monogalactosyldiacylglycerol, digalactosyldiacylglycerol, phosphatidylglycerol, sulfoquinovosyldiacylglycerol and phosphatidylethanolamine in all three algae studied. Earlier studies showed that the fatty acid composition of three species of green algae studied possessed fatty acid composition with palmitic acid, oleic acid and linoleic acid (Bhandari et al., 2012). Palmitic acid was the most abundant fatty acid, amounting to 20% of all fatty acids. These data are in agreement with earlier conclusions that a dominance of C16 and C18 is in green algae (Janieson and Reid 1972; Aknin et al., 1992; Khotimchenko 1993). The majority of green algal species studied up to now have 16:4n-3 acid as their characteristic component (Janieson and Reid 1972; Aknin et al., 1992; Khotimchenko 1993). Only green algae from the genera *Bryopsis and Caulerpa* contain hexadecatrienoic acid as their main C16 PUFA (Aknin et al., 1992; Khotimchenko 1995; Vaskovsky et al., 1996). Different green algal species vary in the ratio of individual C18 PUFA components – linoleic, α-linolenic acid and octadecatetraenoic acids. Fatty acid compositions of twelve algal species from two different classes were determined (Heiba et al., 1997). In this study all the green algae showed the presence of seven different fatty acids such as lauric acid, myristic acid, palmitic acid, stearic, oleic, linoleic and linolenic acid. In previous studies (Bhandari et al., 2012; Khotimchenko 1993) it was reported that *Chaetomorpha linum* contained significant amounts of C16 and C18 PUFAs. Several studies on the fatty acid composition of algae have been carried out by Heiba et al., (1997); Colombo et al., (2006); Aknin et al., (1992); Khotimchenko (1991); Takagi et al., (1985).

Our results also showed presence of neutral lipids such as esters, triglyceride, diglyceride and monoglyceride in three algae studied (Fig. 5, Table no. 5). Khotimchenko (2003) reported that green algae investigated for their fatty acid composition possessed similar profiles of fatty acids. Colombo et al., (2006) reported the presence of palmitic, palmitoleic, oleic, linoleic and α-linolenic acids in *Ulva*. *Ulva pertusa* and *Ulva fenestra* contains high levels of C16 (palmitic acid) and C18 (oleic, linoleic and α-linoleic acids) (Floreto et al., 1993; Sanina et al., 2004). Work done by Ghazala and Shameel (2005) and Cojocaru (2005) on fatty acids of freshwater algae showed occurrence of palmitic, oleic, linoleic and linolenic acids in fresh water members of chlorophyceae. The results obtained during the study show that phosphoglycolpids and neutral lipids of chlorophyceae contain both saturated and unsaturated fatty acids (Bhandari et al., 2012; Fleurence 1994; Helmi 1997). The chlorophyceae comprise the most modern group and this is supported primarily by occurrence of C18 fatty acids typical of the vegetative tissue of higher plants.

Our results showed the presence of phenolic compounds in the three algal samples from fresh water algae namely *Spirogyra neglecta, Pithophora oedogonia* and *Microspora indica* (Fig. 7 & table no. 7). We have observed different types of phenolic compounds such as coumarin, flavonol, flavone, cinnamic acid and anthocyanin in three algae studied. Previous studies have been carried out on production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects *in vitro* toward hepatotoxicity model (Hanaa et al., 2009). Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects *in vitro* toward hepatotoxicity model was also observed (Hanaa et al., 2009).

UV tolerance of some green phytoplankton species was attributed to the cell wall biopolymer phenolic compound sporopollenin (Xiong et al., 1997; Pavia et al., 1997). In tropical algae, enhanced levels of UV-absorbing compounds were detected in tissues from the canopy compared to tissues from understory locations in turf-forming Rhodophytesm (Beach and Smith, 1996). Furthermore, the wide ranges of biological activities associated with micro algae-derived phytoconstituents has the potential to expand their health benefits in food and pharmaceutical industries as an alternative form of synthetic products that can contribute to the well-being of consumers.

The present study highlights the presences and diversity of photosynthetic pigments in *Spirogyra neglecta, Pithophora oedogonia* and *Microspora indica* found similar to that in higher plants. Key pigments like chlorophyll a, chlorophyll b, carotenoids, xanthophylls, β-carotene and lutein were found commonly in all three algae. Besides, there were marked presence of phosphoglycolipids and neutral lipids along with occurrence of varied phenolic compounds which may likely possess antioxidant and biological benefits and thus may be further investigated for micro-algae

chemical ecology to better comprehend the functions of these algae in ecosystems and possible therapeutic benefits to mankind.

