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Research Article

NUTRITIONAL PROFILE, ANTIOXIDANT, ANTIMICROBIAL POTENTIAL, AND BIOACTIVES PROFILE OF *CHLORELLA EMERSONII* KJ725233

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ABSTRACT

Objective: The present study revolved around the nutritional evaluation of *Chlorella emersonii* KJ725233, a novel non-fastidious *Chlorella* strain that was isolated from the Western regions of Maharashtra, India, for a probable use as a food supplement.

Methods: The nutritional composition of *C. emersonii* KJ725233 was evaluated in terms of total protein, total lipids, and total carbohydrates content along with its mineral composition. Furthermore, the effect of different solvents on the extraction of the bioactives and hence the antioxidant and antimicrobial activity of *C. emersonii* KJ725233 was also analyzed. The bioactives extracted in the organic solvents were further identified by gas chromatography–high-resolution mass spectrometry (GC-HRMS).

Results: As reported in the literature for commercial *Chlorella* powder, *C. emersonii* KJ725233 was found to contain an equivalent concentration of protein, three-fold lipid content, i.e. 36.48±0.52% and 43.58±0.72%, respectively; along with 17.17±0.91% carbohydrates and 6.62±0.16% mineral content. Methanolic content was not only found to contain the highest antioxidant, radical scavenging potential as well as total phenolic content but also exhibited a strong antimicrobial potential against the tested Gram-positive, Gram-negative bacterial and fungal strains. Moreover, sterols, hydrocarbons, and fatty acid methyl esters known antimicrobials and antioxidants were identified when the organic extracts of the microalgae dried biomass were subjected to GC-HRMS analysis.

Conclusion: With a well-balanced nutritional composition together with the reservoir of antioxidants and antimicrobials, this native novel isolate of *Chlorella* thus emerges as a potential food supplement whose consumption would not only enhance the dietary value along with providing antiaging benefits.

Keywords: *Chlorella emersonii* KJ725233, Nutritional profile, Antioxidant, Antimicrobial, Gas chromatography–high-resolution mass spectrometry.

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INTRODUCTION

Chlorella is a widely commercialized eukaryotic green alga used as health food and feed supplement as well as in cosmetic and pharmaceutical applications [1]. Over 2500 tonnes of dried *Chlorella* is produced every year by the United States, Japan, China, Taiwan, and Indonesia [2]. For the myriad of its nutrients such as carotenoids, vitamins, and minerals, *Chlorella* biomass is packaged and marketed as tablets, capsules, powders, and liquids [3]. Commercial *Chlorella* production worldwide exceeds by four folds per tonne/year as compared to that of other microalgae like *Dunaliella* [4]. *Chlorella* biomass has been formulated in feed and food products to provide color effect as well as nutritional supplementation. *Chlorella* biomass has recently being used as an additive to fermented milk and yoghurt to enhance the viability of probiotic bacteria as well as in pasta products for improving its nutritional quality [5]. In addition to this, *Chlorella vulgaris* biomass was incorporated into butter cookies to give them an attractive appearance with improved textural characteristics with an unaltered taste [6].

Chlorella is primarily advertised for sale for the water-soluble *Chlorella* growth factor which exhibits a variety of health benefits such as increasing insulin sensitivity, strengthening immune system function, prevention of stress-induced ulcers, and pregnancy-associated anemia [4]. *Chlorella* is known to have the potential to relieve symptoms in people suffering from fibromyalgia, hypertension, and ulcerative colitis [2]. It is also an important health-promoting factor with an antitumor and preventive action against atherosclerosis and hypercholesterolemia [6].

Chlorella strains are cosmopolitan in occurrence due to their ability to adapt to a diverse range of habitats such as soil, freshwater lakes, ponds, marine as well as brackish waters in addition to the polar areas [7,8]. Due to the highest chlorophyll content, a fast growth rate coupled with its distribution over varied environmental conditions, *Chlorella* strains are considered as competent candidates for the synthesis of a diverse array of metabolic compounds [8-12]. To survive the inconsistent physical and chemical parameters, they have developed various adaptive and defensive mechanisms which include synthesis of a diverse array of metabolic compounds [13]. Due to the wide occurrence, rapid growth as well as nutritious qualities of *Chlorella* strains, a novel strain of the microalga was chosen for the present study to explore its potential as a food supplement.

