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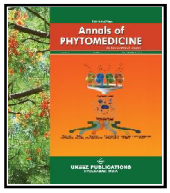

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Antioxidant capacities of crude extract and protein glycation inhibitory activities of bioactive fractions of *Arthrospira* (*Spirulina*) *platensis* Gomont

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Abstract

Under hyperglycaemia, increased non-enzymatic glycation processes are closely associated with the production of ROS, which may contribute to functional complications in degenerative diseases. Nontoxic blue-green microalga, *Arthrospira* (formerly known as *Spirulina*) *platensis* Gomont, composed of a high amount of nutritive ingredients and an immense variety of phytoconstituents, has great potential as medicine. The purpose of this study was to determine the antioxidant potential and to investigate the inhibitory effect of bioactive fractions from the methanolic extract of A. *platensis* in two *in vitro* system, bovine serum albumin-fructose (BSA-fructose) and bovine serum albumin-methylglyoxal (BSA-MG). The quantification of phytochemicals suggests that methanol is the most effective solvent for the extraction, resulting in higher total phenolics (13.50 ± 0.11 mg GAE/g) and flavonoids (6.23 ± 0.06 mg QE/g) compared to other solvents.The antioxidant analysis revealed that the methanolic extract of *A. platensis* shows remarkable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, metal chelating and nitric oxide (NO) scavenging activities with an IC_{50} value 9.58 ± 0.19 mg/ml, 21.44 ± 1.14 mg/ml and 17.14 ± 0.81 mg/ml, respectively. FRAP (ferric reducing antioxidant power) value of extract is 41.26 ± 0.23 mM $FeSO_4$/g. The methanolic extract was further fractionated using column chromatography with nonpolar (hexane), semi-polar (ethyl acetate) and polar (methanol) sequentially and collected five fractions named, F1, F2, F3, F4 and F5. Fraction 4 displays a significantly (p<0.05) lower IC_{50} value in fructosamine, protein carbonyl content and congo red reaction, which indicates the most promising inhibitory effect on various stages of glycation than other fractions. In BSA-fructose model, F4 represents a higher glycation inhibition rate than BSA-MG model. Our study demonstrated the effective glycation inhibitory and antioxidant activities of *A. platensis* which might suggest a potential role as phytomedicine for the treatment of diabetic complications and ageing.

1. Introduction

A non-enzymatic interaction occurs between sugar and protein referred to as protein glycation, which is also known as the Maillard reaction. Protein glycation is a sequential process which takes place in three stages. The first stage initiates with the formation of an unstable reversible product, Schiff base. Later, rearrangement creates Amadori products which are stable ones. The complex cascade of protein glycation ultimately ends with the occurrence of irreversible and harmful, advance glycation end products (AGEs). The progressive rise in the level of AGEs is due to normal ageing and uncontrolled diabetic conditions. Consequently, patients suffering from diabetes mellitus have a twofold higher aggregation of AGEs in the tissues and this is the main cause of complications of diabetes including neuropathy, nephropathy, retinopathy and cardiovascular diseases (Chauhan and Sharma, 2016). Oxidative stress due to increased accumulation of reactive oxygen species (ROS) may also accelerate the non-enzymatic glycation process leading to the development of cardiovascular diseases, atherosclerosis, cancer, hypertension, diabetes mellitus, ischemia, hyperoxaluria, neurodegenerative diseases (Alzheimer's and Parkinson's), ageing, rheumatoid arthritis, *etc*. (Rajeshwari *et al.*, 2013). The rate of AGEs formation is influenced by different parameters such as metal ions and α-dicarbonyls, increasing the formation of free radicals and generation of oxidative stress (Muñiz *et al*., 2018). Fructose consumption in the human diet increasing in the last two decades could possibly contribute to protein glycation and insulin resistance (Teff *et al.*, 2009). Endogenous fructose produced *via* a polyol pathway can be converted to a Heyns rearrangement product and finally produced by irreversible fluorescent AGEs. The rate of protein glycation in the presence of fructose is exceeded than glucose (Gugliucci, 2017). MG, the most reactive α-dicarbonyl compound generated from degradation of glycolytic intermediates, is responsible for AGEs formation. These AGEs play a key role in the structural modification and cross-linking of proteins. They not only change the confirmation of proteins but also lead to dysfunction of different tissues either by direct binding or binding through RAGE (receptor for AGEs). The binding occurs through receptors and triggers a cascade of cellular events underlying inflammation, thrombogenesis and other abnormalities (Yamagishi *et*

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al., 2012). AGEs-related complications could be delayed or prevented by reducing the formation and accumulation of AGEs in tissue. This can be achieved by antiglycation agents which may block the formation of AGEs at various stages of protein glycation. Aminoguanidine (AG) is one of them with a potent antiglycation effect, but due to some side effects and poor efficiency, it was withdrawn during the clinical trial phase 3. However, there is an urge for natural products with higher effectiveness and minimal side effects against protein glycation. Numerous of *in vitro* and *in vivo* studies have confirmed that natural products, specifically the polyphenol family members, are promising AGEs inhibitory agents; their inherent antioxidant capability emphasizes their effectiveness as antiglycation agents (Peng *et al.*, 2011; Banan and Ali, 2016; Kamalakkannan *et al*., 2021).

