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The potent therapeutic effect of novel cyanobacterial isolates against oxidative stress damage in redox rats

O.A.M. Badr[1] , I.I.S. EL-Shawaf[1], H.A.S. El-Garhy[1], M.M.A. Moustafa[1] and O.A. Ahmed-Farid[2]

1 Department of Genetics and Genetic Engineering, Faculty of Agriculture, Benha University, Benha, Egypt
2 Department of Physiology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

Abstract

Aims: Cyanobacteria are immense sources of several pharmacological active compounds such as flavonoids and carotenoids with anti-inflammatory and antioxidant activity. The potential therapeutic effect of two novel cyanobacterial isolates, *Cronbergia siamensis* (KY296358.1) and *Sphaerospermopsis aphanizomenoides* (KU212886.1), against hydrogen peroxide (H_2O_2)-induced oxidative stress damage in the rat model was determined in this study.

Methods and Results: *In vitro* antioxidant activity of the two studied isolates was evaluated by radical scavenging assay and ferric reducing power. The possible prophylactic activity of *S. aphanizomenoides* (KU212886.1) against H_2O_2-induced oxidative stress in the rat model was assessed *in vivo*. Serum alanine transaminase and aspartate transaminase were measured for the liver functions in redox rats. Liver malondialdehyde (MDA), glutathione, oxidized glutathione, nitric oxide, superoxide dismutase (SOD) and catalase (CAT) were assessed as oxidative stress markers. The effect of *S. aphanizomenoides* on the transcripts level of superoxide dismutase (*Mn-SOD*) and catalase (*CAT*) genes in the rat's liver tissues was measured using qRT-PCR. Oral administration of *S. aphanizomenoides* extract in low and high doses (100, 200 mg kg^{-1} b.w) resulted in significant improvement in biochemical parameters of liver functions and oxidative stress markers. Also, the endogenous antioxidant defence enzymes and the expression of their related genes (*Mn/SOD, CAT*) were upregulated. Immunohistochemistry of Caspase-3, an apoptotic marker, showed potent amelioration in the liver tissues.

Conclusions: The novel isolate *S. aphanizomenoides* proved *in vitro* and *in vivo* antioxidant activity against redox rat model.

Significance and Impact of the Study: This isolate provides a new source of pharmacological compounds with great importance in pharmacological and medical fields.

Introduction

Oxidative stress is a common indicator of toxicity that could promote tissue damage of all vital molecules threatened organism health. It occurs when the production of free radicals and reactive oxygen species (ROS) exceed the cellular antioxidant defence system (Mecocci *et al.* 2018). The released free radicals were considered the main endogenous damage in the biological systems (Thajuddin and Subramanian 2005). This damage affects all the essential bio-compounds like proteins, DNA and membrane lipids. The produced ROS correlated directly with over than 100 disease states either as source or outcome like cancer, diabetes, obesity, cardiovascular disease, neurodegenerative diseases, immune function decline and ageing (Wijeratne *et al.* 2005; Gagné 2014; Furukawa *et al.* 2017; Mecocci *et al.* 2018).

Antioxidants work as free radical scavengers that could hunt all ROS and prevent oxidative damage in living organisms. They can interact safely with free radicals to

terminate the chain reaction, enhance the immune system, remove the contaminants and pollutants, and reduce inflammation and hypertension (Safafar *et al.* 2015). In the cell, the antioxidants could be enzymatic factors like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutaredoxins, glutathione reductase and thioredoxins besides nonenzymatic antioxidant factors, such as beta-carotene, carotene, selenium, zinc, taurine, hypotaurine, glutathione and vitamins like vitamin C and E (Agarwal *et al.* 2008; Mulgund *et al.* 2015; Mecocci *et al.* 2018). Despite all organisms have their own antioxidant defence and repair systems, at the time that body ages, antioxidant levels decrease dramatically offering a disturbance in the balance between antioxidants and oxidant factors. As a result of that rising the oxidative stress damage and the shortage of natural repair system that will fail to overcome the entire damage (Chandrika and Kumara 2015).

The crude extracts of vegetables, fruits and plants are the main sources of natural antioxidants (Hossain *et al.* 2014). However, the medical use of these sources is limited as their attainment to optimum growth takes a long time (Nakao *et al.* 2009; Aydaş *et al.* 2013). Hence, these sources must be replaced by nonexploited natural products that can be converted into medical products under high technical condition.

Recently, cyanobacteria have attracted the attention of the scientific community as they represent themselves strongly as new natural sources for numerous pharmacologically active compounds (Hossain *et al.* 2016; Singh *et al.*, 2017). Cyanobacteria produce a variety of secondary metabolites, flavonoids, carotenoids, pigments, phenols, tannins, terpenes and vitamins with antioxidant, antidiabetic, anti-inflammatory, antifungal, anticancer, antibiotic and antiviral activity (Guihéneuf *et al.* 2016; Singh *et al.*, 2017). Interestingly, it is estimated that more than 15 000 innovative compounds have been chemically determined in cyanobacterial biomass (Munir *et al.* 2013). Nowadays cyanobacteria are regarded as good candidates for drug discovery, where they are noncompetitive with economic crops, can multiply in volume nightly and cropped on daily basis. Hence, they are efficient, unconventional and unexplored sources for different antioxidants with multiple applications in the pharmaceuticals and cosmetic sectors (Hossain *et al.* 2016).

The identification and characterization of cyanobacteria have been widely carried out in many previous studies (Gugger *et al.* 2002; Rajaniemi *et al.* 2005; Hayes *et al.* 2007; Nayak *et al.* 2007; Li *et al.* 2008; Zapomělová *et al.* 2010; Galhano *et al.* 2011; Ponnuswamy *et al.* 2013). However, few investigations have been differentiated and quantified the antioxidants in cyanobacteria (Chacón-Lee and González-Mariño 2010; Cirulis *et al.* 2013; Safafar

et al. 2015). The biological and therapeutic efficiency of cyanobacteria have been established in some recent studies (Li *et al.* 2015; Carfagna *et al.* 2015; Bashandy *et al.*, 2016; Hossain *et al.* 2016; Singh *et al.*, 2017). Although, the molecular and therapeutic responses to administration of cyanobacteria received limited attention.

This study aimed to determine *in vitro* and *in vivo* biological activity of two novel cyanobacterial isolates (*Cronbergia siamensis*, KY296358.1, and *Sphaerospermopsis aphanizomenoides,* KU212886.1) against hydrogen peroxide (H_2O_2)-induced oxidative stress in a rat model. The antioxidant effect of *S. aphanizomenoides* (KU212886.1) on liver functions and oxidative stress markers was evaluated and its ability to inhibit Caspase3 interaction was determined. In addition, the mRNA transcripts level of *Mn/SOD and CAT* genes were quantified as an antioxidant biological marker to confirm the therapeutic effect of the potent isolate on redox rats via qRT-PCR.

Materials and methods

Two cyanobacterial isolates, *C. siamensis* (KY296358.1) and *S. aphanizomenoides* (KU212886.1), were used in this study. These isolates were obtained from Badr *et al.* (2018) study as probable new species that were isolated from irrigated agriculture cannels at Kafr Elsheikh governorate, stressed aquatic system. Samples were purified using serial dilutions method according to Lee *et al.* (2014). Where, a nutrient selective media (BG-11) was optimized for the growth of cyanobacterial species (Rippka *et al.*, 1979).