A novel isolate, *Chlorella emersonii* KJ725233, has been isolated from a freshwater source from the Western region of Maharashtra, India [14]. In comparison to *Chlorella vulgaris* and *Chlorella pyrenoidosa,* two of the dominant *Chlorella* species currently in use in food industry which require specific growth requirements such as temperature as well as illumination conditions, *C. emersonii* KJ725233 is non- fastidious growing at temperatures ranging from 25 to 44°C [14-16]. Furthermore, an economical mass culturing method for fostering *C. emersonii* KJ725233 has been developed using low-cost cultivation media which can be reused with minimal energy inputs [14]. Thus, the present study deals with the determination of its nutritional as well as a biochemical composition along with the biological activity of the bioactives of a novel isolate – *C. emersonii* KJ725233.

MATERIALS AND METHODS

Materials

All the chemicals used were of analytical grade and were purchased from SD Fine-Chem Ltd., Mumbai, India. 2,2-diphenyl-2-picrylhydrazyl

(DPPH), Mueller Hinton agar, nutrient broth, nutrient agar, Sabourauds broth (SAB), Sabourauds agar (SAB agar), and gentamicin were obtained from HiMedia, Mumbai, India. Fluka 150 mg (Cipla) was purchased from a local chemist, Mumbai.

Methods

Microalga culturing

C. emersonii KJ725233 was isolated from a freshwater source from the Western region of Maharashtra, India. It was identified morphologically as well as by 18s rDNA sequencing [14]. The alga was mass cultured in 5 L BG-11 medium at 30±1°C with a 12 h photoperiod as well as aeration for a period of 30 days.

Nutritional assessment of C. emersonii KJ725233

After 30 days of growth, the biomass was harvested by centrifuging (Eppendorf, 5810R, Germany) the culture at 5000 rpm for 20 min. Nutritional composition of *C. emersonii* KJ725233 was determined in terms of its elemental composition, protein, lipid, and carbohydrate content. The moisture and ash content were determined gravimetrically by drying the biomass at 110°C and 550°C, respectively, until constant weight was obtained [17]. The ash obtained was then subjected to acid digestion with aqua regia and analyzed by ICP-AES to determine the elemental composition [18]. The total protein content was determined by the Bradford's assay after extraction in 1 N NaOH [19]. The total lipid content was determined gravimetrically as previously described [20]. Carbohydrate levels were determined by anthrone-sulfuric acid method after extraction in 2.5 M HCl at 80°C for 3 h [17-21].

Preparation of algal extracts

Approximately 0.2 g of dried powder was suspended in distilled as well as organic solvents (methanol, acetone, chloroform, diethyl ether, and ethyl acetate) at a concentration of 0.1 g/ml. The suspensions were sonicated (LABMAN sonicator 40 W) for 40 min. The solutions obtained were centrifuged and supernatants pooled after three successive extractions. These were dried at 28±1°C and then reconstituted in absolute dimethyl sulfoxide (DMSO).

Determination of *in vitro* antioxidant activity

Phosphomolybdenum assay

The total antioxidant capacity (TAC) was determined by slight modifications in the previously described method [22]. To 30 µl of the different solvent extracts, 300 µl of TAC reagent (0.6 mM sulfuric acid, 28 mM sodium sulfate, and 4 mM ammonium molybdate) was added in 96-well microtiter plate and incubated in a water bath at 95°C for 90 min. After incubation, the absorbance was read at 695 nm. Ascorbic acid was used as a standard, and the TAC was expressed as mg ascorbic acid equivalent per g dried biomass (mg AAE/g DW). The tests were carried out in triplicates, and the values are expressed as mean ± SD.