A. platensis is well known for its trade name *Spirulina*. It belongs to a photosynthetic bacterial phylum, cyanobacteria and itis widely cultured around the world. *Spirulina* refers to the dried biomass of *A. platensis*, which is considered a superfood due to its high protein composition, essential amino acids, essential fatty acids, vitamins such as vitamin B6, B12, B2, E and A, minerals, *i.e*., Ca, Fe, P, Zn, Cu, Mg, Cr, Mn, Na, K, and Se and enzymes (Guldas and Irkin, 2010). In 2011, the FDA permitted *Spirulina* as a food supplement through GRAS (generally recognized as safe). Apart from its nutritional aspects, *Spirulina* has antioxidant, antidiabetic and antilipidemic activities which are very effective in protecting against oxidative damage in rats with type-I diabetes (Guldas *et al.*, 2021), free radical scavenging and lipid peroxidation inhibitory activity (Boora *et al*., 2014) as well as positive effects on glucose tolerance in rats (Wan *et al.*, 2019) and promotes the regulation of metabolic disorder (Van den Driessche *et al.*, 2018). Several studies have been reported on the dietary abilities, antioxidant capacity and antidiabetic activity of *A. platensis* by various researchers. However, the antiglycation potential of *A. platensis* is not well explored (Guldas *et al.*, 2021). Thus, the present study is focused on antioxidant activity and investigation of antiglycation potential of bioactive fractions of *A. platensis* after *in vitro* glycation by fructose and methylglyoxal for four weeks incubation, examining fructosamine, protein carbonyl content and Congo red binding to amyloid cross β-structure as well as fluorescence intensity.

2. Materials and Methods

2.1 Collection and extraction of *A. platensis*

The nutraceutical-grade *Arthrospira (Spirulina) platensis* Gomont powder used in this study was obtained from Darsh Biotech Private Limited, Ahmedabad (Gujarat). This dried powder (10 g) was subjected to extraction of bioactive phytochemicals with a Soxhlet apparatus using various solvents (distilled water, chloroform, butanol, acetone and methanol), followed by evaporation using a rotary evaporator.The residues were weighed and dissolved in an appropriate amount of dimethyl sulfoxide, which was taken as crude extracts for further experiments. Analytical-grade chemicals were used in the present experiment.

2.2 Determination of total phenolic content

The amount of total phenolic content (TPC) from crude extracts of *A. platensis* was analysed with minor modifications (Singleton *et al.*, 1999). For this, 100 µl of the sample was mixed with 0.2 ml of Folin-Ciocalteu reagent, 3.0 ml of distilled water and 2.0 ml of 20% Na_2CO_3. The mixture was mixed thoroughly and kept in a boiling water bath for exactly 1.0 min. The absorbance was taken at 650 nm wavelength with a UV-visible spectrophotometer. The calibration curve was prepared using gallic acid as a standard. Results were expressed as mg of gallic acid equivalent/g of powder.

2.3 Determination of total flavonoid content

The total flavonoid content (TFC) of crude *A. platensis* extracts was quantified by using the aluminium chloride colorimetric procedure with slight alterations (Chang *et al*., 2002). A mixture of 1.5 ml methanol, 0.1 ml 10% aluminium chloride, 0.1 ml potassium acetate (1.0 M) and 2.8 ml distilled water were added to 100 µl of the sample. After mixing the content well, all tubes were kept at 37°C for half an hour and then the absorbance mixture was measured at 415 nm using a UV-Visible spectrophotometer. The calibration curve was obtained from quercetin and results were expressed as mg of quercetin equivalent/g of powder.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

The scavenging capacity of the extract for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured with some modification in the procedure (Adebiyi *et al*., 2017). Sample extract (100 µl) with various concentrations was added to 1.0 ml of 0.1 mM methanolic DPPH.The mixture was shaken well and allowed to incubate in a dark condition for half an hour and absorbance was taken at 517 nm. Vitamin C was taken at a different concentration as a standard and DPPH solution was taken as a control for this assay. The results were expressed in terms of % inhibition using the following formula:

Inhibition % = Absorbance of control – Absorbance of sample/ absorbance of control × 100

2.4.2 Ferric-reducing antioxidant power (FRAP) assay

The ferric reducing capability of extracts was determined by using the FRAP assay with minor changes to the method described by Benzie and Strain (1996). In brief, 100 µl of sample extract was added to the freshly prepared 3.9 ml of FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ mixed in a ratio of 10:1:1). After incubation of 10 min at 37°C, the absorbance was measured at 593 nm. The calibration curve was obtained using $FeSO_4.7H_2O$ as a standard. The results were expressed in mg equivalents of $FeSO_4$/g.

2.4.3 Metal chelation ability

The capacity of extract to chelate ferrous ions was determined by a method reported earlier (Marcocci *et al.*, 1994). Briefly, 100 µl of sample extract was added to 0.1 ml of $FeCl_2$ (2 mM), and incubated for 30 min at room temperature. Further, 0.2 ml ferrozine (5 mM) was added to the mixture and allowed to incubate for 10 min at room temperature. The absorbance was measured at 562 nm. The results were expressed in terms of % inhibition using the given formula:

Inhibition % = [1– (A1 – A2)/A0] × 100%

A0: Absorbance of the control (the mixture without sample),

A1: Absorbance of the mixture in the presence of the sample,

A2: Absorbance without ferrozine.

2.4.4 Nitric oxide scavenging assay

Nitric oxide scavenging activity of extracts was measured through the method described previously (Bhatt *et al.*, 2019) using Griess reagent. Initially, 100 µl of sample extract was incubated with 0.5 ml 50 mM sodium nitroprusside at room temperature for 1 h. 0.5 ml Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NED) was added to 0.5 ml of the incubated mixture and the absorbance was taken instantly at 540 nm. Ascorbic acid was used as a standard for this scavenging activity. NO scavenging activity was calculated using the following formula:

Scavenging activity (%) = [1– (A1 – A2)/A0] × 100%

A0: Absorbance of the control (the mixture without extract),

A1: Absorbance of the mixture in the presence of the extract,

A2: Absorbance without Griess reagent.

2.5 Fractionation of crude *Spirulina* extract

The crude extract of *A. platensis* was then subjected to fractionation through the column chromatography technique with some modifications (Bajpai *et al.*, 2016). The glass column is prefilled with silica gel (230-400 mesh, 40 g) as a stationary phase. The crude extract was poured on top of the silica gel. The elution was done by applying solvents (with increased polarity order) in a manner initiated with 100% hexane (F1), hexane-ethyl acetate (1:1, F2), ethyl acetate (100%, F3), ethyl acetate-methanol (1:1, F4) and 100% methanol (F5) to achieve fractions. Each fraction (25 ml) was collected and further proceeded for the determination of antiglycation potential.

