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Cultivation and chemical composition of microalgae *chlorella vulgaris* and its antibacterial activity against human pathogens

Abstract

The present study was aimed to isolate green microalgae *Chlorella vulgaris* from the Pichavaram Mangrove Forest, South East coast of India. After being isolated, they were confirmed through morphological structures of microalgae *C. vulgaris* on cultivation of two different Medias. Phytochemicals like phenol, tannins, flavonoids, terpenes, terpenoids, alkaloid and saponins were present in the dried biomass and antibacterial activity was showed better results to control infectious human pathogens. The total biomass of the BBM and the sewage water cultured *C. vulgaris* were found to be 2.034 g/L and 3.615 g/L (dry weight), 0.268 g/L and 0.402 g/L (wet weight), respectively. The physico – chemical parameters of the sewage water were analyzed initially and at the end of the study, to determine the chemical consumption by the microalgae. Likewise, the protein, carbohydrate and lipid content of the BBM and the sewage water cultured *C. vulgaris* were recorded (34.56 ± 1.33 & 36.56 ± 1.28 mg/g), (41.09 ± 0.92 & 42.13 ± 0.85 mg/g) and (28.20 ± 0.89 & 28.68 ± 0.82 mg/g) respectively. The *C. vulgaris* cells were extracted with different solvents like methanol, ethanol, chloroform and diethyl ether, and their antibacterial activity against gram negative and gram positive human pathogenic bacteria was also evaluated. In every respect, the sewage grown green microalgae recorded higher yield and exhibited potential antibacterial activity.

Keywords: Antibacterial activity, BBM, Biochemical compounds, *Chlorella vulgaris*, Phytochemical, Physico chemical, Sewage Water

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Abbreviations: BBM, Bold's Basal Medium; SW, Sewage Water; BOD, Biological Oxygen Demand; DO, Dissolved Oxygen; COD, Chemical Oxygen Demand; DMSO, Dimethyl Sulfoxide; MWC, Modified WC Medium; Chu, Culture media; BG 11, Blue Green Media; TS, Total Solids

Introduction

Microalgae are a group of unicellular or simple multicellular photosynthetic microorganisms and have been explored for their bioactive compounds and their extracellular products also possess with promising applications encompassing antibacterial, antiviral, antifungal, antialgal antiprotozoal and antiplasmodial activities.1-8 They are divided into four groups (red, green, brown and blue green). This taxonomic group, not included in Plant kingdom but rather in the Protista kingdom, shows a high photosynthetic performance. So, algae can have a high reproductive potential and therefore can grow very fast. Now, humans can build a shining future to the next generations in different sectors of our daily life using microalgae for very different applications such as energy source, food, fertilizers, nutraceutical, cosmetics, pharmaceuticals, aquaculture purpose and pollution control. The development in algal therapeutic research has made it possible now a days by their bioactive compounds which have been found effective against most of the pathogens.9-17

The microalgae has immense application in specific to bioactive compounds derived from algae with proven beneficial and much more effective as compared with traditional treatment methods. Fatty acids isolated from *Coelastrella* spp., *R. violacea* and *Chlorella* spp. were found active against human pathogens like *S. aureus* and low in *S. pyogenes*.18 The production of microalgae biomass shows wide valuable uses, in the aquaculture, biotechnology, and food science, among others. However, microalgae show fluctuations in their chemical profile generated mainly by the culture conditions. The previous reports on the assessments of effects was studied through nitrogen starvation and its totally depends on its growth, nutrient uptake, and gross chemical composition of two species *viz. Chlorella* spp. and *Nannochloropsis oculata.*19

Antibacterial substance, named 'chlorellin', was firstly isolated from *Chlorella*. The mixture of fatty acids was found to exhibit inhibitory activity against both Gram-positive and Gram-negative bacteria.11 As microalgae where potentially explored only after 1950s, they were not considered previously for therapeutic purposes. Widespread research is presently undergoing to find the novel therapeutic useful agents to treat infectious diseases because it produces wide range of antibiotics.20-22

Due to the emerging infectious diseases, viral infections and raise in antibiotic resistant bacteria, there is an urgent need for development of alternative treatment therapies against infectious diseases.9 Further, more research is needed to identify the compounds are directly responsible for those antimicrobial features have been made, but are still emergent. Photosynthesis has long been recognized as a means to sequester anthropogenic CO2 and algae have also been identified as fast growing species whose carbon fixing rates are much higher than those of terrestrial plants.23

The continuous cultivation of algae would not only help in biofixation of CO2 but also yield value added products from biomass such as proteins, fatty acids, vitamin A, minerals, pigments, dietary supplements for human, animals, aquaculture and other bio-

compounds.24 Diatoms are also considered for the principal group contributing to primary production and carbon export in coastal areas, dinoflagellates are important contributors to biomass in stratified or silica-limited areas, and cyanobacteria are the dominant group in offshore continental shelf and oceanic waters.25 The chemical composition of microalgae may also vary widely due to differences of the methods of measurement used,26 the physiological state of the microalgae,27 as well as to the experimental conditions applied, like temperature,28 light intensity,29 and culture medium30 especially in batch cultures. The production and accumulation of bio active components are of particular importance if the microalgae are cultivated either to feed marine animals or to produce specific valuable substances.31

Owing to their diverse chemical properties, they can be used as a nutritional supplement or either represents a source of natural food colorants. Some microalgae species are established in the skin care market, the main ones being *Arthrospira* spp. and *Chlorella* spp.32 In view of the above, the present study was undertaken with the aim to evaluate the algal biomass production of *Chlorella vulgaris* in both BBM and sewage water, analysis of its growth performance, evaluation of carbon dioxide sequestration process, and finally estimated its biochemical composition and its antibacterial activity against an array of human pathogens.

