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Green algae *Chlorococcum humicola*- a new source of bioactive compounds with antimicrobial activity

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ABSTRACT

Objective: To analyse the existence of bioactive phytochemicals and their antimicrobial role of green algae *Chlorococcum humicola* (*C. humicola*). **Methods:** The various organic solvents such as acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane and methanol were used for the preparation of the algal extracts then subjected to chemical analysis and further used for the screening of antimicrobial assay. The purified carotenoid pigments and chlorophylls were used for the antimicrobial studies against the harmful pathogens *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumoniae, Vibreo cholerae, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus niger* and *Aspergillus flavus*. **Results:** The chemical analysis showed the existence of bioactive compounds such as carotenoids, alkaloids, favanoids, fattyacids, saponins, aminoacids and carbohydrates. *In vitro* screening of organic solvent extracts of green algae *C. humicola* shows activity in inhibiting the growth of virulent strains of bacteria and fungi pathogenic to human. Eight different extracts showed effective inhibitory action against the selected pathogens. Depends upon their existence of the bioactive compounds the different organic algal extracts shows difference in their inhibitory zone against the microbes. Out of all the organic extracts benzene and ethyl acetate extracts showed excellent effect nearly 80% microbial growth inhibition. The separated carotenoid and chlorophyll fractions of *C. humicola*, also results in the microbial growth inhibition. **Conclusions:** The present study concludes that green algae *C. humicola* are a rich and varied source of pharmacologically active natural products and nutraceuticals. While nutraceutical and pharmaceutical content in the baseline algae strain is very small, they showed excellent effect against the microbial pathogens.

1. Introduction

The macroalgae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, antitumorals, antioxidant and antiinflammatories[1]. Evidence of phytochemical and pharmacological studies on algae is available in the literature with special reference to terpenoids and steroids. Algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes and cyclic polysulphides[2,3].

Marine algae are one of the largest producers of biomass in the marine environments. They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other settling organisms. These active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids, are produced by several species of marine macro and microalgae and have antibacterial, antialgal, and antifungal properties which are effective in the prevention of biofouling and have other uses, *e.g.* in therapeutics[4].

Use of algae, especially the cyanobacteria (blue-green algae), for antibiotics and pharmacologically active compounds has received ever increasing interest. There are a range of pharmaceutical products derived from algae. Chlorophytes were mainly distributed within the crust and the taxa of chlorophytes decrease obviously under the crust.

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In the developing stages of the biological soil crust, species diversity of chlorophytes changed a little, but species composition presented some differences. *Chlorococcum humicola* (*C. humicola*), *Chlorella vulgaris*, *Chlamydomonas ovalis* and *Chlamydomonas* sp. nearly existed in all developing stages of biological crusts[5].

Algae have recently received a lot of attention as a new biomass source for the production of renewable energy. Some of the main characteristics which set algae apart from other biomass sources are that algae (can) have a high biomass yield per unit of light and area, can have a high oil or starch content, do not require agricultural land, fresh water is not essential and nutrients can be supplied by wastewater and CO_2 by combustion gas. The first distinction that needs to be made is between macro algae (or seaweed) versus microalgae[6].

Chlorococcum, currently a distinct genus of chlorophyta, was first described as vegetative cells solitary or in temporary groups of indefinite form, never embedded in gelatin. Cells ellipsoidal to spherical with smooth cell walls and variable size. Extracellular products of *Chlorococcum* were isolated from culture filtrates. Five groups of substances were found in different species: (i) steam-volatile acids; (ii) yellow water-soluble phenolic compounds; (iii) lipophilic substances; (iv) proteins and (v) polysaccharides[7].

Recent studies suggest that seaweeds can produce hundreds to thousands of diverse chemical compounds with different biological activities. These substances can inhibit the growth of micro organisms or kill them. There is a continuous need to discover new antimicrobial compounds with its chemical structures and novel mechanism of action because of the development of resistance to the antibiotics in current clinical use.

In the present study, *C. humicola* – a freshwater green algae has been used to screen for the existence of bioactive compounds and its protective role against the microbial pathogens.

