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RESEARCH PAPER

Microalga *Scenedesmus bajacalifornicus* BBKLP-07, a new source of bioactive compounds with in vitro pharmacological applications

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Abstract

Microalgae are photosynthetic eukaryotes which are primary producers in the food chain and also excellent sources for bioactive compounds such as alkaloids, flavonoids, phenols, saponins and other fine chemicals. In the present study, the microalga *Scenedesmus bajacalifornicus* BBKLP-07 was subjected to soxhlet extraction using solvents like chloroform, acetone, ethanol, methanol and aqueous solvents. All the solvents were tested for the presence of phytochemical constituents such as alkaloids, flavonoids, glycosides, phenols, lignin's, saponins, sterols, tannins, anthraquinone and reducing sugar using the standard procedures. Furthermore, all the crude extracts were subjected to antidiabetic, antioxidant, anti-inflammatory and antimicrobial activities. Antidiabetic activity of the microalgal extracts was observed maximum in Aqueous extract. Methanolic extracts have shown maximum antioxidant activity and chloroform extracts have exhibited highest anti-inflammatory effects. Antimicrobial activities were tested against *E.coli, S, typhi, C.perfringens* and *B.subtilis* bacteria and fungi *A.niger*, and *C. albicans*. Therefore, the green microalga *Scenedesmus bajacalifornicus* BBKLP-07 is a rich source of biological active compounds and nutraceuticals and can be exploited for commercial applications.

Keywords *Scenedesmus bajacalifornicus* · Microalgae · Antidiabetic · Anti-inflamatory · Antimicrobial · Phytochemical screening

Introduction

Microalgae are unicellular, photosynthetic organisms that play a key role in aquatic ecosystems; they grow in fresh water as well as in marine the environment. Approximately 40% of global photosynthesis is due to these microorganisms [15]. Freshwater microalgae are widely dispersed in rivers, lakes and polar water bodies. They exhibit a diverse range of cellular, structural, morphological and biochemical composition [12].

Microalgae are unique reservoirs of bioactive compounds and produce secondary metabolites, which are essential for cell metabolism [52]. These secondary metabolites include phenolic compounds, fatty acids amino acids, carotenoids, tannins, alkaloids, flavonoids and numerous other compounds which have pharmaceutical importance [55]. The secondary metabolites are synthesized in the microalgae due to the microalgal metabolisms. Microalgal metabolism responds to changes in the external environment such as temperature, pH and nutritional composition which in turn changes the intracellular environment of the organism. Thus, the manipulation of the culture conditions, or the presence or absence of certain nutrients, stimulates the biosynthesis of specific bioactive compound [10].

Several reports suggest that the investigation of microalgal metabolites is not only important to understand the nature of the organism but also to search for substances with possible applications to mankind in different fields of interest. Screening of extracts or isolation of metabolites from varieties of microalgae is a universal method for determining the biological activity of these components. Microalgae have been portrayed as rich sources of various bioactive compounds of commercial interest [55].

Among all these microalgae, *Scenedesmus*, which belongs to the order Chlorococcales of the family Scenedesmaceae is frequently dominant in freshwater lakes and rivers [17]. Many species of this genus are being used worldwide for various purposes due to their ability to adapt to harsh environmental conditions, ability to grow rapidly and

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ease of cultivation and handling [21]. Likewise, *Scenedesmus* sp. has been used in many biotechnological applications due to its high nutritional content and bioactivities [16]. Several reports suggest that over 15,000 compounds were extracted from microalgae including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, and flavonoids which are responsible for the source of antioxidant compounds with free radical scavenging activity.

Biologically active substances from several microalgae species have been extracted both as cell extracts and extracellular products which found to possess antimicrobial activities and these include carotenoids, lipids, polysaccharides, terpenoids and chlorophyll [1, 19]. These activities can be antibacterial, antifungal, antialgal and antiprotozoal. Although for several of these activities, the constituents are not yet known as their structures and identities are yet to be discovered [41]. The unique diversity of microalgae is responsible for its capability to be a valuable source of compounds with biotechnological potentials [36, 45]. These valuable compounds are natural substances that attract the attention of both scientists and industrialists due to their use in the development of biotechnology [5, 27]. Novel compounds with antibacterial, antiviral, and antifungal properties have been intensively investigated in microalgae over the last years [17]. Further, the extracts of various unicellular algae (e.g. *Scenedesmus* sp., *Chlorella vulgaris, Chlorella pyrenoidosa*) were proved to have antibacterial activity in vitro against both Gram-positive and Gram-negative bacteria (Chacon et al. 2010). It has also been reported that a wide range of antifungal activities were obtained from extracts of green algae, Diatoms and Dinoflagellates [9]. Brown-algal polyphenols and phlorotannins worked as antioxidants, antibacterial and anti-algal compounds [23]

Antioxidant compounds play an important role against various diseases (e.g., chronic inflammation, atherosclerosis, cancer and cardiovascular disorders) and ageing processes, which explains their considerable commercial potential in medicine, food production and the cosmetic industry. The mechanisms of these anti-activities are carried out by enzymatic, chemical and radioactive digestion of nucleotides. It is reported that the secondary metabolites are responsible for chemical digestion of nucleotides, it may be exonucleolytic or endonucleolytic [19]. Likewise, *Scenedesmus* sp. has also been used in many biotechnological applications due to their high nutritional content and bioactivities (Chacon et al. 2010; Catarina et al. [9]). Therefore, the present study was focused on carrying out the phytochemical analysis of microalga *Scenedesmus bajacalifornicus* BBKLP-07 extracts and to determine the antidiabetic, antioxidant, anti-inflammatory and antimicrobial activity studies using the crude extracts.

Materials and methods

Crude extracts preparation of microalga *Scenedesmus bajacalifornicus* BBKLP-07

The microalgal biomass was dried and finely powdered dried and 50 g of the powdered biomass was subjected to successive solvent extraction using soxhlet apparatus. The extraction of the biomass was carried out using different solvents in their increasing order of polarity which includes chloroform, ethyl acetate, methanol, ethanol and distilled water. Each time, the microalgal biomass was dried and later extracted with next high polar solvents. All extracts were concentrated in Buchi rotary evaporator, followed by removal of traces of solvent using desiccator.

Phytochemical analysis of crude extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

The crude extracts (chloroform, ethyl acetate, methanol, ethanol and distilled water extracts) of microalga *Scenedesmus bajacalifornicus* BBKLP-07 was qualitatively tested for the presence of different phytochemical constituents namely alkaloids, flavonoids, glycosides, phenols, lignin's, saponins, sterols, tannins, anthraquinone and reducing sugar by following the standard procedure reported by Edeoga et al. [14].

Estimation of phenolic content in extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

The total phenolic content of the microalga *Scenedesmus bajacalifornicus* BBKLP-07 extract was estimated using Folin–Ciocalteu method of Singleton et al. [51]. Gallic acid was used as the reference standard. 0.5 ml of plant extract was mixed with 2 ml of the Folin–Ciocalteu Reagent (10 fold) and was neutralized with 4 ml of sodium carbonate solution (8% w/v). The reaction mixture was incubated at room temperature for 30 min for color development. The absorbance of the resulting color was measured at 765 nm using UV–VIS spectrophotometer. The total phenolic content was estimated from the linear equation of standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent.