Cyanobacterial extract preparation

For each cyanobacterial isolate, the fresh biomass was harvested by centrifugation at 2318 *g* for 10 min. The obtained biomass was freeze-dried by Lypohilizer (NANBEI; Model Number: LGJ-10-3; Mainland of China, Zhengzhou, China). The freeze-dried biomass was extracted by homogenization at 11 5216 *g* for 10 min in 70% ethanol. The obtained crude extract was centrifuged at 12 3622 *g* for 10 min, the aqueous supernatant was lyophilized. The dried sample was kept in 5 ml brown sealed bottles, at −20°C, until use. This method was modified from Carvalho *et al.* (2013) methodology.

Chromatographic analysis of phenolic acids, flavonoids, carotenoids and vitamins in cyanobacterial isolates

The phenolic compound was measured by Agilent HPLC 1260 Infinity II, system; the system consisted of autosampler, quaternary pumps, UV detector, an interface, reversed-phase column alternative for LiChrospher 100 RP-c18, 250 mm × 4·0 mm I.D., 5 *μ*m particle size. The

system was controlled by Chemstation HPLC Manager. After lyophilization, the cyanobacterial samples were ground in methanol-dimethyl sulphoxide (DMSO) (v/v) using homogenizer at 14 5216 g then centrifugation was done at 14 1304 g for 10 min. The prepared samples were stored at 4°C till proceeding of analysis.

HPLC conditions

The detector monitored the eluent at 285 nm, two-solvent gradient system was used. The gradient program presented as follow: A (0·01 mol l^{-1} phosphoric acid) and B (methanol), 1st 0–16·5 min, 70–55% (v/v); 2nd 16·5 : 28·5 min, 55–0% A in B; 3rd 28·5 : 30 min, isocratic, 100% B. The resulting chromatographic data on the absorbing peaks was integrated up to 30 min. The flow-rate was 2 ml min^{-1} and the column was operated at 40°C. The sample injection volume was 20 μl.

Flavonoids were determined according to Nogata et al. (1994). The mobile phase contains gradient elution with 0·01 mol l^{-1} phosphoric acid–methanol. The flow rate was adjusted to 0·6 ml min^{-1}, the column was thermostatically controlled at 40°C and the injection volume was kept at 20 μl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elution was changed from 10% to 40% B in a linear fashion for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90% B in 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. HPLC chromatograms were detected using a UV detector 285 nm wave length. Each compound was identified by its retention time and by spiking with standards under the same conditions.

Detection of β-carotene and Zeaxanthin was done by HPLC according to Ahamad et al. (2007). The mobile phase was acetonitrile, dichloromethane and methanol by the ratio of 70 : 20 : 10 respectively. The elution was performed at a flow-rate of 2 ml min^{-1}. The column was reversed-phase C18 (150 mm × 4·6 mm, 5 μm). The injection volume was 20 μl. The analytical column was kept at 25°C and the detection was performed at 452 nm.

Determination of vitamins A and E was done by HPLC according to Gimeno et al. (2000). The mobile phase contains methanol–water (96 : 4, v/v) and the elution was performed at a flow-rate of 2 ml min^{-1}, the column was RP-column (Nucleosil® C18; 10 μm). The injection volume was 20 μl. The analytical column was kept at 45°C. Detection was performed at 325 for vitamin A and 292 nm for vitamin E (Switch the wavelength after 7 min.). While, water-soluble vitamins were detected using Ekinci and Kadakal (2005) methodology. The

mobile phase was 0·1 mol l^{-1} KH$_2$PO$_4$ (pH 7)-methanol, 90 : 10. The elution was performed at a flow-rate of 1 ml min^{-1} the column was reversed-phase Discovery C18 (150 mm × 4·6 mm, 5 μm) from Supelco (Bellefonte, PA). The injection volume was 20 μl. The analytical column was kept at 45°C. Detection was performed using UV detector at 254 nm wavelength.

Antioxidant activity evaluation

Total phenolic content

The total phenolic content of the cyanobacterial extract was determined by the Folin–Ciocalteu method (Kaur and Kapoor 2002). Briefly, 200 μl of the cyanobacterial extract suspension (1 mg ml^{-1}) was adjusted to 3 ml with distilled water, mixed thoroughly with 0·5 ml of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture set for 60 min in the dark and the absorbance was measured at 650 nm (UV-2450, UV-VIS Spectrophotometer; Shimadzu Tokyo, Tokyo, Japan). Total phenolic content was expressed in milligrams equivalents of gallic acid (GAE) per gram of each fraction and it was calculated from the calibration curve.

DPPH radical scavenging assay

The ability of different cyanobacterial extract to act as hydrogen donors was measured by DPPH$^{.}$ (1,1-diphenyl-2-picrylhydrazyl) assay according to Brand-Williams et al. (1995). Different concentrations from cyanobacterial extracts were prepared (50, 100 and 200 μg ml^{-1}). 50 μl of each sample was mixed with 450 μl of Tris-HCl (pH 7) and 1 ml of DPPH then incubated at 37°C for 30 min. Spectrophotometric absorbance was 517 nm and gallic acid was used as standard. Percentage of Inhibition was calculated according to formula:

$$\text{Inhibition (\%)} = \frac{\text{absorbance of DPPH} - \text{absorbance of sample}}{\text{absorbance of DPPH}} \times 100$$

Ferric reducing power assay

Ferric Reducing Antioxidant Power (FRAP) of the tested isolates was determined following Oyaizu (1986) methodology. The FRAP reagent was freshly prepared and the absorbance was measured at 700 nm after 10 min of incubation at 25°C using rutin hydrate as standard.

Experimental design and In vivo study

The in vivo study was established depending on in vitro antioxidant activity results of the obtained cyanobacterial isolates. Where, the most potent isolate S. aphanizomenoides

was used for oral administration in this part of the study. A total of 48 rats (Sprague Dawley) weighed 200 ± 20 g, 9–12 weeks of age were used in this study. Experiments were done following the instructions of the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC). All animals get care in agreement with the rules of the animal care. The animals were protected in a quiet place and allowed free access to water and food during the study period. Rats were randomly separated into eight groups, six each. The 1st group received tap water as a control. The 2nd group received vitamin C as an antioxidant reference drug (250 mg kg^{-1} body weight) according to Paget and Barnes (1964). The 3rd group received a low dose of *S. aphanizomenoides* extract (100 mg kg^{-1} b.w). The 4th group received a high dose of *S. aphanizomenoides* extract (200 mg kg^{-1} b.w). The 5th group received H_2O_2 0·5% as a positive control in drinking water. The 6th group received H_2O_2 (0·5%) in drinking water simultaneously with VC. The 7th group received H_2O_2 (0·5%) as well as low-dose extract of *S. aphanizomenoides*. The 8th group received H_2O_2 (0·5%) simultaneously with high dose of *S. aphanizomenoides* extract. The experiment lasted for 2 weeks and all animals received cyanobacterial extract and other treatments by oral administration. After 2 weeks, blood samples were gathered through 1 h from 8:00 to 9:00 am under light ether anaesthesia by a retro-orbital puncture. Serum was separated for liver function analysis. After general anaesthesia, all animals were immolated by cervical dislocation. The liver was anatomized in ice quickly, cleaned and stored at $-80°C$ till analysis.

