



RESEARCH ARTICLE

# GC-MS profiling, anti-oxidant and anti-diabetic assessments of extracts from microalgae *Scenedesmus falcatus* (KU.B1) and *Chlorella sorokiniana* (KU.B2)

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## Abstract

Microalgae are a potentially valuable source in the food, pharmaceutical and nutraceutical sectors. While biological activities surveys have investigated the pharmaceutical properties of a few microalgae species, there are not many reports covering biological activity studies. This study was carried out to identify the metabolites by gas chromatography-mass spectrometry and evaluate the anti-oxidant, anti-diabetic properties of green algae extracts, *Chlorella sorokiniana* (KU.B2) and *Scenedesmus falcatus* (KU.B1). A total of 51 different chemical constituents were detected and tentatively identified. The primary compounds in both microalgae extracts included (*R*)-2-hexanol (38.67% in *C. sorokiniana* and 23.53% in *S. falcatus*), n-hexadecanoic acid (13.58% in *C. sorokiniana* and 18.94% in *S. falcatus*) and octadecanoic acid (22.30% in *C. sorokiniana* and 32.67% in *S. falcatus*). According to the profiling results, the *C. sorokiniana* extract exhibited greater anti-oxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ( $IC_{50} = 480.30 \pm 14.85 \mu\text{g mL}^{-1}$ ), nitric oxide (NO) radical scavenging ( $562.73 \pm 3.52 \mu\text{g mL}^{-1}$ ) and ferric reducing anti-oxidant power (FRAP) of  $58.51 \pm 2.42 \text{ mgTE g}^{-1}$ . Comparatively, the *C. sorokiniana* extract had higher contents of alpha-glucosidase and alpha-amylase ( $IC_{50} = 491.22 \pm 78.41$  and  $2,817.00 \pm 143.04 \mu\text{g mL}^{-1}$ , respectively) than the *S. falcatus* extract. This first report demonstrated anti-diabetic effect of both extracts on diabetic enzymes. The results confirm microalgae's anti-oxidant and anti-diabetic properties and suggest their potential benefits in cosmeceutical, nutraceutical and pharmaceutical applications.

## Keywords

*Chlorella*, *Scenedesmus*, alpha-amylase, alpha-glucosidase, microalgae

## Introduction

As primary producers in aquatic systems, microalgae have gained increasing interest from the chemical, food and pharmaceutical industries. Various microalgae contain a vast array of biologically active compounds, such as carotenoids (1), fatty acids (2), lipids (3), peptides (4), polyphenols (5), polysaccharides (6) and vitamins (7). Many studies have reported the effects of phytochemicals obtained from microalgae, with specific emphasis on the important bioactive metabolites. Because of such metabolites, microalgae have a wide variety of biological activities, including anti-bacterial (8), anti-diabetic (2), anti-fungal (9), anti-inflammatory (10), anti-tumor (11) and anti-oxidant activities (12), which are promising for drug discovery efforts (13).

Reactive oxygen and nitrogen species (RONS) are produced during physiological processes and are responsible for humans' oxidative cellular damage. RONS have a detrimental role in aging and in various diseases, such as Alzheimer's disease, cardiovascular diseases, cancer, diabetes, inflammatory problems, Parkinson's disease and ischemia/reperfusion disorders (14-18). Several natural antioxidants have exhibited a strong defense against cellular damage caused by free radicals, which may indicate their use in reducing such damage (19, 20).

Type 2 diabetes (T2D), or non-insulin-dependent diabetes, is the most common type of diabetes, accounting for approximately 90% of all cases of diabetes worldwide. It is currently the most prevalent metabolic disease in many modern societies and is becoming a severe disorder on a global scale (2, 13). A cure for diabetes has not been found. Various strategies are used to control the disease, such as diet modification and drug therapy (21, 22). The reduction of postprandial hyperglycemia is highly important in treating T2D (23). Inhibiting carbohydrate-hydrolyzing enzymes, such as alpha-amylase and alpha-glucosidase, can reduce postprandial hyperglycemia. Pancreatic alpha-amylase and intestinal alpha-glucosidase are the primary exo-acting glycoside hydrolase enzymes involved in carbohydrate digestion. Specifically, alpha-amylase is involved in the breakdown of large insoluble starches, while alpha-glucosidase plays a role in breaking down starches and disaccharides into glucose subunits (24). Therefore, enzyme inhibitors can lead to the reduction of postprandial blood glucose levels and act as a potential target for anti-diabetic drugs (25).

In recent years, lipids produced by microalgae have acquired increasing interest because of their chemical constituents and possible applications. Many microalgae have an abundance of valuable products, and interest has focused on lipids. Some reports have suggested that lipids may have potential in the treatment of oxidative stress and T2D (2, 26-28). However, there is little research on microalgae's biotechnological potential as pharmaceutical properties. Few microalgae species have been reported for their biological activity, but not many studies (29). Especially, the study of microalgae extracts against oxidative stress and T2D has been almost totally lacking.

The most common types of microalgae *Chlorella* and *Scenedesmus* have been recognized as potentially good sources for production, popular microalga commercially cultivation, and rich in bioactive components (30-32). Many research studies have reported the potential anti-oxidant and anti-diabetic activities of *Chlorella* and *Scenedesmus* (2, 30, 33-39). However, there is no report anti-oxidant on *S. falcatus* and anti-diabetic alpha-glucosidase and alpha-amylase enzymes on *C. sorokiniana* and *S. falcatus*. Therefore, this study aimed to investigate the chemical profiles and biological properties, including anti-oxidant and anti-diabetic potentials, of the *C. sorokiniana* and *S. falcatus* microalgae extracts that may underlie the observed biological effects.

## Materials and Methods

### General chemicals and materials

The chemicals were purchased as follows: methanol (analytical grade) (Merck, USA), chloroform (analytical grade) (RCL Labscan Limited, Thailand), hexane (analytical grade) (Baker, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Merck, USA), sodium nitroprusside (SNP; Himedia, India), sulfanilamide (Carlo Erba Reagents, France), phosphoric acid (Macron Fine Chemicals, China), N-(1-Naphthyl) ethylenediamine hydrochloride (PanReac AppliChem, Germany), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ; Fluka Chemie, Switzerland), ferric chloride (ChemSupply Australia, Australia), alpha-glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich, USA), 4-nitrophenyl alpha-glucopyranoside (Sigma-Aldrich, USA), alpha-amylase from porcine pancreas (Sigma-Aldrich, USA), starch (Tokyo Chemical Industries, Japan), 3,5-dinitrosalicylic acid (Sigma-Aldrich, USA), potassium sodium tartrate tetrahydrate (Ajax Finechem, Australia) and acarbose (Sigma-Aldrich, USA). Analyses were performed with a Büchi Rotavapor® R-210 (Mumbai, India) 96-well microplate reader (Thermo Scientific, China) and gas chromatography-mass spectrometer (GCMS-QP2020; Shimadzu, Japan).

### Strains and culture conditions

The strains of *C. sorokiniana* (KU.B2) and *S. falcatus* (KU.B1) were isolated and cultured at the Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand. Both microalgae were cultured in a liquid tris-acetate-phosphate (TAP) medium using the followed method from previous report (40) under controlled conditions using a cool white light-emitting diode (200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at a temperature of  $30 \pm 1^\circ\text{C}$  and pH of 7.0. During incubation for 9 days, the culture was shaken on a shaker at 115 rpm for 12 hrs per day.

