In vitro Antioxidant and Anti-Diabetic Potential of Green Microalgae, Chlorella vulgaris

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Abstract: The search for antioxidants with inhibitory effects on the glycation cascade from biological origin offers a promising therapeutic and preventive approach. This study aimed at determining the in vitro antioxidant and anti-diabetic potential of methanolic extract of the microalgae, Chlorella vulgaris. Total phenolics and flavonoids were estimated in the algal extract. Various in vitro antioxidant and anti-diabetic assays were performed using the microalgal extract and compared with standard drugs. Total phenolics and flavonoid contents estimation revealed the presence at 1.27 mg/g GAE and 4.87 mg RE/g respectively. Inhibition percentage of 65.5% by the algal extract indicated the DPPH scavenging potential of the microalgae. Results of hydroxyl radical scavenging activity indicated that the algal extract had higher inhibition percentage (51.3%) than mannitol (48.1%). Both quercetin and algal extract exhibited similar superoxide radical scavenging activity in this study. The percent inhibition of nitric oxide was higher by the algal extract than the standard, curcumin at the concentrations tested. More than 50% of the singlet oxygen was scavenged by the algal extract at the experimental conditions. Inhibition of α-amylase activity was 63.1% at 20 mg l⁻¹ concentration of the algal extract. Glucose at a concentration of 10 mM was used for the glucose uptake assay and the algal extract exhibited 57% inhibition at 250 and 300 mg l⁻¹ concentration. A significant glucose adsorption capacity of the sample was also found which had a directly proportional relationship with the molar concentration of glucose. The results suggested further identification of the phenolic and flavonoid components content in active extract is needed to investigate possibility to isolate new compounds.

Key words: Antioxidant • Anti-Diabetic • Free Radicals • Chlorella vulgaris

INTRODUCTION

Diabetes is usually caused by the interaction of genetic and environmental factors and is characterized by a lack of insulin secretion and insulin resistance, always leading to metabolism disorders of fat, protein and carbohydrate. Insulin supplements and other oral anti-diabetic drugs can be used alone or in combination to improve glycemic regulation. However, some of the available anti-diabetic drugs have either the disadvantage of having low efficacy or serious side effects [1]. For example, synthetic inhibitors or anti-diabetic agents may reduce the absorption rate of glucose by slowing down carbohydrate digestion, causing a decrease in postprandial serum glucose level [2]. However, many of these synthetic hypoglycemic agents are non-specific, fail to prevent the development of diabetic complications and cause side effects [3]. Further, because of the increasing number of diabetic patients and the limited number of anti-diabetic drugs, the search for new compounds has attracted much interest from the scientific community. Thus, there is a continuous search for more effective and safer anti-hyperglycemic agents, especially from natural origins. Anti-diabetes screenings include evaluation of the functioning of specific enzymes involved in sugar metabolism [4]. Several bioactive metabolites produced by cyanobacteria and algae have been discovered by screening programs, employing target organisms quite unrelated to those for which the metabolites evolved. Microalgae have been screened for their anti-diabetic activity and a series of microalgal species with promising anti-diabetes properties have identified [5-12].
Free radicals and oxidative steps are known to get involved in the glycation process which is called glycoxidation and they are closely associated with the development of diabetic complications. One of the problems for many patients with diabetes is oxidative stress; the difference between the generation of oxygen-derived radicals and an organism’s antioxidant potential can lead to oxidative damage to cell components such as proteins, lipids and nucleic acids [13]. Oxidative stress is the trigger that drives various biochemical pathways associated with hyperglycaemia-induced cell damage [14]. Antioxidants are capable of neutralizing free radicals prior to their detrimental physiological effect and the use of synthetic antioxidants has recently been reported to have adverse side effects in human health. The discovery of antioxidants with inhibitory effects on the glycation cascade offers a promising therapeutic and preventive approach. A wide range of antioxidants are produced by microalgae which includes carotenoids, polyunsaturated fatty acids and polysaccharides that are known to protect against oxidative damage [15-21].

This study aimed at determining the in vitro antioxidant and anti-diabetic potential of methanolic extract of the microalgae, Chlorella vulgaris. Total phenolic and flavonoids were determined form the algal extract. Various antioxidant and free radical scavenging assays were performed using the standard drugs.