Materials and methods

Isolation and identification of Microalgae

The water samples were collected during early morning from the Pichavaram Mangrove forest, South East Coast of India. The samples were collected aseptically, filtered with seawater and brought to the laboratory immediately then 10 mL were transferred to a 500 mL conical flask containing 200 mL of sterilized Bold's Basal Medium (BBM)33 and incubated on a rotary shaker for three weeks at 27°C for 100 rpm under continuous illumination using white fluorescent light (maximum 2500 lux). At every two days interval, the flasks were examined for algal growth using optical microscope, with serial dilutions being made in BBM from those flasks that showed growth. Then, subcultures were made by inoculation of 50 µL culture solution onto Petri plates containing BBM and solidified with 1.5% (w/v) of bacteriological agar. These procedures were repeated for each of the original flasks. Then the Petri plates were incubated at 27°C under continuous illumination for two weeks. The purity of the cultures was confirmed by repeated plating and by regular observation under a microscope.34 The microscopic identification was done using botanical approaches35 and the microalgae were identified and authenticated based on a standard manual.36

Estimation of Biomass in BBM and Sewage water

Two strategies were developed for the assessment of the biomass production, using two different culture media. 1) Synthetic (BBM) and 2) Natural media - Sewage Water (SW). The SW (Municipal sewage) was collected at the Parangipettai of Portonovo, India, and the physico-chemical parameters were analyzed as follows. 10mL of *Chlorella vulgaris* were then inoculated in 1,000 mL of sterile BBM medium and also in sewage water. After the inoculation, cultivation of microalgae was checked for every 2-3 days. The BBM flasks were then incubated under stationary conditions at 27oC with a light intensity of 2,500 lux on a 12:12 light/dark cycle; while the SW inoculated flasks were kept under natural environmental conditions to avoid the common lab contaminations. The cultured flasks were then shaken three times a day manually and the process continued until the experiment ended. Every three days, the microalgae cells were harvested by centrifugation and washed twice with deionized water. Finally, the microalgal pellets were dried at 80°C for determining the dry and wet weight measurements.37 The experiments were carried out in triplicate and the average values were recorded to estimate the wet and dry biomass after 28 days.

Physico – chemical parameters

Determination of Dissolved Oxygen

Dissolved oxygen was calculated using the following formula[38]

$$DO(mg/\mathrm{L}) = \frac{K \times N \times 8 \times 1000 \times V_1}{V_2}$$

Where

V1=Volume of Sodium Thiosulphate used

V2= Volume of sample taken

N = Normality of Sodium Thiosulphate

K = Volume of bottle /volume bottle-volume of reagent used

Determination of Biological Oxygen Demand

The Biological Oxygen Demand (BOD) was estimated using the methods proposed[38]

BOD can be calculated using the following formula:

BOD, mg/L= (D0-D5) x dilution factor

Where DO= Initial DO in the sample (mg/L); D5= DO after 5 days (mg/L)

Determination of Chemical Oxygen Demand

Chemical Oxygen Demand (COD)can be calculated using the formula[38]

$$COD(mg/\mathrm{L}) = \frac{(\mathrm{b}-\mathrm{a}) \times N\ of\ FAS \times 1000 \times 8}{Sample}$$

Where a = ml of titre with sample, b = ml of titre with blank, FAS = Ferrous Ammonium Sulphate

Estimation of total solids

Total solids (TS) were determined as the residue after the evaporation of the unfiltered sample. For that purpose, 100 mL capacity evaporating dish was ignited at 550 ± 50ºC in a muffle furnace for about 1 hr then it was cooled down in a desiccators and weighed. Subsequently, 100mL unfiltered sample in the evaporating dish were evaporated on a water bath or a hot plate at 98o C. The residue was finally heated at 103-205oC in an oven for 1 hr and the final weight was taken after being cooled in a desiccators.

Total solids (TS) can be calculated using the formula[38]

$$Total\ solids\ mg/\mathrm{L} = \frac{A - b \times 1000 \times 1000}{V}$$

Where A = final weight of the dish in gm, B = initial weight of the dish in gm, V = volume of the sample taken (mL).