Ferric reducing antioxidant potential (FRAP)

The reducing potential of the alga was evaluated by the potassium ferricyanide method according to standard procedure [23] with slight modifications. 125 µl of 1% potassium ferricyanide was added to 50 µl of the different solvent extracts in a 96-well microtiter plate and incubated at 50°C for 20 min. After incubation, 125 µl of 10% trichloroacetic acid was added and then 100 µl of this reaction mixture was transferred to fresh wells to which an equal volume of distilled water was added. Finally, after addition of 20 µl of 0.1% ferric chloride, the absorbance was read at 700 nm. Ascorbic acid was used as a standard. The reducing potential was expressed as mg AAE per g dried biomass (mg AAE/g DW). The tests were carried out in triplicates, and the values are expressed as mean ± SD.

2,2-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH)

Aliquots of 150 µL of various concentrations (2-10 mg/ml) of the different solvent extracts were mixed with an equal volume of 0.2 mM methanolic DPPH [24]. The reaction mixture was incubated in the dark at 28±1°C for 30 min. After incubation, the absorbance was measured at 520 nm. The free radical scavenging activity was then calculated as percentage inhibition by the following formula:

$$\text{Percent inhibition} = [(A_{(blank)}-A_{(test)})/A_{(blank)}]*100$$

IC50 (mg/ml) for the extracts was determined by plotting a graph of percentage inhibition against extract concentration. The tests were carried out in triplicates, and the values were expressed as mean ± SD.

Determination of total phenolic content

The total phenolic content was studied by Folin–Ciocalteau method [25]. 25 µl of 1 N FCR was added to 50 µl of the extracts followed by the addition of 125 µl of saturated sodium carbonate. The reaction mixture was incubated in the dark at 28±1°C for 30 min after which the absorbance was measured at 765 nm. The phenolic content was expressed as mg gallic acid equivalent per g dried biomass (mg GAE/g DW). The tests were carried out in triplicates and the values are expressed as mean ± SD.

Determination of total flavonoid content

The total flavonoid content was determined by the aluminum trichloride method [26]. In a 96-well microtiter plate, 150 µl of 2% (w/v) $AlCl_3$ was added to an equal volume of the different solvent extracts and incubated in the dark at 28±1°C for 30 min. After incubation, the absorbance was measured at 415 nm. Quercetin was used as a standard with methanol as blank. The total flavonoids content was expressed as mg quercetin equivalent per g dried biomass (mg QE/g DW). The tests were carried out in triplicates and the values are expressed as mean ± SD.

Antimicrobial activity

The antimicrobial activity of *C. emersonii* KJ725233 extracts was determined against *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 6908), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Candida albicans* (MTCC 227), and *Aspergillus niger* (MTCC 1344) by the agar well-diffusion method. 1 mg/ml gentamycin was used as positive control for bacteria whereas 15 mg/ml fluconazole was used for yeast and fungi; DMSO was used as a negative control.

Gas chromatography – high resolution mass spectrometry (GC- HRMS) analysis

Qualitative analysis of all the organic extracts was carried out by GC- HRMS analysis using GC (Agilent Technologies, USA) equipped with AccuTOF. Compounds were separated on HP-5 MS capillary column having 5% phenyl polysiloxane as stationary phase with a column length of 30 m, internal diameter 0.32 mm, and film thickness 0.25 µm. 1 µl of the sample was injected in the split ratio of 10:1, the injector and transfer line temperature was maintained at 250°C and 260°C, while the ion source temperature was 200°C. Oven temperature was programmed from 80 to 280°C with a rise of 10°C/min; flow rate of carrier gas helium was 1 ml/min. Compounds were identified comparing their retention times and mass fragmentation patterns with the data of standards at the NIST library.

Table 1: Nutritional composition of *Chlorella emersonii* KJ725233

Ash (%)	Moisture (%)	Proteins (g%)	Lipids (g%)	Carbohydrates (g%)
6.62±0.16	88.19±0.17	36.48±0.52	43.58±0.47	17.17±0.91

Values are expressed as mean±SD for triplicate experiments. Values are significant at $P<0.05$

RESULTS AND DISCUSSION

Nutritional composition

The nutritional value of *C. emersonii* KJ725233 was evaluated in terms of total lipids, proteins, carbohydrates as well as elemental composition and is presented in Table 1. The lipid content of the microalga was found to be 43.58 g/100 g which is three folds that of a commercial *Chlorella vulgaris* powder as earlier reported. With a protein content equal to that of the commercial product; the alga under consideration scores over with its low carbohydrate content. Since algal carbohydrates are known to provide anticoagulants, antivirals, dietary fibers as well as antioxidants; a higher content is not preferred as greater quantities of carbohydrates correspond to lesser fractions of other macronutrients in the supplement, especially proteins [4].