MATERIALS AND METHODS

Microalgae and Extract Preparation: The green microalgae C. vulgaris used in this study was cultivated in Bristol media in 2L bottles bubbled with air, incubated in a temperature-controlled room (24°C). The bottles were irradiated with daylight fluorescent tubes (light intensity, 54 mMol m<sup>-2</sup> s<sup>-1</sup>) for 10 days (12: 12 photoperiod). The microalgal biomass collected by centrifugation, dried and finely powdered. For solvent extraction, 50 g of the powdered biomass was used along with methanol. The extract was concentrated in Buchi rotary evaporator, followed by removal of traces of solvent using desiccator.

Determination of Total Flavonoid Content: The total flavonoid content was determined by the aluminum chloride colorimetric method as described by Chang et al. [22]. Algal extract (0.1 ml) was mixed with 1.5 ml of methanol, followed by the addition of 0.1 ml of 10% aluminum chloride, 0.1 ml of potassium acetate (1 M) and 2.8 ml of distilled water. The reaction mixture was kept at room temperature for 30 min. Absorbance of the reaction mixture was recorded at 415 nm. The calibration curve (0-8 µg ml<sup>-1</sup>) was plotted using rutin as a standard. The total flavonoids were expressed as mg of rutin equivalent/gram dry weight.

Determination of Total Phenolic Content: The amount of total phenolic content was determined using the Folin-Ciocalteu reagent [23]. Algal extract (0.1 ml) was mixed with 0.75 ml of Folin-Ciocalteu reagent. The mixture was kept at room temperature for 5 min and 0.75 ml of 6% sodium carbonate was added. After 90 min of reaction, its absorbance was recorded at 725 nm. The standard calibration (0-25 µg ml<sup>-1</sup>) curve was plotted using gallic acid. The total phenolics were expressed as mg gallic acid equivalent/gram dry weight. Negative control was prepared by adding 0.1 mL of DMSO instead of extract.

In vitro Antioxidant and Free Radical Scavenging Assays

Total Antioxidant Activity: Total antioxidant activity of microalgal extract was estimated by phosphomolybdenum assay [24]. Methanolic extract of C. vulgaris in different concentrations ranging from 100-500 µg ml<sup>-1</sup> were added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent solution. The tubes were incubated at 95°C for 90 min followed by normalization 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.

Measurement of Reducing Power: The Fe<sup>3+</sup>-reducing power of the extract was determined by a standard method [25]. Different concentrations (0-1.0 mg/ml) of the extract were mixed with equal volume of 0.2 M phosphate buffer (pH 6.6) and 0.1% potassium hexacyanoferrate, followed by incubation for 20 min at 50°C. After incubation, the reaction was terminated with 0.5 ml 10% TCA. Then, 1 ml reaction mixture was diluted with 1 ml distilled water followed by the addition of 0.1 ml FeCl<sub>3</sub> solution (0.01%). The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. Ascorbic acid was used as a standard.

DPPH Radical Scavenging Assay: The complementary study for the antioxidant capacity of the extract was confirmed by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging assay according to Mahakunakorn et al. [26],
with slight modification. Different concentrations (10-160 µg ml⁻¹) of the extract and the standard ascorbic acid were mixed with equal volume of ethanol. Then 50 µl of DPPH solution (1 mM) was added into the mixture and stirred thoroughly. The resulting solution was kept standing for 2 min before the OD was measured at 517 nm. The percentage of scavenging was calculated from the values of the control and the test samples.

**Hydroxyl Radical Scavenging Assay:** The hydroxyl radical scavenging assay was performed using a standard protocol method [27], based on quantification of the degradation product of 2-deoxyribose condensed with thiobarbituric acid. Hydroxyl radical was generated by the Fe³⁺-ascorbate-EDTA-H₂O₂ system (the Fenton reaction). In a final volume of 1 ml, various concentrations of the sample or reference compound was mixed with 2-deoxy-2-ribose (2.8 mM); KH₂PO₄-KOH buffer (20 mM, pH 7.4); FeCl₃ (100 µM); EDTA (100 µM); H₂O₂ (1.0 mM); ascorbic acid (100 µM) and incubated for 1 h at 37°C. 0.5 ml of the reaction mixture was added to 2.8% TCA, followed by 1% TBA and incubated at 90°C for colour development and the absorbance was measured at 532 nm against an appropriate blank solution. Mannitol was used as a positive control. Percentage inhibition was evaluated by comparing the test and blank solutions.

