

Research Article

Comparative Study on the Antioxidant Potency of Some Marine Micro-algal Strains

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Abstract

The daily uptake of food, supplemented with antioxidants shows several health benefits and are advised to take regularly in order to lead a healthy life as well as to avoid so many diseases. However, few natural antioxidants are also generated in human body, but the imbalance between natural antioxidants and free radicals create many health hazards. Hence, synthetic antioxidants can be utilized as health promoters to stop oxidative stress brought on by reactive oxygen species. But the adverse effects of synthetic antioxidants appeal for new natural source of antioxidants. In this regard, microalgae are proved to be the potential source of natural antioxidants. Therefore, an attempt has been taken to study the comparative antioxidant activity of *Chlorella* sp. JD-2016, *Oscillatoria* sp. (MK636802) and *Desertifilum dzianense* (MK634687) isolated from Chilika Lake. Antioxidant activity of the crude extracts from the aforementioned species were conducted by quantifying the total antioxidant activity, DPPH assay, total phenol and total flavonoid content. All the pigments except carotenoids were high in chloroform extract of *Desertifilum dzianense*, while carotenoid was found to be more in *Oscillatoria* sp. The reverse results were observed in methanol extract of the algal isolates. Total antioxidant activity was significantly high in the methanolic extract of the three algae in comparison to the chloroform extract. Maximum DPPH radical scavenging activity was observed in both chloroform and methanol extract of *Desertifilum dzianense*. Phenol content was found to be more in methanol extract of *Oscillatoria* sp. However, methanol extract of *Chlorella* sp. depicts the highest amount of flavonoid content in comparison to other species.

Keywords

Antioxidants, Free Radicals, Micro-algae, Pigments, ROS

1. Introduction

Microalgae are the rich source for various bioactive compounds of commercial interest generated from primary and secondary metabolism [1-3]. Antioxidants are extracted from microalgae are of various types i.e. fat-soluble components

like carotenoids and vitamin E and water-soluble components like polyphenols, vitamins, phycobiliproteins, etc. [4-6].

Production of ROS can be promoted endogenously in cy-

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tochrome P₄₅₀, mitochondria, peroxisomes and by inflammatory cell activation as a consequence of both enzymatic & non-enzymatic reactions. Mitochondria serve as a major site of ROS generation [7]. The generation of superoxide within mitochondria is approximately 2–3nmol/min per mg of protein [8]. In order to detoxify these ROS molecules, mitochondria contain several antioxidants on both sides of their membrane [9, 10].

ROS play several beneficial roles, including phagocytosis, involving apoptosis and necrosis to regulate cell growth and intercellular signaling, or synthesis of biologically significant compounds [11, 12]. But when the intracellular concentration of ROS becomes more, they oxidize cellular macro or micro components causing membrane damages like lipid peroxidation, reduced membrane fluidity as well as DNA mutation [13]. In healthy cells, degenerative effects of ROS are balanced by antioxidant molecule present in the body naturally. But under adverse condition, the antioxidant defense system of a normal human body fails to encounter ROS molecule, due to imbalance between production of free radicals and their detoxification by antioxidants and ultimately, induces oxidative stress within the cell [14]. Generally, oxidative stress plays a major role in development of various diseases like neuro-degenerative disease, cardiovascular disease, premature aging, cancer, atherosclerosis, and respiratory ailments [15, 16].

Unfortunately, antioxidants naturally synthesized by the human body are not enough to neutralize all the free radicals. Therefore, a consistent intake of extra antioxidants should be the part of everyone's daily diet in order to address oxidative stress and relative damages [17, 18]. Thus, use of natural antioxidants from plants and microalgae draw much attention of researchers as an alternative to synthetic antioxidants. Intakes of these natural antioxidants reduce the risk of cancer, cardiovascular diseases, other diseases and show higher biological activity of medicinal value [19, 20].

Algal variety is abundant in Chilka, the biggest brackish water lagoon in Asia (19°28' and 19°54' N latitude and 85°06' and 85°35' E longitude) [21]. Besides that, the seasonal fluctuations of the physico- chemical properties of water also encourages the changes in its algal diversity [22, 23]. Although many algal forms are reported from Chilka but works including their antioxidants effect is poorly documented [24]. Hence, here an attempt has been taken to compare the antioxidant potency of some microalgae isolated from Chilka Lake.