The dietary ash content is important in terms of the minerals and trace elements it can provide; however, a low ash content (<10% of the diet) is recommended in nutraceuticals due to the side effects it can cause. Ash content of *C. emersonii* KJ725233 as represented in Table 1 was found to be 6.6168 g/100 g which falls in the range as recommended for algal products sold in the USA (45% dry weight) [17]. Table 2 represents that to determine the levels of macro as well as micronutrients such as sodium (Na), potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg); trace elements included zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) in *C. emersonii* KJ725233.

When compared with values of commercial products, it is evident that the isolate once again stands apart from the existing ones. Its calcium content was eight folds higher as compared to that reported for another *Chlorella* species. On the other hand, phosphorus was fourfold, and magnesium was found to be six folds greater than that reported for the *Chlorella* species [17]. Similarly, a tenfold difference in potassium content with that reported for *Chlorella* species was also observed [18]. Thus, the consumption of *C. emersonii* KJ725233 could overall contribute to the essential mineral requirements of the body.

Antioxidant properties of *C. emersonii* KJ725233

The total antioxidant potential of *C. emersonii* KJ725233 was determined by the phosphomolybdenum assay based on the reduction of Mo (IV) to Mo (V) by the sample to form a green phosphate/Mo complex with a maximum absorption at 695 nm [27]. The phosphomolybdenum method measures the antioxidant activity due to phenolics, ascorbic acid, α-tocopherol, carotenoids, butylhydroxytoluene as well as reduced glutathione [22,28]. Table 3 shows the antioxidant capacity of the different solvent extracts wherein it can be seen that the maximum activity of 22.4±0.1 mg/g is exhibited by chloroform extract whereas ethyl acetate shows the least antioxidant potential of 8.49±0.51 mg/g AAE.

Table 2: Mineral composition of *C. emersonii* KJ725233

Mineral	mg/100 gDW
Na	0.76±0.04
K	0.46±0.05
P	0.87±0.03
Ca	2.77±0.24
Mg	0.61±0.03
Zn	0.03±0.01
Fe	0.15±0.03
Mn	0.8±0.02
Cu	0.02±0.002

Values are expressed as mean±SD for triplicate experiments. Values are significant at P<0.05. *C. emersonii: Chlorella emersonii*

The reducing power for the extracts was determined by their ability to reduce Fe^{+3} to Fe^{+2} by the action of antioxidants. The water extract exhibited highest reducing power, i.e., 14.86±0.60 mg/g AAE whereas the least was shown by chloroform extract, i.e., 8.08±0.12 mg/g AAE. The reducing power for methanolic extract of *C. emersonii* KJ725233 was found to be 11.02±0.35 mg/g AAE which is twenty folds higher as compared to 0.562±0.172 and 0.73±0.026 mg/g AAE reported for methanolic extract of *Chlorella marina* [23,29].

The hydrogen donating ability of the extracts on DPPH leads to a loss of violet color forming the reduced substance DPPH (non-radical) [30]. IC_{50} values for the different extracts were calculated by plotting a graph of percent radical scavenging against the concentrations of the extract (2-10 mg) and are represented in Table 2. The IC_{50} is inversely proportional to the antioxidant activity, i.e., the lower the IC_{50}, the greater is the antioxidant potential of the extract. Phenols and flavonoids have the ability to donate their hydrogen and thus function as strong free radical scavengers [31]. Methanolic extract, thus, exhibited the lowest IC_{50}, i.e., 6.59±0.33 mg/ml as compared to the other solvent extracts. This, thus, correlates to the concentration of phenols and flavonoids in the methanolic extract as compared to the other solvent extracts. The IC_{50} for the aqueous extract of *C. emersonii* KJ725233 was found to be 7.55±0.09 mg/ml dried biomass which is less than that reported for commercially available tablets of *Chlorella pyrenoidosa* (Sun chlorella), i.e., 9.62 mg/ml as earlier reported [32].