The effect of *S. aphanizomenoides* on rats challenged by H_2O_2

Liver tissues of all groups were homogenized in phosphate buffer saline pH 7·4 and the homogenates were used for the determination of oxidative stress markers malondialdehyde (MDA), glutathione (GSH), oxidized glutathione (GSSG) and nitric oxide (NO) by HPLC according to Karatas *et al.* (2002), Jayatilleke and Shaw (1993) and Papadoyannis *et al.* (1999) respectively. While, endogenous antioxidant enzymes (SOD and CAT) were analysed by spectrophotometer according to Marklund and Marklund (1974) and Aebi (1984) respectively. Hepatic dysfunction was measured by calculating the elevation in serum levels of serum alanine transaminase (ALT), aspartate transaminase (AST) using commercially available kits and the results were expressed in international units/litre (IU l^{-1}) (Reitman and Frankel 1957). For immunohistochemical staining of caspase-3, slide preparation and antigen antibody reaction were performed as described by Abd-Elrazek and Ahmed-Farid (2018). The Adequate amount of haematoxylin stain was

added to the slide to cover the entire tissue surface for counterstaining (Bancroft and Cook 1994).

RNA extraction and differential expression analysis of Mn/SOD and CAT genes

Total RNA was isolated from liver tissues of all groups using SV Total RNA Isolation System (Promega Corporation, Madison, WI). Complementary DNA (cDNA) was synthesized from RNA using High Capacity cDNA reverse transcriptase kit (Thermo Fisher Scientific, Waltham, MA). Conventional PCR was performed to test the primer specificity of target genes using cDNA as a template (data not shown).

Triplicate qRT-PCR was performed for each sample, nontemplate control (NTC) and negative cDNA template. PCR reaction contained of 500 ng per reaction of cDNA (except for NTC and cDNA control), 12·5 μl Maxima SYBR Green qPCR Master Mix (Maxima SYBR Green qPCR; Thermo Fisher Scientific), 0·3 μmol l^{-1} of each forward and reverse primer, 10 nmol l^{-1}/100 Nm ROX Solution, nucleases-free water to a final volume of 25 μl. Reactions were analysed on an AriaMx Real-Time PCR System (Agilent Technologies, Santa Clara, CA), Two-step cycling protocol, under the following conditions: 95°C for 10 min and 40 cycles of 95°C for 15 s followed by 60°C for 60 s. The expression levels of the antioxidant enzyme genes including *Mn/SOD* and CAT were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), the names and oligonucleotide sequences of the primers are listed in Table S1. All changes in the expression of the studied genes were obtained as n-fold changes relative to the corresponding controls. Relative gene expression ratios (RQ) between treated and control groups were calculated using the formula: $RQ = 2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001).

Statistical analysis

All values were stated as a mean $\pm$ SE for the rats in each group. Differences between rat groups were evaluated by one-way analysis of variance using SAS software (SAS, 2004). The obtained data was analysed using the general linear model. Duncan multiple ranges test (Duncan 1955) was used to evaluate the significant differences among means.

Results

Nutritional profile and antioxidant activity of the isolates

Nutritional profile results of the cyanobacterial isolates are presented in Table 1. The isolate *S. aphanizomenoides*

Table 1 Nutritional profile of cyanobacterial isolates powder

Contents concentration (μg g^{-1} powder)	Cronbergia siamensis	Sphaerospermopsis aphanizomenoides
Thiamine HCL	0·03 ± 0·002	0·05 ± 0·003
Riboflavin	0·02 ± 0·001	0·14 ± 0·009
Nicotinamide	0·18 ± 0·011	0·04 ± 0·002
Pyridoxine	0·15 ± 0·009	0·16 ± 0·010
α-tocopherol	0·03 ± 0·002	0·01 ± 0·001
Vitamin A IU g^{-1} powder	211·00 ± 12·83	309·00 ± 19·29
β-carotene	0·15 ± 0·009	0·05 ± 0·003
Zeaxanthin	0·06 ± 0·004	0·20 ± 0·012
Caffeic acid	0·89 ± 0·053	1·34 ± 0·079
Cinnamic acid	ND	0·83 ± 0·050
Coumaric acid	0·23 ± 0·014	0·44 ± 0·027
Ferulic	0·67 ± 0·043	1·21 ± 0·072
Gallic acid	2·11 ± 0·127	4·12 ± 0·257
Salicylic acid	ND	1·39 ± 0·082

Data shown as mean ± SD for five replicates for nutritional profile of different cyanobacterial isolates.

Table 2 Total phenolics content in the extracts of obtained cyanobacterial isolates

Cyanobacterial extracts μg ml^{-1}	Total phenolics content (μg gallic per g dried algae)
Cronbergia siamensis	2·46 ± 0·05
Sphaerospermopsis aphanizomenoides	14·96 ± 0·30

Data shown as mean ± SD for five replicates for total phenolics content of different cyanobacterial isolates.

Table 3 % DPPH radical scavenging activity of cyanobacterial isolates

Cyanobacterial concentration μg ml^{-1}	DPPH scavenging activity %	
	Cronbergia siamensis	Sphaerospermopsis aphanizomenoides
50	5·32 ± 0·15	32·32 ± 0·92
100	8·95 ± 0·32	42·98 ± 1·54
200	13·74 ± 0·49	58·74 ± 2·10

Data shown as mean ± SD for five replicates for scavenging activity of different cyanobacterial isolates.

contained higher levels of caffeic, ferulic, coumaric, gallic, ginnamic, salicylic acids as well as vitamin A, riboflavin, pyridoxine and zeaxanthin, than *C. siamensis* isolate. Consequently, it appeared the highest total phenolic content (Table 2). On another hand, *S. aphanizomenoides* showed the highest scavenging DPPH activity (58·74%) at concentration of 200 μg ml^{-1} compared with *C. siamensis* (Table 3). The reducing powers of the isolates demonstrated that the scavenging ability was positively correlated with cyanobacterial concentrations (Fig. 1). Depending on these findings, the *S. aphanizomenoides* isolate seemed to be the best as an antioxidant compound in the cell, for its higher antioxidant activity than *C. siamensis*. Therefore, its therapeutic effect against H_2O_2-induced oxidative stress in rat model was evaluated *in vivo*.

Effect of S. aphanizomenoides against redox status in rat liver

Oral administration of *S. aphanizomenoides* low dose (100 mg kg^{-1} b.w) and vitamin C revealed no significant differences in all biochemical parameters in comparison with the control group (Table 4). Whereas, the H_2O_2 treatment (P) caused a high toxicity alteration in all biochemical parameters compared with the control group.

On another hand, the groups that received *S. aphanizomenoides* treatments simultaneously with H_2O_2 (P+T1 and P+T2) exhibited a marked amelioration in liver function (AST, ALT), a decrease in oxidative stress markers (MDA, GSSG, NO), increase in endogenous antioxidant

defence enzymes (SOD, CAT) compared with positive control group, $P < 0.05$ (Table 4). Interestingly, the high dose of *S. aphanizomenoides* (200 mg kg^{-1}) showed marked amelioration for all biochemical parameters compared to H_2O_2 group. Similarly, the low dose of *S. aphanizomenoides* (100 mg kg^{-1}) showed marked enhancement for all biochemical parameters in comparison with the H_2O_2 group.