2. Materials and methods

2.1. Collection and culturing of algae

Fresh water, unicellular, nonmotile green algae *C. humicola* was obtained from the culture collected from the department of Algal Biotechnology, Vivekantha Institute, Chennai, India. Algal culturing was carried out with 100 mL Bold's basal medium[8], supplemented with sterile compressed air and kept under fluorescent light (20 μmol $m^{-2}s^{-1}$) with 16 h light period and at 25 ± 2 ℃ temperature. Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris.

2.2. Preparation of algal crude extracts

The algal samples were centrifuged at 2 500 rpm for 10 minutes to remove the water content. 25 g of fresh algae was extracted for 15 minutes with 50 mL of organic solvents, acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane and methanol. All the crude extracts were used for the antimicrobial screening assays with varying concentrations.

2.3. Chemical composition analysis

Preliminary phytochemical analysis was carried out by the standard method to identify the available chemicals in the various organic extracts of *C. humicola*. The benzene and ethyl acetate extracts of *C. humicola* were subjected to the analysis of chemical components by gas ghromatography-mass spectrometer (GC-MS) in Indian Institute of Technology, chennai, India. Identification of the chemical constituents of extracts were made using Hewlett Packard HB 5890 gas liquid chromatography (GLC) coupled with 5989 B series mass spectrometer (MS). Identification of the individual components was performed by comparison of mass spectra with the profiles from the Wiley GC-MS 275 libraries.

2.4. Pigment extraction and separation

2.4.1. Determination of chlorophyll

One gram of *C. humicola* was homogenized in acetone (20 mL, 80%) and allowed to stand overnight in dark at 4 ℃ for complete extraction followed by centrifugation at 10 000 Xg for 5 min. The contents of total chlorophyll (TChl), chlorophyll a (Chl-a) and chlorophyll b (Chl-b) in the supernatant were spectophotometrically determined[9].

2.4.2. Determination of carotenoids

5 g of algal samples were used for carotenoid extraction by acetone. Carotenoid pigments were separated by open column chromatographic (OCC) method with neutral alumina column deactivated with methanol using petroleum ether as mobile phase and by thin layer chromatography (TLC). The absorption maxima (λ_{max}) of the collected pigments were recorded in UV-VIS spectrophotometer from 200-1 500 nm. These purified pigments were also used for further antimicrobial screening assays.

2.4.3. Fractionation of pigments by TLC

TLC was carried out on 10 × 20 cm silica gel plates, 5 μL of extracted chlorophyll and carotenoid pigments were applied to 1 cm of the base of plate and developed with hexane/acetone (75:25 v/v). The separated compounds were located and identified by visualizing plates stained with 0.5 mmol/L DPPH in methanolic solution and observed pigments colors bands. The Rf values of pigments were measured and compared with the reports available for standard pigments.

2.5. Test microorganisms

Gram negative bacterial strains *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhimurium* (*S. typhimurium*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Vibreo cholerae* (*V. cholerae*) Gram positive bacterial strains *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and fungus *Candida albicans*, *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*) were used for the study. The parent cultures were obtained from microbiology department and the subcultures were maintained once in 15 days.

2.6. Agar well diffusion method

All the extracts were dissolved in their respective solvents at the concentration of 10 mg/mL. The antibacterial activity was performed by well diffusion method. The respective bacterial culture was poured into the Muller Hinton agar plates for uniform distribution of microorganisms. Using sterile well puncture 3 mm wide well was made on each agar plates. Various concentrations of organic crude extracts (25, 50, 75 μg/well) from the stock were poured into each well using a sterile micropipette[10]. Penicillin, ampicillin, methicillin (30 μg/well) were used as standards. The plates were incubated for 24 hours at 37 ℃. At the end of incubation period, the zone of inhibition was measured.

2.7. Minimum inhibitory concentration (MIC)

The antibacterial activity and MIC was calculated according to the standard reference method[11]. MIC was defined as the lowest extract concentration showing no viable microbial growth after incubation time for 24 h at 37 ℃.

