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 (IC50 = 480.30 ± 14.85 µg ml⁻¹), nitric oxide (NO) radical scavenging (562.73 ± 3.52 µM⁻¹) and ferric reducing anti-oxidant power (FRAP) of 58.51 ± 2.42 mgTE g⁻¹. Comparatively, the C. sorokiniana extract had higher contents of alpha-glucosidase and alpha-amylase (IC50 = 491.22 ± 78.41 and 2,817.00 ± 143.04 µg 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) (RCI 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-dinitosalicylic 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 μmol photons m⁻² s⁻¹) at a temperature of 30 ± 1°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°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 N₂ stream for 1 min. The mixture was incubated at 80°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).

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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 µl 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⁻¹, 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⁻¹ 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. falcatus 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⁻¹ 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% H₃PO₄ 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 FeCl₃·6H₂O (10 ml). The extracts of C. sorokiniana and S. falcatus (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⁻¹, using the Trolox line equation (concentration at 0 to 250 mg l⁻¹): y = 0.01x + 0.2046, R² = 0.9917.

### Anti-diabetic activity

Alpha-glucosidase activity

The inhibition of alpha-glucosidase 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⁻¹) 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 incubated for 10 min, at 25°C, then 200 µl of alpha-amylase (15 U ml⁻¹) was added and incubated at 25°C for 10 min. Subsequently, 400 µl of DNS solution, containing 1 g dinitrosalicylic 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. falcatus were evaluated and shown in Fig. 1. Fifty-one compounds were identified, while 34 compounds from C. sorokiniana, 28 from S. falcatus and 11 were found in both species (Table 1). The following major components (>3%) were found in both extracts: (R)-2-hexanola (4; 38.67% in C. sorokiniana and 23.53% in S. falcatus), n-hexadecanoic acid (33; 13.58% in C. sorokiniana and 18.94% in S. falcatus), and octadecanoic acid (43; 22.30% in C. sorokiniana and 32.67% in S. falcatus). 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-heptan-one, 6-methyl (12; 3.55%), and epiceredol (20; 10.44%) in S. falcatus. All major chemical compositions are shown in Fig. 2. The chemical compounds identified by GC-MS were based on the reten-
tion time (Rt) and molecular weight, while the fragmenta-
tion patterns and data comparisons with the NIST14 li-
brary provide further structural identification. The C. soroki-
niana and S. falcatus extracts also contained some alco-
hols, fatty acids and straight-chain hydrocarbon com-
ounds associated with various biological activities.

Microalgae can produce numerous volatile compo-
unds, and they can be used as an alternative source for

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Rt</th>
<th>Compounds</th>
<th>Molecular weight</th>
<th>Ratio C. sorokiniana</th>
<th>S. falcatus</th>
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<td>1</td>
<td>3.236</td>
<td>3-hydroxy-3-methyl-2-butane</td>
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<td>0.50</td>
<td>0.37</td>
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<td>2</td>
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<td>4</td>
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<td>(R)-2-hexanol</td>
<td>102</td>
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<td>23.53</td>
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<td>5</td>
<td>5.455</td>
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<td>-</td>
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<td>11</td>
<td>15.530</td>
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<td>-</td>
<td>3.95</td>
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<td>13</td>
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<tr>
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<td>Benzene, 1,3-bis(1,1-dimethyl)ethyl-</td>
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<td>-</td>
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<td>128</td>
<td>0.77</td>
<td>-</td>
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<tr>
<td>20</td>
<td>17.530</td>
<td>Epicedrol</td>
<td>222</td>
<td>-</td>
<td>10.44</td>
</tr>
</tbody>
</table>
| 21     | 17.980 | Acetic acid, 4-(7-methyl) 
7-nenebicyclo[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-t-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 | n-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                 | -           |
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 C16-C18 (>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 an 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 (IC50 = 480.30 ± 14.85 µg ml⁻¹ in DPPH and IC50 = 562.73 ± 3.52 µg ml⁻¹ in NO) exhibited better radical scavenging activity than the *S. falcatus* extract (IC50 = 693.53 ± 30.32 µg ml⁻¹ in DPPH and IC50 = 728.67 ± 38.54 µg ml⁻¹ in NO). A significant difference was observed between the DPPH and NO scavenging activities in both microalgae extracts, except at 125 µg ml⁻¹ in NO activity. Similarly, the FRAP was significantly higher in the *C. sorokiniana* extract (58.51 ± 2.42 mgTE g⁻¹) than the *S. falcatus* extract (32.67 ± 1.75 mgTE g⁻¹) (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 major 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 (IC50 = 491.22 ± 78.41 µg ml⁻¹) and alpha-amylose (IC50 = 2,817.00 ±143.04 µg ml⁻¹) more effectively than the *S. falcatus* extract (IC50 = 689.71 ± 38.99 µg ml⁻¹ in alpha-glucosidase and IC50 = 3,370.6 ± 85.98 µg ml⁻¹ in alpha-amylose). 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⁻¹ of *C. sorokiniana* extract on alpha-amylose, 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-amylose. On the other hand, *n*-hexadecanoic acid and oc-
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 models 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-
over, this paper presents novel findings of *C. sorokiniana* and *S. falcatus* 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. falcatus* 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. falcatus* 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

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