2. Materials and Methods

2.1. Selection Of Study Sites and Collection of the Samples

The water and soil samples were aseptically collected from Balugaon of Chilika Lake and were kept in sterile zipper bag and taken to the laboratory for further studies. At the site of

sampling the salinity, water temperature and pH were recorded using a salinity meter, thermometer and pH meter respectively. A green alga *Chlorella* sp. JD-2016 was collected previously from Chilika Lake and identified by the Department of Microbiology, OUAT, Bhubaneswar which was also used for this study.

2.2. Isolation, Identification and Maintenance of a Pure Culture of the Algae

Soil samples were submerged in distilled water and then cleaned thoroughly with tap water then by distilled water to get rid of debris and then observed under microscope. Water samples were serially diluted and used for isolation. To isolate the organisms, BG-11, modified Chu-10 and F/2 medium and solid medium using the above said media were used [25]. Repeated culture and sub culture practice was conducted to obtain the pure culture of two algae (S1 and S2). To maintain the pure culture of isolates, *Chlorella* sp. was grown in f/2 media. S-1 and S-2 were maintained in BG11 medium. Isolates were observed under Phase Contrast microscope (Leica DM 3000) for their preliminary morphological identification. They are incubated at 28 ± 2°C and 2400lux light intensity under laboratory condition [25]. Growth was determined using the dry weight method [26].

2.3. Photosynthetic Pigment Estimation

The pigment concentration (Chl-*a*, Chl-*b* and carotenoids) of *Chlorella* sp., S1 and S2 extracted in methanol and chloroform was determined following the method described by El-Tablawy et al. 2020 with slight modifications in the method applied [27]. Phycobiliprotein concentrations of the isolates S-1 and S-2 in the solvents (methanol and chloroform) determined by following the methods mentioned by Bounnit et al. 2022 and Shawer et al., 2023 using spectrophotometer (Perkin-Elmer; LAMBDA 365) [28, 29].

$$\text{Chl}_a = 16.29 A_{665} - 8.54 A_{652} \quad (1)$$

$$\text{Chl}_b = 30.66 A_{652} - 13.58 A_{665} \quad (2)$$

$$\text{Carotenoid} = (1000 A_{470} - 1.63 \text{Chl}_a - 104.9 \text{Chl}_b) / 221 \quad (3)$$

$$\text{Allophycocyanin (APC)} = (A_{652} - 0.208 (A_{615})) / 5.09 \text{ mg/ml} \quad (4)$$

$$\text{Phycocyanin (PC)} = (A_{615} - 0.474 (A_{652})) / 5.34 \text{ mg/ml} \quad (5)$$

$$\text{PE} = (A_{562} - 2.41 (\text{PC}) - 0.849 (\text{APC})) / 9.62 \text{ mg/ml} \quad (6)$$

2.4. Preparation of Algal Extracts for Measurement of Antioxidant Activity

Samples were harvested at their log phase of growth and

filter dried. A specified amount of sample was weighed, wrapped with a filter paper and dipped inside various organic solvents i.e. methanol and chloroform in the dark. Then the extracts were evaporated, putting them in water bath at 37°C for 6 to 8 hours. After complete evaporation, the dried extract was weighed and stored at -4°C till further use [30].

2.5. DPPH Radical Scavenging Activity

The antioxidant activity through DPPH radical scavenging was determined [31]. The three organisms' methanol and chloroform extracts were diluted in distilled water to achieve concentrations of 100-500 µg/ml. Each 2ml of extract combined with 1ml of DPPH solution (0.2mM in methanol) and mixed thoroughly. This mixture was then incubated in dark for 40 minutes at 20 °C. The absorbance was read at 517nm against methanol as a blank. The experiment was replicated for thrice. The crude extract is not present in the blank solution.

Results were converted in to % antioxidant activity using following equation.

$$\text{Scavenging effect (\%)} = [1 - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) / \text{Abs}_{\text{control}}] \times 100 \quad (7)$$

The percentage of scavenging activity obtained from equation were plotted against ascorbic acid standard concentrations. A linear regression ($R^2 = 0.989$) was used to calculate the scavenging capacity of the samples which is expressed in terms of mg ascorbic acid equivalents AAE per 100g sample.

2.6. Estimation of Total Antioxidant Activity

The crude extracts' total antioxidant activity (TAA) was calculated [32]. Briefly, 3.0mL reagent solution; 0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) were combined with 0.3mL of sample solution; 0.1mg/mL and incubated at 95 °C for 90min using a water bath. At 695 nm, the absorbance of each sample combination was measured. Ascorbic acid equivalents per gram are used to express total antioxidant activity.