3. Results

3.1. Phytochemical analysis

The various organic extracts of *C. humicola* differ in colour based on the components what extracted. The tested phytochemicals are listed on Table 1. Fatty acids and chief colouring pigment carotenoids are present at higher concentrations. Saponins and alkaloids are also found at the major concentration. Carbohydrates and flavanoids were also identified. Tannins and glycosides are absent.

3.2. GC-MS analysis

GC-MS analysis of benzene and ethyl acetate extracts revealed the presence of mixture of standard fatty acids including saturated and unsaturated, and it also displayed variable relative percentage of fatty acids (Figure 1; Table 2). The major components were palmitic acid, oleic acid, linoleic acid, myristic acid and stearic acid.

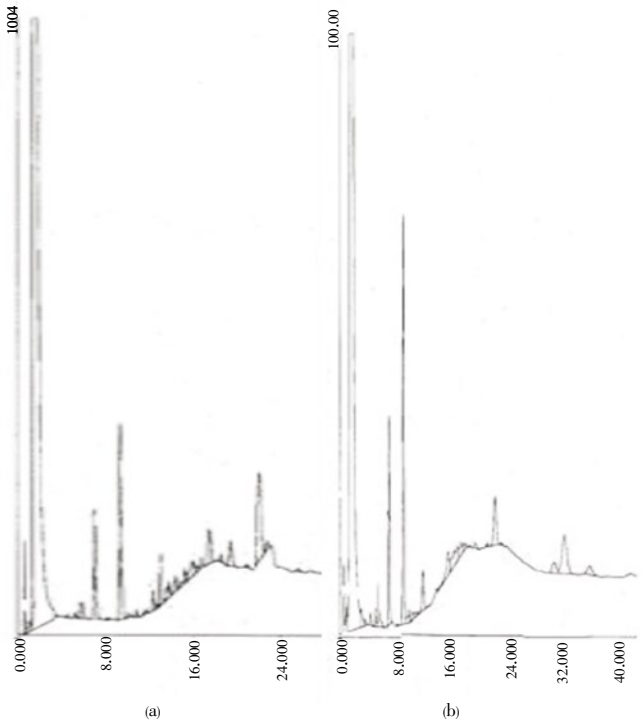

Figure 1. Chromatographic separation by GLC of antimicrobial active compounds of benzene (a) and ethyl acetate (b) extract of *C. humicola*.

3.3. Separation and characterization of pigments

The results of pigments separated by TLC and column

Table 1
Phytochemical existence in various organic extracts of *C. humicola*.

Identified compounds	Acetone	Benzene	Chloroform	Diethyl ether	Ethyl acetate	Ethanol	Hexane	Methanol
Carbohydrates	+	+	+	+	+	+	+	+
Fatty acids	+	+	+	+	+	+	+	+
Aminoacids	+	+	+	+	+	+	+	+
Saponins	+	–	+	+	+	+	+	–
Tannins	–	–	–	–	–	–	–	–
Carotenoids	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+
Glycosides	–	–	–	–	–	–	–	–

+ Present, – Absent.

chromatography confirm the existence of β-carotene and chlorophyll (Figure 2). The algae were found to contain two important pigments which were confirmed to their absorption maxima. The orange yellow colour compound that showed maximal absorption at 468 nm was identified as β-carotene. The second compound spectrum showed absorption of two different compounds, one at 425 nm and 660 nm and the other at 428 nm and 645 nm which was correlated with pigments of chlorophyll a and b (Figure 3).