Estimation of flavonoids content in extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

The flavonoids content in the microalgal extract was estimated according to Chang et al. [11] with quercetin as the reference standard. It is an Aluminum chloride colorimetric method is used in which each extract (0.5 ml) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% Aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm using a UV–VIS Spectrophotometer. The value of optical density was used to calculate the flavonoids content present in the sample and the calibration curve was plotted using quercetin solutions at concentrations 12.5–100 µg/ml in methanol.

Evaluation of the antidiabetic activity of extracts of microalga Scenedesmus bajacalifornicus BBKLP-07using in vitro assays

Alpha-amylase inhibitory assay

The alpha-amylase inhibitory assay for different solvent extracts of microalga *Scenedesmus bajacalifornicus BBKLP-07* was evaluated according to a previously described method by Singh et al. (50). 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 mL of dinitrosalicylic acid color reagent. Test tubes were incubated in a water bath at 100 °C for 5 min. and cooled until room temperature was reached. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following equation:

$$\%\ \text{inhibition of}\ \alpha - \text{Amylase} = \left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\left(\text{Abs}_{\text{control}}\right) \times 100$$

where Abs control corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution and Abs sample corresponds to the solution with extract and α -amylase solution.

Studies on glucose uptake in yeast cells

The commercial baker's yeast was suspended in distilled water and subjected to repeated centrifugation at 3000×*g* for 5 min until clear supernatant fluids were obtained. 10% (v/v) of the suspension was prepared in distilled water. Different concentrations of solvent extracts of microalga *Scenedesmus bajacalifornicus BBKLP-07* were added to 1 mL of glucose solution (5 mM) and incubated together for 10 min at 37 °C. The reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2500×*g*, 5 min) and the amount of glucose was estimated in the supernatant. Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake}\ (\%) = \frac{\text{Abs sample} - \text{Abs control} \times 100}{\text{Abs sample}}$$

where Abs sample is the absorbance of the test sample and Abs control is the absorbance of the control reaction (containing all reagents except the test sample).

Determination of antioxidant activity of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 using in-vitro assays

Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu [18]. Methanol, ethanol and aqueous extracts microalga *Scenedesmus bajacalifornicus* BBKLP-07 in different concentrations ranging from 100–500 µl were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1%, w/v ferric chloride were added to the mixture, which was incubated for 10 min. Finally, the absorbance was measured at 700 nm using a UV–VIS Spectrophotometer. Ascorbic acid was used as the reference standard.

Phosphomolybdenum assay

Total antioxidant activity was estimated by Phosphomolybdenum assay (PM) using standard procedure of Prieto et al. [20]. Methanol, ethanol and aqueous extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 in different concentrations ranging from 100–500 µl were added to each test

tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm using a UV–VIS Spectrophotometer. Ascorbic acid was used as the reference standard.

DPPH (1, 1-diphenyl 2-picrylhydrazyl) free radical-scavenging assay

Radical scavenging activities of methanol, ethanol and aqueous extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 were determined using the DPPH radical reagent. A known quantity of a DPPH radical solution in ethanol was mixed with different concentrations of extracts in ethanol to make the final concentration of 10, 20, 30, 40 and 50 µg/ml. The mixture was incubated for 30 min in the dark at room temperature and then absorbance was measured at 517 nm using UV–VIS Spectrophotometer. Ascorbic acid was used as a reference standard. The DPPH scavenging activity of each sample was calculated using the following equation:

$$\text{Scavenging effect } (\%) = A_c - A_t / A_c \times 100$$

where A_c is the absorbance of the control reaction and A_t is the absorbance of the test sample. The experiment was done in triplicate.

Determination of in vitro anti-inflammatory activity of extracts of microalgal *Scenedesmus bajacalifornicus* BBKLP-07

Anti-inflammatory activity of methanol, ethanol and aqueous extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 was evaluated by protein denaturation method. Diclofenac sodium, a powerful non-steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 ml of different concentrations of methanol, ethanol and aqueous extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 (500 µg/ml) with standard Diclofenac sodium (500 µg/ml) and 2.8 ml of phosphate buffered saline (pH 6.4) was mixed with 2 ml of egg albumin (from fresh hen's egg) and incubated at (27 ± 1) °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm using double distilled water as a blank. Each experiment was done in triplicate. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = A_t - A_c / A_c \times 100$$

where A_t =absorbance of test sample; A_c=absorbance of control.

Determination of antimicrobial Assay extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 extracts

Antimicrobial bioassay was performed against microbial pathogenic strains which include two Gram-negative (*Escherichia coli* and *Salmonella typhi*), two Gram-positive (*Bacillus subtilis* and *Clostridium perfringens*) and two pathogenic fungi (Aspergillus niger and Candida albicans). The strains were procured from ATCC culture collection center. Antimicrobial activity of chloroform, acetone, ethanol, methanol and aqueous extracts were evaluated using the agar well diffusion method as described by Patra et al. [1]. The wells were then filled with 100 µl of extract, and Amoxicillin was used as a standard for bacterial cultures and Flucanazole was used as a standard for fungal cultures. All bacterial plates were incubated for 24 h at 37 ± 1 °C and fungal plates were incubated for 72–120 h at 25 ± 2 °C for fungal strains. The diameter of the inhibition zones was measured.

Statistical analysis

All experiments were performed in five replicates ($n = 5$) and the data are presented as the mean ± standard deviation and standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software 20 Version (IBM). The significance of differences was defined at these $P < 0.05$.

Results

Phytochemical analysis of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

In the present study, the qualitative phytochemical screening of bioactive compounds was performed using chloroform, acetone, ethanol, methanol and aqueous solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 (Table 1). Alkaloid, flavonoid, oils and fat contents were present in all the extracts, alkaloids were in present moderate quantity, flavonoids were maximum in all the extracts. Oils and fats were moderate in chloroform and acetone extract and maximum in ethanol, methanol and aqueous extracts. Phenolic compounds were also present in high quantity in all extracts; similarly, sterols were present in all extracts except

Table 1 Phytochemical constituents of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Tests	Extracts				
	Chloroform	Acetone	Ethanol	Methanol	Aqueous
Alkaloids					
Iodine test	+	+	+	+	+
Waganer's test	−	+	+	+	−
Dragendroff's test	+	−	−	−	+
Flavonoids					
Shinoda test	++	+++	+++	+++	++
Pew's test	++	++	++	++	+++
NaOH test	++	+++	++	++	++
Saponins					
Foam test	−	+	+	+	−
Haemolysis test	−	+	+	++	+
Tannins					
Gelatin test	−	−	−	−	−
Lead acetate test	−	−	−	−	−
Lignin test					
Lignin test	−	−	−	−	−
Lobat test	−	−	−	−	−
Terpenoids	−	−	−	−	−
Glycosides					
K-K test	+	−	−	+	++
Glycoside test	+	−	−	−	+++
Sulphuric acid test	+	−	+	−	+
Molish's test	+	−	−	−	++
Reducing sugar	−	−	−	−	−
Phenols					
Ellagic acid test	+	++	++	++	++
Phnols test	++	++	++	++	++
Sterols					
Salkowsk's test	+	+	+	−	+
L-B test	+	−	−	−	+
Anthraquinone					
Borntranger's test	−	++	−	−	++
Oils and fats					
Filter paper test	+	+	++	++	++
Saponification test	+	+	+	++	++
Volatile oil	−	−	−	−	−

(−) absent, *(+)* moderate, *(++/+++)* high presence

Table 2 Total yield of solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Solvent extracts	Yield in percentage (%)
Chloroform	2.5
Acetone	3.2
Ethanol	4.1
Methanol	4.3
Aqueous	5.8

The data represent the percentage (%) yield

methanolic extracts. Glycosides were present in high quantity in aqueous extracts and moderately in chloroform extract and absent in acetone, ethanol and methanolic extracts. Anthraquinones were abundant in acetone and aqueous extracts and saponins were present in all extracts except chloroform extracts. Tannins, lignins, terpenoids, reducing sugars and volatile oils are absent in all the solvent extracts.