### Preparation of crude extract

*C. sorokiniana* and *S. falcatus* were collected and dried, then mechanically ground to a coarse powder. The powder samples of *C. sorokiniana* (20 mg) and *S. falcatus* (20 mg) were macerated with chloroform and methanol (2:1 v/v; 50 ml) at room temperature. After 7 days, the resulting extracts were filtered through Whatman® grade 1 filter paper. The extracts were evaporated to obtain the crude extracts of *C. sorokiniana* (1.16 mg; 5.8% of yield) and *S. falcatus* (1.98 mg; 9.9% of yield), then extracts were kept at  $-20^\circ\text{C}$  in the dark until analysis.

### Gas chromatography-mass spectrometry (GC-MS) analysis

To prepare the samples, the crude extracts of *C. sorokiniana* (2 mg) and *S. falcatus* (2 mg) were separately mixed with 1 ml of 1 M HCl in methanol under a constant  $\text{N}_2$  stream for 1 min. The mixture was incubated at  $80^\circ\text{C}$  for 40 min and then, cooled at room temperature. After adding 1 ml of 0.9% sodium chloride and 1 ml of hexane to the mixtures, they were centrifuged at 3000 rpm for 3 min. Subsequently, the hexane phase was transferred into a glass tube and dried once (41).

The samples were analyzed using gas chromatography-mass spectrometry (GCMS-QP2020; Shimadzu, Japan) equipped with an SH-RXI-5SIL-MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Spectroscopic detection by GC-MS involved an electron ionization at 70 eV. The flow rate of the column carrier gas was set at 1 ml min<sup>-1</sup>, and 1 µl samples were injected into the columns, which were set at 250 °C in split mode. The temperature gradient of the GC oven was maintained at an initial temperature of 40°C, was increased to 300 °C at a rate of 5 °C min<sup>-1</sup> for 52 min, and was then held at 300 °C for 8 min. Mass data analysis was conducted using an enhanced GC-MS post-run analysis programme fitted with the National Institute of Standards and Technology NIST14 library database.

### Antioxidant activity

#### 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH scavenging capacity was demonstrated using a DPPH test, according to a previously described procedure (42). Separately, 150 µl of each extract of *C. sorokiniana* and *S. falcatius* was mixed with 150 µl of 0.2 mM DPPH solution in methanol (150 µl). After an incubation period of 30 min at 25 °C, the absorbance was measured at 520 nm.

#### Nitric oxide (NO) radical scavenging activity

The capacity to scavenge NO was evaluated based on the standard method (18). First, 10 mmol l<sup>-1</sup> sodium nitroprusside in phosphate buffered saline (125 µl) and the sample (25 µl) were mixed. After 150 min, the Griess reagent containing 1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% N-(1-Naphthyl)ethylenediamine hydrochloride (50 µl) was added. The absorbance was measured at 546 nm.

#### Ferric reducing antioxidant power (FRAP) activity

The ferric reducing anti-oxidant power (FRAP) activity was evaluated according to a procedure described (17). Fresh FRAP solution was prepared with 300 mM acetate buffer (100 ml), 10 mM TPTZ solution (10 ml) and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O (10 ml). The extracts of *C. sorokiniana* and *S. falcatius* (15 µl) and the FRAP solution (285 µl) were mixed in the dark and incubated for 30 min. The absorbance was measured at 593 nm. The FRAP content in the samples was reported as milligrams of the Trolox equivalent (TE) g extract<sup>-1</sup>, using the Trolox line equation (concentration at 0 to 250 mg l<sup>-1</sup>):  $y = 0 + 0.01x + 0.2046$ ,  $R^2 = 0.9917$ .

### Anti-diabetic activity

#### Alpha-glucosidase activity

The inhibition of alpha-glycosidase was assessed following a previously reported procedure (43). Fifty microliters of the sample, 130 µl of the buffer, and 20 µl of the alpha-glucosidase solution (0.28 U ml<sup>-1</sup>) were mixed and incubated at 37°C for 10 min, and then 100 µl of 0.5 mM 4-nitrophenyl alpha-glucopyranoside was added. The absorbance was measured at 405 nm. and compared with acarbose as the positive control.

#### Alpha-amylase activity

The inhibition of alpha-amylase was assessed according to the procedure described (43). First, 1% of the starch solution (200 µl) and the extract (200 µl) were mixed and incu-

bated for 10 min, at 25°C, then 200 µl of alpha-amylase (15 U ml<sup>-1</sup>) was added and incubated at 25°C for 10 min. Subsequently, 400 µl of DNS solution, containing 1 g di-nitrosalicylic acid, 20 ml 2 M NaOH, and 30 g potassium sodium tartrate tetrahydrate in 100 ml water, was added. The mixtures were incubated at 100°C for 5 min and cooled at room temperature, and then 80 µl water was added. The absorbance was measured at 540 nm and compared with acarbose as the positive control.

### Statistical analysis

The data were expressed as a mean of three analyses. The statistical approaches were conducted using GraphPad Prism 6.01 software (San Diego, CA, USA). Tukey's multiple comparisons test was used to compare the statistical significance, where *P*-values < 0.05 were considered statistically significant.

## Results

The GC-MS profile of the extracts from *C. sorokiniana* and *S. falcatius* were evaluated and shown in Fig. 1. Fifty-one compounds were identified, while 34 compounds from *C.*

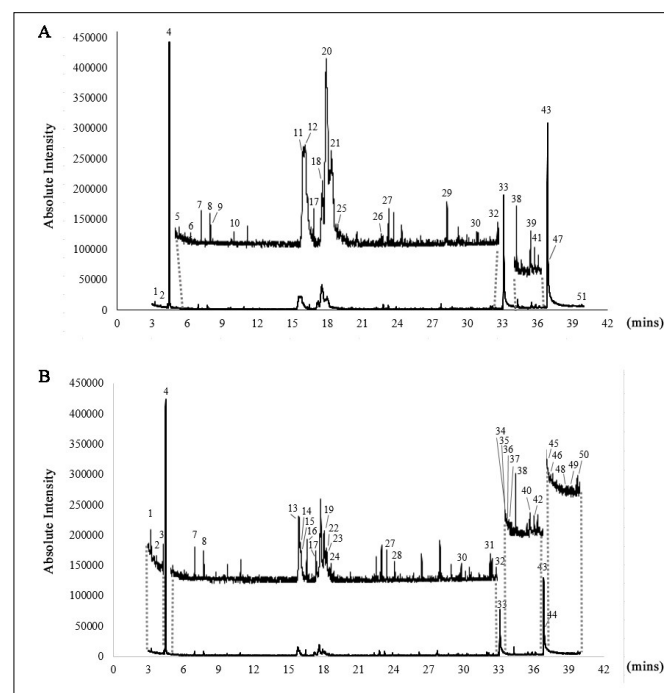


Fig. 1. GC-MS profiles of (A) *Chlorella sorokiniana* and (B) *Scenedesmus falcatius* extracts.