3.4. Antimicrobial assay and MIC

The antimicrobial activity of *C. humicola* in different organic extracts were assayed against seven bacterial strains and three fungal strains by evaluating the inhibition zones, zone diameter and MIC values. Generally, almost all *C. humicola* organic extracts were found to be effective against all tested microbes and these antimicrobial activities was found to be dose depended. This phenomenon was in agreement with previous findings[12,13]. The data in Table 3 and Figure 4 showed that the most susceptible bacteria to the ethyl acetate extracts were *S. typhimurium* and *V. cholerae* with the highest inhibition zones of 17 mm at concentration of 25 μg/well. Thus, the antibacterial activity may be attributed to presence of some active components in all organic extracts such as lipophilic and phenol compounds. Acetone extracts of *C. humicola* showed lower inhibition zones ranged from 7 mm to 10 mm, compared to the values of other organic extracts with MIC of 10 mg/mL. Among all extracts of the *C. humicola*, benzene and ethyl acetate extract were the most potent against all bacteria with MIC values of 22 and 17 mg/mL, as compared with extracts of acetone with the value of 6 mg/mL. The maximal inhibition of microbial strains from benzene and ethyl acetate extracts. The standard antibiotics were in range of 17 mm/well to 22 mm/well. Table 4 indicates that both the β-carotene and the chlorophyll fractions showed the effective inhibition against the various microbes in the concentration gradient.

Table 2
Fatty acid content (saturated and unsaturated) in the extract of *C. humicola*, their retention times and relative concentrations as analyzed by GLC and compared with a mixture of standard fatty acids.

Fatty acids		Benzene extract		Ethyl acetate extract	
		Retention time (min)	Relative %	Retention time (min)	Relative %
Saturated fatty acids	Caprylic acid	1.83	64.80	1.96	60.70
	Capric acid	7.10	3.53	7.08	5.78
	Undecyclic acid	8.27	0.06	8.23	0.06
	Lauric acid	9.42	6.09	9.09	11.41
	Tridecyclic acid	–	–	9.85	0.46
	Myristic acid	11.77	0.90	11.88	1.54
	Pentadecyclic acid	12.37	1.55	15.43	1.74
	Palmitic acid	16.68	0.34	16.77	0.86
	Stearic acid	20.82	1.13	20.87	1.42
Unaturated fatty acids	Palmitooleic acid	17.42	2.42	17.83	0.36
	Oleic acid	22.17	4.86	22.00	2.61
	Linolic acid	27.75	2.19	24.00	0.30
	Linolenic acid	30.67	3.09	30.45	0.99
	Stearidonic acid	32.24	4.70	31.92	4.20
	Arachidonic acid	35.85	1.44	35.52	1.05
	Eicosapentaenoic acid	–	–	40.23	3.20

Table 3
Antimicrobial activities around the wells of different organic extracts (25 μg/well) of *C. humicola*. inhibition zone in diameter (mm).

Microorganism		Acetone	Benzene	Chloroform	Diethyl ether	Ethyl acetate	Ethanol	Hexane	Methanol
Bacteria	*B. subtilis*	8	18	16	10	16	15	8	9
	S. aureus	7	14	–	9	13	–	10	11
	E. coli	8	12	18	11	15	6	7	8
	P. aeruginosa	9	18	14	11	15	–	12	–
	S. typhimurium	10	12	13	8	17	13	9	11
	K. pneumoniae	7	10	12	12	13	13	11	10
	V. cholerae	11	15	16	10	17	14	13	12
Antibiotics	Amphicillin	18	17	20	18	18	20	19	19
	Penicillin	22	22	19	19	20	21	18	20
Fungi	*C. albicans*	–	11	–	6	15	–	11	–
	A. niger	8	18	12	10	13	7	8	12
	A. flavus	9	17	14	9	12	6	7	12
Antibiotics	Fluconazole	20	22	21	20	21	22	20	20

Values represent the mean of triplicates.

Both the pigments showed the better inhibitory effect against *E. coli*, *B. subtilis* and *S. aureus* and no activity against *P. aeruginosa*. β-carotene alone showed inhibitory action on the selected fungi *C. albicans*.

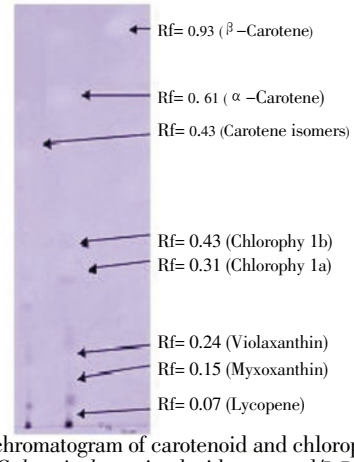

Figure 2. TLC chromatogram of carotenoid and chlorophyll extracts separated from *C. humicola*, stained with 0.5 mmol/L DPPH in methanolic solution.