2.6 Preparation of *in vitro* glycated bovine serum albumin model

2.6.1 BSA-fructose model

Glycated BSA was prepared by the previously described method with necessary changes to determine the inhibition of protein glycation at an early stage (Ardestani and Yazdanparast, 2007). In a phosphate buffer saline (200 mM, pH 7.4) containing 0.02% sodium azide, 400 ml of fructose (500 mM) and 500 ml of BSA (40 mg/ml) were incubated together. This mixture is considered glycated BSA-fructose for further procedures.

2.6.2 BSA-methylglyoxal model

The assessment of the inhibition of middle-stage protein glycation was carried out according to the previously reported method (Peng *et al.*, 2008). For this, 500 µl of BSA (40 mg/ml) was incubated with 400 µl of methylglyoxal (MG) (500 mM), in phosphate buffer saline (200 mM, pH 7.4) and 0.02% sodium azide. This mixture is further used as glycated BSA-MG.

2.7 Antiglycation potential

Bioactive fractions of *A. platensis* were added at different concentrations to the prepared glycated models. Glycated BSA-fructose and glycated BSA-MG were incubated separately, with and without test samples, at 37°C for 4 weeks. As a positive control, aminoguanidine (AG) was used in place of test samples and for the negative control, only BSA was taken. All the reaction mixtures were assessed for various glycation parameters, *i.e.*, fructosamine (early stage), protein carbonyl content (intermediate stage), amyloid β-aggregation by Congo red binding and formation of AGEs (late stage).

2.7.1 Estimation of fructosamine

Nitro blue tetrazolium (NBT) was used to determine fructosamine as per the previously described method of Baker *et al.* (1994). In brief, 0.2 ml of each reaction mixture was treated with 0.8 ml of NBT reagent (0.3 mM), prepared in sodium carbonate buffer (100 mM, pH 10.5). After incubation at 37°C for 15 min, the absorbance was taken at 530 nm. For this experiment, the results were expressed in terms of % inhibition calculated using the given formula:

Inhibition % = [(A0 – A1)/A0] × 100

A0: Absorbance of the control (the reaction mixture without a test sample)

A1: Absorbance of the mixture with a test sample.

2.7.2 Determination of protein carbonyl content

The carbonyl content was analysed as per a method reported earlier with minor modification (Uchida *et al.*, 1998). The experiment started by incubating 0.1ml of every reaction mixture with 0.4 ml of 2,4-dinitrophenylhydrazine (DNPH, 10 mM) in 2.5 M HCl for about 60 min at room temperature. This was followed by precipitation using 20% trichloroacetic acid (TCA). After 5 min of incubation on ice, the mixtures were centrifuged at 10,000 g for 10 min at 4°C. The pellet was washed thrice using 0.5 ml of ethanol and ethyl acetate mixture (1:1). Finally, the pellet was dissolved in 6.0 M guanidine hydrochloride and the absorbance was measured at 370 nm. The concentration of protein carbonyl was calculated using the absorption coefficient and results were expressed as % inhibition.

2.7.3 Determination of amyloid β-aggregation

The assay was performed to determine the binding ability of a glycated protein to Congo red dye (Klunk, 1994). Reaction mixture (0.5 ml) was incubated with 0.1 ml of Congo red (100 mM) in phosphate buffer saline with 10% ethanol at room temperature for 20 min and the absorbance was recorded at 530 nm. The results were expressed in terms of % inhibition of Congo red binding.

2.7.4 Spectrofluorometric measurement of AGEs

The development of AGEs is also measured using fluorescence intensity at an excitation of 370 nm and an emission wavelength of 440 nm using a fluorescence spectrophotometer (Perkin Elmer) (Ashraf *et al.*, 2016). The percentage of inhibition of AGEs is calculated using the following formula:

Inhibition of AGEs formation = [(F1 – F2)/F1] × 100

F1: Fluorescence intensities of glycated BSA-fructose or BSA-MG,

F2: Fluorescence intensities of glycated BSA treated with sample or AG

2.8 Statistical analysis

Each experiment was carried out in triplicate and the results are presented as the mean ± standard deviation. Statistical analyses were performed using ANOVA.Values of p less than 0.05 were considered to be statistically significant.

3. Results

3.1 Quantitative analysis of phytochemicals

The total phenolic content and total flavonoid content extracted from the dried biomass of *A. platensis* with various solvents are

presented in Figure 1. These data indicate that the methanolic extract has the highest total phenolic (13.50 ± 0.11 mg GAE/g) and flavonoid (6.23 ± 0.06 mg QE/g) content, which was followed by the aqueous, acetone and butanol extracts of *A. platensis.* Chloroform is considered as a poor solvent to extract phytochemicals as it presents significantly lower phenolic content (4.54 ± 0.05 mg GAE/g) and total flavonoid (1.92 ± 0.08 mg QE/g) levels.The order for phytochemical content is the following; Methanol>D/W>Acetone>Butanol>Chloroform.

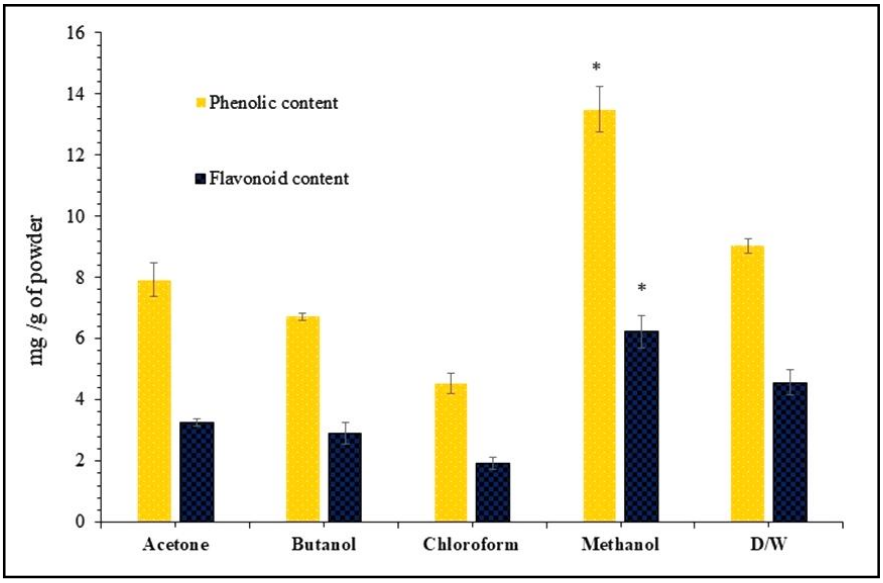

Figure1: Total phenolics and total flavonoids content of extract of *A. platensis*.
The data are mean ± SD of independent experiments (n=3). *p<0.05 significance difference