Immunohistochemistry of Caspase-3 in the liver tissue

Histopathological examination of liver tissues of normal rats in control groups showed normal hepatic tissue without any injury and no interaction of Caspase 3 (Fig. 2a,b). Liver tissues treated with H_2O_2 showed strong positive interaction of Caspase 3 (Fig. 2c). Whereas, oral administration of high and low doses (200, 100 mg kg^{-1}) of *S. aphanizomenoides* extract strongly reduced the interaction of Caspase 3 in redox rat livers (Fig. 2d–f). Remarkably, the reduced patterns of Caspase 3 caused by the high dose of *S. aphanizomenoides* (200 mg kg^{-1}) was nearly reached to that created by the vitamin C, standard drug, (Fig. 2e,f).

Effect of S. aphanizomenoides on the expression of Mn/SOD and CAT

The effects of H_2O_2 and *S. aphanizomenoides* extract on the mRNA levels of two antioxidant enzyme encoding genes, manganese superoxide dismutase (*Mn/SOD*) and
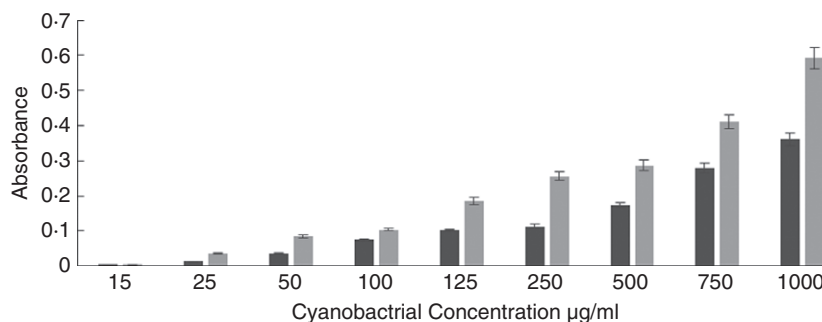
Figure 1 Reducing power of cyanobacterial isolates at different concentration (■ *C. siamensis*; ■ *S. aphanizomenoides*).

catalase (*CAT*), were determined by qRT-PCR (Fig. 3a,b). There were no significant differences in gene expression levels of *Mn/SOD* and *CAT* among the groups that received vitamin C (VC), low and high doses of *S. aphanizomenoides* (T1 and T2). In contrast, the groups that received hydrogen peroxidase (P) reduced *Mn/SOD* and *CAT* transcripts significantly by 1·89- and 1·45-fold, respectively, compared to untreated groups. Where, Treatments of low and high doses of *S. aphanizomenoides* upregulated mRNA levels of *Mn/SOD* by 2·42- and 2·89-fold, respectively, compared with the positive control group (P). Similarly, the transcripts amount of *CAT* gene increased by 2·62- and 2·92-fold in response to treatments of low and high doses *S. aphanizomenoides* respectively.

Discussion

Cyanobacteria are enormously diverse group of prokaryotic organisms that live in a widespread environmental systems (Hu *et al.* 2008). They are rich sources of secondary metabolites with potential biotechnological applications in the field of pharmacology (Vijayakumar and Menakha 2015; Singh *et al.*, 2017). It is clearly known that environmental pollution and diverse stress factors elicit cyanobacteria to produce high levels of secondary metabolites (Jimenez-Garcia *et al.* 2013). The cyanobacterial isolates in this study obtained from irrigated wastewater cannels that characterized with the presence of various types of contaminants. The continuous environmental stresses in this site may be promoting the development of its cyanobacterial community through upregulating of secondary metabolites production. Consequently, these isolates were expected to have upregulated secondary metabolites with probable antioxidant activity.

Carotenoids and phenolic compounds are major contributors of antioxidant capacities for microalgae (Jiménez-Escrig *et al.* 2001; Li *et al.* 2007; Goh *et al.* 2010; Hajimahmoodi *et al.* 2010). Additionally,

flavonoids are capable of inhibiting and reducing free radicals to terminate the radical chain reactions (Rahul *et al.* 2016). Nutritional profile results of the cyanobacterial isolates revealed that both isolates contained different antioxidant constituents like phenolic compounds, flavonoids, carotenoids and vitamins. The highest levels of vitamin A, gallic acid, salicylic acid, ferulic acid (FA), cinnamic acid, coumaric acid, riboflavin, pyridoxine and zeaxanthin appeared in the crude extract of *S. aphanizomenoides*. Interestingly, it recorded high levels of caffeic acid in disagreement with Goiris *et al.* (2014) who investigated flavonoids in different cyanobacterial species and reported that the flavanone eriodyctiol and its precursor caffeic acid were not detected in the algal biomass samples. However, the recent study of Singh *et al.* (2017) evidenced that cyanobacteria have a wide range of carotenoids, flavonoids and phenolic compounds.

The DPPH radical scavenging assay is used usually for estimating natural antioxidants due to its constancy, simplicity and reproducibility (Kuda *et al.* 2007). The DPPH scavenging ability of a compound depend on its ability to pair with the unpaired electron of a radical (Park *et al.* 2004). Hence, The DPPH scavenging ability is a reliable and effective indicator of possible antioxidant activity (Hossain *et al.* 2016). Jerez-Martel *et al.* (2017) investigated the antioxidant activity of *Nostoc* sp., *L. protospira, N. spumigena* and *Phormidiochaete* sp., where DPPH radical ranged from 7·65% (*L. protospira*) to 27·89% (*Nostoc* sp.). While, other investigations reported that *S. marginatum* and *T. conoides* scavenged DPPH by 11 and 17·2%, respectively (Chandini *et al.* 2008) as well as *F. vesiculosus* and *A. nodosum* scavenged DPPH by 31·2 and 25·6% respectively (O'sullivan *et al.* 2011). In comparison with all previous results, *S. aphanizomenoides* in this study appeared the highest scavenging DPPH activity (58·74%) at concentration of 200 μg ml^{-1}. These findings approved that, *S. aphanizomenoides* could be a promising natural source of antioxidants with high pharmacological and medical importance. Depending on

Table 4 Effect of *Sphaerospermopsis aphanizomenoides* dose level on some biochemical parameters (ALT, AST, MDA, NO, GSH, GSSG, SOD and CAT) of rats treated with hydrogen peroxide

Parameters	Groups							
	Control	VC	T1	T2	P	P+VC	P+T1	P+T2
ALT	22·6 ± 0·69	20·8 ± 0·60	24·2 ± 0·80	27·2 ± 0·84a	42·5 ± 1·35a	29·4 ± 0·88ab	36·3 ± 1·10ab	38·1 ± 1·17ab
AST	51·3 ± 1·51	54·5 ± 1·59	56·8 ± 1·74	60·8 ± 1·90a	87·1 ± 2·51a	58·5 ± 1·87ab	81·2 ± 2·55ab	77·5 ± 2·28ab
MDA	33·31 ± 0·99	28·95 ± 0·90	36·51 ± 1·18	26·72 ± 0·77	76·16 ± 2·32a	57·09 ± 1·72a	71·75 ± 2·16a	49·50 ± 1·57ab
NO	0·37 ± 0·01	0·33 ± 0·01	0·37 ± 0·01	0·38 ± 0·01	0·89 ± 0·03a	0·52 ± 0·015ab	0·69 ± 0·02ab	0·66 ± 0·02ab
GSH	23·99 ± 0·70	27·83 ± 0·79a	21·80 ± 0·72	26·75 ± 0·81a	9·56 ± 0·28a	16·20 ± 0·52ab	12·42 ± 0·39ab	18·26 ± 0·58b
GSSG	0·39 ± 0·01	0·31 ± 0·01	0·36 ± 0·01	0·38 ± 0·01	1·02 ± 0·03a	0·64 ± 0·02ab	0·95 ± 0·03a	0·71 ± 0·02ab
SOD	47·7 ± 1·43	52·3 ± 1·60a	44·6 ± 1·42	50·9 ± 1·66	27·1 ± 0·78a	36·0 ± 1·19ab	31·8 ± 0·98a	34·5 ± 1·07ab
CAT	24·9 ± 0·75	26·7 ± 0·79	20·4 ± 0·59	27·2 ± 0·83	11·5 ± 0·36a	19·8 ± 0·63ab	13·4 ± 0·40ab	20·2 ± 0·59b