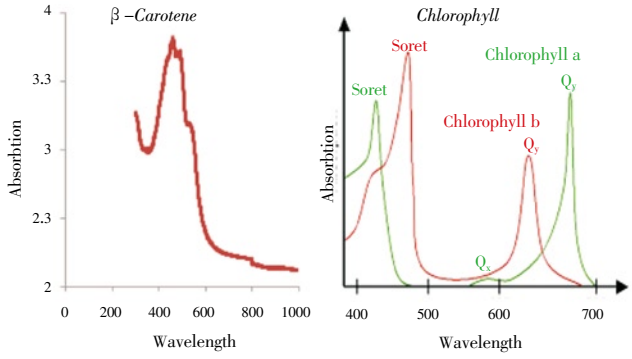

Figure 3. UV-Vis spectrum of chromatographic algal pigments shows the λ max 462 for β-carotene, λ max 425, 660 for chlorophyll a and λ max 458, 645 for chlorophyll b.

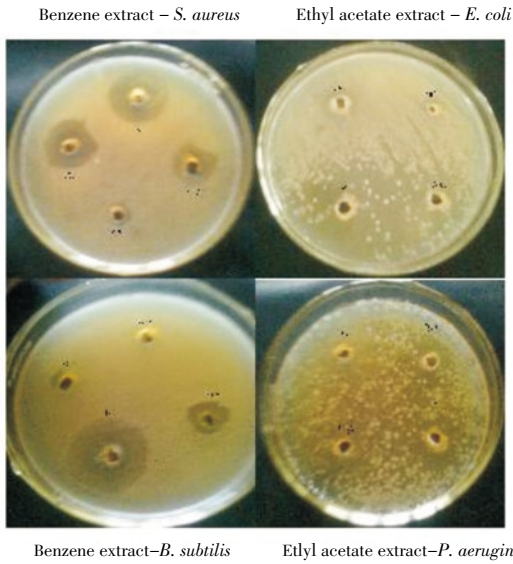

Figure 4. The plates showed antimicrobial activity of various organic extracts of *C. humicola*. Zone of inhibition was measured in mm. A-Amphicillin, M-Methicillin, P-Penicillin.

Table 4
Inhibitory effect of purified pigments (25 μg/well) β-carotene and chlorophyll of *C. humicola* on selected microorganism (mm).

Microorganism		β-carotene	Chlorophyll a	Chlorophyll b
Bacteria	*B. subtilis*	7	12	12
	S. aureus	9	7	8
	E. coli	15	14	10
	P. aeruginosa	–	–	–
	S. typhimurium	11	8	7
	K. pneumoniae	9	10	9
	V. cholerae	10	11	11
Fungi	*C. albicans*	11	–	–
	A. niger	9	11	7
	A. flavus	8	7	9

Values are mean of triplicates.

4. Discussion

Algae and microalgae have become part of complementary medicine worldwide, because of their potential health benefits. Algae have been used for years in medicinal practices mostly in therapy of diverse pathologists. Our aim in this study is to identify the beneficiary effect of bioactive compounds produced by the selected green algal species *C. humicola*.

The organic extracts of *C. humicola* also showed a powerful effect against the pathogenic fungal strains. As similar to bacterial strains fungal strains *A. niger* and *A. flavus* are also more susceptible to benzene extract and *C. albicans* with maximal inhibition zones and MIC values. It seems that the antimicrobial activity is related to the amounts of lipophilic, lipid soluble phenol compounds and pigments available in the *C. humicola* organic extracts. The present results agreed with the previous results[12,13] which explained that the antibacterial effects of phenolic compounds are concentration dependent. Similar results were obtained with the antibacterial activity of *Spirulina maxima* (*S. maxima*) organic extracts that were assayed against six bacterial strains (*B. subtilis, B. cereus, S. aureus, Micrococcus luteus, K. pneumoniae, Serratia marcescens*) by evaluating the inhibition zones, zone diameter and MIC values. Generally, all *S.maxima* extracts were found to be effective against all tested bacteria and these antibacterial activities was found to be dose depended[14,15].