3.2 Antioxidant activities

The antioxidant capacity of phenolic and flavonoid-rich methanolic crude extract of *A. platensis* was investigated by DPPH radical scavenging assay, metal chelation and nitric oxide scavenging activity and the result is depicted in Figure 2. The antioxidant activity presented in the result is dose dependent. The IC_{50} value is used for the interpretation of the antioxidant capacity of methanolic crude extract of *A. platensis* shown in Table 1. IC_{50} values of DPPH radical scavenging assay, nitric oxide scavenging and metal chelation activity were found to be slightly higher than ascorbic acid and EDTA as reference standard. It is well known that antioxidant ability is inversely correlated with IC_{50} values. A remarkable antioxidant potential of methanol extract of *A. platensis* was obtained. Ferric reducing power is an essential aspect of measuring the antioxidant activity. The ferric reducing ability of *A. platensis* extract is increased linearly with the concentration of extract shown in Figure 3.

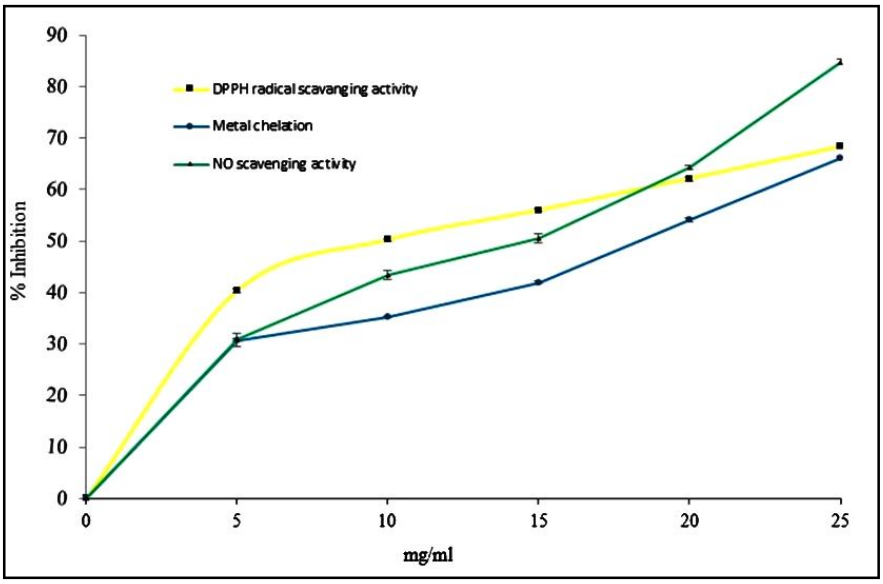

Figure 2: Antioxidant potential of methanolic extract of *A. platensis*.
The data are mean ± SD of independent experiments (n=3).

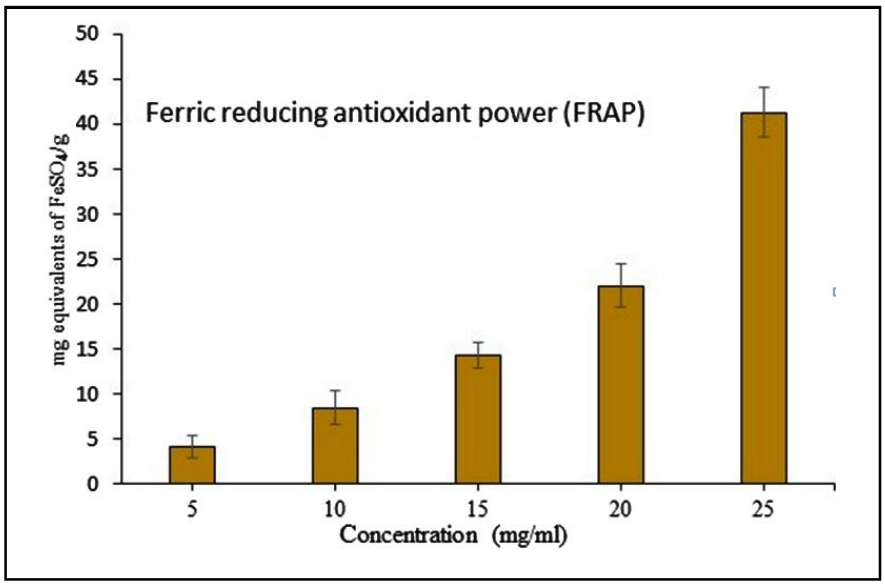

Figure 3: FRAP activity of methanolic extract of *A. platensis*.
The data are mean ± SD of independent experiments (n = 3).

Table 1: IC_{50} value of antioxidant parameters for methanolic extract of *A. platensis*

Antioxidant parameter	IC_{50} value(mg/ml)
DPPH radical scavenging activity	9.58 ± 0.19
Metal chelation activity	17.44 ± 0.14
Nitric oxide scavenging activity	13.14 ± 0.11

3.3 Antiglycation potential of bioactive fractions of *A. platensis*

When amethanolic extract of *A. platensis* was subjected to column chromatography, five fractions were collected based on increasing polarity. Each five fractions were investigated for their phenolic and flavonoid content. Figure 4 indicates that significantly higher amounts of phenolics and flavonoids are present in F5 (methanol), followed by F4 (ethyl acetate-methanol) compared to F3 and F4. F1 (hexane) shows negligible amounts of phenolics and flavonoid not significantly considered. Data indicates fraction 5 contains the highest amount of phenolics and flavonoids.