**Superoxide Radical Scavenging Assay:** This activity was measured by the reduction of nitro blue tetrazolium (NBT) according to an earlier method [28]. The 1 ml reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations of sample and standard quercetin solution. After incubation for 5 min at 25°C, quantity of generated formazan was measured at 562 nm against an appropriate blank.

**Hydrogen Peroxide Scavenging Assay:** H₂O₂ scavenging property of algal extract was evaluated using Ferrous oxidation-xylene orange (FOX) reagent with reference to sodium pyruvate [29]. An aliquot of 50 mM H₂O₂ and various concentrations of samples were mixed (1:1 v/v) and incubated for 30 min at room temperature. 90 µl of the incubated reaction mixture was mixed with 10 µl HPLC-grade methanol followed by 0.9 ml FOX reagent and incubated at ambient temperature for 30 min. The absorbance of the ferric-xylene orange complex was measured at 560 nm.

**Nitric Oxide Radical Scavenging Assay:** Nitric oxide radical scavenging activity was measured by adding 10 mM sodium nitroprusside, phosphate buffered saline (pH 7.4) and various doses of algal extract (5-50 µg ml⁻¹). Curcumin was used as a standard compound. After incubation for 150 min at 25°C, 1 ml sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 ml of the incubated solution and again after 5 min, 1 ml N-(1-Naphthyl) ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated for 30 min at 25°C. The resulting pink colour was measured spectrophotometrically at 540 nm against a blank sample [30].

**Peroxynitrite Radical Scavenging Assay:** The peroxynitrite scavenging activity of the extract and reference gallic acid was measured by Evans’ blue bleaching assay according to a standard method [31]. In a 1 ml reaction mixture contained 50 mM phosphate buffer (pH 7.4), 0.1 mM DTPA, 90 mM NaCl, 5 mM KCl, 12.5 µM Evans Blue, various doses algal extract (0–200 µg ml⁻¹) and 1 mM peroxynitrite. After incubation at 25°C for 30 min the absorbance was measured at 611 nm. The percentage scavenging of peroxynitrite was calculated by comparing the results of the test and blank samples.

**Singlet Oxygen Radical Scavenging Assay:** The production of singlet oxygen was determined by monitoring N, N-dimethyl-4-nitrosoaniline bleaching [32]. The final reaction mixture (2 ml) contained 45 mM phosphate buffer (pH 7.1), 50 mM NaOCl, 50 mM H₂O₂, 50 mM histidine, 10 µM N, N-dimethyl-4-nitrosoaniline and various concentrations (0-200 µg ml⁻¹) of sample. After incubation at 30°C for 40 min the decrease in N, N-dimethyl-4-nitrosoaniline absorbance was measured at 440 nm. Lipoic acid was used as reference compound for comparing the scavenging activity of the sample.

**Hypochlorous Acid Scavenging Assay:** The reaction mixture (1 ml) contained 50 mM phosphate buffer (pH 6.8), catalase (7.2 µM), freshly prepared HOCl (8.4 mM) and increasing concentrations (10-250 µg ml⁻¹) of extract. The assay mixture was incubated at 25°C for 20 min and the scavenging activity of the extract and the standard ascorbic acid was evaluated by measuring the decrease in absorbance of catalase at 404 nm [33].

**In vitro Anti-Diabetic Assay**

**Alpha-Amylase Inhibitory Assay:** The α-amylase inhibition assay was performed by some modification in
the method proposed by Giancarlo et al. [34]. Starch solution (1% w/v) was obtained by boiling and stirring 1 g of potato starch in 100 ml of sodium phosphate buffer for 30 min. The enzyme solution (50 U/ml) was prepared by mixing 0.01 g of α-amylase in 10 ml of sodium phosphate buffer (pH 6.9) containing 0.0006 mM sodium chloride. The extracts were dissolved in dimethyl sulfoxide (DMSO) to give concentrations from 2.5 to 25 mg/mL (2.5, 5, 10, 15 and 20 µg ml⁻¹). The color reagent was a mixture of 0.1 g of 3, 5-dinitrosalicylic acid, 2.99 g sodium potassium tartrate in 0.16 g sodium hydroxide and phosphate buffer (10 ml).