Total phenolic as well as flavonoid content of *C. emersonii* KJ725233 different organic extracts is shown in Table 3. The yield varied with the different organic solvents used for the extraction aqueous extracts showed a higher amount of phenols 6.60±0.06 mg/g GAE as compared to organic extracts, for which the phenolic content ranged from 2.20±0.21 to 5.19±0.10 mg/g GAE. *C. emersonii* KJ725233 contains an appreciable amount of phenols wherein the phenolic content in the methanolic extract 5.19±0.10 mg/g GAE is eight folds higher than that reported for the methanolic extract of *C. marina* 0.647 mg/g GAE [29]. In the present investigation, the total flavonoid content ranged from 1.20±0.09 to 24.60±1.18 mg/g quercetin equivalence.

Antimicrobial activity

The antimicrobial activity of the solvent extracts of *C. emersonii* KJ725233 was evaluated against selected bacterial as well as fungal clinical isolates by the agar well-diffusion method. Except the aqueous, all the organic extracts exhibited considerable antimicrobial activity ranging from 11.46±0.15 to 19.36±0.51 mm against selected bacteria as well as the yeast while no effect on other fungal species as seen in Table 4. Results indicated that the methanolic, chloroform, and ethyl acetate extracts were effective against both Gram-positive as well

Table 3: Comparison of the antioxidant activity (TAC, FRAP and DPPH), total phenolic content and total flavonoid content of *C. emersonii* KJ725233 in different solvents

Solvents	TAC[a]	FRAP[b]	DPPH[c]	TPC[d]	TFC[e]
Water	17.53±0.35	14.86±0.60	7.8±0.08	6.60±0.06	1.20±0.09
Methanol	16.8±0.26	11.02±0.35	6.59±0.33	5.19±0.10	24.60±1.18
Acetone	13.63±1.33	8.20±1.13	13.82±0.35	2.20±0.21	4.33±0.29
Chloroform	22.4±0.1	8.08±0.12	6.98±0.80	4.624±0.31	15.62±1.07
Ethyl acetate	8.49±0.51	9.53±0.38	16.25±0.73	2.75±0.19	8.70±0.34
Diethyl ether	11.9±0.44	8.59±0.51	13.38±0.25	2.96±0.14	4.79±0.47

Values are expressed as mean±standard deviation for triplicate experiments. Values are significant at P<0.05. [a]TAC expressed as mg AAE/g DW, [b]FRAP expressed as mg AAE/g DW, [c]DPPH: IC50 expressed as mg [D]W/ml, [d]Total phenolic content expressed as mg GAE/g DW, [e]Total flavonoids expressed as mg QE/g DW

as Gram-negative organisms with a zone of inhibition ranging from 11.46±0.15 mm to 15.36±0.45 mm, whereas the acetone extract and the diethyl ether extract showed activity only against the Gram-positive and Gram-negative organisms, respectively. This could be attributed to the presence of antimicrobial compounds such as phytol, hexadecane, and 1-docosene in methanolic, chloroform, and ethyl acetate extracts as reported in Table 5. The inability of the acetone extract to inhibit the growth Gram-negative and diethyl ether extract to inhibit Gram- positive

Table 4: Comparison of the antimicrobial activity of *C. emersonii* KJ725233 in different solvents

Extracts/ control	*B. subtilis* MTCC 441	*E. coli* MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 6908	*C. albicans* MTCC 227	*A. niger* MTCC 1344
Methanol	11.46±0.15	12.56±0.20	15.3±0.36	13.8±0.3	18.1±0.86	-
Acetone	12.23±0.35	-	-	19.36±0.51	14.3±0.66	-
Chloroform	11.66±0.51	13.83±0.25	15.36±0.45	12.46±0.25	13.66±0.57	-
Ethyl acetate	14.23±0.35	12.5±0.4	12.5±0.4	13.06±0.15	16.42±0.59	-
Diethyl ether	-	12.4±0.36	11.13±0.15	-	12.8±0.32	-
Gentamycin[a]	22.23±0.25	25.66±0.58	25±0.1	30.33±0.58	NA	NA
Fluconazole[b]	NA	NA	NA	NA	12.9±0.45	25.5±0.86
DMSO[c]	-	-	-	-	-	-