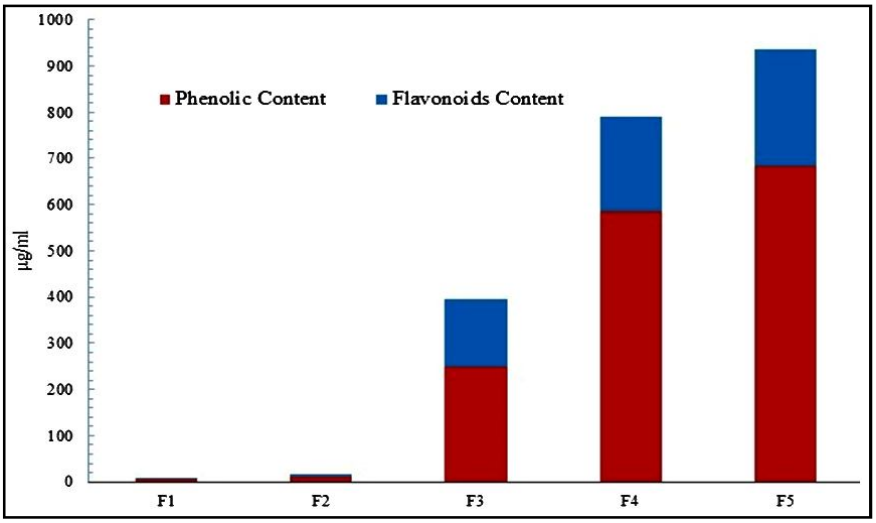

Figure 4: Phenolics and flavonoids content obtained in five fraction of *A. platensis* by column chromatography.

The experiment was designed to examine the antiglycation potential of each fraction and reference compound, aminoguanidine based capacity to inhibit the formation of AGEs at multistage glycation markers-fructosamine (early stage), protein carbonyls (intermediate stage) and β aggregation of albumin using amyloid-specific dyes-Congo red (late stage) in the BSA-fructose and BSA-MG model. Figure 5 presents an increase in the percentage of inhibition for fructosamine formation in the presence of each fraction at various concentrations, except for F1and F2.

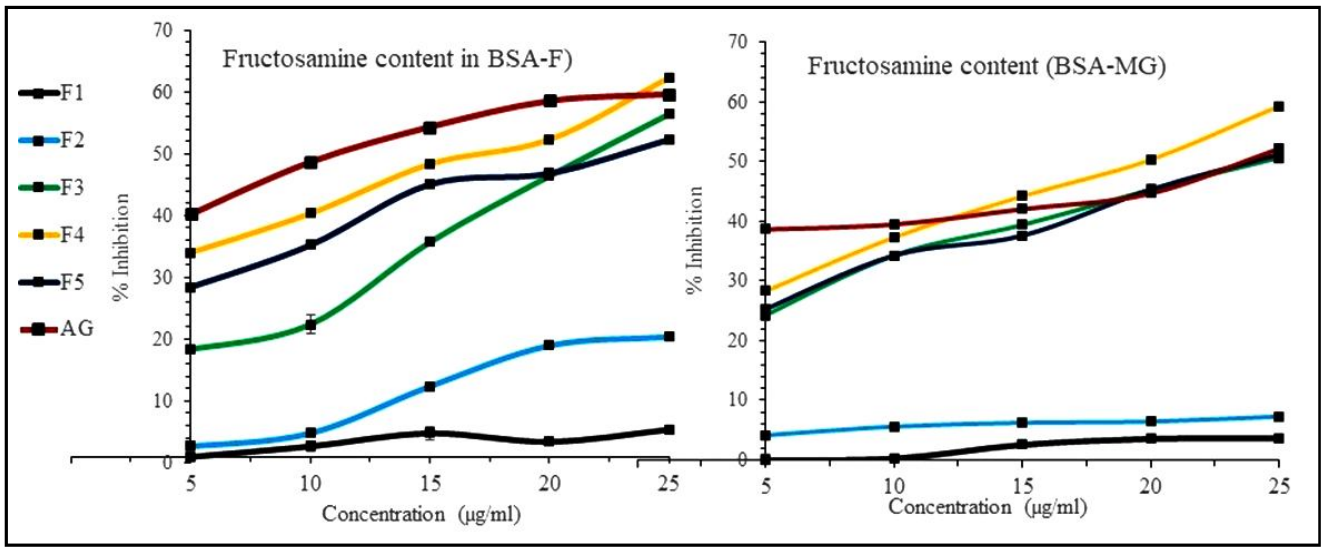

Figure 5: Inhibition of fructosamine by each fraction obtained from methanolic extract of *A. platensis* in the BSA-fructose and BSA-MG model.

The data are mean ± SD of independent experiments (n=3).

The findings of the current study showed that F4 exhibits significantly higher inhibition of fructosamine formation in BSA-fructose (63.46 ± 2.14%) and in BSA-MG (59.12 ± 3.47%) at its maximum concentration compared to other fractions. It was found that F1 was not able to inhibit fructosamine formation in both glycation models. The process of glycation causes protein oxidation, for which the percentage of inhibition of protein carbonyl content was measured as a marker for the intermediate stage of glycation and shown in Figure 6.