*sorokiniana*, 28 from *S. falcatius* and 11 were found in both species (Table 1). The following major components (>3%) were found in both extracts: (*R*)-2-hexanol (4; 38.67% in *C. sorokiniana* and 23.53% in *S. falcatius*), *n*-hexadecanoic acid (33; 13.58% in *C. sorokiniana* and 18.94% in *S. falcatius*), and octadecanoic acid (43; 22.30% in *C. sorokiniana* and 32.67% in *S. falcatius*). Other identified components included 1,3,6-heptatriene, 2,5,5-trimethyl- (13; 4.14%) in *C. sorokiniana* and 1,5-heptadiene, 2,5-dimethyl-3-methylene- (11; 3.95%), 5-hepten-1-yne, 6-methyl (12; 3.55%), and epicedrol (20; 10.44%) in *S. falcatius*. All major chemical compositions are shown in Fig. 2. The chemical compounds identified by GC-MS were based on the reten-

tion time ( $R_T$ ) and molecular weight, while the fragmentation patterns and data comparisons with the NIST14 library provide further structural identification. The *C. sorokiniana* and *S. falcatius* extracts also contained some alco-

hols, fatty acids and straight-chain hydrocarbon compounds associated with various biological activities.

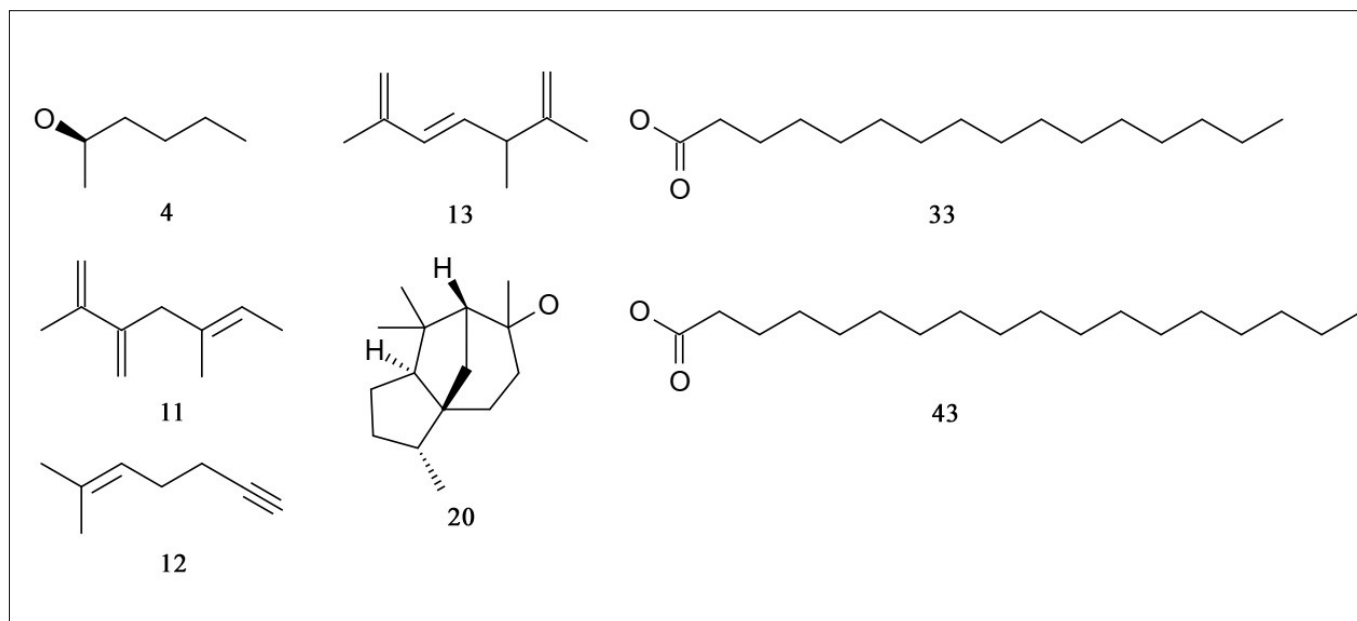
Microalgae can produce numerous volatile compounds, and they can be used as an alternative source for

**Table 1.** Chemical constituents of *Chlorella sorokiniana* and *Scenedesmus falcatius* extracts.

Sl. No.	$R_T$	Compounds	Molecular weight	Ratio	
				<i>C. sorokiniana</i>	<i>S. falcatius</i>
1	3.236	3-hydroxy-3-methyl-2-butanone	102	0.50	0.37
2	3.706	2-propenoic acid, 2-methyl-, ethenyl ester	112	0.52	0.15
3	4.422	Propanoic acid, 2-hydroxy-2-methyl-, methyl ester	118	0.89	-
4	4.472	( <i>R</i> )-2-hexanol	102	38.67	23.53
5	5.455	3-butyric acid	84	-	0.21
6	6.059	1-butene, 2,3-dimethyl	84	-	0.08
7	6.949	2-butenal, 3-methyl-	84	0.57	0.36
8	7.719	Diisooamyl ether	158	0.42	0.35
9	7.767	4-octen-3-one	126	-	0.20
10	9.723	Methacrolein	70	-	0.13
11	15.530	1,5-heptadiene, 2,5-dimethyl-3-methylene-	136	-	3.95
12	15.785	5-hepten-1-yne, 6-methyl	108	-	3.55
13	15.794	1,3,6-heptatriene, 2,5,5-trimethyl-	136	4.14	-
14	15.94	Acetoxyacetic acid, 2-(1-adamantyl) ethyl ester	280	0.74	-
15	15.98	Ethanol, 2-methoxyphenyl-	152	0.35	-
16	16.474	Benzene, 1,3-bis(1,1-dimethylethyl)-	190	0.88	-
17	16.468	<i>p</i> -Pentylacetophenone	190	-	0.45
18	17.055	Propanedioic acid, oxo-, bis(1-methylethyl) ester	202	-	0.09
19	17.229	4-heptanone, 3-methyl-	128	0.77	-
20	17.530	Epicedrol	222	-	10.44
21	17.980	Acetic acid, 4-(7-methyldenebicyclo[3.3.1]non-2-en-3-yloxy)butyl ester	264	-	0.32
22	18.165	Crotyl methacrylate	140	0.36	-
23	18.385	4-hexen-2-one, 3-methyl-	112	0.45	-
24	18.497	Heptane, 3,3-dimethyl-	128	0.39	-
25	18.500	3-hexanone, 2,2-dimethyl-	128	-	0.18
26	22.804	Nonane, 5-methyl-5-propyl-	184	-	0.53
27	23.236	Pentanoic acid, 5-hydroxy-, 2,4-di- <i>t</i> -butylphenyl esters	306	0.72	0.39
28	23.916	3-hexanone, 2,5-dimethyl-	128	0.35	-
29	27.745	3,5-dimethyl-4-octanone	156	-	0.56
30	30.267	Oxalic acid, butyl propyl ester	228	1.20	0.19
31	32.156	4-heptanone, 2-methyl-	128	0.52	-
32	32.999	Phthalic acid, 4-cyanophenyl nonyl ester	393	0.68	0.32
33	33.113	<i>n</i> -hexadecanoic acid	256	13.58	18.94
34	33.255	Valeric anhydride	186	0.82	-
35	33.280	2-methylbutanoic anhydride	186	1.02	-
36	33.395	1,2,4-benzenetricarboxylic acid, 1,2-dimethyl ester	238	0.43	-
37	33.910	Propane, 2-methoxy-2-methyl-	88	0.53	-
38	34.312	14-heptadecenal	252	1.31	0.83
39	35.493	1-undecene, 9-methyl-	168	-	0.53
40	35.499	1-tridecyn-4-ol	196	0.60	-
41	35.856	3-hepten-2-one	112	-	0.24
42	35.868	Oxalic acid, allyl heptyl ester	228	0.46	-
43	36.863	Octadecanoic acid	284	22.30	32.67
44	37.005	1-piperidin-1-ylpropan-2-yl acetate	185	1.88	-
45	37.080	4-pentadecanol	228	1.24	-