[a]Positive control against bacterial strains, [b]positive control against fungi and yeast, [c]negative control, "-" no zone of inhibition, NA: Not applicable

Table 5: Bioactives extracted in different organic solvent extracts of *C. emersonii* KJ725233

Compounds	Extract	RT[a]	% area
Phytol	Methanol	21.51	64.77
	Acetone	27.34	12.77
	Chloroform	21.89	46.73
	Ethyl acetate	21.92	23.03
	Diethyl ether	21.94	4.45
1-Docosene	Methanol	24.74	1.26
		26.37	8.04
	Acetone	32.95	20.30
		35.15	10.12
	Chloroform	28.07	16.19
	Ethyl acetate	28.07	13.28
	Diethyl ether	28.12	16.19
Hexadecane	Methanol	14.48	2.88
	Ethyl acetate	14.45	3.96
	Chloroform	14.45	1.86
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Acetone	21.78	9.50
		22.51	2.73
	Chloroform	16.35	9.50
		16.98	3.30
	Diethyl ether	16.38	3.05
2 – Propanol, 1,1,1-trichloro-2-methyl	Acetone	4.45	26.59
Butylhydroxytoluene	Diethyl ether	11.85	9.45
2-Butanone, 4-hydroxy-	Ethyl acetate	3.17	9.26
1-Octacosanol	Chloroform	30.34	6.67
9,12 -Octadecadienoic acid, methyl ester, (E, E')-	Methanol	21.05	5.76
2-Isopropyl-5-methyl-1-heptanol	Diethyl ether	8.47	5.5
1-Heptanol, 2, 4 – dimethyl-, (2S,4R)-(-)-	Ethyl acetate	4.84	5.39
n-Hexadecanoic acid	Diethyl ether	18.48	5.05
Hexane, 3,3,4,4-Tetramethyl-	Ethyl acetate	3.83	4.6
Bis [3-cycloehexylidene-2-oxocyclohexyl] methane	Methanol	25.05	4.6
		25.17	0.03
Octatriacontylpentafluoropropionate	Ethyl acetate	30.35	4.23
Phenol, 2,4-bis (1,1-dimethyethyl)-	Chloroform	11.78	2.98
Pentadecanoic acid, 14 – methyl –, methyl ester	Methanol	17.72	2.83
		20.93	0.02
Tetradecane	Diethyl ether	9.98	2.75
1-Nonene, 4,6,8 – trimethyl	Chloroform	8.59	2.45
		4.85	0.28
Nonadecane	Diethyl ether	14.47	2.33
Decane, 4 – methyl	Chloroform	3.85	2.29
Heptadecane	Chloroform	14.45	1.35
1,2-Benzenedicarboxylic acid, diisooctyl ester	Chloroform	28.73	2.24
1-Octene, 3,7-dimethyl-	Diethyl ether	4.85	0.07
1-Iodo-2-methylundecane	Diethyl ether	15.23	0.04
Undecane-5,7-dimethyl-	Diethyl ether	5.13	0.02
Dodecane	Diethyl ether	6.69	0.02
Oxalic acid, allyl tetradecyl ester	Diethyl ether	15.55	0.02
Dodecane, 4,6 – dimethyl-	Diethyl ether	7.44	0.01

[a]Retention time in minutes

could be due to the absence of hexadecane in these extracts (Table 5). The synergistic and antagonistic effect of the bioactives may play a role in the antimicrobial potential, and hence the presence of hexadecane may be mandatory for the extracts to be antibacterial against both Gram-positive as well as Gram-negative bacteria. Since the positive controls were broad-spectrum antibiotics, their antimicrobial activity against the tested micro-organisms was more as compared to that of the crude algal extracts.