Estimation of Nitrogen content

Nitrogen was estimated by Microkjedhal method as described.39 The total N was calculated by the following formula and the results were tabulated.

$$\%\ \text{of}\ Nitrogen = \frac{14 \times 0.02 \times titration\ value - Blank\ value}{Weight\ of\ the\ sample}$$

Determination of Total Biomass

On the last day of growth, the biomass obtained was harvested by flocculation using alum and followed by filtration and it was allowed to dry under room temperature. The weight of the Petriplates was calculated initially to avoid numerical errors. The filtered biomass was kept in sterile dried Petriplates which were weighed initially (fresh weight or wet biomass). Then, it was allowed to dry under sun light and the dried biomass in the Petriplate was weighed (dry weight). Finally, the total biomass could be calculated using the formula as follows40 Total biomass (g/L) = dry weight (g/L) - Initial weight (wet biomass (g/L))

Biochemical composition of dry biomass

Various biochemical parameters were analyzed like protein content,41 carbohydrate (CHO) analysis,42 chlorophyll,43 carotenoids44 and Total lipid content by.45

Quantitative phytochemical analysis

The dry biomass of the sewage water cultured *C.vulgaris* was estimated for the quantitative phytochemicals analysis. Total phenolic content,46 total flavonoid content,47 quantification of alkaloid content48 and quantification of tannin content49 were measured.

Estimation of carbon content & Carbon dioxide fixation rate

Dried algal (0.2 mg) samples were placed in 500 ml conical flask and 10 ml of 1N potassium dichromate and 20 ml of Conc. H2SO4 mixture was diluted with 200 ml of distilled water and 10 ml of hypophosphate (H3PO4) and 1 ml of diphenyl amine was added. Finally it was titrated against 4N Ferrous Ammonium Sulphate (FAS). The end point was the appearance of brilliant green colour. The carbon content was estimated using the following formula.

A=3.951/g (1-T/S)

Where, A is carbon content, g is weight of the sample, T is FAS with blank (ml) and S is FAS with sample (ml). The amount of carbon dioxide fixation rate was estimated using formula.50

R CO2=Cc×μL (Mco2/Mc)

Where, R CO2 and μL are the CO2 fixation rate (g CO2 m-3 h-1) and the volumetric growth rate (g dry weight m-3 h-1) respectively in the linear growth phase. MCO2 and MC represented the molecular weights of CO2 and elemental carbon respectively, CC is average carbon content (algal dry weight/ g).

Elemental analysis

The filtered sample was collected and subjected to elemental analysis for Calcium (Ca), Potassium (K) and Sodium (Na). The estimation of calcium was determined by the method developed for Potassium,52 Sodium51 and the presence were finally measured in flame photometer.

Antimicrobial activity

A certain amount of dry biomass of *Chlorella vulgaris* was allowed to air dry at room temperature and then was pulverized using a blender. The powder obtained (5g) was placed in sterile tubes and extracted with different solvents like methanol, ethanol, chloroform and diethyl ether using a rotary evaporator at the temperature of 40oC for 12h. From the solvent extracts, 5 mL were isolated separately, allowed to dry at room temperature and weighed to estimate. The dry extracts were completely dissolved in 5 ml of 0.5% Tween 80 and preserved at 5oC in airtight screw cap bottles until further use53 for the respective antimicrobial studies. Dimethyl sulfoxide (DMSO) was mixed with double distilled water and served as control for all the plates. All the experiments were carried out in triplicates. The antimicrobial activity was carried out by using disc diffusion method (NCCLS, 1993), testing the microalgal extract effectiveness against gram negative pathogens viz. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Vibrio cholerae*, *Salmonella typhi*, *Escherichia coli*and some gram positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus* sp., *Clostridium botulini* and *Nocardia* sp. The cultures were procured from Microbial Culture Maintenance Laboratory, Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamil Nadu, India.

Statistical analysis

All the experiments were carried out in triplicates and average values were recorded, expressed in Mean ± Standard Deviation (SD).

Physico - Chemical characteristics of Sewage Water (SW)

The physical and chemical components of the SW collected at Portonovo (Parangipettai, Tamil Nadu, India) were analyzed at the beginning and at the end. The values were recorded for various parameters like i.e. Total Solids (486 mg/L), Total Nitrogen (40.6 mg/L), COD (74.8 mg/L), BOD (96.1mg/L) and Dissolved Oxygen (77.84 mg/L) were found higher (Table 1) and after 25 days they were reduced gradually. As the green microalgae utilizes almost all the parameters in natural conditions and final values are measured as Total Solids (287mg/L), Total Nitrogen (7 mg/L), COD (394 mg/L), BOD (119mg/L) and DO (120.68 mg/L).

Table 1 Physicochemical parameters of SW

Parameter	Initial concentration untreated sewage sample (mg/l)	Final concentration treated sewage sample (mg/l)
Total Solids	486	287
Total Nitrogen	40.6	7
COD	74.8	394
BOD	96.1	119
DO	77.84	120.68

Total Biomass estimation in BBM and SW medium

The total biomass of the green micro algae, *Chlorella vulgaris* was analyzed in both media and the wet & dry weight were calculated. The wet weight of the BBM was found to be 2.03g/L, whereas in the case of the SW it yields 3.61g/L. The dry weight of the BBM showed 0.26g/L and 0.40g/L was recorded in SW (Table 2).