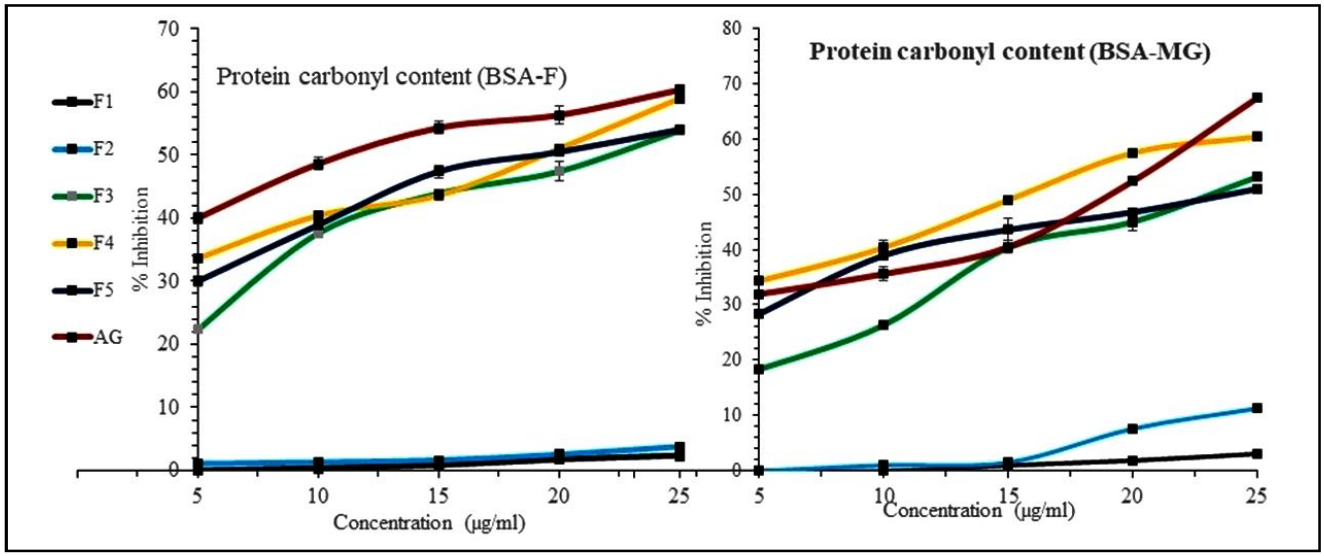

Figure 6: Inhibition of protein carbonyl content by each fraction obtained from methanolic extract of *A. platensis* in the BSA-fructose and BSA-MG model.

The data are mean ± SD of independent experiments (n=3).

The results for the BSA-fructoseand BSA-MG models revealed that F4 had the greatest inhibition with 58.07 ± 4.76% and 62.27 ± 2.17%, respectively. It is observed that F2 had the lowest inhibition with 11.45 ± 0.29% and 3.65 ± 0.34% in protein carbonyl formation at the end of 28 days for both glycation models, respectively. The inhibition of protein carbonyl formation was not detected for fraction 1 in the BSA-fructose and the BSA-MG models indicating an absence of antiglycation ability. Glycation reaction carries out conformational change in native protein and leads to formation of amyloid β-aggregation, which was investigated using a specific dye Congo red.

As evident from Figure 7, F4 exhibits maximum potential to inhibit Congo red binding with 68.72 ± 2.53% in the BSA-fructose model and 65.65 ± 1.64% in the BSA-MG model when compared to F3 and F5. The absence of a line in Figure 7 for the F1 and F2 fractions indicates that these fractions could not prevent the formation of amyloid β-aggregation in both glycated models. The comparative total antiglycation potency of each fraction of *A. platensis* isindicated from an IC_{50} value for the fructosamine, protein carbonyl and Congo red assays shown in Table 2.

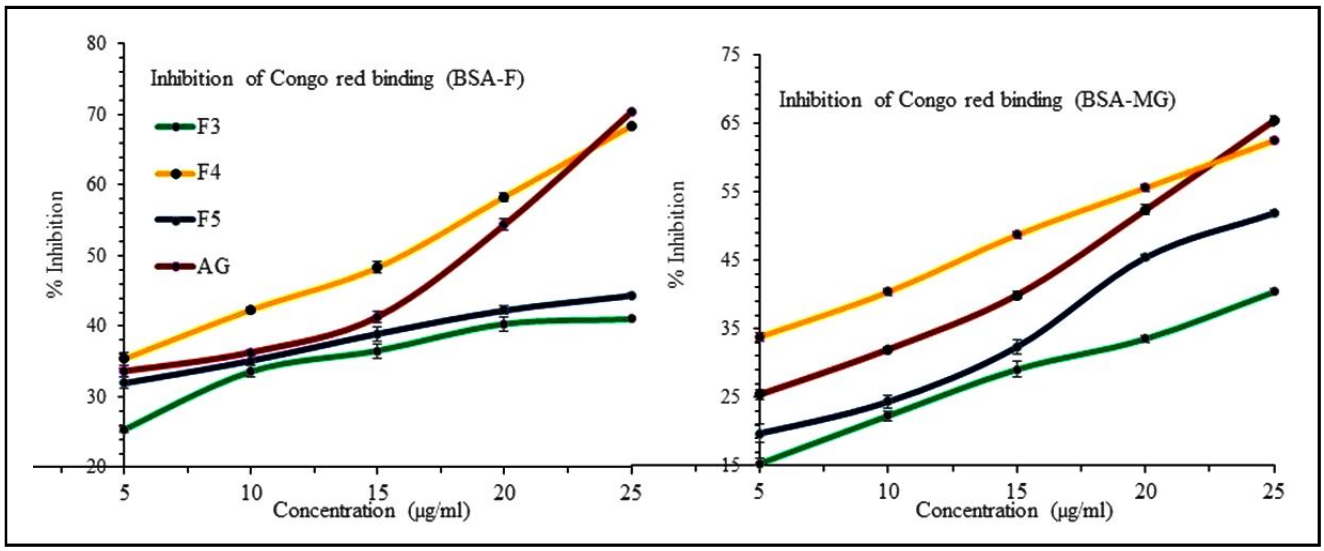

Figure 7: Inhibition of Congo red binding by fractions obtained from methanolic extract of *A. platensis* in the BSA-fructose and BSA-MG model.