2.7. Estimation of Total Phenolic Content

The total phenolic content was determined using the technique described by Jerez-Martel et al. (2017) using various solvents [33]. For this, 0.5ml of each extract was diluted to 3ml with distilled water before being combined with 0.5ml of Folin-Ciocalteus reagent. After 5 minutes, 2ml of 2% Na_2CO_3 solution was added to the solution and stirred thoroughly. Absorbance was taken at 650nm after being held at 30 °C for 60 minutes in the dark. The phenolic content is reported as Gallic acid equivalent per gram.

2.8. Determination of Total Flavonoid Content

Total flavonoid content was determined by spectrometric technique [34]. An appropriate amount of extract (0.5ml) was

taken and diluted up to 1ml with methanol. A 0.4ml volume of distilled water was added to it followed by 0.3ml of a 5% NaNO_2 solution and left for 5 minutes. Then, 0.3ml of 10% AlCl_3 solution was added into it and settle down for 6minutes. Again, 2ml of 1M NaOH solution was added to this mixture and the final volume was made up to 10ml with distilled water. Then the reaction mixture was well mixed and left for 15 minutes. Absorbance of the reaction mixture was read at 510 nm and the concentration of total flavonoids was calculated as mg of Quercetin equivalents per gram of dry weight.

2.9. Statistical Analysis

Data analysis was done using the Statistical Analysis System software. Analyses of variance were performed using ANOVA as mean $\pm$ standard deviation (SD).

3. Results

In order to compare the antioxidants potential of marine microalgae from Chilika Lake, two microalgae S1 and S2 were collected from Balugaon of Chilka Lake between the months of January to March 2016 for this study (Figure 1 and Figure 2). Apart from this, *Chlorella* sp. (*Chlorella* sp. JD-2016) was revived which was already available in the Dept. of Microbiology, OUAT, previously isolated from Chilka Lake (Figure 3).

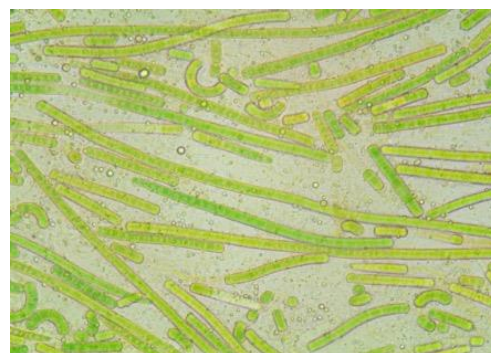


Figure 1. S-1. *Oscillatoria* sp.



Figure 2. S-2. *Desertiifilum dzianense*.

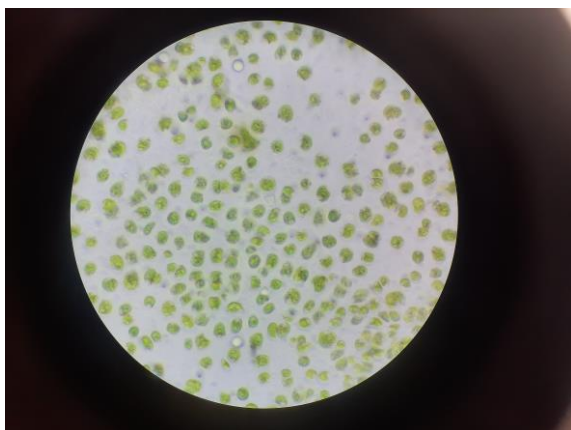


Figure 3. S-3. *Chlorella* sp. JD-2016.

3.1. Identification of the algal isolates

The microscopic observations revealed that S-1 isolate was a filamentous alga belonging to the genus *Oscillatoria* sp. (MK636802) and S-2 isolate was *Desertifilum dzianense* (MK634687). The algae were grown in different medium and it was observed that the growth of the *Chlorella* sp. was more in f/2 medium, while *Oscillatoria* sp. and *Desertifilum dzianense* showed luxuriant growth in BG11 medium. The microscopic pictures of all the strains are given in Figures 1, 2 and 3. The FASTA sequence of both the organisms are given below:

>MK636802.1 *Oscillatoria* sp. S1 16S ribosomal RNA gene, partial sequence

```
CTCCCGGGGGGGGGGGGGGGGAGACGCGTGAGAA
TCTGTTTTCTAGGATGGGGATAACGACTGGAAACGA
GCTCGTTAATACCCGATGTGCCGAGAGGTGAAAGA
TTGTATCGCCTGAAGATGAGCTCGCGTCCGATTAGC
TAGTTGGTAGGGTAAGAGCCTACCAAGGCGACGAT
CGGTAAGTGGTCTGAGAGGATGACCAGTCACACTG
GGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATTTTCCGCAATGGGCGCAAGCCTG
ACGGAGCAAGACCGCGTGGGGGAAGAAGGTTTGTG
ATTGTAAACCCCTTTTATCAGGGAAGAAGCTCTGAC
GGTACCTGATGAATCAGCCTCGGCTAACTCCGTGCC
AGCAGCCGCGGTAATACGGAGGGGGCAAGCGTTAT
CCCGAAATTTTGGGCGTAAAGCTTCCCAGGGTGG
CTATACGTGTCAGTTGTTAAAGAATGGGGCTTAAC
CCATATGGGCGATTGAAACTGTATAGCTAGAGAGT
GGTAGGGGCAAGGGGAATTTCCAGTGTAGCGGTGA
AATGCGTAGATATTTGGGAAGAACACCAGGCTGGC
GAAAGCGCTCTTGCTGGGCCATCACTGACGCTGAG
AGACGAAAGCTATGAAGCGTTGGATAATCTCCTCTT
TTGTAACACCACC
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>MK634687.1 *Desertifilum dzianense* S2 16S ribosomal RNA gene, partial sequence

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CTACCTTCAGGTCGGGGATAACCCCTGGAAACGG
GGGCTAATACCGGATAGGTGCGAAAGATGAAAGGTT
AACTGCCTGAAGAGGAGCTCGCGTCTGATTAGCTA
GTAGGTGTGGTAAGAGCGCACCTAGGCGACGATCA
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GTAGCTGGTCTGAGAGGATGAGCAGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATTTTCCGCAATGGGCGAAAGCCTGAC-
GGAGCAAGACCGCGTGGGGGAGGAAGGCCTTTGGG
TTGTAAACCCCTTTTGTAGGGAAGAAAAAATGAC
GGTACCTAACGAATCAGCCTCGGCTAACTCCGTGCC
AGCAGCCGCGGTAATACGGAGGAGGCAAGCGTTAT
CCGGAATTATTGGGCGTAAAGCGTCCGTAGGTGGC
CCGGCAAGTCTGCTGTCAAAGACCACAGCTCAACT
GTGGAAAGGCAGTGGAAACTGCAAGGCTAGAGTGC
GGTAGGGGTAGAGGGAATTTCCAGTGTAGCGGTGA
AATGCGTAGAGATTGGGAAGAACACCGGTGGCGAA
AGCGCTCTACTGGGCCGCAACTGACACTGAATGGA
CGAAAGCTAGGGGAGCGAATGGGATTAGATACCCC
A
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3.2. Characterization of Growth

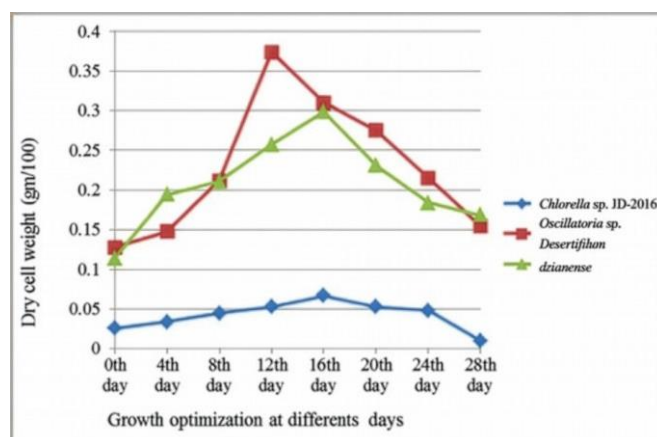


Figure 4. Growth curve of the organisms as calculated from dry cell weight (DCW).

The growth was studied by culturing the three organisms under adequate light, optimum growth media and temperature. In each four-day interval the dry weight of all three organisms was taken. The initial dry weight of the *Chlorella* sp., *Oscillatoria* sp. and *Desertifilum dzianense* were found to be 0.01, 0.128 and 0.114g/100ml, respectively. From the growth curve (Figure 4), it was noticed that *Oscillatoria* sp. grows rapidly showing maximum growth on 12th day after which growth was retarded. Similarly, the exponential growth of the *Chlorella* sp. continued till 14 days and then slowed down. But in case of *Desertifilum dzianense*, the log phase continued till 17 days and then gradually decreased.

3.3. Analysis of Photosynthetic Pigments

Table 1 indicates the number of pigments present in the methanol and chloroform extracts of *Chlorella* sp., *Oscillatoria* sp. and *Desertifilum dzianense* at their early stationary phase of growth. In both methanol and chloroform extracts of