46	37.147	2,2'-oxybis(ethane-2,1-diyl) dipentanoate	274	0.74	-
47	37.160	5-isoxazolecarboxylic acid, 4,5-dihydro-3,5-dimethyl-, methyl ester, (S)-	157	-	0.15
48	39.150	Propane, 2-methoxy-2-methyl-	88	0.38	-
49	39.314	Propanoic acid, ethenyl ester	100	0.51	-
50	39.805	Butanal, 4-[(tetrahydro-2H-pyran-2-yl)oxy]-	172	0.53	-
51	39.805	Pentanoic acid, 1,1-dimethylpropyl ester	172	-	0.30



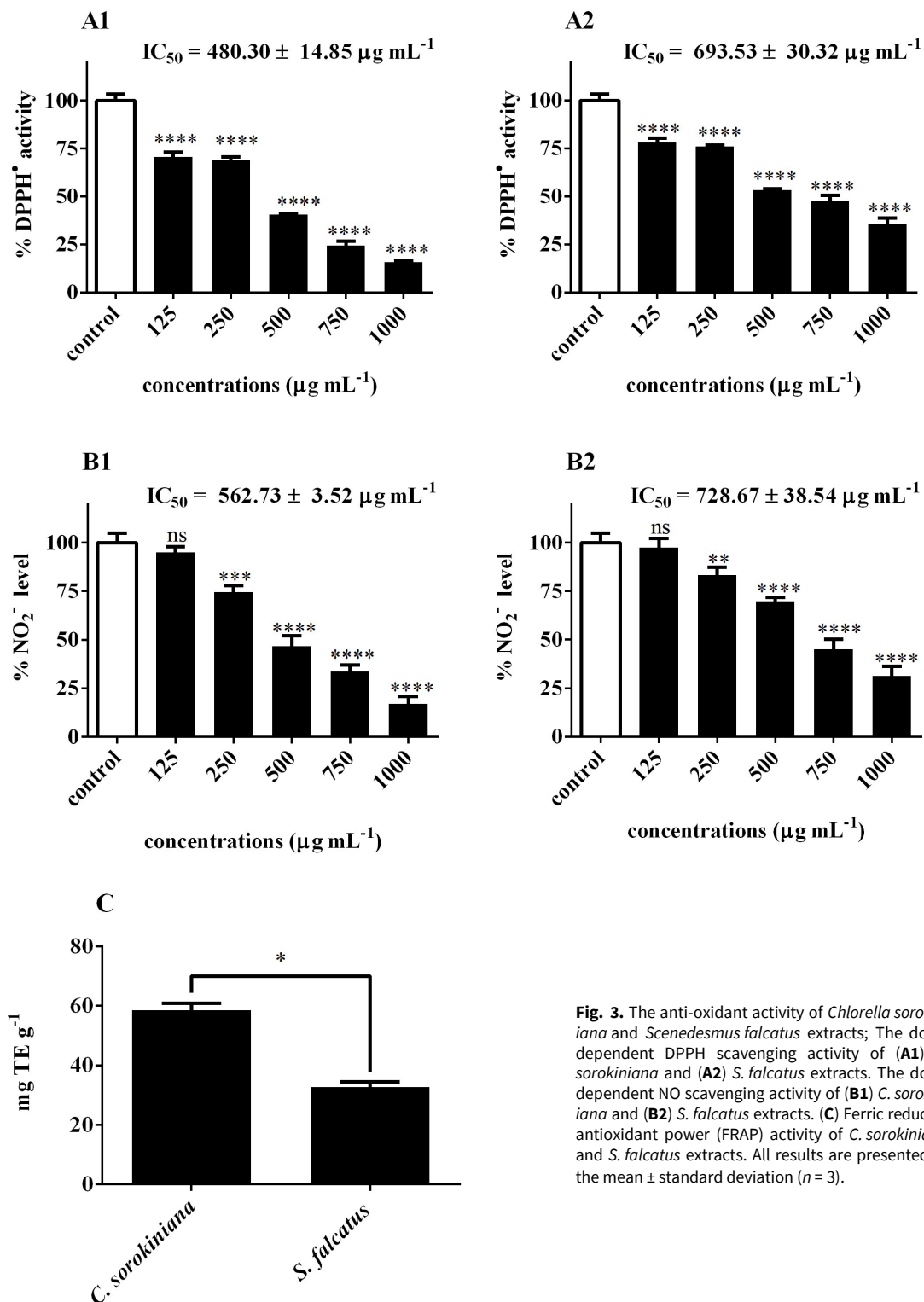
**Fig. 2.** Structure of major compounds of microalgae extracts: (*R*)-2-hexanol (**4**), 1,3,6-heptatriene, 2,5,5-trimethyl- (**13**), epicedrol (**20**), *n*-hexadecanoic acid (**33**) and octadecanoic acid (**43**).

some specific advantages. For instance, hexanol, which has received commercial interest, was found in *S. obliquus* and *C. vulgaris* microalgae (44-47). Other studies have also identified fatty acid components in various microalgae (48), such as *n*-hexadecanoic acid and octadecanoic acid in a *C. sorokiniana* extract (49). Results of other studies also revealed that the profile of the fatty acid composition in *Chlorella* and *Scenedesmus* extracts is mostly composed of C<sub>16</sub>-C<sub>18</sub> (>92%) (31, 47). Moreover, hydrocarbon compounds have been found in green microalgae, such as *S. obliquus*, *S. dimorphus* and *C. vulgaris* (50-53). Therefore, the chemical constituents obtained from *C. sorokiniana* and *S. falcatus* may play important role in the biological activities and pharmacological properties.

Fig. 3. shows the anti-oxidant activities of *C. sorokiniana* and *S. falcatus*, including the DPPH, NO and FRAP. The results show that the *C. sorokiniana* extract (IC<sub>50</sub> = 480.30 ± 14.85 µg ml<sup>-1</sup> in DPPH and IC<sub>50</sub> = 562.73 ± 3.52 µg ml<sup>-1</sup> in NO) exhibited better radical scavenging activity than the *S. falcatus* extract (IC<sub>50</sub> = 693.53 ± 30.32 µg ml<sup>-1</sup> in DPPH and IC<sub>50</sub> = 728.67 ± 38.54 µg ml<sup>-1</sup> in NO). A significant difference was observed between the DPPH and NO scavenging activities in both microalgae extracts, except at 125 µg ml<sup>-1</sup> in NO activity. Similarly, the FRAP was significantly higher in the *C. sorokiniana* extract (58.51 ± 2.42 mgTE g<sup>-1</sup>) than the *S. falcatus* extract (32.67 ± 1.75 mgTE g<sup>-1</sup>) (Fig. 3. C). These findings indicated that *C. sorokiniana* has greater anti-oxidant activity than *S. falcatus*, a finding that is attributed to the presence of major constituents. For instance, some studies reported that hexanol and other ma-

ior derivatives found in various extracts, such as pomegranates, mung bean, ripe coffee beans and soybeans, are responsible for anti-oxidant properties (54-56). In the case of saturated fatty acids, extracts of *Annona muricata* L. *Labisia pumila* Benth, sea buckthorn, and *Trifolium* species also exhibited anti-oxidant activity owing to large percentages of *n*-hexadecanoic acid and octadecanoic acid (57-60). In addition, fatty acids such as *n*-hexadecanoic acid and octadecanoic acid were reported to exert effects against oxidative stress (61, 62). Our results were consistent with those of previous findings that demonstrated anti-oxidant activities of hexanol, *n*-hexadecanoic acid and octadecanoic acid.