Phytochemicals are non-nutritive chemicals that have protective or disease preventive properties; they are produced during the metabolic activities of microalgae. Phytochemical screening microalga *Scenedesmus bajacalifornicus* BBKLP-07 was found to be the good source of secondary metabolites. The total yield of the phytochemical constituents of chloroform, acetone, ethanol, methanol and aqueous extracts were 2.5, 3.2, 4.1, 4.3 and 5.8% of the total biomass respectively (Table 2). Aqueous extracts yielded a maximum quantity of phytochemical constituents, whereas minimum quantity of extract was obtained by chloroform extract.

Phenol content of the extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Phenols are the aromatic compounds having –OH functional group attached to the benzene ring; phenolic compounds have been associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. Total phenolic content of different extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 was determined by the FCR method [51] and expressed as gallic acid equivalents (GAE) per gram of plant extracts. Phenolic compounds were extracted in abundant quantity using all the extracts where the quantity of extracts were 42.25, 38.13, 20.58, 32.12 and 58.63 mg/g in chloroform, acetone, ethanol, methanol and aqueous extracts respectively (Fig. 1). The maximum quantity of phenolic compounds was observed in aqueous extract and minimum quantity was observed in ethanol extract.

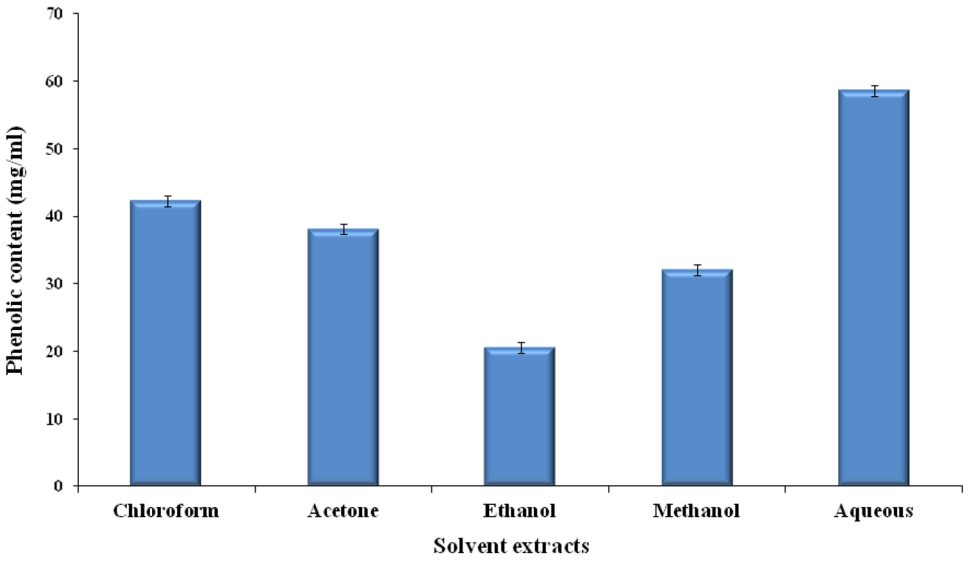

Fig. 1 Phenolic content in solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Flavonoid content of the extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Flavonoids are widely distributed in microalgae, fulfilling many functions. Flavonoids are the most important compounds which act as chemical messengers, physiological regulators, cell cycle inhibitors and performs various other physiological roles. Total flavonoids content of different extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 was determined by Aluminum chloride colorimetric method and expressed as quercetin equivalents (QE) per gram of biomass extracts. The quantities of flavonoid contents in chloroform, acetone, ethanol, methanol and aqueous extracts were 34.14, 32.15, 41.76, 30.32 and 70.34 mg/g respectively (Fig. 2). The highest quantity of flavonoids was observed in aqueous extracts.

Antidiabetic activity of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion. Food habits and genetic factors are responsible for diabetes. Antidiabetic activity of the extracts was studied using

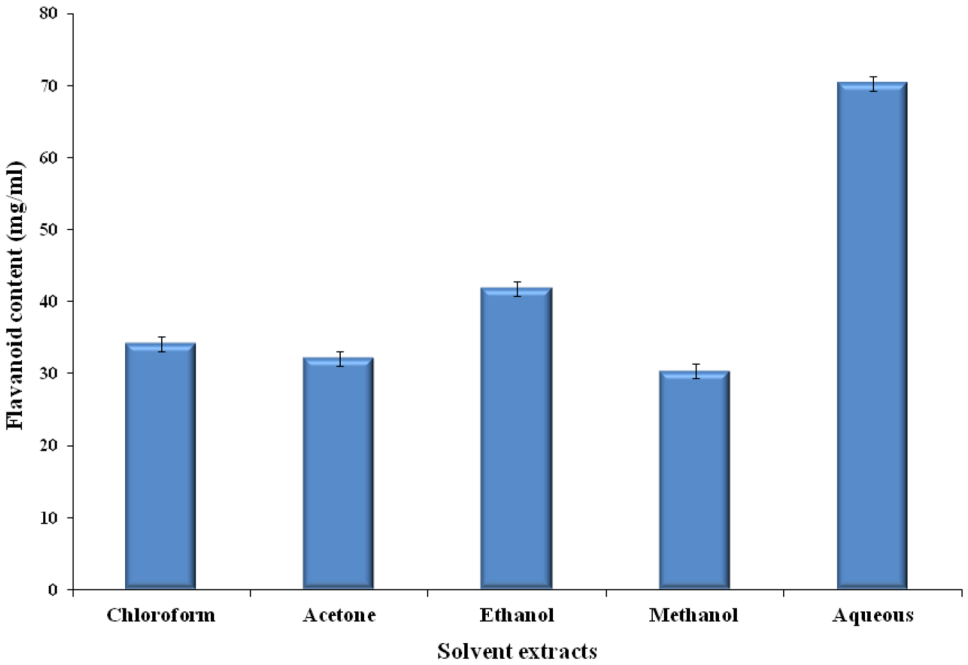

Fig. 2 Flavonoid content in solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

two methods; one was α-amylase inhibition test method and the second one was glucose uptake method. The inhibition α-amylase enzyme involved in the digestion of carbohydrates, can significantly reduce the post-prandial increase of blood glucose, and therefore, can be an important strategy in the management of blood glucose level diabetic patients and glucose uptake method indirectly assesses diabetes caused by the deficiency of insulin.