the organisms, their chlorophyll, carotenoid, allophycocyanin, phycocyanin and phycoerythrin content were calculated. The maximum amount of chlorophyll (15.081mg/l) was observed in the chloroform extract of the *Desertifilum dzianense* and the minimum (14.253mg/l) in *Chlorella* sp. The carotenoid content was found to be maximum (3.061mg/l) in *Oscillatoria* sp. and minimum in *Chlorella* sp. The allophycocyanin and phycocyanin content were high in *Desertifilum dzianense*, followed by *Oscillatoria* sp. The phycoerythrin was more in *Oscillatoria* sp. In the case of the methanolic extracts of the algae, a high chlorophyll pigment was found in *Chlorella* sp. and low (6.221mg/l) in *Desertifilum dzianense*. The maximum amount of carotenoid was observed in *Desertifilum dzianense*, while *Oscillatoria* sp. exhibited the minimum number of carotenoids. The allophycocyanin content and phycoerythrin were more in *Oscillatoria* sp. in comparison to *Desertifilum dzianense*.

3.4. Total Antioxidant Activity

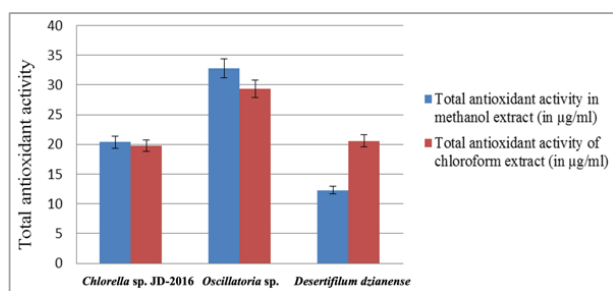


Figure 5. Comparative study of the total antioxidant activity of methanol and chloroform extracts of *Chlorella* sp., *Oscillatoria* sp. and *Desertifilum dzianense*.

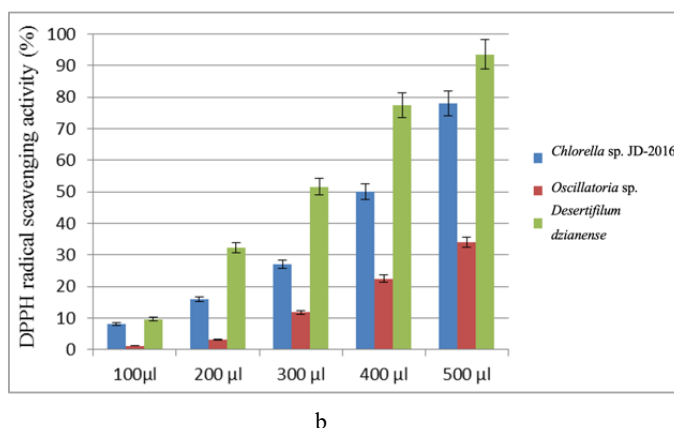
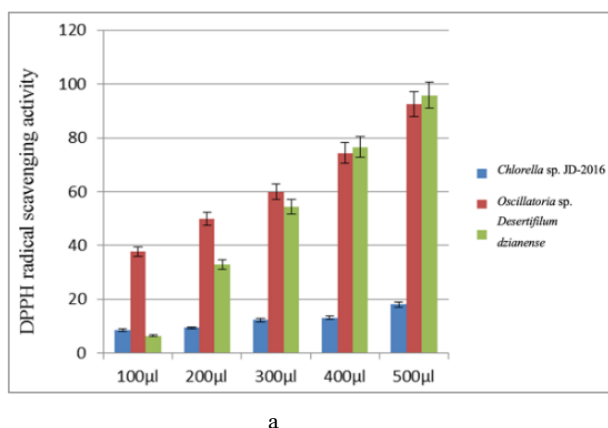


Figure 6. a. DPPH free radical scavenging activity of the chloroform extract; b. DPPH freed radical scavenging activity of the methanol extract.

Figure 5 depicts the total antioxidant activity of different solvent extracts of all the strains. The highest antioxidant activity was observed in methanol extract of *Oscillatoria* sp. (0.720 ± 0.1414 µg/g ascorbic acid equivalent). The lowest activity was noticed in the chloroform extract of *Chlorella* sp. (0.073 ± 0.017 µg/g ascorbic acid equivalent).

3.5. DPPH Free Radical Scavenging Activity

The reaction capability or the scavenging percentage of the Reactive oxygen species “DPPH free radical” was determined by the decrease in its absorbance at 517nm i.e. induced by antioxidants. DPPH radical scavenging assay at 100-500 µg/ml concentration of chloroform extracts of *Chlorella* sp., *Oscillatoria* sp. and *Desertifilum dzianense* was found to be 8.49-18.12%, 37.7-92.4% and 6.38-95.7%, respectively (**Figure 6**). The extracts exhibited concentration dependent radical scavenging activity, i.e. higher the concentration of the extract higher was the scavenging activity. Here ascorbic acid was taken as the standard. *Desertifilum dzianense* showed the highest percentage of antioxidant activity (95.74%) against DPPH, whereas in *Chlorella* sp., it was the lowest (18.12%) at 500 µg/ml chloroform extract. But surprisingly, at the lower chloroform extract concentration, i.e., 100 µg/ml, *Oscillatoria* sp. showed a higher percentage of antioxidant activity (37.7%) than all other organisms. Similar trend was found in the methanolic extract of all the organisms (**Figure 6**). *Desertifilum dzianense* showed the highest percentage of antioxidant activity, i.e. 93.54% at 500 µg/ml concentration and 9.67% at 100 µg/ml concentration. *Oscillatoria* sp. indicated the least antioxidant activity among all.

3.6. Total Phenolic Content

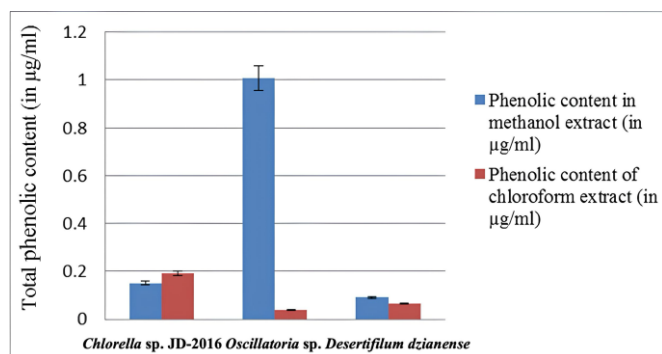


Figure 7. Phenolic content of the isolates in methanol and chloroform extract.

The methanol extract of *Oscillatoria sp.* exhibited the highest phenolic content, i.e. 0.7925 ± 0.0415 µg/ml (Figure 7). The

lowest amount of phenolic content was observed in the methanol (0.042 ± 0.0016 µg/ml) and chloroform extracts (0.055 ± 0.01 µg/ml) of *Desertifilum dzianense*. The phenolic content was 0.1623 ± 0.0023 µg/ml in the methanol extract and 0.1933 ± 0.018 µg/ml in the chloroform extract of *Chlorella sp.* (Figure 7).

3.7. Total Flavonoid Content