The inhibitory effects of *C. sorokiniana* and *S. falcatus* extracts on diabetic enzymes compared with acarbose as the positive control are shown in Fig. 4. The *C. sorokiniana* extract inhibited alpha-glucosidase (IC<sub>50</sub> = 491.22 ± 78.41 µg ml<sup>-1</sup>) and alpha-amylase (IC<sub>50</sub> = 2,817.00 ± 143.04 µg ml<sup>-1</sup>) more effectively than the *S. falcatus* extract (IC<sub>50</sub> = 689.71 ± 38.99 µg ml<sup>-1</sup> in alpha-glucosidase and IC<sub>50</sub> = 3,307.6 ± 85.98 µg ml<sup>-1</sup> in alpha-amylase). Nevertheless, the inhibitory effect of acarbose was greater than that of both extracts (Fig. 4. A3 and B3). Both microalgae extracts in different concentrations also significantly impeded the diabetic enzymes compared with the control, except for 1000 µg ml<sup>-1</sup> of *C. sorokiniana* extract on alpha-amylase, which had no effect. According to a previous study, hexanol was reported to have hypoglycemic potential via insulin secretion (63) but no activity against alpha-glucosidase and alpha-amylase. On the other hand, *n*-hexadecanoic acid and oc-

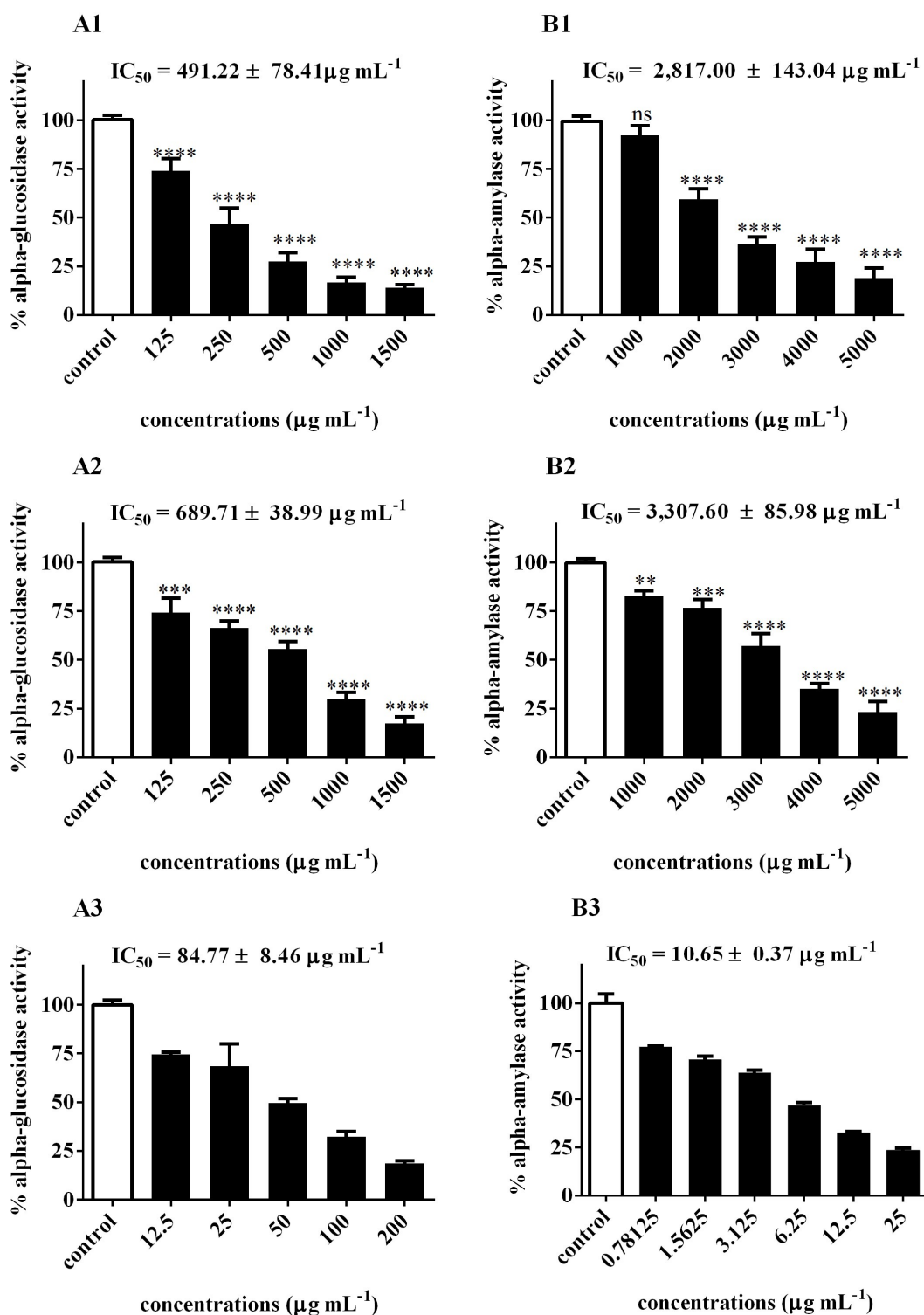


**Fig. 3.** The anti-oxidant activity of *Chlorella sorokiniana* and *Scenedesmus falcatus* extracts; The dose-dependent DPPH scavenging activity of (**A1**) *C. sorokiniana* and (**A2**) *S. falcatus* extracts. The dose-dependent NO scavenging activity of (**B1**) *C. sorokiniana* and (**B2**) *S. falcatus* extracts. (**C**) Ferric reducing antioxidant power (FRAP) activity of *C. sorokiniana* and *S. falcatus* extracts. All results are presented as the mean  $\pm$  standard deviation ( $n = 3$ ).

tadecanoic acid had a potential capacity for glucose reduction (64). Moreover, the *n*-hexadecanoic and octadecanoic fatty acids also demonstrated potent inhibition of alpha-glucosidase and alpha-amylase (65-68). Accordingly, our findings were similar to those of previous reports.