Inhibition of α -amylase activity

A known concentration (100 µg/ml) of different solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 were subjected to α-amylase inhibitory assay along with Acarbose as a standard. Inhibition of α-amylase activity of chloroform, acetone, ethanol, methanol and aqueous extracts were 12.87, 21.65, 28.63, 36.32, 60.21% with IC_{50} values 220.18, 249.71, 193.33, 98.74 and 150.32 µg/ml, respectively, whereas the control (acarbose) was showing 71.2% inhibition with IC_{50} value of

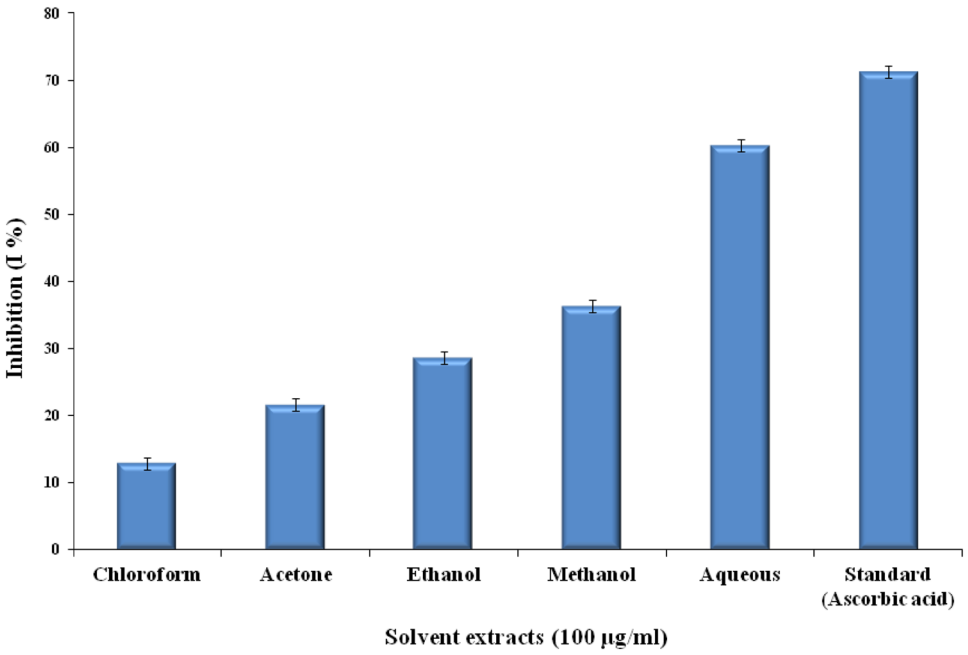

Fig. 3 Alpha-amylase inhibitory activities of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

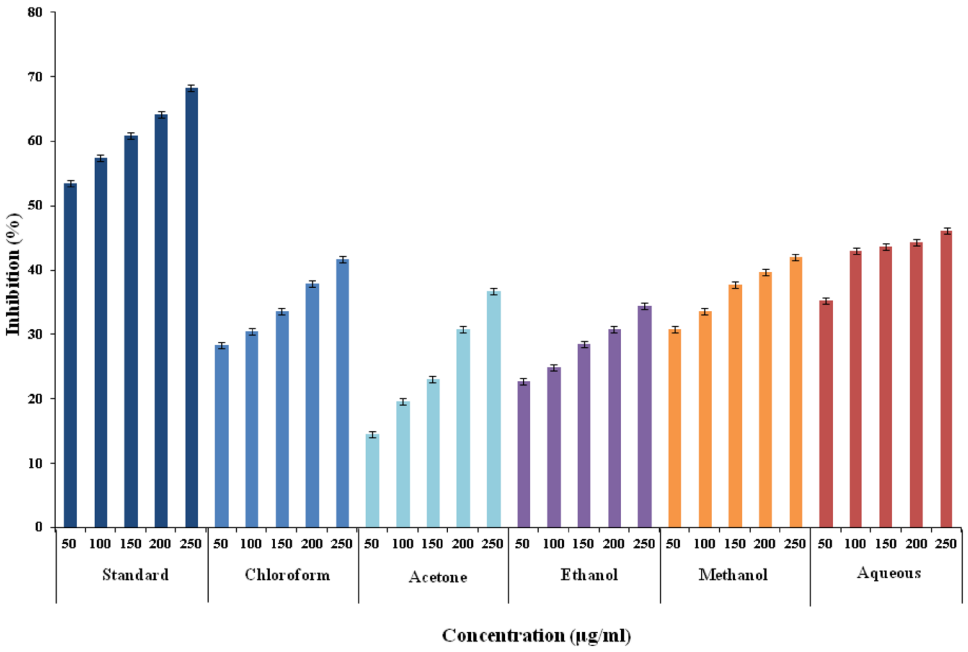

Fig. 4 Percentage of glucose uptake in yeast cells treated with extracts of Microalga *Scenedesmus bajacalifornicus* BBKLP-07

80.21 µg/ml. α-amylase inhibitory activity was found to be increasing with the increasing polarity of the solvents. Least polar solvent chloroform was showing the lowest percentage of inhibitory activity, whereas highly polar solvent aqueous extract was showing the highest inhibitory activity (Fig. 3).

Glucose uptake assay

Glucose uptake assay was carried out using Yeast cells as model. All the solvent extracts were treated at varying concentrations (50, 100, 150, 200 and 250 µg/ml) for the in vitro glucose uptake assay. The percentage of glucose uptake in yeast cells by the extract was compared with Metronidazole standard drug (Fig. 4). Among all the solvent extracts, aqueous extracts had shown maximum glucose uptake activity of 46.12% at 250 µg/ml concentration which was expressed in inhibition percentage and IC_{50} value was found to be 223.58 µg/ml. Acetone extract had shown minimum glucose uptake activity of 14.57% at 50 µg/ml with IC_{50} value 235.65 µg/ml; chloroform, ethanol and methanol extracts have shown moderate glucose uptake activity.

Determination of antioxidant activity of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

A paradox in metabolism is that, while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, reactive oxygen species also have useful cellular functions, such as redox signaling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level. The antioxidant activities of the microalgal extracts were studied using the following methods.

FRAP assay

In FRAP assay, different concentrations of chloroform, acetone, ethanol, methanol and aqueous extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 were subjected to the assay with Ascorbic acid as a standard. Absorbance was measured at 700 nm. Increasing optical density indicates higher reducing power. Among all the extracts obtained, methanolic extracts have shown the maximum activity followed by ethanol, acetone, chloroform and aqueous extracts. Highest activity was exhibited by methanolic extracts with absorbance value 0.91 OD at 500 µg/ml concentration and lowest was exhibited by aqueous with absorbance value 0.21 OD at 100 µg/ml concentration (Fig. 5).