The data seemed to indicate that the antimicrobial activity of the compound extracts was related to the concentration of lipophilic and phenolic compounds containing in *C. humicola* extracts. A large number of algal extract products have been found to have antimicrobial activity, many of the structures were identified as fatty acids and hydroxyl unsaturated fatty acids, glycolipid, steroid, phenolics and terpenoids[16,17]. Lauric acid, palmitic acid, linolenic acid, oleic acid, stearic acids are known to be potential antibiotic or antifungal agents[18-20]. There are various reports says that linolenic acid was active against *Mycobaterium*

smegmatis and *Mycobacterium fortuitum*. Results showed that gram positive bacteria were more susceptible than the gram negative bacteria. These difference of the fatty acid sensitivities between gram positive and gram negative bacteria may result from the permeability of the outer membrane of gram negative bacteria are more resistant to inactivation by medium and long chain fattyacids than gram positive bacteria[21,22].

The seaweeds are the rich source of a number of antimicrobial compounds. Many authors have found antibacterial activities of micro algae are due to fatty acids. The fatty acids (PUFA) in litter fall of mangroves might have positive role on the growth of fishes and shrimps. Seaweeds are widely used by all sections like pharmaceutical industry, medicinal applications *etc*. The fatty acids from *Borticoccus braunii*, has previously been reported to have significant antimicrobial activity , says there were a mixture of free fatty acids including linolenic, oleic, linolin, and hexadecanoic acid[23-25]. They suggested that fatty acids exhibit cytotoxic activity against microbial planktons through damaging the plasma membranes. When these organisms were treated with deleterious concentrations of fatty acids, a remarkable elevation of extracellular potassium (K) was detected in the culture medium; indicating leakage of intracellular K as a result of damage to the plasma membranes. The suggested sequence of cytotoxic effects is that plasma membranes are affected, leading to a change in membrane permeability. Severe damage to the plasma membranes would give rise to a disruption of the stressed cells. Similarly various results suggested that fatty acids are able to change the permeability of the cell membrane, interact with proteins and lipids of the cell membrane, inhibit special enzymes or form a layer around the cells[13,26].

All these mechanisms could result in bacteriostatic and/or bactericidal activity and improve the survival of microalgae in their environment. In context with these reports, showed that lysozyme can be modified to be highly potent antimicrobial agent against gram negative bacteria that possess a large lipopolysaccharide layer thus hindering the entry of the lytic action of lysozyme. Consistently, it is reported that the antibiotic activity of some algal species could be attributed to the presence of a mixture of organic acids. The free fatty acids were also reported to be potent allelopathic agents[27,28].

The findings of *Ulva lactuca* grow under laboratory conditions are rich in non- and moderate-polar compounds including: carotenoids, chlorophyll derived and phenolic compounds and possesses antioxidant and antibacterial activity. The production of these components might promote in algae by changing the culture conditions to overproduce the targeted molecules[14,29]. Consequently, they are valuably increasing shelf life of food stuffs and replacing synthetic antioxidants as well as preventing cellular damage, cause of aging and human diseases. In addition, antibacterial activity and food colorant properties can therefore be used as natural preservative ingredient in food and in pharmaceutical industry[30-36].

The reason for antimicrobial activity of carotenoids was still poorly understood. Reports suggested that β-carotene could lead to the accumulation of lysozyme, and antibacterial immune enzyme that digests bacterial cell walls, therefore generate the antibacterial activity[37]. As mentioned above, the composition of other carotenoids such as β-cryptoxanthin, violaxanthin isomers, lycopene, β-carotene etc, it could be inferred that its antimicrobial activities might be highly with these compounds[38].

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals.