REFERENCES

Aknin, M., Moellet-Nzaou, R., Cisse, E., Kornprobst, J. M., Gaydoudou, E. M, Samb, A., and Miralles, J. 1992. Fatty acid composition of twelve species of chlorophyceae from the Snegalese coast. *Phytochem.* **31**: 2739-2741.

Beach, K. S. and Smith, C. M. 1996. Ecophysiology of tropical rhodophytes. I. Microscale acclimation in pigmentation. *J Phycol*. **32**: 701–710.

Benson, H. and Cobb, A. H., 1981. The separation, identification and quantitative determination of photopigments from the siphonaceous marine alga *Codium fragile*. *New phytol*. **88**: 627-632.

Bhandari, R., Talvar, S., Sadanandan, S., and Sharma, P. K. 2012. Photosynthetic pigments and fatty acid composition of four marine green algae from the coastal zones of Goa. *Ind Hydrobiol.* **14**: 181-191.

Boyd, B. 2003. Introduction to algae. *Aimee Durrant.*

Cojocaru, M. 2005. Gas chromatographic/mass spectrometric analysis of fatty acids found in aquatic algae. *Biol. Mass Spectro*. **16**: 477- 480.

Colombo, M. L., Rise, P., Giavarini, F., De Angelis, Galli, C., and Bolis, C.L. 2006. Marine macroalgae as sources of polyunsaturated fatty acids. *Plt Foods for Human Nutri*. **61**: 67-72 .

Dere, S., Gunes, T., and Sivaci, R. 1998. Spectrophotometric determination of chlorophyll - A, B and total carotenoid contents of some algae species using different solvents. *Trop. J of Bot.* 22: 13-17.

Fabregas, J. 1998. Induction of astaxanthin by nitrogen and magnesium deficiencies in *Haematococcus pluvialis*. Biotech. Lett. **20:** 623-626

Fleurance, J., and Kaas, R. 1995. Les algues marines: une source meconnue de proteines vegetales. *Equinox*. **56:** 12–17.

Fleurence, J. 1994. Fatty acids from 11 marine macroalge of the French Brittany coast. *J of Appl. Phycol.* **6 :** 527-532.

Floreto, E.A., Hirata, H., Yamasaki, S., and Castro, S. C. 1993. Effect of salinity on the growth and fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyata). *Bot. Mar.* **37:** 151-156.

Ghazala, B. and Shameel, M. 2005. Phytochemistry and bioactivity of some freshwater green algae from Pakistan. *Pharmaceutical Biol.* **43**: 358-369.

Hanaa, H., Abd, El-B., Farouk, K. Baz, E. and El-Baroty, G. S. 2009. Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects. *Afr J of Biotech*. **8:** 7059-7067.

Hegazi, M. M., Pérez-Ruzafa, A., Almela, L. and Candela, M. E. 1998. Separation and identification of chlorophylls and carotenoids from *Caulerpa prolifera*, *Jania rubens* and *Padina pavonica* by reversed-phase high-performance liquid chromatography. *J of Chromat*. **829:** 153-159.

Heiba, H.I., Al-Easa, H.S., Rizk, A. F. M. 1997. Fatty acid composition of twelve algae from the coastal zones of Qatar. *Plt Foods for Human Nutri*. **51**: 27-34.

Heiba H. I., Al-Nagdy, S. A., Rizk, A. M. and Durgham, M. M. 1993. The amino acid composition of some common marine algae from Qatar (Arabian Gulf). *Qatar Uni Sci J*. **13:** 219-225.

Helmi, H. 1997. Fatty acid composition of 12 algae from the coastal zones of Qatar. *Plt foods for Human Nutr.* **51**: 27-34.

Jamieson, G. R. and Reid, E. H. 1972. The component fatty acids of some algal lipids. *Phytochem.* **11**: 1423-1432.

Khotimchenko, S. V. 1991. Fatty acid composition of seven *Sargassum* species. *Phytochem.* **30:** 2639-2641.

Khotimchenko, S. V. 1993. Fatty acids of the genus *Codium*. *Bot Mar.* **46:** 456-460.

Khotimchenko, S. V. 1995. Fatty acid composition of green algae of the genus *Caulerpa*. *Bot Mar* **38**:509–512.

Khotimchenko, S. V. 2003. Fatty acids, of species in the genus *Codium*. *Bot. Mar.* **46**:456-460.

Liljenberg, C. and Kates, M. 1985. Changes in lipid composition of Oat root membranes as a function of water-deficit stress. *Can J Biochem Cell Biol*. **63**: 77-84.