Microalgae extracts are increasingly considered an excellent natural anti-oxidant and anti-diabetic sources concerning the effects of phytochemicals. Previous studies on *C. sorokiniana* extracts reported the presence of anti-oxidant inhibitors, radical scavenging inhibition (69) and the construction of cell-based mod-

els and a *Caenorhabditis elegans* survival assay under oxidative stress (70), reduced ROS release in the mitochondria of a hyperthyroid rat liver (71) and reversible physiological oxidative perturbation (72). Green microalgae, *Chlorella* and *Scenedesmus*, also contain various anti-oxidant enzymes, including ascorbate peroxidase (APX), glutathione reductase (GR), glutathione S transferase (GST), peroxidase (POX) and superoxide dismutase (SOD) (30, 71-72). These anti-oxidant enzymes increase the anti-oxidant activity and reduce oxidative stress in *Chlorella* (26). Until now, no work has reported the anti-oxidant activity of *S. falcatus* extract. More-



**Fig. 4.** The anti-diabetic activity of *Chlorella sorokiniana* and *Scenedesmus falcatum* extracts; Alpha-glucosidase activity of (A1) *C. sorokiniana* and (A2) *S. falcatum* extracts. Alpha-amylase activity of (B1) *C. sorokiniana* and (B2) *S. falcatum* extracts. (A3, B3) Acarbose was used as the standard for the positive control. All results are presented as the mean  $\pm$  standard deviation ( $n = 3$ ).

over, this paper presents novel findings of *C. sorokiniana* and *S. falcatum* on diabetic enzymes, alpha-glucosidase and alpha-amylase.

## Conclusion

This investigation revealed that microalgae possess promising pharmaceutical and nutraceutical properties for a range of applications and are primarily attributable to lipid extracts. According to the results, the extracts of

*C. sorokiniana* and *S. falcatum* contained 34 and 28 different compounds respectively; (*R*)-2-hexanol, n-hexadecanoic acid, and octadecanoic acid had the highest percentages. Significant differences were observed between individual biological activities in the extracts; specifically, *C. sorokiniana* extract showed higher antioxidant and anti-diabetic activities. This study's impact is worth mentioning that *C. sorokiniana* and *S. falcatum* are potentially interesting natural sources in the alternative food health market.

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## Authors contributions

RS cultivated microalgae, investigated experiments, analyzed data and wrote the first draft of the manuscript. SK and RS investigated experiments and analyzed the data. SD provided resources and guidelines for the experiments and reviewed the manuscript. NS provided conceptualizations and resources and reviewed and edited the manuscript. All authors have approved the manuscript and agree to its publication.

## Compliance with ethical standards

**Conflict of interest:** The authors declare no conflict of interest.

**Ethical issues:** None

## References

- Sathasivam R, Ki JS. A review of the biological activities of microalgal carotenoids and their potential use in healthcare and cosmetic industries. *Mar Drugs*. 2018;16:26. <https://doi.org/10.3390/md16010026>
- Wan XZ, Li TT, Zhong RT, Chen HB, Xia X, Gao LY et al. Anti-diabetic activity of PUFAs-rich extracts of *Chlorella pyrenoidosa* and *Spirulina platensis* in rats. *Food Chem Toxicol*. 2019;128:233-39. <https://doi.org/10.1016/j.fct.2019.04.017>
- Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnol Adv*. 2012;30:709-32. <https://doi.org/10.1016/j.biotechadv.2012.01.001>
- Chakrabarti S, Guha S, Majumder K. Food-derived bioactive peptides in human health: a challenges and opportunities. *Nutrients* 2018;10:1738. <https://doi.org/10.3390/nu10111738>
- Jerez-Martel I, García-Poza S, Rodríguez-Martel G, Rico M, Afonso-Olivares C, Gómez-Pinchett JL. Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *J Food Qual*. 2017;1:8. <https://doi.org/10.1155/2017/2924508>
- Xu SY, Huang X, Cheong KL. Recent advances in marine algae polysaccharides: isolation, structure, and activities. *Mar. Drugs*. 2017;15:388. <https://doi.org/10.3390/md15120388>
- Hosseini TA, Shariati M. *Dunaliella* biotechnology: methods and applications. *J Appl Microbiol*. 2009;107:14-35. <https://doi.org/10.1111/j.1365-2672.2009.04153.x>
- Al-Saif SS, Abdel-Raouf N, El-Wazanani HA, Aref IA. Antibacterial substances from marine algae isolated from Jeddah coast of red sea, Saudi Arabia. *Saudi J Biol Sci*. 2014;21:57-64. <https://doi.org/10.1016/j.sjbs.2013.06.001>
- Bhagavathy S, Sumathi P, Bell IJS. Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pac J Trop Biomed*. 2011;1:S1-S7. [https://doi.org/10.1016/s2221-1691\(11\)60111-1](https://doi.org/10.1016/s2221-1691(11)60111-1)
- Choo WT, Teoh ML, Phang SM, Convey P, Yap WH, Goh BH et al. Microalgae as potential anti-inflammatory natural product against human inflammatory skin diseases. *Front Pharmacol*. 2020;11: 1086. <https://doi.org/10.3389/fphar.2020.01086>
- Wang HM, Pan JL, Chen CY, Chiu CC, Yang MH, Chang HW et al. Identification of anti-lung cancer extract from *Chlorella vulgaris* C-C by antioxidant property using supercritical carbon dioxide extraction. *Proc Biochem*. 2010;45:1865-72. <https://doi.org/10.1016/j.procbio.2010.05.023>
- Assunção MFG, Amaral R, Martins BC, Ferreira DJ, Ressurreição S, Santos DS et al. Screening microalgae as potential sources of antioxidants. *J Appl Phycol*. 2017;29:865-77. <https://doi.org/10.1007/s10811-016-0980-7>
- Zhao C, Yang C, Wai STC, Zhang Y, Portillo M, Paoli P et al. Regulation of glucose metabolism by bioactive phytochemicals for the management of type 2 diabetes mellitus. *Crit Rev Food Sci Nutr*. 2018;59:830-47. <https://doi.org/10.1080/10408398.2018.1501658>
- DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32 Suppl. 2009;2:157-63. <https://doi.org/10.2337/dc09-S302>
- Emily J, Leroith D, Karnieli E. Insulin resistance in obesity as the underlying cause for the metabolic syndrome. *Mt Sinai J Med*. 2010;77:511-23. <https://doi.org/10.1002/msj.20212>
- Migdal C, Serres M. Reactive oxygen species and oxidative stress. *Méd sci*. 2011;27:405-12. <https://doi.org/10.1051/medsci/2011274017>
- Suksungworn R, Duangrisai S. Phytochemical contents and antioxidant activity of medicinal plants from the Rubiaceae family in Thailand. *Plant Science Today*. 2021;8:24-31. <https://doi.org/10.14719/pst.2021.8.1.882>
- Suksungworn R, Andrade PB, Oliveira AP, Valentao P, Duangrisai S, Gomes NGM. Inhibition of proinflammatory enzymes and attenuation of il-6 in lps-challenged raw 264.7 macrophages substantiates the ethnomedicinal use of the herbal drug *Homalium bhamaense* Cubitt and W.W.Sm. *IntJ Mol Sci*. 2020;21: 2421. <https://doi.org/10.3390/ijms21072421>
- Salman KA, Ashraf S. Reactive oxygen species: a link between chronic inflammation and cancer. *Asia Pacific J Mol Biol & Biotechnol*. 2015;21:42-49.
- Roy N, Laskar RA, Sk I, Kumari D, Ghosh T, Begum NA. A detailed study on the antioxidant activity of the stem bark of *Dalbergia sissoo* Roxb., an Indian medicinal plant. *Food Chem*. 2011;126:1115-21. <https://doi.org/10.1016/j.foodchem.2010.11.143>
- Dall TM, Yang W, Halder P, Pang B, Massoudi M, Wintfeld N, Semilla AP, Franz J, Hogan PF. The economic burden of elevated blood glucose levels in 2012: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. *Diabetes Care*. 2014;37:3173-79. <https://doi.org/10.2337/dc14-1036>
- Verspohl EJ. Novel pharmacological approaches to the treatment of type 2 diabetes. *Pharmacol Rev*. 2012;64:188-237. <https://doi.org/10.1124/pr.110.003319>
- Hinnen DA. Therapeutic options for the management of postprandial glucose in patients with type 2 diabetes on basal insulin. *J Clin Diabetes*. 2015;33:175-80. <https://doi.org/10.2337/diaclin.33.4.175>
- Ofosu FK, Elahi F, Daliri EB, Chelliah R, Ham HJ, Kim JH et al. Phenolic profile, antioxidant and antidiabetic potential exerted by millet grain varieties. *Antioxidants*. 2020;9:254. <https://doi.org/10.3390/antiox9030254>
- Nair SS, Kavrekar V, Mishra A. In-vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extractso. *European J Experimen Bio*. 2013;3:128-32.