Our goal in this study is to reveal the biological production of bioactive compounds by some green algal species. This study confirms that the green algae *C. humicola* possess biological active compounds. The phytochemicals in the selected algae shows various beneficial effects which was supported by variety of literature. In the present study the used organic solvents are capable to extract the various bioactive compounds from the algae. Similarly the major colouring pigments of green algae, β-carotene and Chlorophyll, also act as an effective microbial growth inhibitors. From previous studies in addition to the current results, it could be concluded that the antibiotic production is largely dependent on the algal species.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Patra JK, Rath SK, Jena K, Rathod VK, Thatoi HN. Evaluation of antioxidant and antimicrobial activity of seaweed (Sargassum sp.) extract: A study on inhibition of Glutathione-Stransferase activity. *Turkish J Biol* 2008; **32**: 119-125.

[2] Taskin E, Ozturk M, Taskin E, Kurt O. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *Afr J Biotechnol* 2007; **6**: 2746-2751.

[3] Rao M, Malhotra S, Fatma T, Rattan A. Antimycobacterial activity

from cyanobacterial extracts and phytochemical screening of methanol extract of *Hapalosiphon*. *Pharm Biol* 2007; **45**: 88–93.

[4] Bhadury P, Wright PC. Exploitation of marine algae: biogenic compounds for potential antifouling applications. *Planta* 2007; **219**: 561– 578.

[5] Zhao J, Zhang B, Zhang Y. Chlorophytes of biological soil crusts in Gurbantunggut Desert, Xinjiang Autonomous Region. *Front Biol China* 2005; **23**(2): 189–193

[6] Spolare PC, Joannis E, Duran, Isambert A. Commercial applications of microalgae. *J Biosci Bioeng* 2006; **101**; 87–96.

[7] Bhat VB, Madyastha KM. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. *Biochem Biophys Res Commun* 2001; **285**: 262–266.

[8] Bischoff HW, Bold HC. Psychological studies. 4– Some soil algae from Enchanted rock and related algal species. *Univ Texa Publ* 1963; **6318**: 32–36.

[9] Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembrane. *Methods Enzymol* 1987; **147**: 350–382.

[10] Srinivasan KN, Devarajan C, Mohanasundari V, Chinthambi Nandakumar N. Antibacterial, preliminary phytochemical and pharmocognostical screening on the leaves of *Vicoa indica* (L) DC. *Iran J Pharmacol therepeutics* 2007; **6**: 109–113.

[11] National Committee for Clinical, Laboratory Standards. R*eference method for broth dilution antifungal susceptibility testing of filamentous fungi; Apporved standard.* Pennsylvania: Wayne; 2002.

[12] Ozdemir G, Karabay NU, Dalay CM, Pazarbasi B. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Phytother Res* 2004; **18**: 754–757.

[13] Mundt S, Kreitlow S, Jansen R. Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. *J Appl Phycol* 2003; **15**: 263–267.

[14] Hanaa H Abd El-Baky, Farouk K El Baz1, Gamal S El-Baroty. Characterization of nutraceutical compounds in blue green alga Spirulina maxima. *J Med Plants Res* 2008; **2**(10): 292–300.

[15] Seenivasan R, Indu H, Archana G, Geetha S. The antibacterial activity of some marine algae from south east coast of India. *J Phar Res* 2010; **8**: 1907–1912.

[16] Thillairajasekar K, Duraipandiyan V, Perumal P, Ignacimuthu S. Antimicrobial activity of *Trichodesmium erythraeum* (Ehr.) (microalgae) from South East coast of Tamil Nadu, India. *Int J Integr Biol* 2009; **5**(3): 167–170.

[17] Luesch H, Yoshida WY, Moore RE, Paul VJ, Mooberry SL. Isolation, structure determination and biological activity of Lyngbyabellin A from the marine Cyanobacterium Lyngbya majuscula. *J Nat Prod* 2000; **63**: 611–615.

[18] MacMillan JB, Ernst-Russell MA, De Roop JS, Molinski TF. Lobocyclamides A–C, lipopeptides from a cryptic cyanobacterial mat containing *Lyngbya confervoides*. *J Org Chem* 2002; **67**: 8210–8215.

[19] Tan LT. Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochem* 2007; **68**: 954–79.