Figure 8 indicates the flavonoid content of the three algal isolates. The highest amount of flavonoid, i.e. 0.1503 ± 0.0154 µg/ml, was obtained in the methanol extract and 0.0546 ± 0.0056 µg/ml in the chloroform extract of *Oscillatoria sp.* On the other hand, 0.0825 ± 0.0047 µg/ml flavonoids in the methanol extract and 0.0025 ± 0.0005 µg/ml in the chloroform extract of *Chlorella sp.* were observed. However, in *Desertifilum dzianense*, the flavonoid content in methanol and chloroform extract was found to be 0.0115 ± 0.0015 and 0.0045 ± 0.0005 µg/ml, respectively.

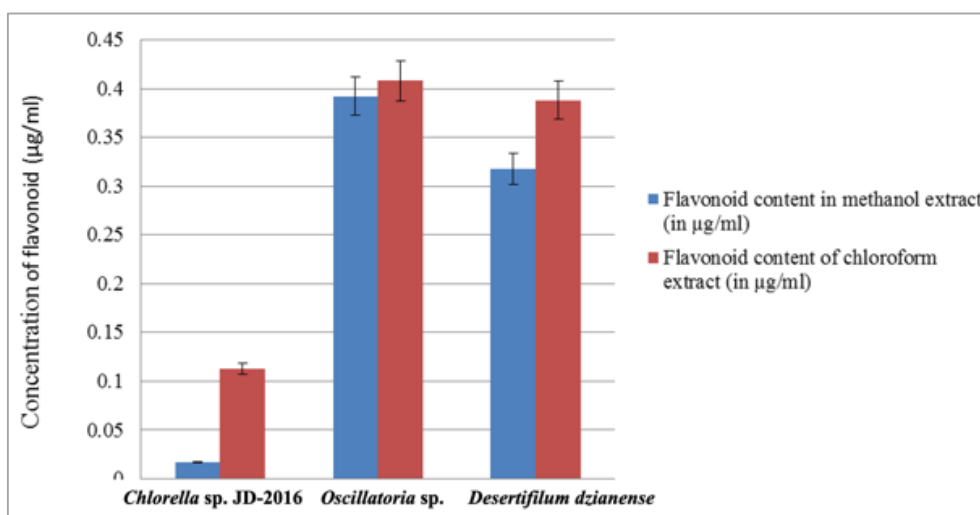


Figure 8. Flavonoid content of the isolates in methanol and chloroform extract.

3.8. Estimation of Photosynthetic Pigments

Quantitative estimation of photosynthetic pigments in chloroform and methanol extract of *Chlorella sp.*, *Oscillatoria sp.* and *Desertifilum dzianense* revealed significant variation when compared between varieties of species and extraction solvents given in the Table 1.

Chlorophyll a was the dominant pigment in all the organisms. In chloroform extracts, the highest concentration was recorded in *Desertifilum dzianense* (15.081mg/ml), followed by *Chlorella sp.* (14.253mg/ml) and *Oscillatoria sp.* (13.476mg/ml). Similarly, Chlorophyll b content was varied

in all the different species while the highest content was estimated in methanol extract of *Oscillatoria sp.* i.e. 6.331mg/ml. Carotenoid content was notably observed high in the chloroform extract of *Oscillatoria sp.* (3.061mg/ml).

In addition to this, phycobiliproteins were also detected in all species, but were relatively high in cyanobacteria (*Oscillatoria sp.* and *Desertifilum dzianense*). The highest phycocyanin (0.102mg/ml) and phycoerythrin (0.018mg/ml) were accumulated in chloroform extracts of *Desertifilum dzianense* and *Oscillatoria sp.*, respectively. On this contrary, methanol extract of allophycocyanin showed a slightly improved amount in *Oscillatoria sp.* (0.106mg/ml).

Table 1. Quantification of pigments extracted through chloroform and methanol.

Pigments	Chloroform Extract			Methanol Extract		
	<i>Chlorella</i> sp.	<i>Oscillatoria</i> sp.	<i>Desertifilum dzianense</i>	<i>Chlorella</i> sp.	<i>Oscillatoria</i> sp.	<i>Desertifilum dzianense</i>
Chlorophyll a	14.253	13.476	15.081	10.933	10.294	6.221
Chlorophyll b	0.241	2.928	2.883	0.844	6.331	1.772
Carotenoid	0.101	3.061	0.33	0.388	2.494	2.828
Allophycocyanin	0.002	0.05	0.097	0.011	0.106	0.05
Phycocyanin	0.003	0.063	0.102	0.001	0.008	0.004
Phycoerythrin	0.001	0.018	0.012	0.003	0.01	0.001

4. Discussion

Two algal species collected from Chilika Lake and were identified by following the Monograph of Desikachary (1959) [35]. *Chlorella* sp. (*Chlorella* sp. JD-2016) previously isolated from Chilka was also collected from Dept. of Microbiology, OUAT for the study. The antioxidant potency of these three algal isolates was studied.