Biochemical and elemental analysis

The essential components were analyzed and compared among

the dry biomass obtained from *Chlorella vulgaris* in the BBM and the Sewage Water, respectively. They were subjected to biochemical analysis and their composition was calculated and compared to check their yield. Comparing all the parameters except carbon content, it was found that the dry biomass from the sewage water showed satisfactory results in the protein content as (36.56 ± 1.28 mg/g), carbohydrates content (42.13 ± 0.85 mg/g), total chlorophyll (35.76 ± 0.61 mg/g), Carotenoids (32.14 ± 0.66 mg/g) in SW. The amount of carbon present in the dry biomass was calculated in SW (20.42 ± 0.33 mg/g), being higher under laboratory conditions than in the sewage water. Carbon fixation rate (RCO2) in the SW sample (25.88 ± 0.12 mg/g) followed by lipid content was found to be very similar in the BBM (28.20 ± 0.89 mg/g), and the SW samples (28.68 ± 0.82 mg/g). The presence of calcium was found to be (24.64 ± 0.36 mg/g), Potassium (19.09 ± 0.14 mg/g, Sodium (13.21 ± 0.18 mg/g), Nitrogen content 16.32 ± 0.33 (Table 3) in the SW samples.

All the values are Mean ± Standard Deviations of three determinations.

Table 2 Estimation of total Biomass in BBM and SW

Algae	BBM medium at laboratory condition (G/L)		SW at outdoor condition (G/L)	
Chlorella vulgaris	Wet wt	Dry wt	Wet wt	Dry wt
	2.034	0.268	3.615	0.402

Table 3 Biochemical and elemental analysis of green algae, *Chlorella vulgaris*

S.No	Biochemical Composition	Concentration (mg/g) on Dry Biomass in BBM Medium	Concentration (mg/g) on Dry Biomass in SW Medium
1	Protein	34.56 ± 1.33	36.56 ± 1.28
2	Carbohydrate	41.09 ± 0.92	42.13 ± 0.85
3	Total chlorophyll	32.76 ± 0.78	35.76 ± 0.61
4	Carotenoids	29.63 ± 0.79	32.14 ± 0.66
5	Carbon	21.73 ± 0.21	20.42 ± 0.33
6	Carbon fixation rate (CO2)	26.03 ± 0.08	25.88 ± 0.12
7	Lipids	28.20 ± 0.89	28.68 ± 0.82
8	Calcium	21.03 ± 0.43	24.64 ± 0.36
9	Potassium	18.92 ± 0.17	19.09 ± 0.14
10	Sodium	13.08 ± 0.21	13.21 ± 0.18
11	Nitrogen	15.61 ± 0.73	16.32 ± 0.33

Screening of Phytochemicals

Phytochemical were screened by using four solvents *viz*. ethanol, methanol, chloroform and diethyl ether for their potential activities. In ethanol extracts, all the Phytochemical failed to exhibit such activities (Table 4). Flavonoids, terpenes and alkaloids were noticed while other Phytochemical were not found in the solvent screening. In methanol and chloroform extracts the flavonoids were present in higher quantity, whereas in the diethyl ether extracts were noticed at trace levels. Moderate amount of terpenes and carbohydrate were noticed and alkaloids were also found at traces levels in the methanolic extracts. Traces of alkaloid were also noticed in the diethyl extracts.

Antibacterial activity of crude extracts

The methanolic, ethanolic, chloroform and diethyl ether extracts were tested for antibacterial activity against gram negative and positive human pathogens. As for the *C. vulgaris* solvent extraction, the maximum zone recorded for *S.typhi* was about 9 mm in gram negative bacteria followed by 7 mm for *K.pneumoniae* of diethyl ether extract; the pathogen *P.mirabilis* showed a 6 mm zone in methanolic extracts; *V.cholerae in* chloroform extract showed an 8 mm zone whereas *E.coli* showed a maximum zone of 6 mm in the ethanol & methanol extracts. In *C vulgaris* solvent extraction, the maximum zone was recorded for the gram positive bacteria, *Bacillus subtilis* of about 9 mm (Table 5) followed by 8 mm in *Staphylococcus aureus* in the ethanol extract; the pathogen *Enterococcus* sp showed a 7 mm zone in the methanolic extracts, *Clostridium botulini*in the ethanol extract showed a 6 mm zone and finally *Nocardia* sp showed a maximum zone of 3 mm in the chloroform extract.