[20] Shanab SMM. Bioactive Allelo-chemical compounds from *Oscillatoria* Species (Egyptian isolates). *Int J Agric Biol* 2007; **9**(4): 617– 621.

[21] Prashantkumar P, Angadi SB, Vidyasagar GM. Antimicrobial activity of blue-green and green algae. *Ind J of Pharm Sci* 2006; **68**(5): 647–648

[22] Robinson I, Junqua G, Coillie RV, Thomas O. Trends in the detection of pharmaceutical products, and their impact and mitigation in water and wastewater in North America. *Anal Bioanal Chem* 2007; **387**: 1143–1151.

[23] Benkendorff K, Davis AR, Rogers CN, Bremner JB. Free fatty acids 1 and 2 sterols in the benthic spawn of aquatic molluscs, and their associated antimicrobial 3 properties. *J Exp Mar Biol Ecol* 2005; **316**: 29–44.

[24] Abd El-Baky HH, El Baz FK, El-Baroty GS. Over-production of lipid rich in _-linolenic acid by blue green alga spirulina maxima and its Inhibitory effect on carcinoma cells. *Adv Food Sci* 2006; **4**: 206–212.

[25] Athukorale DP. The health giving benefits of seaweed. Colombo: Leader Publication (PVT) Ltd; 2002.

[26] Mar Drugs, Kaushik P, Chauhan A. *In vitro* antibacterial activity of laboratory grown culture of *Spirulina platensis*. *Ind J Microbiol* 2008; **48**: 348–352.

[27] Ramsewak RS, Nair MG, Murugesan S, Mattson WJ, Zasada J. Bioactive natural products and phytoceuticals. *J Agric Food Chem* 2001; **49**(12): 5852–5856.

[28] Smit AJ. Medicinal and pharmaceutical uses of seaweed natural products: A review. *J Appl Phycol* 2004; **16**: 245–262.

[29] Awad NE. Biologically active steroid from the green alga *Ulva lactuca*. *Phytother Res* 2000; **14**: 641–643.

[30] Ajit Kandale, Meena AK, Rao MM, Panda P, Mangal AK, Reddy G, et al. Marine algae: An Introduction, food value and medicinal uses. *J Pharm Res* 2011; **4**(1): 219–221.

[31] Zubia M, Fabre MS, Kerjean V, Le Lann K, Stiger-Pouvreau V, Fauchon M, et al. Antioxidant and antitumor activities of some Phaeophyta from Brittany Coasts. *Food Chem* 2001; **116**: 693– 701.

[32] Saad S, Taher M, Susanti D, Qaralleh H, Rahim NABA. Antimicrobial activity of mangrove plant (*Lumnitzera littorea*). *Asian Pac J Trop Med* 2011; **4**(7): 523–525.

[33] Raja RDA, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. Antibacterial activity of selected ethnomedicinal plants from South India. *Asian Pac J Trop Med* 2011; **4**(5): 375–378.

[34] Bragadeeswaran S, Priyadharshini S, Prabhu K, Rani SRS. Antimicrobial and hemolytic activity of fish epidermal mucus *Cynoglossus arel* and *Arius caelatus*. *Asian Pac J Trop Med* 2011; **4**(4): 305–309.

[35] Madhumitha G, Saral AM. Preliminary phytochemical analysis, antibacterial, antifungal and anticandidal activities of successive extracts of *Crossandra infundibuliformis*. *Asian Pac J Trop Med* 2011; **4**(3): 192–195.

[36] Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L. *Asian Pac J Trop Med* 2011; **4**(3): 196–200.

[37] Cucco MB, Guasco G, Malacarne Oltonelli R. Effects of β -carotene on adult immune condition and antibacterial activity in the eggs of *Grey partridge*, *Perdix perdix*. *Comp Biochem Physiol A Mol Integr Physiol* 2007; **147**: 1038–1046.

[38] Nengguo Tao, Yumci Gao, yucjin Liu, Fei Ge. Carotenoids from the peel of Shatian Pummelo (*Citrus grandis* Osbeek) and its antimicrobial activity. *American-Euresian J Agri Environ Sci* 2010; **7**(1): 110–115.