Data shown as mean ± SE for six rats/group.
[a]Significant from control group at the same raw with one-way ANOVA at $P < 0.05$.
[b]Significant from P group at the same raw with one-way ANOVA at $P < 0.05$.

these results, *S. aphanizomenoides* was selected to study its antioxidant effect against H_2O_2-induced oxidative stress in the rat model.

Cyanobacteria antioxidant defence system consists of both enzymatic (SOD, CAT, glutathione reductase and ascorbate peroxidase) and nonenzymatic compounds (ascorbic acid, reduced glutathione, tocopherols, carotenoids and phycocyanin) (Ohki *et al.* 2008). In this study, the oral administration of *S. aphanizomenoides* significantly reduced free radical damage induced by H_2O_2 which is an extremely reactive free radical, can damage almost every molecule in living cells (Hochstein and Atallah 1988). There was a marked amelioration on liver function (AST, ALT), a decrease in oxidative stress markers (MDA, GSSG, NO), increase in endogenous antioxidant defence enzymes (SOD, CAT). These results supported by the study of El-Tantawy (2016) who revealed that *Spirulina* oral administration revealed a significant decrease in serum ALT, AST activities as compared to lead treated animals. The effect of *Spirulina* administration was strong as the effect of vitamin C and these results are similar to the obtained results in this study. Moreover, the study of El-Baz *et al.* (2018) reported that the antioxidant activity of *Dunaliella salina* and *Haematococcus pluvialis* against colon, breast, lung and liver cancer cell lines could be due to the carotenoid fraction in the crude extracts of these isolates. While, the study of Chen *et al.* (2016) clearly confirmed that glutathione has the ability of scavenging free radicals and protecting the liver from oxidative stress.

The antioxidant activity of *S. aphanizomenoides* could be due to high levels of FA that appeared in the crude extracts of the isolate. Ferulic acid is a phenolic compound biosynthesized from caffeic acid by the action of the enzyme caffeate O-methyltransferase. It exhibits a strong antioxidant activity via donating one hydrogen atom from its phenolic hydroxyl group (Kikuzaki *et al.* 2002; Ou and Kwok 2004; Rosa *et al.* 2013a, 2013b). Its antioxidant activity probably due to the specific structure of the compound, the hydroxyl group, the location —a couple, a methoxy group in the meta- position of the phenyl ring, (Mielczarek *et al.* 2010). Some investigations revealed that the regular absorption of FA to provide protection associated with a range of oxidative stress related diseases. Jung *et al.* (2009) reported that ferulate effectively suppresses redox-sensitive, proinflammatory NF-kappaβ activation via NF-kappa-β-inducing kinase/I-kappa-β kinase and MAPKs by reducing oxidative stress in aged rats. Moreover, Khanduja *et al.* (2006) indicated that FA helped in reduction in lipid peroxidation in peripheral blood mononuclear cells induced by H_2O_2. Hence, the presence of FA and other phenolic compounds in the crude extract of *S.*
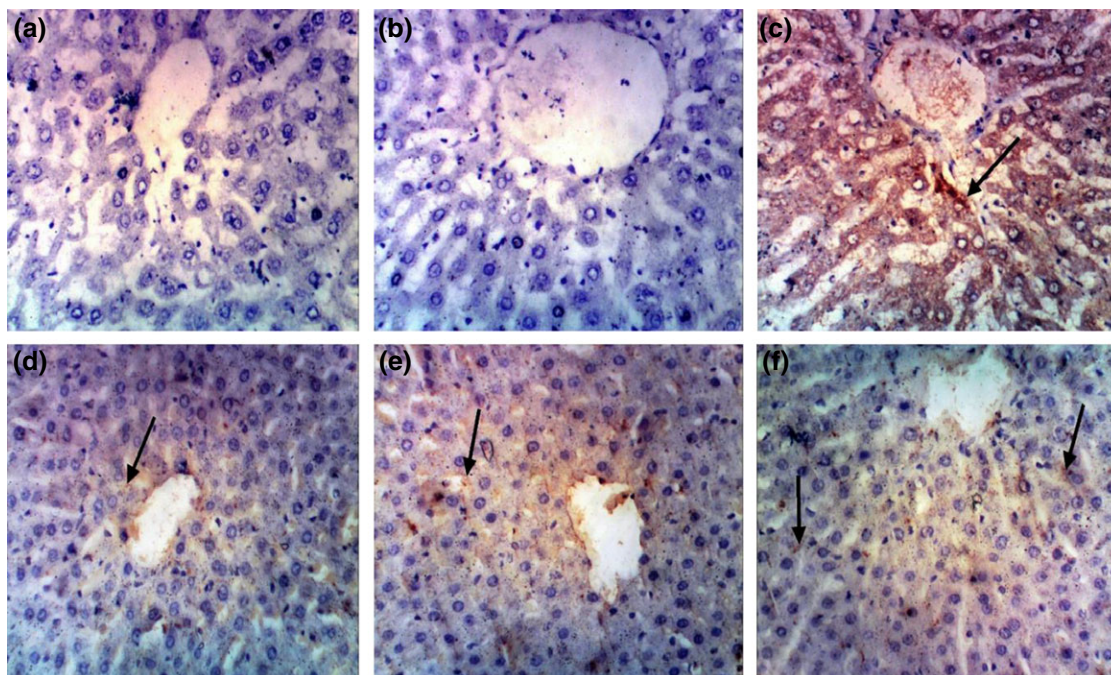
Figure 2 Immunohistochemical staining of Caspase 3 in liver of rats from the studied groups, (a) control group (tap), (b) vitamin C group (VC), (c) H_2O_2 group (P), (d) the group of H_2O_2 with VC (P + VC). (e) The group of H_2O_2 with low dose of algae (P + T1). (f) The group of H_2O_2 with high dose of algae (P + T2), immunopositivity indicated by brown colour, (X 400).
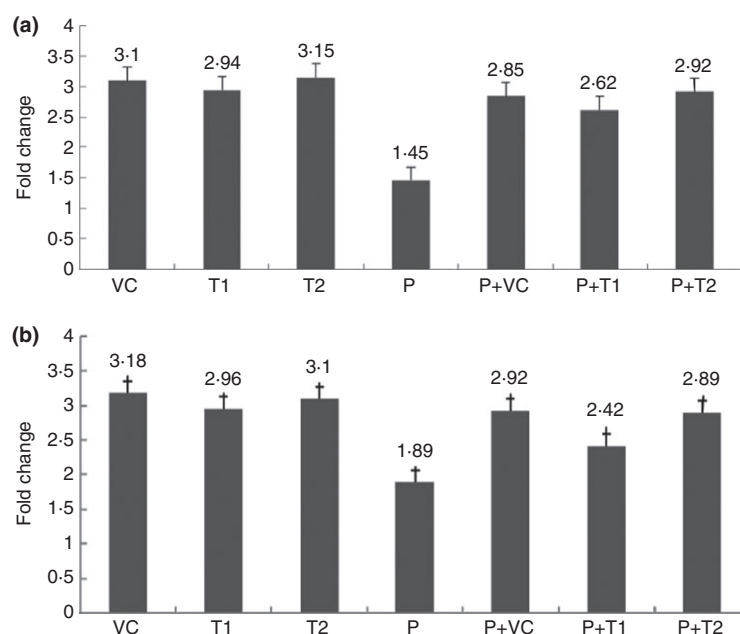