Discussion

According to our result, *Chlorella vulgaris* fixing higher level of CO2 from sewage indicated that this organism could be suggested as the best microalgae for CO2 sequestration. Under waste water stabilization conditions, the algae produced the higher levels of oxygen as a by- product of photosynthesis. From the present study, the carbon fixation rate was found higher in the BBM medium i.e. (26.03 ± 0.08 mg/g) and in SW it showed (25.88 ± 0.12 mg/g). This oxygen is used by the bacteria as they bio-oxidize the organic compounds present in the waste water. The end product, CO2 is fixed into cell as an organic compound by the algae during photosynthesis54,55 reported that the batch cultures of *C. vulgaris* grown in the BG 11 (Blue Green Medium), reached a maximum chlorophyll *a* concentration of 5µg/ mL-1 approximately within the first 10 days. The increase in CO2 sequestration is very efficient by maneuvering chemically aided biological sequestration of CO2. *Chlorella* sp. and *Spirulina platensis* showed 46% and 39% mean fixation efficiency, respectively, at input CO2 concentration of 10%. The effect of acetazolamide, a potent carbonic anhydrase inhibitor, on CO2 sequestration efficiency was studied to demonstrate the role of carbonic anhydrase in calcite deposition.56 In the present study, total chlorophyll amount was analyzed in the Sewage Water inoculated with *C.vulgaris* showing a value of (35.76 ± 0.61mg/g) after 25 days of culture period.

The highest dry weight (16.82 pg.cell-1) concentration achieved using LC Oligo (lowest in Chu medium) can be due to its composition, as this medium had the highest N and P concentrations. Similar to the present study, the dry biomass from the SW was found to be 0.402gL-1.30 Obtained a dry weight production of about 50 µg.mL-1 during

the exponential phase of *C. vulgaris* grown at 0.036% CO2 and 30°C, which falls within the values (13-54 µg.mL-1). A possible explanation for this trend results from the consumption of internal pools of inorganic nitrogen, presumably created due to the luxuriant uptake of nitrate.57 Large pools of nitrate, nitrite and ammonia/ammonium are accumulated in cell vacuoles during the exponential growth of batch cultures run with sufficiency in nitrate.58 The protein production of 50% (7.0 mgL-1) in the Chu (Culture medium) and 6.8 mg.L-1 in the MWC media (Modified WC Medium) were higher than those obtained in other studies, but similar to the results.59 The highest carbohydrates production by *C.vulgaris* under the present study conditions was obtained in the LC Oligo cultures (7.36 µg.mL-1) but, the SW proves to be suitable for CHO production (42.13 ± 0.85 mg/g). In *Chlorella* sp., increments of the carbohydrate content from the exponential to the stationary growth phase were more intense than in *N. oculata*, with a peak concentration of 54.5% of the dry matter (d.m.) in the N –experiment.19 Reported that for semi-continuous grown cells, once appropriate culture conditions are reached a high productivity can be sustained for long periods of time. The biomass of microalgae obtained will show constant biochemical composition that can be further controlled by manipulating environmental/culture parameters in order to increase its nutritive value.60 In the present experiment, under autotrophic conditions the healthy growth was noticed in the SW and it is usually above 90% of the total lipids while under heterotrophic, the value of healthy growth is found lower.61

Table 4 Screening the phytochemicals of different solvent extract from *Chlorella vulgaris*

Name of the species	Extracts	Phenolic	Tannins	Flavonoids	Terpenes	Terpenoids	Alkaloid	Saponins
Chlorella vulgaris	Ethanol	-	-	-	-	-	-	-
	Methanol	+	-	+++	++	-	+	-
	Chloroform	-	-	+++	-	-	+	-
	Diethylether	-	-	+	-	-	+	-

Present in High amount (+++), Moderate amount (++), trace amount (+) and absent (-).

Table 5 Antibacterial activity of various extracts in *Chlorella vulgaris*

S.No	Name of the Bacterial Strains	Concentration/Zone of Inhibition (mm)					
		Ethanol Extract	Methanol Extract	Chloroform Extract	Diethyl Ether Extract	+Ve	-Ve
1	*K.pneumoniae*	7.0±0.5	6.0±0.3	7.0±0.4	5.0±0.5	16.0±0.2	-
2	*P. mirabilis*	5.0±0.6	6.0±0.5	-	8.0±0.5	14.0±0.9	-
3	*V. cholera*	-	2.0±0.1	8.0±0.5	-	15.0±0.1	-
4	*S. typhi*	9.0±0.7	4.0±0.1	3.0±0.1	-	18.0±0.11	-
5	*E. coli*	8.0±0.6	8.0±0.5	-	5.0±0.6	21.0±0.03	-
6	*S.aureus*	8.0±0.4	7.0±0.5	7.0±0.4	3.0±0.1	17.0±0.8	-
7	*B. subtilis*	9.0±0.5	2.0±0.2	6.0±0.6	3.0±0.1	22.0±0.1	-
8	*Enterococcus* sp	7.0±0.3	8.0±0.5	8.0±0.4	4.0±0.4	17.0±0.02	-
9	*C. botulini*	6.0±0.7	-	4.0±0.5	-	15.0±0.07	-
10	*Nocardia* sp	-	-	3.0±0.1	-	11.0±0.6	-

The maximum growth rate was achieved by the marine green micro algae *C. vulgaris* at the 25th day with no decline phase during the study period. A common feature of the biomass of the algal species currently produced commercially (i.e. *Chlorella* sp, *Spirulina* sp and *Dunaliella* sp) that grow in open air cultures and still remain relatively free from contamination by other algae. The present study revealed that during the cultivation period the main part of the organic pollutants were consumed by the algae; - the total nitrogen was highly up taken by *C. vulgaris* (80%). The level of BOD was reduced in the BBM and found higher in the sewage water. Compared to the results of other works, the removal of total nitrogen from sewage was found better in the present study.62