Phosphomolybdenum assay

Phosphomolybdenum assay (PM) assay was performed to evaluate total antioxidant activity of extracts of microalga

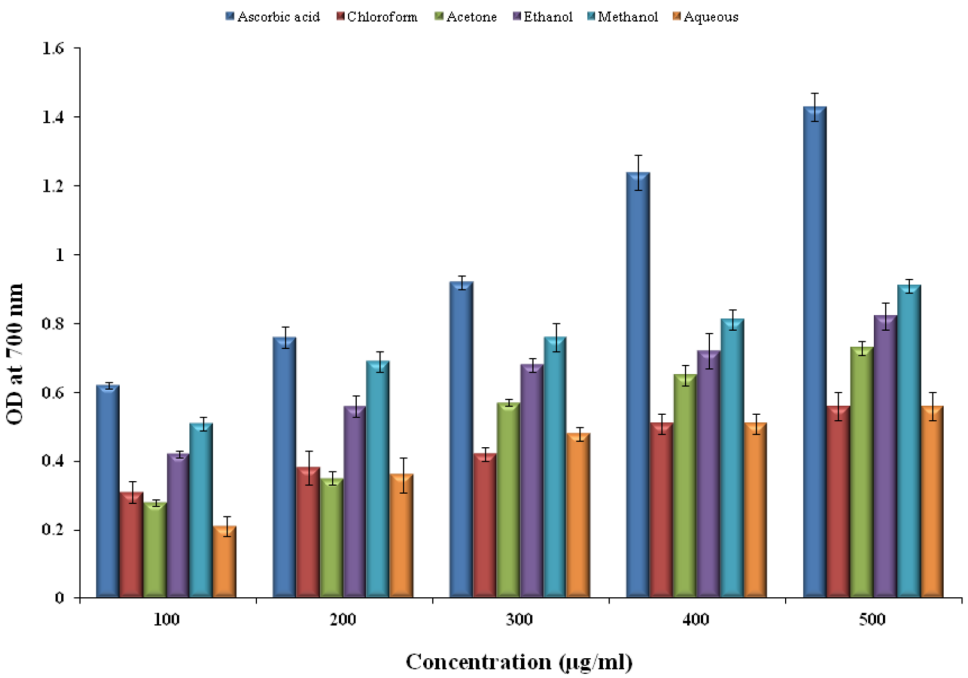

Fig. 5 FRAP Assay of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

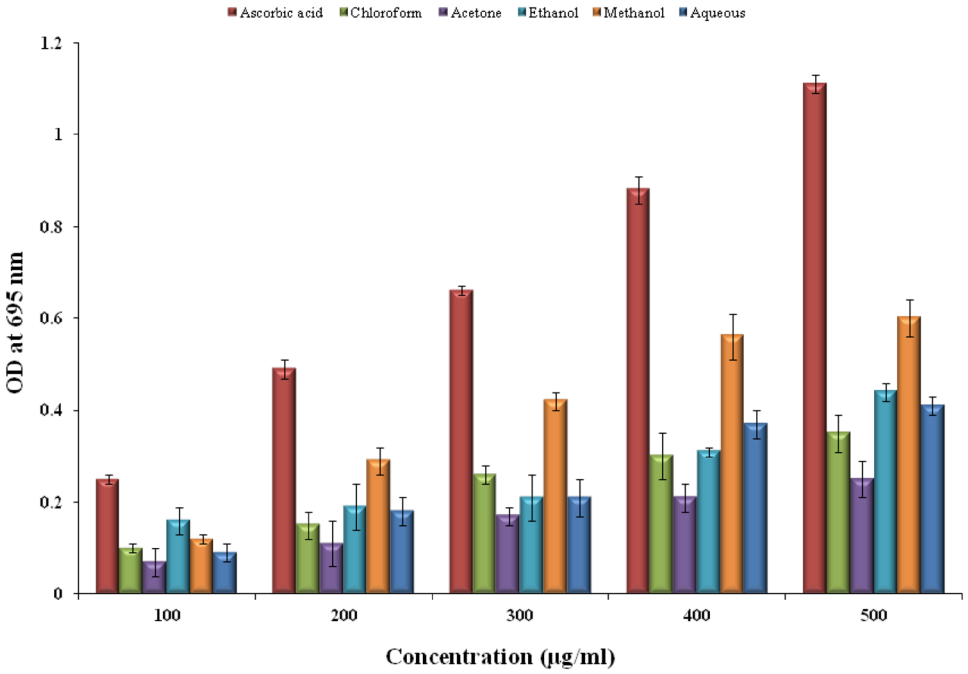

Fig. 6 PM assay of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Scenedesmus bajacalifornicus BBKLP-07 using Ascorbic acid as standard. The antioxidant activity depends on the reducing power of extracts in reduction of phosphate-Mo (VI) at acidic pH. In the present study different concentrations of chloroform, acetone, ethanol, methanol and aqueous extracts of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 using Ascorbic acid as a standard. Among all the extracts obtained, methanolic extracts have shown the maximum activity followed by ethanol, aqueous, chloroform and acetone extracts. Highest activity was exhibited by methanolic extracts with absorbance value 0.60 OD at 500 µg/ml concentration and lowest was exhibited by acetone with absorbance value of 0.07 OD at 100 µg/ml concentration (Fig. 6).

DPPH free radical-scavenging assay

In DPPH free radical-scavenging assay, the different concentrations of chloroform, acetone, ethanol, methanol and aqueous extracts (10, 20, 30, 40 and 50 µg/ml) of microalga

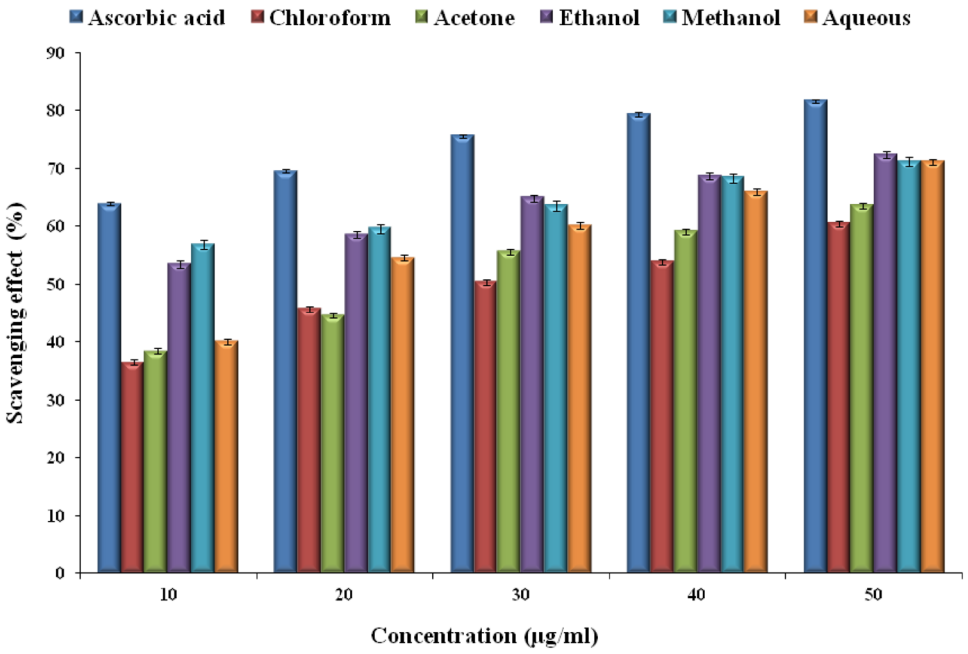

Fig. 7 Percentage inhibition of DPPH free radical of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Scenedesmus bajacalifornicus BBKLP-07 were evaluated for their free radical scavenging property using DPPH free radical scavenging assay. The maximum radical scavenging effects of 72.42, 71.25 and 71.12% were observed at 50 µg/ml concentrations of ethanol, methanol and aqueous, respectively, whereas chloroform and acetone extracts have shown radical scavenging effects of 60.45 and 63.57% at 50 µg/ml concentration. Lowest radical scavenging effects of 36.51 and 38.45% were observed in chloroform and acetone extracts at 10 µg/ml concentration (Fig. 7).