Totally, 50 µl of algae extract and 150 µl of starch solution, as well as 10 µl of enzyme, were mixed in a 96 well plate and incubated at 37°C for 30 min. Then, 20 µl of sodium hydroxide and 20 µl of color reagent were added and the closed plate was placed into a 100°C water bath. After 20 min, the reaction mixture was removed from the water bath, cooled, thereafter α-amylase activity was determined by measuring the absorbance of the mixture at 540 nm using ELISA reader. Blank samples were used to correct the absorption of the mixture in which the enzyme was replaced with buffer solution. In control, the algae extract was replaced with 50 µl of DMSO and the maximum enzyme activity was determined. Acarbose solution at the same concentrations was used as a positive standard. The inhibition percentage of α-amylase was assessed by the following formula:

\[
\% \text{ inhibition of } \alpha \text{-amylase} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

where \( A_{\text{control}} \) corresponds to the absorbance of the solution without extract (buffer instead of extract) and with \( \alpha \)-amylase solution and \( A_{\text{sample}} \) corresponds to the solution with extract and \( \alpha \)-amylase solution.

Determination of Glucose Uptake Capacity by Yeast Cells: Glucose uptake assay was performed according to the method of Cirillo [35]. 1% commercial baker’s yeast suspension was prepared in distilled water and kept overnight at 25°C. This was followed by centrifugation at 4200 rpm for 5 minutes and the process was repeated to obtain the clear supernatant. Different concentrations of algal extract in solvent were added to 1 ml of glucose solution (10 mM) and incubated for 10 min at 37°C. To initiate the reaction, 100 µl of yeast suspension (10% v/v) was added in the mixture of glucose and extract, vortexed and incubated for another 60 minutes at 37°C. After incubation, the tubes were centrifuged for 5 minutes at 3800 rpm and glucose was estimated by using a spectrophotometer at 520 nm. Absorbance for the respective control was also recorded on the same wavelength. The percent increase in uptake was calculated by the formula:

\[
\% \text{ increase in glucose uptake} = \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{sample}}} \times 100
\]

where control is the solution having all reagents except the test sample. Metronidazole was used as standard drug.

Glucose Adsorption Assay: The glucose adsorption capacity of the extract was determined by the method of Ou et al.,[36]. Approximately, 1 gram of extract was added to 100 ml of glucose solution of five different concentrations (5, 10, 15, 20 and 30 mM). Each of these mixtures was mixed well, stirred and incubated in a shaker water bath at 37°C for 6 hours, respectively. After incubation, the mixture was centrifuged at 4800 rpm for 20 minutes and finally the glucose content was determined in the supernatant by using glucose oxidase peroxidase diagnostic kit. The amount of bound glucose was determined by the given formula:

\[
\text{Glucose bound} = \frac{G1 - G6}{\text{Weight of sample}} \times \text{volume of sample}
\]

G1 is the glucose concentration of the original solution, while G6 is the glucose concentration after 6 hours.

Statistical Analyses: All experiments were performed in three replicates and the data are presented as the mean ± standard deviation and standard error. To evaluate the statistical significance of the data obtained in the analyses, the one-way ANOVA test (p<0.05) was applied.

RESULTS AND DISCUSSION

Total Phenolics and Flavonoids Content: Quantitative total flavonoid determination was performed by precipitating the extract with aluminum chloride have an intense yellow fluorescence when observed by UV spectrophotometer. Total flavonoids content were expressed as mg rutin equivalent (RE) per gram dry extract.
concentrations of sample and 1 concentration of BHT whereas it was 1 concentration which was 1 such as Fe²⁺ and Cu²⁺ [38]. 2+ 2+

attributed to the inhibition of OH by chelating metal ions demonstrate that the OH scavenging activity may be conditions. Several studies of antioxidant capacity generated in the human body under certain physiological reactive free radical. This free radical can be formed from The OH generated by the Fenton reaction is a highly 4.87 mg RE/g. The quantitative determination of total phenolic was carried out using Folin–Ciocalteu reagent in terms of gallic acid equivalent. Total phenolic content is expressed as mg gallic acid equivalent per gram dry extract weight. The highest amount of total phenolics present in the methanolic extract was found as 1.27 mg/g GAE.

In-vitro Antioxidant Activity: Different concentrations of algal extracts were tested for its total antioxidant activity and compared with the ascorbic acid activity. An increase in absorbance was observed with higher concentrations of the extract. Highest absorbance of 0.74 and 1.19 were found at 500 µg ml⁻¹ concentrations of sample and standard drug (Fig. 1). Butylated hydroxytoluene (BHT) was used to compare the reducing power activity of algal extract in vitro. Highest absorbance of 0.68 was recorded with 0.8 µg ml⁻¹ concentration of BHT where as it was 0.51 in the case of algal extract (Fig. 2).