The growth was characterised on dry biomass basis. Maximum growth was observed in 12days, 14days and 17days in *Oscillatoria* sp., *Chlorella* sp. and *Desertifilum dzianense*, respectively. The growth was gradually reduced. The results obtained in the present study were corroborated with findings of Sharma et al. (2012) [36]. Dry biomass (15.2mg/50ml) of *Chlorella vulgaris* at 25-30 °C and natural daylight conditions was observed during second to third week. The pigment profile of the isolates using chloroform as organic solvent indicates all the pigments except carotenoid were high in *Desertifilum dzianense* while carotenoid was found to be higher in *Oscillatoria* sp. Significantly, reverse observations were noticed in the methanol extract of the three algal isolates. The findings were in agreement with other workers. According to Lall and John (2017), *Anabaena* sp., *Nostoc* sp., *Microcystis* sp. and *Desertifilum dzianense* were used in the extraction of pigments, where methanol, ethanol and acetone were used as extraction solvents but methanol was found to be the most suitable one [37]. To examine the antioxidant potency of the algae total antioxidant activity, DPPH free radical scavenging assay and total phenolics and flavonoid contents were carried out. The total antioxidant activity of these three algae was more in methanol extract in comparison to the chloroform extract. Significant variation was found in the values which may be due to the polarity of the different kinds of solvents used by El-Aty et al. 2014 [38]. The total antioxidant activity was studied earlier by Sharathchandra et al. (2013) and found to be

highest in the methanol extract of *Lyngbya limnetica* in comparison with three other cyanobacteria [39]. The findings of Hemalatha et al. (2013) suggested that the TAC in the methanol extract of *Chlorella salina* is 1.5 ± 0.05 mg/g ascorbic acid equivalent, which also supports the resulted values [40].

DPPH free radical decaying activity of chloroform and methanol extracts of all the three algal species was raised with increasing the amount of extract from 100 to 500 µg/ml. Maximum amount of scavenging activity was observed in the chloroform extractive of *Desertifilum dzianense*, followed by *Oscillatoria* sp. and *Chlorella* sp. However, DPPH radical scavenging activity in the methanol extract was found to be highest in *Desertifilum dzianense* and lowest in *Oscillatoria* sp. Rai et al. 2015 reported maximum DPPH radical scavenging activity in the methanol extract of *Oscillatoria freymii* than the other strains, i.e. *O. geminata*, *Lyngbya confervoides*, *O. sancta*, *Nostoc commune*, *Phormidium corium*, *P. tenue* and *Spirulina major* [41]. There is a significant difference in the phenolic content of the algal isolates. It was found to be highest in the methanol extract of *Oscillatoria* sp. On this contrary, *Chlorella* sp. depicts the highest amount of flavonoid content in the methanol extract in comparison to the other organisms studied. El-Aty et al. 2014 studied that among the three different solvents methanol, acetone and water, the highest phenolic content was found in methanol extract (20.91 ± 0.21 mg/g GAE) of *Oscillatoria agardhii* as compared to other aqueous extract [38]. Such variations can be attributed to the type of phenolic; the polarity of the solvents and flavonoids present in each species [40]. From our observation, methanol was found to be the best solvent for the extraction of total phenolics and total flavonoids. Manivannan et al. (2012) also stated that the methanol extract of *Chlorella marina* showed higher activity against diethyl ether and hexane extracts, which is due to the differences in the polarity of the solvents used [42].