In-vitro anti-inflammatory activity of microalgal extracts

Anti-inflammatory activity of crude extracts (100 µg) of chloroform, acetone, ethanol, methanol and aqueous extracts microalga *Scenedesmus bajacalifornicus* BBKLP-07 were performed using protein denaturation assay. The in-vitro anti-inflammatory activities of the extracts were compared with Diclofenac sodium, a reference drug. A significant difference was observed among all extracts in the denaturation of protein. Protein denaturation of chloroform, acetone, ethanol, methanol and aqueous extracts were found to be 20.54, 24.75, 21.65, 67.35 and 30.45% respectively. Among all tested solvent extracts, methanolic extract has shown highest protein denaturation of 67.35%, whereas the chloroform extract has shown lowest protein denaturation of 20.54%, however, in the control (diclofenac sodium) the protein denaturation was found to be 95.14% (Fig. 8).

Antimicrobial assay of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Antimicrobial bioassay was performed against microbial pathogenic strains which include two Gram-negative (*Escherichia coli* and *Salmonella typhi*), two Gram-positive (*Bacillus subtilis* and *Clostridium perfringens*) and two pathogenic fungi (Aspergillus niger and Candida

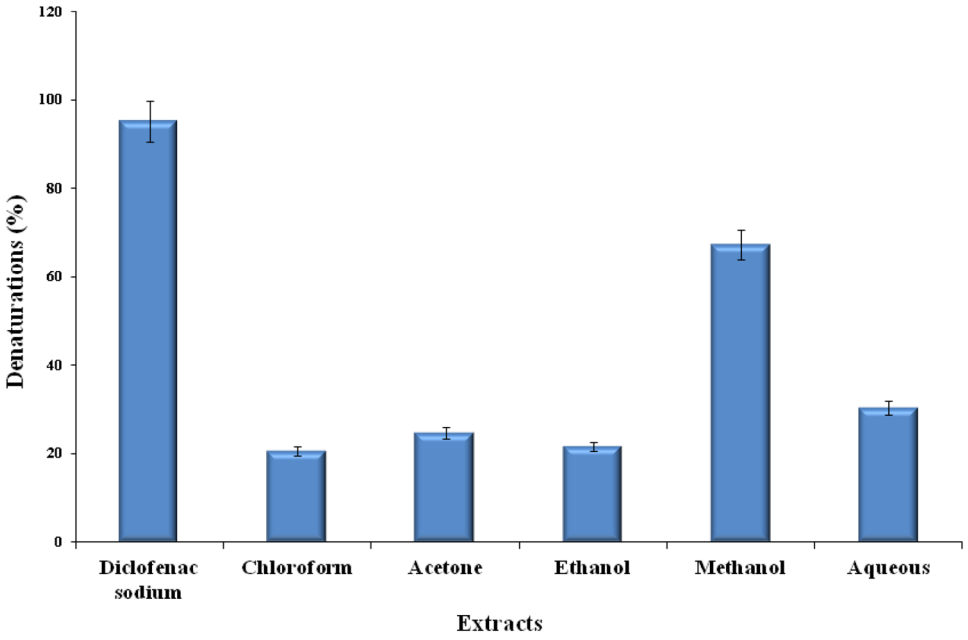

Fig. 8 Anti-inflammatory assay of different solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Table 3 Antimicrobial activity of different solvent extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Species	Zone of inhibition (mm) of microalgal extracts					
	Amoxicillin/ Flucanazole	Chloroform	Acetone	Ethanol	Methanol	Aqueous
E. Coli	21.4 ± 0.10	12.2 ± 0.07	11.7 ± 0.05	12.6 ± 0.03	14.1 ± 0.07	15.1 ± 0.09
Salmonella typhi	20.4 ± 0.05	10.0 ± 0.08	12.0 ± 0.07	13.1 ± 0.06	14.2 ± 0.10	16.0 ± 0.05
Bacillus subtilis	19.8 ± 0.06	17.9 ± 0.10	13.5 ± 0.09	18.6 ± 0.08	17.9 ± 0.05	14.8 ± 0.08
Clostridium perfringens	18.7 ± 0.12	NA	NA	NA	NA	10.2 ± 0.07
Aspergillus niger	20.4 ± 0.08	17.2 ± 0.06	14.1 ± 0.09	15.2 ± 0.04	18.4 ± 0.06	19.0 ± 0.07
Candida albicans	21.2 ± 0.09	19.1 ± 0.09	18.0 ± 0.10	19.1 ± 0.05	19.8 ± 0.08	20.1 ± 0.06

The data represent Mean ± SE

albicans) (Table 3). All microbial strains have exhibited activity against all the microalgal extracts except *Clostridium perfringens*, which possessed activity against aqueous extracts only with inhibition of 10.2 mm. *E. coli* exhibited inhibition zone of 12.2, 11.7, 12.6, 14.1 and 15.1 mm against chloroform, acetone, ethanol, methanol and aqueous extracts respectively. *Salmonella typhi* exhibited inhibition zone of 10.0, 12.0, 13.16, 14.2 and 16.0 mm. Similarly, Gram-positive bacteria *Bacillus subtilis* exhibited inhibition zones of 17.2, 14.1, 15.2, 18.4 and 19.0 mm against chloroform, acetone, ethanol, methanol and aqueous extracts, respectively, whereas *Clostridium perfringens* had shown activity only against aqueous extracts. Both fungi *Aspergillus niger* and *Candida albicans* have shown inhibition of 17.2, 14.1, 15.2, 18.4, 19.0 mm and 19.1, 18.0, 19.1, 19.8 and 20.1 respectively. Compared to all the extracts, aqueous extracts have shown maximum inhibition zones against all the solvent extracts.

Discussion

Extraction from the microalgal biomass is usually not selective and the extracts are complex mixtures of the major compounds present in microalgae: polysaccharides, phenolic compounds, polyunsaturated fatty acids, proteins, peptides, pigments, vitamins, terpenoids and sterols. Their content varies with season, age, species, geographical location and environmental factors. In the present study, the biomass of green microalga *Scenedesmus bajacalifornicus* BBKLP-07 was subjected to soxhlet extraction using five different solvents such as chloroform, acetone, ethanol, methanol and aqueous solvents on the basis of their polar nature. Successive extractions were performed beginning from non-polar solvents followed by the next polar solvent. Bioactive compounds from microalgae belong to various chemical groups such as tannins, alkaloids, glycosides, lignans, terpenoids, etc which are soluble in various solvents.

Most of the bioactive compounds are hydrophobic in nature and extraction with different solvents with increasing polarity influences the phytoconstituents present in the microalgae so the extraction was carried out using non-polar solvents in the beginning followed by the next polar solvent. Similar studies were reported previously where solvents such as benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol and distilled water were used for the extraction of bioactive compounds by microalga *Chlorella vulgaris* [23]. Acetone, methanol, ethanol and chloroform extracts were used to assess the phytochemical constituents of five different microalgae such as *Tetraselmis* sp., *Dunaliella* sp., *Chlorella* sp., *Synechocystis* sp., and *Oscillatoria* sp., [42]. Several plants species such as *Kandelia candel* and *Rhizophora apiculata* were also screened for the bioactive compounds using chloroform, ethyl acetate, methanol, ethanol and distilled water solvents [49].