Positive result in DPPH radical scavenging activity of microalgal extract was observed however, the activity was lower than the ascorbic acid standard. Inhibition percentage of 65.5% by the algal extract indicated the DPPH scavenging potential of the microalgae (Fig. 3). DPPH is a radical scavenged by antioxidants through a donation of a proton forming reduced DPPH which changes the colour from purple to yellow after reduction. DPPH radical scavenging activity increased with increasing percentage of free radical inhibition. DPPH is a stable radical. In the assay, the DPPH is scavenged by the antioxidant present in extract with a suitable reducing agent. The electrons now pair off and the solution loses the colour depending upon the electrons taken up [37]. It can be assumed that microalgal extract acts as hydrogen donors with good antioxidant principles.

Results of hydroxyl radical scavenging activity indicated that the algal extract had higher inhibition percentage than mannitol (Fig. 4). At 200 µg ml⁻¹ concentration, 51.3% inhibition was recorded which was higher than mannitol at the same concentration (48.1%). The OH generated by the Fenton reaction is a highly reactive free radical. This free radical can be formed from hydrogen peroxide and the superoxide anion and may be generated in the human body under certain physiological conditions. Several studies of antioxidant capacity demonstrate that the OH scavenging activity may be attributed to the inhibition of OH by chelating metal ions such as Fe²⁺ and Cu²⁺ [38].

Both quercetin and algal extract exhibited similar superoxide radical scavenging activity in this study. More than 50% inhibition was seen at the concentrations tested indicating the free radical scavenging activity of the test sample (Fig. 5). Superoxide dismutase catalysis the disputation of reactive superoxide anion to oxygen and H₂O₂ [39]. The superoxide scavenging is determined in terms of inhibition of generation of superoxide anions.

Hydrogen peroxide scavenging assay revealed the moderate activity of algal extract as the reference standard sodium pyruvate exhibited higher activity of 53.2 % scavenging at its highest concentration. The formation of ferric-xylenol orange complex was lower in algae treated samples which was reflected in the lower scavenging activity (Fig. 6). H₂O₂ scavenging by an extract is due to the presence of their phenolics, which can donate electrons to H₂O₂ and to neutralize it to water [40, 41].

Increase in the nitric oxide radical scavenging activity as indicated by the percent inhibition was seen with the algal extract. The percent inhibition was higher by the algal extract than the standard, curcumin at the concentrations tested. At 50 µg ml⁻¹, 49.3% inhibition was recorded for algal extract whereas it was 43% for curcumin (Fig. 7). Nitric oxide (NO) is a pro-inflammatory mediator involved in various physiological events and its production is extremely important to defend the body. However, its overproduction can lead to tissue damage and activation of pro-inflammatory mediators associated with acute and chronic inflammation [42]. The continuous exposure to free radicals generated from chronic inflammation causes more cancers than environmental chemicals [43]. Therefore, excessive generation of NO needs to be checked by using certain agents and the inhibition of nitric oxide synthesis by the algal extract makes it suitable to control the pro-inflammatory mediators.

Lower peroxynitrite radical scavenging activity was exhibited by the algal extract in this study (Fig. 8). Gallic acid was used as standard and 57.2% scavenging was observed at 250 µg ml⁻¹ concentration which was quite higher than the algal extract (37.1%). Nitric oxide toxicity is heightened on reaction with superoxide radical to form a second reactive compound peroxynitrite anion. Furthermore, the protonation of peroxynitrite forms a dangerous and highly reactive compound peroxynitrous acid [44, 45]. The algal extract inhibited the process by scavenging peroxynitrite.