Liljenberg, C. and Von Arnold, S. 1987. Effects of physiological and ontogenetical ageing on membrane lipid levels in pea leaves (*Pisum sativum*). *J Plant Physiol.* **130:** 497-509.

Lorquin, J., Molouba, F. and Dreyfus, B. L. 1997. Identification of the carotenoid pigment canthaxanthin from photosynthetic *Bradyrhizobium* Strains. *Appl Environ Microbiol*. **63:** 1151-1154.

Naidu, K. A., Tiwari, A., Joshi, H. V., Viswanath, S., Ramesh, H. P. and Rao, S. V. 1993. Evaluation of nutritional quality and food safety of seaweeds of India. *J Food Saf*. **13:** 77-90.

Pavia, H., Cervin, G., Lindgren, A. and Aberg, P. 1997. Effects of UV-B radiation and simulated herbivory on phorotannins in the brown alga *Ascophyllum nodosum*. *Mar Ecol Prog Ser.* **157**:139-146.

Ramazanov, A. and Ramazanov, Z. 2006. Isolation and characterization of a starchless mutant of *Chlorella pyrenoidosa* STL-PI with a high growth rate, and high protein and polyunsaturated fatty acid content. *Phycol Res*. **54**: 255-259.

Ravikumar, S., Inbaneson, S. J., Suganthi, P., Ramasamy, G. and Venkatesan, M. 2010. In vitro antiplasmodial activity of ethanolic extracts of seaweed macroalgae against *Plasmodium falciparum*. *Parasitol Res.* **10**: 2185-2193.

Sanina, N. M., Goncharova, S. N. and Kostetsky, E. Y. 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. *Phytochem.* **65**: 721-730.

Sankhalker, S. 2000. Photoinhibition of photosynthesis and possible role of xanthophyll cycle in protection against photodamage in sorghum seedlings. Ph. D. thesis, Goa University, Goa.

Schagerl, M., Pichler, C. and Donabaum, K. 2003. Patterns of major photosynthetic pigments in freshwater algae, Dinophyta, Euglenophyta, Chlorophyceae and Charales. *Ann Limnol Int J Lim*. **39**: 49-62.

Sharma, P. K. and Hall, D.O. 1996. Effect of photoinhibition and temperature on carotenoids in young seedlings and older sorghum leaves. *Ind J Biochem Biophys.* **33**: 471-477.

Sharma, P. K., Anand, P., Sankhalkar, S. and Shetye, R. 1998. Photochemical and biochemical changes in wheat seedlings exposed to supplementary UV-B radiation. *Plant Sci*. **132**: 21-30.

Shie, D. Y., Han, L. J., Sun, J., Yang, Y. C., Shi, J. G. and Fan, X. 2005. Studies on chemical constitutes of green alga *Chaetomorpha basiretorsa* and their bioactivity. *Zhongguo Zhong Yao Za Zhi.* **30**:1162-1165.

Srivastava, A., Villalobos, M. B. and Singh, R. K. 2020. Engineering Photosynthetic Microbes for Sustainable Bioenergy Production. In *Contemporary Environmental Issues and Challenges in Era of Climate Change*. Springer, Singapore. pp. 183-198.

Strain, A. 1965. Chloroplast pigments and the classification of some siphonalean green algae of Australia. *Biol Bull*. **129**: 366-370.

Takagi, T., Asahi, M. and Itabashi, Y. 1985. Fatty acid composition of twelve algae from Japanese waters. *Yukagaki.* **34**: 1008-1012.

Takaichi, S. and Mimuro, M. 1998. Distribution and geometric isomerism of neoxanthin in oxygenic phototrophs: 9'-cis, a sole molecular form. *Plt Cell Physiol.* **39:** 968–977.

Turnham, E. and Northcote, D. H. 1984. The incorporation of (1-14C) acetate in to lipids during embryogenesis in oil palm tissue cultures. *Phytochem.* **23**: 35-39.

Vaskovsky, V. E., Khotimchenko, S. V., Xia, B. and Hefang, L. 1996. Polar lipids and fatty acids of some marine macrophytes from the yellow sea. *Phytochem.* **42**: 1347–1356.

Xiong, F., Komenda, J., Kopecky, J. and Nedbal, L. 1997. Strategies of ultraviolet-B protection in microscopic algae. *Physiol Plant.* **100**:378-388.

Yoshii,Y., Hanyuda, T., Wakana, I., Miyaji, K., Arai, S., Ueda, K. and Inouye, I. 2004. Carotenoid compositions of *Cladophora balls* (*Aegagropila linnaei*) and some members of the cladophorales (ulvophyceae, chlorophyta): their taxonomic and evolutionary implication. *J of Phycol*. **40**: 1170-1177.