S. typhi is a gram negative rod shaped bacteria; causative agent for enteric fever, sepsis and infectious diarrhea in human beings. The ethanolic extracts of *C.vulgaris* showed an antibacterial activity against the pathogen *S. typhi* and hence, it should be recommended as an additive to the available drugs, and there for the extracts of *C.vulgaris* could be used against the enteric fever, septis and infectious diarrhea or gastroenteritis. The antimicrobial property of *C.vulgaris* were found to control the three gram-positive bacterias like *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 12344, *Enterococcus faecalis* ATCC 29212, and two gram-negative bacteria were *Pseudomonas aeruginosa* ATCC 29212 and *Escherichia coli* ATCC 11230.63

It is also suggested that the extracts of *C.vulgaris* may be used to treat urinary tract infections, diarrhea, pyogenic infections and septicemia. The ethanol extract of *C.vulgaris* alsoshowed an antibacterial activity against *B.subtilis.* Hence, the extract can be suggested to treat infections like those produced by *Bacillus subtilis*, *S. typhi* and for others. Generally, *C.vulgaris* extracts were found to be effective against only two pathogens and the antibacterial activities were responds in a dose dependent manner. The previous results are in accordance with the present findings.64

From this study, the phytochemical also revealed that the presence of metabolites like flavonoids, terpenes and carbohydrates might be responsible for the antibacterial activity of these extracts against these types of pathogenic bacteria. However, more research is needed in this particular aspect to prove the benefits of the traditional methods by using materials from autotrophic organisms instead of those from synthetic drugs to cure diseases caused by bacteria. Hence, from the present study it is revealed that the presence of competent antibacterial compounds in the marine algae has been assessed. From this, wastewater may prove as a potential sustainable growth medium for algae feedstock, which corresponds with a wide range of studies which have also reported microalgal growth in wastewaters including municipal sewage wastewater and agricultural manure wastewater.65

Further research is under trail in nursery fields, to check the efficacy of dried biomass obtained from Sewage water for the production of beneficial plant crops. Form our results, it is suggested that the algal dried biomass can be used to treat human pathogens, effluent water purifying systems and beneficial cost effects for large scale effective production.

Conclusion

The study clearly shows that the sewage water is very effective for the fast growth of microalgae compared to BBM. The biomass production also found to be good and the production cost is very cheap, when compared to synthetic media used. Compared to synthetic media, green marine micro algae show a higher biomass yield cultured in sewage under outdoor environmental condition as they can grow effectively in nutrient-rich environments and efficiently accumulate nutrients and metals from the wastewater. In addition to that, further research is needed in order to increase the lipid concentration for the enhancement and production of high nutritional value added products and for the production of a better and cheaper, bio diesel.

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Conflicts of interest

None.