GC-HRMS analysis of crude organic extracts of *C. emersonii* KJ725233

HRMS analysis is reported to provide twice the identification as compared to that of a low-resolution mass spectrometer [33]. Hence, even though gas chromatography–mass spectrometry (GC-MS) has been used to analyze biomass of micro as well as macroalgae, the present study utilized GC-HRMS for the purpose of analysis [32,34].

In the present study, 29 compounds were identified in the five different organic extracts as presented in Table 5. However, a majority of them (seventeen) contributed <5% to the total peak area of the respective chromatograms, and hence only the major compounds are referred herewith. Of these compounds, phytol and 1-docosene were common in all the organic extracts. Phytol, a major contributor (12-65%) of all the organic extracts, has been reported to be an antioxidant, antimicrobial, anti-inflammatory, anticancer, diuretic, anti-malarial as well as antimycobacterial whereas 1-docosene is a known anticancer as well as antimicrobial [35-38]. Hexadecane identified in methanol, chloroform, and ethyl acetate extracts is reported for antibacterial as well as antioxidant activities [39]. Butyl hydroxyl toluene, an excellent antioxidant, contributed 9.45% to the diethyl ether extract [40]. A variant of phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol has contributed about 2.73–9.5% in acetone, chloroform and diethyl ether extract. This compound is known antimicrobial as well as anti- inflammatory in nature [41].

In addition to these, a phenolic antioxidant, phenol,2,4- bis(1,1- dimethylethyl)-, identified in the chloroform extract is reported as an ultraviolet stabilizer as well as an antioxidant for hydrocarbon- based products such as the plastic coatings used for food packaging [42]. Phenol,2,4-bis(1,1-dimethyethyl)- has been identified as an additive in plastic packaging like Hostanox PAR 24 FF by GC-MS analysis [43]. 1,2-Benzenedicarboxylic acid, diisooctyl ester – another plasticizer was also detected in the chloroform extract [44].

In the present study, the acetone extract revealed an unusual peak of 2-propanol, 1,1,1-trichloro-2-methyl (chloretone) with a retention time 4.45 min that contributes to the extent of 26% of the total peak area. Chloretone is reported to have a mild anesthetic potential in addition to, the antibacterial as well as germicidal effect and hence used in pharmaceutical formulations. Literature reports the chemical synthesis of this compound by addition of chloroform and acetone in the presence of powdered potassium hydroxide as a catalyst [45]. Production of chloroform by microalgae under conditions of hypochlorite stress at temperatures 20°C and 25°C as well as under alkaline conditions is reported [46]. Production of chloroform by freshwater *Chlorella* species and blue green alga A*nabaena flos-aquae* has also been reported by Wachter and Andelman, 1984. [47]. Therefore, with the growth conditions employed for the present study, *Chlorella* might have synthesized chloroform which is further converted to chloretone during the extraction with acetone. This is further supported by the fact that the compound is detected only in the acetone extract and none of the other solvents. This hypothesis, however, requires a further extensive study to investigate the regulation of chloroform synthesis in *C. emersonii* KJ725233.

CONCLUSION

With economically attractive growth conditions, non-fastidious growth characteristics, and a commercially attractive nutrient profile *C. emersonii* KJ725233 presents itself as an inexpensive, commercially exploitable source of bioactives with potential applications in food industry. The nutritional as well as biological profile of this isolate when compared to existing commercial *Chlorella* powders as well as the wild type *Chlorella* strains reported in literature, clearly indicates the advantage of this isolate not only as protein-lipid but also as an antioxidant and antimicrobial source. The strain, thus, harbors a potential to substitute their synthetic equivalents currently used. Its use, therefore, as a potential food supplement and/or as a complete food would be able to improve bodily functions enhancing animal health.

AUTHOR'S CONTRIBUTION

Ms. Sneha Sunil Sawant – Experimentation, Results evaluation, Manuscript writing, Editing, final manuscript approval.

Dr. Mrs. Varsha Kelkar Mane – Experiment designing, result evaluations, manuscript revisions, final manuscript approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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