Our study revealed that chloroform was more efficient for chlorophyll a recovery, while methanol favored the extraction of accessory pigments like chlorophyll b and a few carote-

noids. This species-specific variation aligns with various previous findings in algal pigment research, where the polarity of the solvent directly affects pigment yield. Wet biomass of a green alga, *Chlorella thermophila*, extracted using ethanol, showed a significantly high amount of chlorophyll and carotenoids when compared to the extraction process in terms of cell dispersion and solvent penetration for pigment recovery [43]. Similarly, cyanobacteria pigment profiles in recent studies show remarkable variability in phycobiliprotein as well as other pigment content. An *Oscillatoria* spp. was well profiled for its chlorophyll and phycobilin content [44].

5. Conclusion

All the three microalgal sp. were examined for their total antioxidant activity, carotenoids and chlorophyll content and total phenolics as well as flavonoid content, using methanol and chloroform as extraction solvents. The crude extracts in methanol showed maximum antioxidants activity than the same of chloroform extract. In brief, it is concluded that the chloroform extract of *Desertifilum dzianense* showed the highest DPPH scavenging activity while the total antioxidant activity was found to be more in both the methanol and chloroform extract of *Oscillatoria* sp. Similarly, methanol extract of *Oscillatoria* sp. and *Desertifilum dzianense* were found to produce more amount of phenolic and flavonoid contents respectively. In the other hand the chlorophylls and carotenoids are found in a very good amount in the methanol extracts and the phycobilin is better observed in chloroform extracts. Hence, this study showed that all the three native microalgal cultures have some amount of antioxidant property in more or less amount for which they can be considered as potent strains and ultimately used as therapeutic agents in near future if further study will be done properly and carefully.

Abbreviations

APC	Allophycocyanin
DCW	Dry Cell Weight
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
TAA	Total Antioxidant Activity
TAC	Total Antioxidant Capacity
UV	Ultraviolet

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Author Contributions

Pritikrishna Majhi: Writing – original draft, Data curation,

Formal Analysis, Investigation, Methodology

Saubhagya Manjari Samantaray: Conceptualization, Writing – review & editing, Supervision

Suraj Kumar Samal: Editing of the manuscript, Visualization

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Data Availability Statement

The data supporting the outcome of this research work have been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography



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Pritikrishna Majhi: Microbiology, Bioremediation, Microalgae, Biofuel, Conservation Biology

Saubhagya Manjari Samantaray: microalgal and cyanobacterial diversity, stress physiology, photoprotective and antioxidant compounds, lipid and polyunsaturated fatty acid (PUFA) production, nutraceutical applications, and green synthesis of metal nanoparticles

Suraj Kumar Samal: Farm machinery, Ergonomics, Renewable energy, Life cycle analysis, Biofuel from lignocellulosic biomass