Figure 3 (a) Expression profiling of *CAT* gene in response to cyanobacterial treatments. (b) Expression profiling of *Mn-SOD* gene in response to cyanobacterial treatments. Changes in gene expression were denoted as relative change (ratio of target gene/reference gene). *GAPDH* was used as reference gene.

aphanizomenoides may be the main cause of decreasing the levels of ALT, AST, MDA, GSSG, NO and increasing GSH, SOD, CAT in redox rats.

In this study, immunohistochemical examination for caspase-3 (proapoptotic marker) of liver revealed pathological alteration in the H_2O_2 group and ameliorative

effect of *S. aphanizomenoides* at low and high doses. H_2O_2-induced apoptosis was associated with activation of caspase-3 as assessed by cleavage of specific fluorogenic substrate peptide, processing of procaspase-3 and poly (ADP-ribose) polymerase. However, the treatment of *S. aphanizomenoides* caused a decrease in proapoptotic markers and this may be due to the presence of FA in the isolate crude extract which could increase the stability of cytochrome *c*, and hence inhibits the apoptosis. These results agreed with the results of Wang *et al.* (2016) who studied the effect of FA on the expression of Bcl-2, an anti-apoptotic Bcl-2 family protein, and Bax, a proapoptotic Bcl-2 family protein, in osteosarcoma cells using immunostaining. The Bax/Bcl-2 ratio affects caspase 3 and controls the cell apoptosis. He reported that FA upregulated Bax, downregulated Bcl-2, and subsequently enhanced caspase-3 activity.

These results were confirmed by the expression analysis of antioxidant enzyme genes, *Mn/SOD* and *CAT*. The mRNA transcript levels of *Mn/SOD* and *CAT* raised significantly in the H_2O_2 induced groups that received *S. aphanizomenoides* extract. Thus, the induction of mRNA patterns was in accordance with the changes in levels of antioxidant enzymes that observed in the liver. Storz (2011) results stated that *Mn/SOD* and *CAT* enzymes are directly induced by FOXO3a which an isotype of FOXO transcription factors. FOXO3a binding sites have been identified in the promoters of *Mn/SOD* and *CAT* genes (Kops *et al.* 2002; Li *et al.* 2008; Tan *et al.* 2008). Thus, the induction of *Mn/SOD* and *CAT* transcripts may be due to the activation of PI3K/Akt/FOXO3a pathway via administration of *S. aphanizomenoides* that contain high levels of FA.

In conclusion, the newly identified isolate *S. aphanizomenoides* can be of pharmacological and medical importance. The observed antioxidant activity of *S. aphanizomenoides* may be due to a presence of different antioxidant constituents. Especially, the presence of high contents of FA in its crude extract could be the main factor responsible for the antioxidant activity through induction of PI3K/Akt/FOXO3a pathway. Consequently, increasing the levels of Mn/*SOD* and *CAT* transcripts and recovering caspase3 in redox rats.

Acknowledgements

Availability of data and materials

The datasets used and/or analysed during this study are available on request from the corresponding author.

Author contributions

Omnia A. Badr, Mahmoud M. Moustafa and Omar Farid performed the *in vitro* and *in vivo* tests to measure the biochemical and physiological parameters and analysed the related data. Omnia A. Badr, I.I.S. EL-Shawaf, Hoda A. El-Garhy and Mahmoud M. Moustafa quantitatively examined the expression of *SOD* and *CAT* genes and performed the immunohistochemical examination. All authors wrote and drafted the corresponding sections of the manuscript and revised the complete manuscript.

Ethics approval and Consent to participate

The protocol for the conducted animal experiments was approved by the Research Ethics Committee of the Faculty of Pharmacy, Ain Shams University which followed the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication no. 85–23, revised 1996).

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no compact of interests.

References

Abd-Elrazek, A.M. and Ahmed-Farid, O.A.H. (2018) Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. *Andrologia* **50**, e12806.

Aebi, H. (1984) Catalase in vitro. *Methods Enzymol* **105**, 121–126.

Agarwal, A., Gupta, S., Sekhon, L. and Shah, R. (2008) Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. *Antioxid Redox Signa* **10**, 1375–1404.

Ahamad, M.N., Saleemullah, M., Shah, H.U., Khalil, I.A. and Saljoqi, A.U.R. (2007) Determination of beta carotene content in fresh vegetables using high performance liquid chromatography. *Sarhad J Agric* **23**, 767.

Aydaş, S.B., Ozturk, S. and Aslım, B. (2013) Phenylalanine ammonia lyase (PAL) enzyme activity and antioxidant properties of some cyanobacteria isolates. *Food Chem* **136**, 164–169.

Badr, O.A., El-Shawaf, I.I., El-Garhy, H.A. and Moustafa, M.M. (2018) Isolation and molecular identification of two novel cyanobacterial isolates obtained from a stressed aquatic system. *Gene Rep* **13**, 110–114.

Bancroft, J. and Cook, H. (1994) Immunocytochemistry. In *Manual of Histological Techniques and Their Diagnostic Application*, ed. Turner, D.R. 2nd ed. pp. 263–325. Edinburgh, New York: Churchill Livingstone.

Bashandy, S.A., El Awdan, S.A., Ebaid, H. and Alhazza, I.M. (2016) Antioxidant potential of Spirulina platensis mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats. *Oxid Med Cell Longev* **2016**, 8.

Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T. (1995) Use of a free radical method to evaluate antioxidant activity. *Lebenson Wiss Technol* **28**, 25–30.

Carfagna, S., Napolitano, G., Barone, D., Pinto, G., Pollio, A. and Venditti, P. (2015) Dietary supplementation with the microalga *Galdieria sulphuraria* (Rhodophyta) reduces prolonged exercise-induced oxidative stress in rat tissues. *Oxid Med Cell Longev* **2015**, 1–11.

Carvalho, L.R., Costa-Neves, A., Conserva, G.A., Brunetti, R.L., Hentschke, G.S., Malone, C.F., Torres, L., Sant'Anna, C.L. *et al.* (2013). Biologically active compounds from cyanobacteria extracts: in vivo and in vitro aspects. *Revista Brasileira de Farmacognosia* **23**, 471–480.