Phytochemical analysis of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

In recent years, the use of microalgae for extraction of metabolites, bioactive molecules and other industrial important products has been gaining lots of importance. Some algal species have been used for therapeutical practices against various pathogens both in medicine and agriculture for decades. In the present study, preliminary phytochemical screening was carried out to determine the existence of natural bioactive molecules in the biomass of microalga *Scenedesmus bajacalifornicus* BBKLP-07. Preliminary phytochemical screening showed the presence of alkaloids, saponins, glycosides, flavonoids, phenols, anthraquinones and oils and fats in most of the solvent extracts, chloroform, acetone, ethanol, methanol and aqueous extracts.

The presence of flavonoids, oils, fats and phenols was observed in large quantity, alkaloids, saponins, sterols and anthraquinones in moderate quantity and the presence of lignin and terpenoids in negligible quantity was observed in all the solvent extracts of *Desmococcus olivaceous* and *Chlorococcum humicola* [53]. It is reported that the freshwater microalga *Scenedesmus acutus* also possess the phytochemical constituents such as alkaloids, flavonoids, sterols, anthraquinones, saponins and carbohydrates [22]. Similarly C. vulgaris and green alga *Pithophora oedogonia* showed the presence of alkaloids, saponins, lignins, flavonoids, phenols, proteins, carotenoids, sugars, fatty acids, carbohydrates, volatile oils and glycosides in most of the organic solvent extracts, hexane and chloroform extracts [23, 50] However, it has been also reported several plant species such as *Curcuma pseudomontana* were also screened for the phytochemical constituents in which alkaloid, flavonoid, tannin, steroids, saponins, carbohydrate, proteins, and amino acids were observed in abundant quantity [20].

Phenol content of the extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Phenols are the aromatic compounds having –OH functional group attached to the benzene ring; phenolic compounds have been associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen

and free radicals. The maximum amount of phenolic compounds were extracted using aqueous solvent of the microalga *Scenedesmus bajacalifornicus* BBKLP-07 and lowest amounts were observed in methanol and acetone extracts. Similar reports suggest that aqueous extracts were generally preferred for the extraction of phenolic compounds. The highest extraction yield of phenolic compounds were recorded for the aqueous extract of seaweeds *Ulva lactuca* and *Chondrus crispus*, whereas the lowest yield was recorded in acetone extracts [52]. Li *et al*. (2015) determined the quantity of phenolic compounds in 23 different microalgal species using Folin–Ciocalteu method and elucidated the role of phenolic compounds in antioxidant activities [21].

Phenolic compounds are the major primary compounds which are acting as free radical scavengers and they also play a role in antioxidant activity. The phenolic contents present in methanol extracts of microalgae *Nostoc caeruleum, Spirulina platensis, Cylindrospermum majus, Oscillatoria formosa* and *Chlorella vulgaris* was found to exhibit antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus pyogenes*) and three Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*) as well as for their antifungal activity against (*Aspergillus fumigatus, Candida albicans, Geotrichum candidum* and *Trichophyton mentagrophytes*) [30].

Flavonoid content of the extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

The flavonoids are considered to be a diverse and broad group of natural components which possess a broad spectrum of biological activities including antioxidant activity. In the present study, chloroform, acetone, ethanol, and methanol extracts of the microalga *Scenedesmus bajacalifornicus* BBKLP-07 have shown minimum quantity of flavonoid contents, whereas highest flavonoid content was observed in aqueous extracts. Flavonoids are responsible for the multiple activities such as anti-microbial, anti-cancer, anti-inflammatory and Anti-diabetic activities of the microalgal extracts [4]. Flavonoids play a vital role in protecting the cells from premature aging and disease by shielding DNA, proteins, and lipids from oxidative damage [34]. Flavonoid content is also responsible for antioxidant activity of many plants and algal species [12].

Antidiabetic activity of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Diabetes mellitus, commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Insulin is the hormone that regulates the glucose level of blood of the body. Improper secretions of insulin in β-cells of pancreatic islets may lead to type 1 or type 2 diabetes. It is suggested that various bioactive compounds such as flavonoids, vitamins, and carotenoids present in algae and plants or bioactive compounds altogether may affect directly on enhancing insulin secretion and preventing beta-cell apoptosis, and some bioactive compounds can also modulate beta-cell proliferation [29]. Zhang et al. [55] evaluated the capability of extracts of Dinoflagellates which inhibited the protein tyrosine phosphatase 1B, a negative regulator of insulin receptor signal transduction and a drug target for the treatment of type 2-diabetes. In the present study, anti-diabetic activity was determined by studying the α-amylase inhibition and glucose uptake activities. α- amylase is an enzyme which breaks down the carbohydrate molecule and converts it into absorbable monosaccharide units by the cells, the bioactive compounds such as flavonoids, alkaloids and glycosides inhibit the α-amylase thus preventing the catabolism of carbohydrates which intern decrease absorption of glucose by the cells. Aqueous extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 have shown maximum α-amylase inhibitory activity.

Similarly, regulation of glucose level in the blood of a diabetic patient can prevent the various complications associated with the disorder. The maintenance of plasma glucose concentration for a longer time under variation in dietary condition is one of the most important and closely regulated process observed in the mammalian species especially type II diabetes characterized by a deficiency of insulin, causing increased levels in blood glucose level and it depends on the uptake of glucose by the cells. In the present study, Percent increase in glucose uptake in yeast cells by the action of microalga *Scenedesmus bajacalifornicus* BBKLP-07 extracts was compared with the standard drug Metronidazole. There was a concentration-dependent increase in the percentage of glucose uptake with increasing concentration. Among all the extracts, aqueous extract exhibited the highest percentage of glucose uptake with increasing concentrations of aqueous. The increased concentration of extracts correspondingly increased the percentage of glucose uptake in yeast cells. This result indicated that the increasing concentrations of extracts exhibited high glucose uptake. Several reports suggest that biologically active components and phlorotannins, marine polyphenols are responsible for the antidiabetic activities [25, 35].

Antioxidant activity of microalga *Scenedesmus bajacalifornicus* BBKLP-07 extracts

Reactive oxygen species (ROS) are chemically reactive molecules produced within biological systems [13]. Naturally generated antioxidants, such as superoxide dismutase (SOD), are able to scavenge excess oxidants and convert

them into less harmful molecules [43]. The generation of ROS is shown to be either beneficial or harmful depending on various conditions. In regard to the beneficial aspects of ROS, it has been revealed that ROS at normal levels are involved in mediating many cellular responses, including cell growth and immunity [26]. Indeed, ROS occurs naturally as a result of basic metabolic processes; however, the presence of excess ROS can lead to lipid peroxidation, DNA damage and even induce cell death [18].

Antioxidant mechanisms in biological tissues are extremely complex and the only one method that is difficult to decide the antioxidant capacity of crude extracts [8]. Guedes et al. [16] tested the antioxidant capacity of 23 microalgae cultured in only one growth condition. A large number of in-vitro methods are available to evaluate the antioxidant activity of pure compound or extracts. Hence, in the present study, three in-vitro assays viz., FRAP, PM and DPPH assay are employed.