References

1. Mayer AM, Hamann MT. Marine pharmacology in marine compounds with antihelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti–inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comp Biochem Physiol C Toxicol Pharmacol*. 2005;140(3–4):265–286.
2. Cardozo KH, Guaratini T, Barros MP, et al. Metabolites from algae with economical impact. *Comp Biochem Physiol C Toxicol Pharmacol*. 2007;146(1–2):60–78.
3. Kellan SJ, Walker JM. Antibacterial activity from marine microalgae. *British Journal of Phycology*. 1989;24(2):191–194.
4. Ozdemir G, Karabay NU, Dalay MC, et al. Antibacterial activity of volatile components and various extracts of Spirulina platensis. *Phytother Res*. 2004;18(9):754–757.
5. Herrero M, Ibáñez E, Cifuentes A, et al. Dunaliella salina microalga pressurized liquid extracts as potential antimicrobials. *J Food Prot*. 2006;69(10):2471–2477.
6. Ghasemi Y, Yazdi M, Shafiee A, et al. a novel antimicrobial substance from Fischerella ambigua. *Pharmaceutical Biology*. 2004;42(4–5):318–322.
7. Mendiola JA, Torres CF, Martín Alvarez PJ, et al. Use of supercritical CO_2 to obtain extracts with antimicrobial activity from Chaetoceros muelleri microalga. A correlation with their lipidic content. *Eur Food Res Technol*. 2007;224(4):505–510.
8. Metting B. Biologically active compounds from microalgae. *Enzyme and Microbial Technology.* 1986;8(7):386–394.
9. Swapnil S, Benedict B, Udhaya R, et al. Bioactive Compounds Derived from Microalgae Showing Antimicrobial Activities. *J Aquac Res Development*. 2014;5(224):3.
10. Kamalnizat I, Ramliza R, Abdul Halim AR, et al. Antimicrobial Property of Water and Ethanol Extract Chlorella vulgaris: A Value–Added Advantage for a New Wound Dressing Material. *International Medical Journal*. 2015;22:399–401.
11. Pratt R, Daniels TC, Eiler JJ, et al. *Chlorellin, an antibacterial substance from Chlorella. Science*. 1944;99(2574):351–352.
12. Danyal A, Mubeen U, Malik K.A. Investigating Two Native Algal Species to Determine Antibiotic Susceptibility Against some Pathogens. *Curr Res J Biol Sci*. 2013;5(2):70–74.
13. Akgul R, Suerdem TB, Akgul F. Antimicrobial Activities of Some Marine Algae and Some Cyanobacteria from Canakkale J. *Algal Biomass Utln*. 2013;4:35–40
14. Rosaline XD, Sakthivelkumar S, Rajendran K, et al. Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*. 2012;2(1):S140–S146.
15. Genovese G, Faggio C, Gugliandolo C, et al. In vitro evaluation of antibacterial activity of Asparagopsis taxiformis from the Straits of Messina against pathogens relevant in aquaculture. *Mar Environ Res*. 2012;73:1–6.
16. Bhagavathy S, Sumathi P, Bell JS. Green algae Chlorococcum humicola–a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*. 2011;1(1):S1–S7.
17. Najdenski HM, Gigova LG, Iliev II, et al. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *International Journal of Food Science and Technology*. 2013;48(7):1533–1540.
18. Orhan I, Sener B, Atici T, et al. Turkish freshwater and marine macrophyte extracts show in vitro antiprotozoal activity and inhibit FabI, a key enzyme of Plasmodium falciparum fatty acid biosynthesis. *Phytomedicine*. 2014;13(6):388–393.
19. Caroline RPS Paes, Gabrielle R, Faria, et al. Growth, nutrient uptake and chemical composition of Chlorella sp. and Nannochloropsis oculata under nitrogen starvation, Lat. Am. *J. Aquat. Res*. 2016;44(2):275–292.
20. Mendes RL, Nobre BP, Cardoso MT, et al. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chimica Acta*. 2003;356:328–334.
21. Mayer AMS, Hamann MT. Marine compounds with antihelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comp Biochem Physiol C Toxicol Pharmocol*. 2005;140:265–286.
22. Cardozo KH, Guaratini T, Barros MP, et al. Metabolites from algae with economical impact. *Comp Biochem Physiol C Toxicol Pharmacol*. 2007;146(1–2):60–78.
23. Jeong MJ, Gillis JM, Hwang, JY. Carbon dioxide mitigation by microalgal photosynthesis. *Bull. Korean Chem. Soc*. 2003;24(12):1763–1766.
24. González López CV, Acién Fernández FG, Fernández Sevilla JM, et al. Utilization of the cyanobacteria Anabaena sp. ATCC 33047 in carbon dioxide removal processes. *Bioresour Technol*. 2009;100(23):5904–5910.
25. Silva AF, SO Lourenço RM, Chaloub. Effects of nitrogen starvation on the photosynthetic physiology of a tropical marine microalga Rhodo–monas sp. (Cryptophyceae). *Aquacult Bot*. 2009;91(4):291–297.

26. Barbarino E, Lourenço SO. An evaluation of methods for extraction and quantification of protein from marine macro– and microalgae. *J Appl Phycol*. 2005;17(5):447–460.

27. Geider R, La Roche J, Greene R, et al. Response of the photosynthetic apparatus of Phaeo–dactylum tricornutum (Bacillariophyceae) to nitrate, phosphate, or iron starvation. *J. Phycol*. 1993;29(6):755–766.

28. Durmaz Y, Donato M, Monteiro M, et al. Effect of temperature on α–tocopherol, fatty acid profile, and pigments of Diacronema vlkianum (Haptophyceae) Aquacult. Int. 2009;17(4):391–399.

29. Lourenço SO, Vieira AAH. Culture collec–tions of microalgae in Brazil: progress and constraints. *Nova Hedwigia*. 2004;79(1–2):149–173.

30. Huerlimann R, de Nys R, Heimann K. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale–up production. *Biotechnol Bioeng*. 2010;107(2):245–257.

31. Machado RR, Lourenço SO. Propriedades nutricionais de microalgas usadas como alimento de moluscos bivalves: uma revisão. *Museu Nacional Série Livros*. 2008;30:281–304.

32. Stolz P, Obermayer B. Manufacturing microalgae for skin care. *Cosmetics Toiletries*. 2005;120:99–106.

33. Kanz T, Bold HC. *Physiological studies, morphological and taxonomical investigation of Nostoc and Anabaena in Culture*. Austin (TX), University of Texas. 1969;pp.6924

34. Prabakaran P, David Ravindran A. Lipid extraction and CO_2 mitigation by microalgae. *J Biochem Tech*. 2013;4(1):469–472.

35. John DM, Whitton BA, Brook AJ. The freshwater algal flora of the British Isles an identification guide to freshwater and terrestrial algae. *Cambridge, Cambridge University Press*. 2003;p.39–43.

36. Prescott GW. How to Know the Fresh Water Algae. Michigan: Cranbrook Press. 1959.

37. Borowitzka MA. Microalgae as sources of pharmaceuticals and other biologically active compounds. *J Appl Phycol*. 1995;7(1):3–15.

38. APHA. Standard methods for examination of water and wastewater (17th edn), Washington DC, APHA, AWWA, WPCF, USA. 1990.