Table 2: IC_{50} value for various parameters of antiglycation potential for fractions obtained from extract of *A. platensis* (Value indicates in µg/ml); AG-aminoguanidine, ND: Not detected

Fractions	BSA-F			BSA-MG		
	Fructosamine inhibition	Protein carbonyl content	Inhibition of amyloid β-aggregation	Fructosamine inhibition	Protein carbonyl content	Inhibition of amyloid β-aggregation
F1	ND	ND	ND	ND	ND	ND
F2	ND	ND	ND	ND	ND	ND
F3	23.57 ± 0.46	24.24 ± 0.32	ND	24.76 ± 0.14	23.97 ± 0.38	ND
F4	18.47 ± 0.13	20.44 ± 0.05	17.71 ± 0.19	19.63 ± 0.10	17.78 ± 0.18	19.03 ± 0.69
F5	23.90 ± 0.11	22.94 ± 0.33	ND	24.92 ± 0.06	24.74 ± 0.14	24.80 ± 0.26
AG	18.99 ± 0.29	13.11 ± 0.28	18.90 ± 0.32	24.79 ± 0.13	19.67 ± 0.75	19.48 ± 0.34

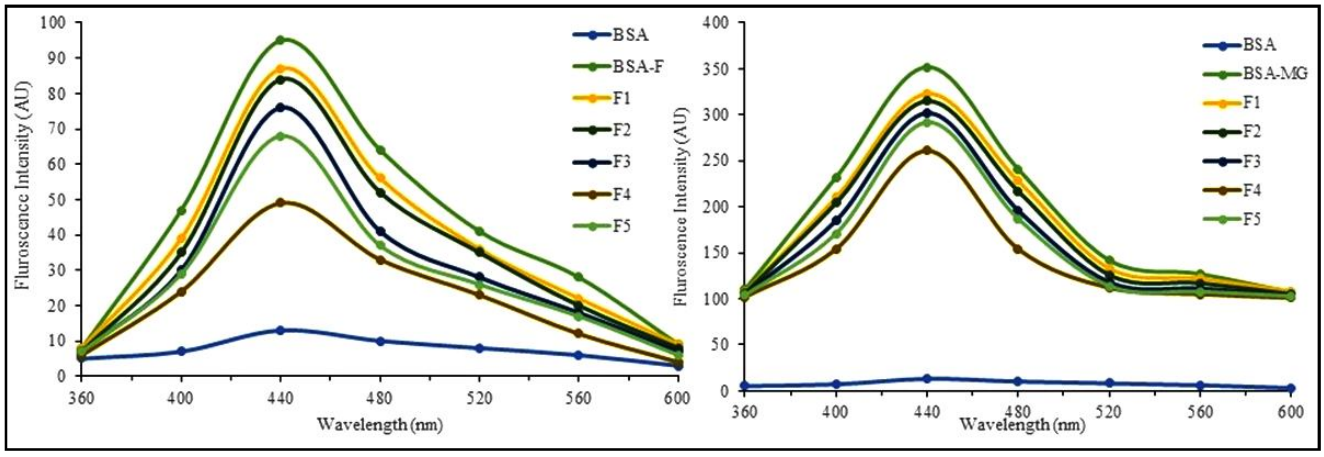

Figure 8: Different fraction of *A.platensis* methanolic extract inhibited fluorescence intensity of AGEs (λex=370 nm; λem=450 nm)produced inBSA-fructose and BSA-MG model.

Fraction 4 displayed the lowest IC_{50} value (18.47 ± 0.13 µg/ml, 20.44 ± 0.05 µg/ml and 17.71 ± 0.19 µg/ml in the BSA-fructose model) 19.63 ± 0.10 µg/ml, 17.78 ± 0.18 µg/ml and 19.03 ± 0.69 in the BSA-MG model for the inhibition of fructosamine, protein carbonyl content and Congo red content, respectively) among all fractions. The protective effect of bioactive fractions from *A. platensis* was examined by their ability to inhibit the fluorescence of AGEs, which were formed during the incubation of BSA with fructose and MG

depicted in Figure 8. After 28 days of incubation, fluorescence intensity of control having BSA-fructose and BSA-MG had elevated by 10 fold and 6 fold when compared with BSA only. The results demonstrated that F4 of *A. platensis* most effectively inhibited the fluorescent AGE formation indicated from significant decline in fluorescent intensity inglycated BSA-fructose and BSA-MG model.

4. Discussion

The non-enzymatic protein glycation contributes to the development of an unstable Schiff base in the early stage, followed by rearrangement which further converts to Amadori and reactive carbonyl product in the intermediate stage. In the last stage, reactive carbonyl products react with endogenous protein producing advanced glycation end products (AGEs). The accumulation of AGEs into tissue is considered the most responsible factor for the development of long-term complications in diabetes and other degenerative disorders (Singh *et al.*, 2014). *A. platensis* is a medicinally important microalgae used as functional food and also reported its antidiabetic activity (Hannan *et al.*, 2020). The current study investigated the *in vivo* antioxidant and antiglycation potential of methanolic extract of *A. platensis* in the BSA-fructose and BSA-MG glycated model.