26. Hamed I. The evolution and versatility of microalgal biotechnology: A Review. *Compr Rev Food Sci Food Saf.* 2016;15:1104-23. <https://doi.org/10.1111/1541-4337.12227>
27. Hartweg J, Perera R, Montori V, Dinneen S, Neil HA, Farmer JA. Omega-3 polyunsaturated fatty acids (PUFA) for type 2 diabetes mellitus. *Cochrane Database Syst Rev.* 2008;23:CD003205. <https://doi.org/10.1002/14651858.CD003205.pub2>
28. Levasseur W, Perre P, Pozzobon V. A review of high value-added molecules production by microalgae in light of the classification. *Biotechnol Adv.* 2020;41:1-21. <https://doi.org/10.1016/j.biotechadv.2020.107545>
29. Lauritano C, Andersen JH, Hansen E, Albrigtsen M, Escalera L, Esposito F et al. Bioactivity Screening of Microalgae for Antioxidant, Anti-Inflammatory, Anticancer, Anti-Diabetes and Antibacterial Activities. *Frontiers in Marine Science.* 2016;3. <https://doi.org/10.3389/fmars.2016.00068>
30. Patil L, Kaliwal BB. Microalga *Scenedesmus bajacalifornicus* BBKLP-07, a new source of bioactive compounds with in vitro pharmacological applications. *Bioprocess Biosyst Eng.* 2019;42:979-94. <https://doi.org/10.1007/s00449-019-02099-5>
31. Pantami HA, Ahamad Bustamam MS, Lee SY, Ismail IS, Mohd Faudzi SM, Nakakuni M, Shaari K. Comprehensive GCMS and LC-MS/MS Metabolite Profiling of *Chlorella vulgaris*. *Mar. Drugs.* 2020;18: 367. <https://doi.org/10.3390/md18070367>
32. Dolganyuk V, Belova D, Babich O, Prosekov A, Ivanova S, Katserov D et al. Microalgae: A promising source of valuable bioproducts. *Biomolecules.* 2020; 10:1153. <https://doi.org/10.3390/biom10081153>
33. Napolitano G, Fasciolo G, Salbitani G, Venditti P. *Chlorella sorokiniana* Dietary supplementation increases antioxidant capacities and reduces ROS release in mitochondria of hyperthyroid rat liver. *Antioxidants.* 2020;9(9):883. <https://doi.org/10.3390/antiox9090883>
34. El-Fayoumy EA, Shanab SMM, Gaballa HS, Tantawy MA, Shalaby EA. Evaluation of antioxidant and anticancer activity of crude extract and different fractions of *Chlorella vulgaris* axenic culture grown under various concentrations of copper ions. *BMC Complement Med Ther.* 2021;21:51. <https://doi.org/10.1186/s12906-020-03194-x>
35. Bito T, Okumura E, Fujishima M, Watanabe F. Potential of *Chlorella* as a dietary supplement to promote human health. *nutrients.* 2020;12:2524. <https://doi.org/10.3390/nu12092524>
36. Miranda MS, Sato S, Mancini-Filho J. Antioxidant activity of the microalga *Chlorella vulgaris* cultured on special conditions. *Boll Chim Farm.* 2001;140:165-68.
37. Afify AEMR, El Baroty GS, El Baz FK, Abd El Baky HH, Murad SA. *Scenedesmus obliquus*: Antioxidant and antiviral activity of proteins hydrolyzed by three enzymes. *J Genet Eng Biotechnol.* 2018;16:399-408. <https://doi.org/10.1016/j.jgeb.2018.01.002>
38. Zaharieva MM, Zheleva-Dimitrova D, Rusinova-Videva S, Ilieva Y, Brachkova A, Balabanova V et al. Antimicrobial and antioxidant potential of *Scenedesmus obliquus* microalgae in the context of integral biorefinery concept. *Molecules.* 2022;27(2):519. <https://doi.org/10.3390/molecules27020519>
39. Qi, J., Kim, S.M.  $\alpha$ -Glucosidase inhibitory activities of lutein and zeaxanthin purified from green alga *Chlorella ellipsoidea*. *J Ocean Univ. China.* 2018;17:983-89. <https://doi.org/10.1007/s11802-018-3465-2>
40. Kaeoboon, S, Suksungworn, R, Sanevas N. Toxicity response of *Chlorella* microalgae to glyphosate herbicide exposure based on biomass, pigment contents and photosynthetic efficiency. *Plant Science Today.* 2021;8:293-300. <https://doi.org/10.14719/pst.2021.8.2.1068>
41. Benning C, Somerville RC. Isolation and genetic complementation of a sulfolipid-deficient mutant of *Rhodobacter sphaeroides*. *J Bacteriol.* 1992;174:2352-60. <https://doi.org/10.1128/jb.174.7.2352-2360.1992>
42. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958;181:1199-1200. <https://doi.org/10.1038/1811199a0>
43. Ferreres F, Andrade C, Gomes NGM, Andrade PB, Gil-Izquierdo A, Pereira DM et al. Valorisation of kitul, an overlooked food plant: Phenolic profiling of fruits and inflorescences and assessment of their effects on diabetes-related targets. *Food Chem.* 2021;342: 128323. <https://doi.org/10.1016/j.foodchem.2020.128323>
44. Santos AB, Fernandes AS, Wagner R, Jacob-Lopes E, Zepka LQ. Biogenesis of volatile organic compounds produced by *Phormidium autumnale* in heterotrophic bioreactor. *J Appl Phycol.* 2016;28:1561-70. <https://doi.org/10.1007/s10811-015-0740-0>
45. Jacob-Lopes E, Franco TT. From oil refinery to microalgal biorefinery. *J CO2 Utilization.* 2013;2:1-7. <https://doi.org/10.1016/j.jcou.2013.06.001>
46. Wathnani AH, Ara I, Tahmaz RR, Al-Dayel HT, Bakir AM. Bioactivity of natural compounds isolated from cyanobacteria and green algae against human pathogenic bacteria and yeast. *J Medicinal Plants Res.* 2012;6:3425-33. <https://doi.org/10.5897/jmpr11.1746>
47. Nascimento TC, Nass PP, Fernandes AS, Vieira KR, Wagner R, Jacob-Lopes E et al. Exploratory data of the microalgae compounds for food purposes. *Data in Brief.* 2020;29: 105182. <https://doi.org/10.1016/j.dib.2020.105182>
48. Hong JW, Jo SW, Kim OH, Jeong MR, Kim H, Park KM et al. Characterization of a korean domestic cyanobacterium *Limnothrix* sp. KNUA012 for biofuel feedstock. *J Life Sci.* 2016;26:460-67. <https://doi.org/10.5352/jls.2016.26.4.460>
49. Yun HS, Kim YS, Yoon HS. Characterization of *Chlorella sorokiniana* and *Chlorella vulgaris* fatty acid components under a wide range of light intensity and growth temperature for their use as biological resources. *Heliyon.* 2020;6: e04447. <https://doi.org/10.1016/j.heliyon.2020.e04447>
50. Patterson WG. The effect of culture conditions on the hydrocarbon content of *Chlorella vulgaris*. *J Phycol.* 1967;3:22-23. <https://doi.org/10.1111/j.1529-8817.1967.tb04623.x>
51. Kalhor XA, Movafeghi A, Mohammadi-Nassab AD, Abedi E, Bahrami A. Potential of the green alga *Chlorella vulgaris* for biodegradation of crude oil hydrocarbons. *Mar Pollut Bull.* 2017;123:286-90. <https://doi.org/10.1016/j.marpolbul.2017.08.045>
52. Jamil TS, Abdel Aty AM, Ghafar Hany HA, Abdo SM. Separation and identification of hydrocarbons and other organic compounds from *Scenedesmus obliquus* and three cyanobacterial species. *Desalination Water Treat.* 2014;57: 1-8. <https://doi.org/10.1080/19443994.2014.972987>
53. Habibi Z, Imanpour NJ, Ramezanzpour Z. Evaluation of antimicrobial activities of microalgae *Scenedesmus dimorphus* extracts against bacterial strains. *Caspian J Environ Sci.* 2018;16:25-36. <https://doi.org/10.22124/CJES.2018.2779>
54. Lee GK, Shibamoto T. Antioxidant properties of aroma compounds isolated from soybeans and mung beans. *J Agr Food Chem.* 2000;48:42090-44293. <https://doi.org/10.1021/jf000442u>
55. Escarcega HG, Sánchez-Chávez E, Alvarez PS, Caballero SM, Parra SJM, Flores-Córdova MA et al. Determination of antioxidant phenolic, nutritional quality and volatiles in pomegranates (*Punica granatum* L.) cultivated in Mexico. *Int J Food Prop.* 2020;23:979-91. <https://doi.org/10.1080/10942912.2020.1760879>
56. Kulapichitr F, Borompichaichartkul C, Pratontep S, Lopetcharat K, Boonbumrung S, Suppavorasatit I. Differences in volatile compounds and antioxidant activity of ripe and unripe green