39. Jones, MM. Marine organisms transported in ballast water: a review of the Australian scientific position. Bureau of Rural Resources Bulletin No. 11, *Australian Government Publishing Service, Canberra*. 1991;pp.48.

40. Moheimani NR, Borowitzka MA, Isdepsky A, et al. Standard methods for measuring growth of algae and their composition. In: Borowitzka MA, Moheimani NR (Eds.), *Algae for Biofuels and Energy*. 2013;pp 265–284.

41. Lowry PH, Rosebrough NJ, Furr AL, et al. Protein measurement with Folin phenol reagent. *J Biol Chem*. 1951;193(1):265–274.

42. Dubois M, Gilles KA, Hamilton JK, et al. Calorimetric method for determination of sugars and related substances. *Anal Chem*. 1956;28(3):350–356.

43. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenol oxidase in Beta vulgaris L. *Plant Physiol*. 1949;24(1):1–15.

44. Kirk JT, Allen RL. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. *Biochem Biophysic Res Commun*. 1965;21(6):523–530.

45. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959;37(8):911–917.

46. Hammuel C, Abdullahi MS, Mankilik M, et al. The phytochemical and antimicrobial activities of oil from the seed of thevetia peruviana plant. *J Appl Environ Biol Sci*. 2011;1(12):597–601.

47. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondias pinnata. *BMC Complement Altern Med*. 2008;8:63–71.

48. Ghate NB, Chaudhuri D, Mandal N. In vitro antioxidant and free radical scavenging assessment of Tinospora cordifolia stem with DNA protective potential. *Int J Pharm Bio Sci*. 2013;4:373–388.

49. Chaudhuri D, Ghate NB, Sarkar R, et al. Phytochemical analysis and evaluation of antioxidant and free radical scavenging activity of Withania somnifera root. *Asian J Pharm Clin Res*. 2012;5:193–199.

50. Yun YS, Lee SB, Park JM, et al. Carbon dioxide fixation by algal cultivation using wastewater nutrients. *J Chem Technol Biotechnol*. 1997;69(4):451–455.

51. Watson ME, Isaac RA. Analytical instruments for soil and plant analysis. In: Westerman RL (Ed.), Soil Testing and Plant Analysis. (3rdedn), *Soil Science Society of America, Madison, WI*. 1990;pp.691–740.

52. Natusch DS, Hopke PK. Analytical aspects of environmental chemistry. John Wiley & Sons, USA. 1983.

53. Dineshkumar R, Narendran R, Sampathkumar P. Phytochemical and antimicrobial activity of green microalgae from Vellar Estuary, southeast coast of India. *Journal of Coastal Life Medicine*. 2016;4(5):374–376.

54. Wang B, Li Y, Wu N, et al. CO_2 bio–mitigation using microalgae. *Appl Microbiol Biotech*. 2009;79(5):707–718.

55. Chinnasamy S, Ramakrishnan B, Bhatnagar A, et al. Biomass Production Potential of a Wastewater Alga Chlorella vulgaris ARC 1 under Elevated Levels of CO_2 and Temperature. *Int J Mol Sci*. 2009;10(2):518–532.

56. Rishiram R, Krishnamurthi K, Ashok D, et al. Enhanced algal CO_2 sequestration through calcite deposition by Chlorella sp. and Spirulina platensis in a mini–raceway pond, *Bioresource Technology*. 2010;101(8):2616–2622.

57. Lourenço SO, Barbarino E, Lanfer–Marquez UM, et al. Distribution of intracelluar nitrogen in marine microalgae: basis for the calculation of specific nitrogen–to–protein conversion factors. *J Phycol*. 1998;34(5):798–811.

58. Lomas MW, Glibert PM. Comparisons of nitrate uptake, storage, and reduction in marine diatoms and dinoflagellates. *J Phycol*. 200;36(5):903–913.

59. Bertoldi FC, Sant'anna E, Oliveira JLB. Chlorophyll content and mineral profile in the microalgae Chlorella vulgaris cultivated in hydroponic wastewater. *Cienc Rural*. 2008;38(1):54–58.

60. Ferreira M, Coutinho P, Seixas P, et al. Enriching Rotifers with "premium" microalgae Nannochloropsis gaditana. *Mar. Biotechnol*. 2009;11(5):585–595.

61. Hu Q, Sommerfeld M, Jarvis E, et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J*. 2008;54(4):621–639.

62. Scarsella M, Belotti G, De filippis P, et al. Study on the optimal growing conditions of Chlorella vulgaris in bubble column photo bioreactors. *Chem Eng*. 2010;20:85–90.

63. Kamalnizat I, Ramliza R, Abdul Halim AR, et al. Antimicrobial Property of Water and Ethanol Extract Chlorella vulgaris: *A Value–Added Advantage for a New Wound Dressing Material International Medical Journal*. 2015;22(5):399–401.

64. Vlachos V, Critchley AT, Von Holy A. *Establishment of a Protocol for Testing Antimicrobial Activity in Southern African Macroalgae. Microbios*. 1996;88(355):115–123.

65. Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol*. 2011;102(1):17–25.