Phytochemicals are powerful therapeutic agents that can deal with pathogenic conditions and diseases with fewer side effects. Hence, the phytochemical principles contain higher amounts of phenolics, alkaloids, flavonoids, tannins, sterols, glycosides and terpenoid are in demand due to their medicinal properties, which can possibly inhibit oxidative stress (Malik *et al.*, 2020; Singh and Singh, 2021). In the present study, methanolic extract of *A. platensis* shows a significantly higher content of total phenolic and flavonoids content compared to the extract of other solvent in the present study. Previously, it was well reported that methanol is most extensively used as a solvent for the extraction of phytochemicals due to its polarity (Balyan and Ali, 2022). *A. platensis* comprises certain phenolic compounds such as catechin, epicatechin, pyrocatechol, pyrogallol, gallic, protocatechouic and salicylic acid (Machu *et al.*, 2015). Flavonoids are the most studied group of polyphenolic compounds, which are found in methanolic extract with higher concentrations than in other solvents. Because of its high phenolic and flavonoid content, methanolic extract of *A. platensis* was chosen to investigate antioxidant capacities. A significant association was reported between the antioxidant capacities of extracts and their phenolic and flavonoid contents (Saeed *et al.*, 2012). An increased generation of free radicals, *i.e.*, hydroxyl radical, nitrogen oxide radical, superoxide radical and other reactive oxygen species contribute toward oxidative stress,accelerating protein glycation and subsequent progress of diabetes and its complications (Chen *et al.*, 2013). The results showed that the methanolic extract of *A. platensis* has potent antioxidant effects reported in DPPH radical scavenging, metal chelating and nitric oxide radical scavenging activity. *A. platensis* is believed to scavenge DPPH radicals due to its hydrogen donating capability contributed by chlorophyll, phycocyanin, carotenoid and vitamin E (Zaid *et al.*, 2015). The results of a current study showed the powerful metal chelation activity of methanolic extract of *A. platensis* that captures a ferrous ion and blocks the formation of the ferrozine-Fe^{+2} complex. Previously, studies suggested that *Spirulina* acts as a metal concentrator (Choudhary *et al.*, 2007), catalysing many processes, leading to the disappearance of free radicals. In the present study, the potent antioxidant activity of *A. platensis* might due to the presence of a mixture of antioxidant pigments including carotenoids, xanthophylls and chlorophyll. Phycocyanin is the unique blue pigment present in *A. platensis* which is 20 times more powerful as a major antioxidant than vitamin C (Chopra *et al.*, 2008). It has been suggested that C-phycocyanin and carotene present in *A. platensis* are key contributors to FRAP activity (Madhyastha *et al.*, 2009). It has been studied that flavonoid components act as powerful scavengers for nitric oxide radicals (Lakhanpal and Rai, 2007). The remarkable NO scavenging property of the methanolic extract of *A. platensis*is contributed by its higher flavonoid content. It is well reported that the antioxidant activity of *A. platensis* may be due to the presence of higher quercetin and rutin flavonoids (Ahmad *et al.*, 2022). Antioxidant compounds present in the extract act as scavengers of nitric oxides, and thus decreases the production of nitrite ions (Boora *et al.*, 2014). The methanolic crude extract of *A. platensis* was fractionated with increasing polarity of solvent hexane<hexane+ethyl acetate<ethyl acetate< ethyl acetate+methanol <methanol named F1 to F5. A significantly higher amount of phenolic and flavonoid content was recorded in F5 due to the high polarity of solvent. An earlier study describing a positive correlation between the antioxidant and antiglycation potential suggested that an ideal medicine should possess free radical scavenging activity which contributes in inhibitory activity for AGEs formation (Jha *et al.*, 2018). Each fraction isolated from *A. platensis* crude extract was tested for its antiglycation potential in three glycation steps: (1) formation of fructosamine (early), (2) formation of dicarbonyl compound (intermediate) and (3) aggregation of amyloid-β (late). According to our results, interestingly, F4 displayed maximum antiglycation properties in both the *in vitro* BSA-fructose and BSA-MG model systems of protein glycation. This might be due to a mixture of polar methanol and semi-polar ethyl acetate likely to dissolve polar as well as low-polarity flavonoids such as isoflavones, flavanones, methyl flavones and flavonols (Muhridja *et al.*,2016). In all experiments, the order of antiglycation activities of fraction from highest to lowest was as follows: F4>F5>F3>F2. The antiglycation properties were not found for the F1 due to the absence of phenolic and flavonoid compounds. In addition, our results suggest that the F4 showed potent antiglycation ability in the BSA-fructose model rather than in the BSA-MG model. It is believed that MG is a dicarbonyl molecule, a very reactive molecule and contributes more rapidly and efficiently to the formation of AGEs than fructose. It is assumed that the presence of various phenolic and flavonoid compounds in fraction 4 is responsible for potent antiglycation activities. Further more, the presence of the semi-polar nature of various flavonoids in the F4 may contribute to more potent antiglycation properties than inthe F5. The protective effect of phycocyanin present in *A. platensis* against the formation of glycated haemoglobin has been documented. (Ou *et al.*, 2016). *A. platensis* contains 5,7-dihydroxyflavone (Pinocembrin) that has hepatoprotective, anti-inflammatory and microcidal effects (Shen *et al.*, 2019). Pinocembrin, a bioactive compound from *Spirulina* possessed methylglyoxal trapping activity which reduced the enzymatically-induced glycation (Potipiranun *et al.*, 2018). Although, previous evidence suggests two flavonoids, quercetin and rutin, had an inhibitory effect on formation of AGEs. Our results of the Congo red assay suggest that F4 effectively attenuates the formation of protein aggregation in the late stage of BSA glycation. The effect of protecting BSA from structural modification was similar to that observed in an earlier study on a flavonoid-rich extract of *A. platensis* using BSA-glucose as a model

(Kumar *et al.*, 2022). An increase in the fluorescence intensity when fructose or MG is incubated with BSA suggests protein glycation formed at the end stage. The declining percentage of fluorescence intensity in BSA-fructose and BSA-MG treated with the F4 suggests that it is a powerful fraction to deal with the end stage of glycation as well.

5. Conclusion

The current study revealed the remarkable action of the F4 from *A. platensis* extract at an early stage by reducing fructosamine formation, a decrease in protein carbonyl content at the intermediate stage, inhibiting the binding of amyloid-specific dye Congo red and hindering the generation of fluorescent AGEs at the last stage. Thus, the results of the present study suggest that the F4 and F5 of methanolic extract of *A. platensis* might be potential sources of natural antiglycation agentsand could be further developed as therapeutic agents in the treatment of AGEs related complications. More analytical work is required to identify and characterise the bioactive compounds found in fraction 4 from *A. platensis* crude extract.The results obtained in this study are valuable to design further research and investigate antiglycation agents for the control of diabetic complication.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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