Chacón-Lee, T.L. and González-Mariño, G.E. (2010) Microalgae for "healthy" foods—possibilities and challenges. *Compr Rev Food Sci Food Saf* **9**, 655–675.

Chandini, S.K., Ganesan, P. and Bhaskar, N. (2008) In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chem* **107**, 707–713.

Chandrika, U.G. and Kumara, P.A.P. (2015) Gotu kola (*Centella asiatica*): nutritional properties and plausible health benefits. *Adv Food Nutr Res* **76**, 125–157.

Chen, L., Li, S., Guo, X., Xie, P. and Chen, J. (2016) The role of GSH in microcystin-induced apoptosis in rat liver: involvement of oxidative stress and NF-κB. *Environ Toxicol* **31**, 552–560.

Cirulis, J.T., Scott, J.A. and Ross, G.M. (2013) Management of oxidative stress by microalgae. *Can J Physiol Pharmacol* **91**, 15–21.

Duncan, D.B. (1955) Multiple range and multiple F tests. *Biometrics* **11**, 1–42.

Ekinci, R. and Kadakal, C. (2005) Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high-performance liquid chromatography. *Acta Chromatogr* **15**, 289.

El-Baz, F.K., Hussein, R.A., Mahmoud, K. and Abdo, S.M. (2018) Cytotoxic activity of carotenoid rich fractions from *Haematococcus pluvialis* and *Dunaliella salina* microalgae and the identification of the phytoconstituents using LC-DAD/ESI-MS. *Phytother Res* **32**, 298–304.

El-Tantawy, W.H. (2016) Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *J Tradit Complement Med* **6**, 327–331.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M. *et al.* (2017) Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* **114**, 1752–1761.

Gagné, F. (2014) Oxidative stress. In *Biochemical Ecotoxicology-Principles and Methods* pp. 103–115. London, UK: Elsevier Inc.

Galhano, V., De Figueiredo, D.R., Alves, A., Correia, A., Pereira, M.J., Gomes-Laranjo, J. and Peixoto, F. (2011) Morphological, biochemical and molecular characterization of Anabaena, Aphanizomenon and Nostoc strains (Cyanobacteria, Nostocales) isolated from Portuguese freshwater habitats. *Hydrobiologia* **663**, 187–203.

Gimeno, E., Castellote, A.I., Lamuela-Raventos, R.M., De La Torre, M.C. and Lopez-Sabater, M.C. (2000) Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography. *J Chromatogr A* **881**, 251–254.

Goh, S.H., Yusoff, F.M. and Loh, S.P. (2010) A comparison of the antioxidant properties and total phenolic content in a diatom, *Chaetoceros* sp. and a green microalga, *Nannochloropsis* sp. *J Agric Sci*, **2**, 123.

Goiris, K., Muylaert, K., Voorspoels, S., Noten, B., De Paepe, D., E Baart, G.J. and De Cooman, L. (2014). Detection of flavonoids in microalgae from different evolutionary lineages. *J Phycol* **50**, 483–492.

Gugger, M., Lyra, C., Suominen, I., Tsitko, I., Humbert, J.F., Salkinoja-Salonen, M.S. and Sivonen, K. (2002) Cellular fatty acids as chemotaxonomic markers of the genera Anabaena, Aphanizomenon, Microcystis, Nostoc and Planktothrix (cyanobacteria). *Int J Syst Evol Microbiol* **52**, 1007–1015.

Guihéneuf, F., Khan, A. and Tran, L.S.P. (2016) Genetic engineering: a promising tool to engender physiological, biochemical, and molecular stress resilience in green microalgae. *Front Plant Sci* **7**, 400.

Hajimahmoodi, M., Faramarzi, M.A., Mohammadi, N., Soltani, N., Oveisi, M.R. and Nafissi-Varcheh, N. (2010) Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. *J Appl Phycol* **22**, 43–50.

Hayes, P.K., El Semary, N.A. and Sʋnchez-Baracaldo, P. (2007). The taxonomy of cyanobacteria: molecular insights into a difficult problem. *Syst Assoc Spec*, **75**, 93.

Hochstein, P. and Atallah, A.S. (1988) The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat Res* **202**, 363–375.

Hossain, M.A., Al Kalbani, M.S.A., Al Farsi, S.A.J., Weli, A.M. and Al-Riyami, Q. (2014) Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L. *Asian Pac J Trop Dis* **4**, 378–383.

Hossain, M.F., Ratnayake, R.R., Meerajini, K. and Wasantha Kumara, K.L. (2016) Antioxidant properties in some selected cyanobacteria isolated from fresh water bodies of Sri Lanka. *Food Sci Nutr* **4**, 753–758.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. and Darzins, A. (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J* **54**, 621–639.

Jayatilleke, E. and Shaw, S. (1993) A high-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. *Anal Biochem* **214**, 452–457.

Jerez-Martel, I., Garcьa-Poza, S., Rodrьguez-Martel, G., Rico, M., Afonso-Olivares, C. and Gómez-Pinchetti, J.L. (2017) Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *J Food Qual* **2017**, 1–8.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R. and Saura-Calixto, F. (2001) Antioxidant activity of fresh and processed edible seaweeds. *J Sci Food Agric* **81**, 530–534.

Jimenez-Garcia, S.N., Vazquez-Cruz, M.A., Guevara-Gonzalez, R.G., Torres-Pacheco, I., Cruz-Hernandez, A. and Feregrino-Perez, A.A. (2013) Current approaches for enhanced expression of secondary metabolites as bioactive compounds in plants for agronomic and human health purposes–a review. *Pol J Food Nutr Sci* **63**, 67–78.

Jung, K.J., Go, E.K., Kim, J.Y., Yu, B.P. and Chung, H.Y. (2009) Suppression of age-related renal changes in NF-κB and its target gene expression by dietary ferulate. *J Nutr Biochem* **20**, 378–388.

Karatas, F., Karatepe, M. and Baysar, A. (2002) Determination of free malondialdehyde in human serum by high-performance liquid chromatography. *Anal Biochem* **311**, 76–79.

Kaur, C. and Kapoor, H.C. (2002) Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Technol* **37**, 153–161.

Khanduja, K.L., Avti, P.K., Kumar, S., Mittal, N., Sohi, K.K. and Pathak, C.M. (2006) Anti-apoptotic activity of caffeic acid, ellagic acid and ferulic acid in normal human peripheral blood mononuclear cells: a Bcl-2 independent mechanism. *Biochim Biophys Acta Gen Subj* **1760**, 283–289.

Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K. and Taniguchi, H. (2002) Antioxidant properties of ferulic acid and its related compounds. *J Agric Food Chem* **50**, 2161–2168.

Kops, G.J., Dansen, T.B., Polderman, P.E., Saarloos, I., Wirtz, K.W., Coffer, P.J., Huang, T.T., Bos, J.L. *et al.* (2002) Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* **419**, 316.

Kuda, T., Kunii, T., Goto, H., Suzuki, T. and Yano, T. (2007) Varieties of antioxidant and antibacterial properties of Ecklonia stolonifera and Ecklonia kurome products

harvested and processed in the Noto peninsula, Japan. *Food Chem* **103**, 900–905.

Lee, K., Eisterhold, M.L., Rindi, F., Palanisami, S. and Nam, P.K. (2014) Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources. *J Nat Sci Biol Med* **5**, 333.