- coffee beans (*Coffea arabica* L. 'Catimor'). *Acta Horticulturae*. 2017;261-68. <https://doi.org/10.17660/ActaHortic.2017.1179.41>
57. Elagbar ZA, Naik RR, Shakya AK, Bardaweel SK. Fatty acids analysis, antioxidant and biological activity of fixed oil of *Annona muricata* L. seeds. *J Chem*. 2016;1-6. <https://doi.org/10.1155/2016/6948098>
  58. Karimi E, Jaafar ZH, Ghasemzadeh A, Ebrahimi M. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of *Labisia pumila* Benth. *Biol Res*. 2015;48: 9 <https://doi.org/10.1186/0717-6287-48-9>
  59. Saeidi K, Alirezalu A, Akbari Z. Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (*Hippophae rhamnoides* L.) grown wild in Iran. *Natural Prod Res*. 2016;30. <https://doi.org/10.1080/14786419.2015.1057728>
  60. Sabudak T, Ozturk M, Goren AC, Kolak U, Topcu G. Fatty acids and other lipid composition of five *Trifolium* species with antioxidant activity. *Pharm Biol*. 2009;47: 137-41. <https://doi.org/10.1080/13880200802439343>
  61. Zadeh HE, Khodadadi M, Asadi F, Koohi MK, Eslami M, Hasani-Dizaj S et al. The antioxidant activity of palmitoleic acid on the oxidative stress parameters of palmitic acid in adult rat cardiomyocytes. *Annals Military Health Sci Res*. 2016;14: e11467. <https://doi.org/10.5812/amh.11467>
  62. Wang ZJ, Liang CL, Li GM, Yu CY, Yin M. Stearic acid protects primary cultured cortical neurons against oxidative stress. *Acta Pharmacol Sinica*. 2007;28:315-26. <https://doi.org/10.1111/j.1745-7254.2007.00512.x>
  63. Bharti SK, Krishnan S, Kumar A, Kumar A. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. *Ther Adv Endocrinol Metab*. 2018;9: 81-100. <https://doi.org/10.1177/2042018818755019>
  64. Chelladurai, MRG, Chinnachamy C. Alpha amylase and alpha glucosidase inhibitory effects of aqueous stem extract of *Salacia oblonga* and its GC-MS analysis. *Braz J Pharm Sci*. 2018;54: e17151. <https://doi.org/10.1590/s2175-97902018000117151>
  65. Wang Y, Liu F, Liang Z. Nutritional composition,  $\alpha$ -Glucosidase Inhibitory and antioxidant activities of *Ophiopogon japonicus* tubers. *J Chem*. 2015;1-7. <https://doi.org/10.1155/2015/893074>
  66. Balongun O, Oladosu I, Akinnusi A, Zhiqiang L. Fatty acids composition,  $\alpha$ -glucosidase inhibitory potential and cytotoxicity activity of *Oncoba spinosa* Forssk. *Appl Chem*. 2013;59:15630-641.
  67. Ahmad Z, Zamhuri KF, Yaacob A, Siong CH, Selvarajah M, Ismail A, Nazrul HM. In vitro anti-diabetic activities and chemical analysis of polypeptide-k and oil isolated from seeds of *Momordica charantia* (bitter gourd). *Molecules*. 2012;17:9631-40. <https://doi.org/10.3390/molecules17089631>
  68. George OL, Radha RH, Somasekriah VB. *In vitro* anti-diabetic activity and GC-MS analysis of bioactive compounds present the methanol extract of *Kalanchoe pinnata* Indian *J Chem*. 2018;57: 1213-21.
  69. Olasehinde TA, Odjajare EC, Mabinya LV, Olaniran AO, Okoh AI. *Chlorella sorokiniana* and *Chlorella minutissima* exhibit antioxidant potentials, inhibit cholinesterases and modulate disaggregation of  $\beta$ -amyloid fibrils. *Electronic J Biotechnol*. 2019;40:460-69. <https://doi.org/10.1016/j.ejbt.2019.03.008>
  70. Petruk G, Gifuni I, Illiano A, Roxo M, Pinto G, Amoresano A et al. Simultaneous production of antioxidants and starch from the microalga *Chlorella sorokiniana*. *Algal Res*. 2018;34:164-74. <https://doi.org/10.1016/j.algal.2018.07.012>
  71. Napolitano G, Fasciolo G, Salbitani G, Venditti P. *Chlorella sorokiniana* dietary supplementation increases antioxidant capacities and reduces ROS release in mitochondria of hyperthyroid rat liver. *Antioxidants*. 2020;9:883. <https://doi.org/10.3390/antiox9090883>
  72. Salbitani G, Vona V, Bottone C, Petriccione M, Carfagna S. Sulfur deprivation results in oxidative perturbation in *Chlorella sorokiniana* (211/8k). *Plant and Cell Physiol*. 2015;56: 897-905. <https://doi.org/10.1093/pcp/pcv015>

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