FRAP assay includes use of ferricyanide and ferric ions as chromogenic oxidants. It gives an immediate result of a large range of individual antioxidants in a dose-dependent manner and intensity of color directly proportional to the reducing power of antioxidants. The color of the reaction mixture which is measured at 700 nm and higher the absorbance indicates higher antioxidant activity. In the present study, methanol and ethanol extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07 have exhibited higher activity than chloroform, acetone and aqueous extracts.

PM assay is a quantitative method used to evaluate redox reaction by antioxidant, oxidants with the involvement of ligand molybdenum. It involves the longer incubation period at higher temperatures, which influences the auto-oxidation reaction in the mixture. It is directly estimated the reducing potential of the extracts. The reaction mixture forms, green phosphomolybdenum colored complex at acidic pH which is measured at 695 nm. The reduction activity of extracts and standard drug was increased with increase in concentrations. In the present study, the methanolic extract of microalga *Scenedesmus bajacalifornicus* BBKLP-07 possessed higher activity compared to chloroform, acetone, ethanol and aqueous extracts.

DPPH assay is most widely accepted method for evaluating antioxidant activity of many algae-based drugs and crude extracts [42]. It is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavengers. The concentration of free radical scavengers is proportional to scavenging DPPH with the absorbance at 517 nm. The reducing potential is measured by decreasing in absorbance by the action extracts. In the present study, the ethanol, methanol and aqueous extract of microalga *Scenedesmus bajacalifornicus* BBKLP-07 showed higher antioxidant activity compared to chloroform and acetone extracts.

In-vitro anti-inflammatory activity of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

Inflammation is a common reaction of living tissues towards infection and injuries [32]. Inflammation is a protective response that involves immune cells, blood vessels, and different molecular mediators (e.g., TNFα, IL1, nitric oxide, and prostaglandins). Anti-inflammatory properties were previously found for other microalgae, such as the green algae *Dunaliella bardawil* [24], the diatoms *Porosira glacialis, Attheya longicornis* and *P. tricornutum* [46], and the dinoflagellate A. carterae [46]. Steroidal anti-inflammatory agents are available but they induce damage to the lymphocytes and causes severe side effects, therefore, the anti-inflammatory agents from natural sources like microalgae are gaining importance and are more promising agent with less side effect. In the present study, in vitro protein denaturation method was used in which methanol extract of microalga *Scenedesmus bajacalifornicus* BBKLP-07 showed highest anti-inflammatory activity compared to chloroform. Acetone, ethanol and aqueous extracts.

Antimicrobial assay of extracts of microalga *Scenedesmus bajacalifornicus* BBKLP-07

The search for antimicrobials from natural sources has recently received much attention and efforts are on to identify compounds that can act as suitable antimicrobial agents to replace synthetic ones. These compounds have significant therapeutic application against human pathogens, including bacteria and fungi. Numerous studies have been conducted with the extracts of various microalgae for the discovery of new antimicrobial compounds [2, 9, 34]. The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes—including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [28, 29]; for instance, the antimicrobial activity of supercritical extracts obtained from the microalga *Chaetoceros muelleri* were related to its lipid composition [30]. However, the antimicrobial activity detected in several pressurized extracts from *Dunaliella salina* may be explained not only by several fatty acids, but also by such compounds as α- and β-ionone, β-cyclocitral, neophytadiene and phytol [19].

Efforts to identify the compounds directly responsible for those antimicrobial features—e.g. chlorellin [31], have been on the run, but are still relatively incipient owing to some new classes of compounds found. To date, microalgae extracts and bioactive compounds have found their way into pharmaceuticals, nutraceutical and food supplements. In the present study the crude chloroform, acetone, ethanol, methanol and aqueous extracts of *Scenedesmus bajacalifornicus* BBKLP-07 were found to exhibit inhibitory activities

against the foodborne pathogenic bacteria *E. coli, S.typhi*, and *B. subtilis* aureus, whereas *Clostridium perfringens* had not shown any activity except aqueous extracts. All extracts were also found to exhibit activity against fungal species *Aspergillus niger* and *Candida albicans*. Similar findings were reported in which various organic solvents such as acetone, benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane and methanol extracts of *Chlorococcum humicola, Scenedesmus* sp and a plant species *Curcuma pseudomontana* were tested against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumoniae, Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus niger* and *Aspergillus flavus* [6, 7]. The present study concludes that green algae *Scenedesmus bajacalifornicus* BBKLP-07 are rich source of pharmacologically active natural products.

Conclusion

Microalgae are photosynthetic eukaryotes which are primary producers in the food chain and also the excellent sources of bioactive compounds. The microalga *Scenedesmus bajacalifornicus* BBKLP-07 was subjected to soxhlet extraction using solvents like chloroform, acetone, ethanol, methanol and aqueous solvents. All the solvents were tested for the presence of phytochemical constituents such as alkaloids, flavonoids, glycosides, phenols, lignin's, saponins, sterols, tannins, anthraquinone and reducing sugar using the standard procedures. Quantification of phenolic and flavonoids content was also performed. Further, all the extracts were subjected to verities of assays such as antidiabetic, antioxidant and anti-inflammatory and anti-microbial activities. Among all the solvent extracts, aqueous extract of microalga *Scenedesmus bajacalifornicus* BBKLP-07 was found to exhibit a maximum number of bioactive compounds with a maximum quantity of yield. The maximum phenolic (58.63 mg) and flavonoid (70.34 mg) contents were observed in aqueous extracts. Antidiabetic activity of the microalgal extracts was assessed using two methods viz., α-amylase amylase inhibition assay and glucose uptake assay. Maximum α-amylase amylase inhibition activity of 60.21% and glucose uptake of 46.12% by yeast was exhibited by aqueous extracts. Minimum α-amylase amylase inhibition activity of 12.87% and glucose uptake of 28.33% by yeast cell were observed in case of chloroform extracts.

Antioxidant activities were determined by Ferric ion reducing antioxidant power assay (FRAP) assays, phosphomolybdenum Assay (PM) and DPPH (1, 1- diphenyl 2-picrylhydrazyl) free radical-scavenging assay. FRAP assay and PM assay showed maximum absorption of 0.91 and 0.60 OD at 700 and 695 nm respectively for methanol extracts. Anti-inflammatory were studied using *in vitro* protein denaturation method in which methanol extract of microalga *Scenedesmus bajacalifornicus* BBKLP-07 showed highest anti-inflammatory activity. Furthermore, all the extracts of *Scenedesmus bajacalifornicus* BBKLP-07 were found to exhibit inhibitory activities against the pathogenic bacteria *E. coli, S. typhi*, and *B. subtilis* as well as fungal species *A. niger* and *C. albicans*, whereas *C. perfringens* had not shown any activity except aqueous extracts. The present study suggests that the green microalga *Scenedesmus bajacalifornicus* BBKLP-07 is a rich source of biologically active compounds and nutraceuticals. Further study is required to identify and purify the specific compounds responsible for the anti-activities and to explore the medicinal and pharmacological importance of the microalga.

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Compliance with ethical standards

Conflict of interest Authors do not have any conflict of interest related to the manuscript.

Ethical approval This article does not contain any studies related to animals and human participants.

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