The decrease in N, N-dimethyl-4-nitrosourea absorbance was observed in both the sample and reference compound tested. More than 50% of the singlet
Fig. 1: Total antioxidant activity

Fig. 2: Reducing power activity

Fig. 3: DPPH radical scavenging activity (%)
Fig. 4: Hydroxyl radical scavenging activity (% (%)

Fig. 5: Superoxide radical scavenging activity (%)

Fig. 6: Hydrogen Peroxide radical scavenging activity (%)
Fig. 7: Nitric Oxide radical scavenging activity (%)

Fig. 8: Peroxynitrite anion scavenging activity (%)

Fig. 9: Singlet oxygen scavenging activity (%)
Fig. 10: Hypochlorous acid scavenging activity (%)

Oxidative stress has been the predominant cause for the pathogenesis of diseases and disorders like cardiovascular diseases, diabetes mellitus, cancer, aging and many inflammatory conditions. Cell damage caused by generation of free radicals appears to be a major contribution to this oxidative stress. So, antioxidants can offer protection and combat against this oxidative stress by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. Compounds with antioxidant activity may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents and quenchers of single-oxygen formation or reactive oxygen species. Antioxidants play a crucial role in delaying or preventing the oxidation of lipids or other cellular compounds by inhibiting the initiation or perpetuation of oxidative chain reactions [46]. Phenolic components such as flavonoids, phenolic acids and phenolic diterpenes have shown antioxidative properties that decrease free radicals, quench singlet and triplet oxygen or decompose peroxides.

The positive relationship between phenolic compounds and total antioxidant capacity is reported long ago [47]. Goiris et al. [48] reported that both carotenoid and phenolic content significantly contributed to the antioxidant capacity of microalgae. Similarly, Hajimahmoodi et al. [49] reported the positive correlation between antioxidant capacity and phenolic content.

In-vitro Anti-Diabetic Activity: 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. A known concentration of methanolic extracts of C. vulgaris was subjected to α-amylase inhibitory assay along with Acarbose as a standard. Inhibition of α-amylase activity was 63.1% at 20 mg l⁻¹ concentration whereas acarbose was showing 76.7% inhibition (Fig. 11).

Glucose uptake assay was carried out using yeast cells as model using metronidazole as standard drug. Glucose at a concentration of 10 mM was used and the algal extract exhibited 57% inhibition at 250 and 300 mg l⁻¹ concentration. Inhibition percentage of 65% was recorded with metronidazole (Fig. 12). The results indicated that increasing the concentration of extract will increase the capability of yeast cells to uptake more glucose from the environment. Transport of glucose across yeast membrane may involve facilitated diffusion rather mediation of a phosphotransferase enzyme system or any other unknown process. The extract could bind glucose effectively and transport it across the cell membrane for further metabolism.

The effect of algal extract on in vitro glucose adsorption has been shown in Fig. 13. The results of the present study indicated that the extract possessed a significant glucose adsorption capacity at all tested concentrations. The glucose adsorption capacity of the sample was also found to have a directly proportional
Fig. 11: α-amylase inhibition assay

Fig. 12: Glucose Uptake Capacity by Yeast Cells

Fig. 13: Glucose adsorption assay
relationship with the molar concentration of glucose. Hence the minimum adsorption was recorded at 5 mM glucose concentration and maximum at 30 mM. It was also seen that the extract is capable of binding the glucose even at lower concentrations.

Flavonoids are the most important compounds which act as chemical messengers, physiological regulators, cell cycle inhibitors and performs various other physiological roles. Flavonoids and their polymers are important members of polyphenols which have been extensively studied in the last decades. Flavonoids have long been recognized to possess biological activities [50] and natural flavonoids are promising compounds as a potential source of exogenous antioxidants. Functional constituents of algae have been increasingly used as food supplements as well as for anti-diabetic purposes [51]. Microalgae are considered preventive nutrients and are used for their hypoglycemic and therapeutic effects [52]. Recently, anti-diabetes peptides from *Spirulina platensis* were identified by Hu *et al.* [53]. The inhibitory effect of *C. pyrenoidosa* against key enzymes relevant for type-2 diabetes was reported by Sun *et al.* [54]. In this study, the presence of flavonoids in the algal extract and its anti-diabetic activity suggests the biological potential of microalgae.

It is well-known that microalgae could produce a wide range of antioxidant compounds including carotenoids, phenolic compounds, polysaccharides and long-chain polyunsaturated fatty acids. Besides them, also other compounds should contribute to the bio-activities of microalgae. Further characterization of phenolic and flavonoid components is needed to better understand the contribution to the antioxidant capacities of microalgae.

**CONCLUSION**

The microalgae extract exerting the high antioxidant activities are potential new source of natural antioxidants with anti-diabetic activity. Further identification of the phenolic and flavonoid components content in active extract is needed to investigate possibility to isolate new compounds.

**REFERENCES**