Li, H.B., Cheng, K.W., Wong, C.C., Fan, K.W., Chen, F. and Jiang, Y. (2007) Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem* **102**, 771–776.

Li, R., Wilhelm, S.W., Carmichael, W.W. and Watanabe, M.M. (2008) Polyphasic characterization of water bloom forming Raphidiopsis species (cyanobacteria) from central China. *Harmful Algae* **7**, 146–153.

Li, S., Tan, H.Y., Wang, N., Zhang, Z.J., Lao, L., Wong, C.W. and Feng, Y. (2015) The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci* **16**, 26087–26124.

Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2 - \Delta\Delta CT$ method. *Methods* **25**, 402–408.

Marklund, S. and Marklund, G. (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *FEBS J* **47**, 469–474.

Mecocci, P., Boccardi, V., Cecchetti, R., Bastiani, P., Scamosci, M., Ruggiero, C. and Baroni, M. (2018) A long journey into aging, brain aging, and Alzheimer's disease following the oxidative stress tracks. *J Alzheimers Dis* **62**, 1319–1335.

Mielczarek, M., Kołodziejczyk, J. and Olas, B. (2010) Właściwości lecznicze chmielu zwyczajnego (Humulus lupulus L.). *Postępy Fitoterapii* **4**, 205–210.

Mulgund, A., Doshi, S. and Agarwal, A. (2015) The role of oxidative stress in endometriosis. In *Handbook of Fertility* ed. Watson, R. pp. 273–281. London, UK: Elsevier Inc.

Munir, N., Sharif, N.A.D.I.A., Shagufta, N., Saleem, F.A.I.Z.A. and Manzoor, F.A.R.K.H.A.N.D.A. (2013) Harvesting and processing of microalgae biomass fractions for biodiesel production (a review). *Sci Tech Dev* **32**, 235–243.

Nakao, M., Okamoto, S., Kohara, M., Fujishiro, T., Fujisawa, T., Sato, S., Tabata, S., Kaneko, T. *et al.* (2009) CyanoBase: the cyanobacteria genome database update 2010. *Nucleic Acids Res* **38**(suppl_1), D379–D381.

Nayak, S., Prasanna, R., Prasanna, B.M. and Sahoo, D.B. (2007) Analysing diversity among Indian isolates of Anabaena (Nostocales, Cyanophyta) using morphological, physiological and biochemical characters. *World J Microbiol Biotechnol* **23**, 1575–1584.

Nogata, Y., Ohta, H., Yoza, K.I., Berhow, M. and Hasegawa, S. (1994) High-performance liquid chromatographic determination of naturally occurring flavonoids in Citrus with a photodiode-array detector. *J Chromatogr A* **667**, 59–66.

Ohki, K., Kamiya, M., Honda, D., Kumazawa, S. and Ho, K.K. (2008) Morphological and phylogenetic studies on unicellular diazotrophic cyanobacteria (Cyanophytes)

isolated from the coastal waters around Singapore. *J Phycol* **44**, 142–151.

O'sullivan, A.M., O'Callaghan, Y.C., O'Grady, M.N., Queguineur, B., Hanniffy, D., Troy, D.J., Kerry, J.P. and O'Brien, N.M. (2011) In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. *Food Chem* **126**, 1064–1070.

Ou, S. and Kwok, K.C. (2004) Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J Sci Food Agric* **84**, 1261–1269.

Oyaizu, M. (1986) Studies on products of browning reaction. *Jpn J Nutr Diet* **44**, 307–315.

Paget, G.E. and Barnes, J.M. (1964) Toxicity tests. In *Evaluation of Drug Activities: Pharmacometrics* ed.Laurence, D. R. and Bacharach, A. L. pp. 135–165. London and New York: Academic Press.

Papadoyannis, I.N., Samanidou, V.F. and Nitsos, C.C. (1999) Simultaneous determination of nitrite and nitrate in drinking water and human serum by high performance anion-exchange chromatography and UV detection. *J Liq Chromatogr Relat Technol* **22**, 2023–2041.

Park, P.J., Shahidi, F. and Jeon, Y.J. (2004) Antioxidant activities of enzymatic extracts from an edible seaweed *Sargassum horneri* using ESR spectrometry. *J Food Lipids* **11**, 15–27.

Ponnuswamy, I., Madhavan, S. and Shabudeen, S. (2013) Isolation and characterization of green microalgae for carbon sequestration, waste water treatment and bio-fuel production. *Int J Biosci Biotechnol* **5**, 17–25.

Rahul, V., Agrawal, P., Sharma, M. and Shukla, S. (2016) Total phenolics, flavonoids and antioxidant potential of organic extract of fresh water algal sample collected from a marine lake. *Indian J Geomarine Sci* **45**, 1320–1326.

Rajaniemi, P., Hrouzek, P., Ka̦tovska, K., Willame, R., Rantala, A., Hoffmann, L., Kom̹rek, J. and Sivonen, K. (2005) Phylogenetic and morphological evaluation of the genera Anabaena, Aphanizomenon, Trichormus and Nostoc (Nostocales, Cyanobacteria). *Int J Syst Evol Microbiol* **55**, 11–26.

Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* **28**, 56–63.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology* **111**, 1–61.

Rosa, N.N., Dufour, C., Lullien-Pellerin, V. and Micard, V. (2013a) Exposure or release of ferulic acid from wheat aleurone: impact on its antioxidant capacity. *Food Chem* **141**, 2355–2362.

Safafar, H., Van Wagenen, J., Møller, P. and Jacobsen, C. (2015) Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Mar Drugs* **13**, 7339–7356.

SAS (2004) *SAS Procedure Guide "Version 6.12 Ed."* Cary, NC: SAS Institute Inc.

Singh, R., Parihar, P., Singh, M., Bajguz, A., Kumar, J., Singh, S., Singh, V.P. and Prasad, S.M. (2017) Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: current status and future prospects. *Front Microbiol* **8**, 515.

Storz, P. (2011) Forkhead homeobox type O transcription factors in the responses to oxidative stress. *Antioxid Redox Signal* **14**, 593–605.

Tan, W.Q., Wang, K., Lv, D.Y. and Li, P.F. (2008) Foxo3a inhibits cardiomyocyte hypertrophy through transactivating catalase. *J Biol Chem* **283**, 29730–29739.

Thajuddin, N. and Subramanian, G. (2005) Cyanobacterial biodiversity and potential applications in biotechnology. *Curr Sci* **89**, 47–57.

Vijayakumar, S. and Menakha, M. (2015) Pharmaceutical applications of cyanobacteria—a review. *J Acute Med* **5**, 15–23.

Wang, T., Gong, X., Jiang, R., Li, H., Du, W. and Kuang, G. (2016) Ferulic acid inhibits proliferation and promotes apoptosis via blockage of PI3K/Akt pathway in osteosarcoma cell. *Am J Transl Res* **8**, 968.

Wijeratne, S.S., Cuppett, S.L. and Schlegel, V. (2005) Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco-2 human colon cells. *J Agric Food Chem* **53**, 8768–8774.

Zapomělová, E., Řeháková, K., Jezberov̹, J. and Kom̹rkov̹, J. (2010) Polyphasic characterization of eight planktonic Anabaena strains (Cyanobacteria) with reference to the variability of 61 Anabaena populations observed in the field. *Hydrobiologia* **639**, 99–113.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers used for qPCR amplification.