Screening of four green microalgae potentially used as feedstock for biodiesel and nutraceuticals

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Abstract

Detection of oleaginous microalgae with high lipid productivity is a crucial feature for biofuel and nutraceutical production. Therefore, screening of microalgae from different habitats has become highly significant to face the request on these bioresources. Accordingly, the present study aimed to screen the efficiency of biodiesel feedstocks and the ability to accumulate essential fatty acids of four green microalgae (*Chlorolobion braunii, Tetradesmus obliquus, Monoraphidium miutum*, and *Asterarcys quadricellulare*), isolated from soil samples of Benha Egypt. Although *T.obliquus* was the highest biomass productive species (689 $mgL^{-1}d^{-1}$), *M. minutum* recorded the top of lipid productivity (29 $mg\ L^{-1}d^{-1}$). Fatty acid profiles of the four microalgae confirmed their suitability for biodiesel production where it recorded biodiesel properties within the limits of international standards. Also, species selection through PROMETHEE and GAIA analysis revealed that *C. braunii* is the best promising as a potential source for biodiesel production because it has a high saturated fatty acids proportion (39.75%) and shows cetane number (54.99) and low iodine value (100.57) and many other biodiesel properties within the standards limit. In addition, the screened microalgae showed a high percentage of omega fractions in their total fatty acids. *Asterarcys quadricellulare* and *M. minutum* recorded high omega-3 fatty acids content of 34.4% and 28.9%, respectively.

Introduction

Microalgae include a varied group of photoautotrophic microorganisms that inhabit aquatic habitats and terrestrial ecosystems. A long time ago, the screening of microalgae attracted attention in respect of their high potency for biotechnological applications including food supplements to prevent many diseases, and biodiesel production [1, 2]. Microalgae show great potential as feedstocks in replacing fossil fuels to ensure energy security, and to avoid environmental pollution globally [3]. Biodiesel raw materials obtained from microalgae appeared to be one of the foremost promising alternative sources for biodiesel generation due to their rapid growth rates, high biomass productivity and high lipid contents over the terrestrial crop plants. Microalgae produce a significantly higher yield of lipids, about 150 000 L of oil per hectare, which is three times higher than other oil crops [4]. In addition, microalgae can grow on wastewaters and adapt themselves to adverse environmental conditions [5]. Also, with their photosynthetic effectiveness and CO_2 sequestration, they can be grown successfully with high lipid content appropriate as biodiesel feedstock and do not compete with human nutrition [6]. A large number of oleaginous microalgae species have already been substituted for a small part of petroleum-based fuels in some countries [7]. Typical microalgae for biodiesel generation require not only high lipid content but also reasonable fatty acids composition. The generation of saturated and monounsaturated fatty acids by microalgae is ideal for biodiesel production, where it achieves the equilibrium between cetane number and cold-flow properties [8, 9].

As a result of algal growth in diverse and extreme habitats, they produce a group of unique bioactive compounds. PUFAs are highly beneficial bioactive products for human health. Algae have a useful effect on human nutrition and health, because of their high polyunsaturated fatty acids [10]. Algal lipids are of

an extraordinary commercial value as sources of nutritiously important polyunsaturated fatty acids (PUFAs) and are therefore broadly utilized as ingredients in the nutraceutical industry [11]. Replacing saturated lipids with unsaturated is effective in preventing cardiovascular and cancer diseases [12]. The human body cannot generate essential fatty acids, and hence, these must be consumed in the diet. They are satisfactory for the convenient growth. Omega-3 fatty acids alpha-linolenic acid, docosahexaenoic acid and eicosapentaenoic acid provide substantial protection against chronic diseases. Therefore, adjustable intake is advisable [13]. Dietary omega-3 PUFAs enhance the body's functions to fight against inflammatory conditions that lead to initiating diseases like cancer, kidney malfunction, diabetes, and cardiovascular disorders [14]. DHA is a vital bioactive lipid that is suggested for keeping up physiological health. DHA contributes to the upregulation of intracellular antioxidants and drives oxidative stress reduction. Other than this, DHA is additionally reported to display an anti-inflammatory effect [15]. In addition, valuable omega-6 fatty acids are linoleic acid (LA), gamma-linolenic acid (GLA), arachidonic acid (AA) and docosapentaenoic acid, (DPA) are the major fatty acids of this group. They play a significant part in numerous physiological functions and molecular pathways [16]. Linoleic acid reduces the risk of blood pressure and activates prostaglandins synthesis. It leads to the composition of arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid that play principal roles in regulating body homeostasis [17, 15].

Recently, microalgae biorefinery models have been applied to reduce biodiesel production costs through biomass valorization. For example, lipid-free biomass residue after lipid extraction can be utilized as a nourishing supplement [18]. This study intended to isolate some local soil microalgae and evaluate their potential ability for utilizing them in biodiesel production and food supplements. Also, determination of the most suitable species as biodiesel feedstock by PROMETHEE and GAIA software.

Materials And Methods

Microalgae isolation and growth conditions

The soil samples have been collected from a local agricultural land in Benha city, Egypt. Ten grams of soil were mixed with 50 mL of sterilized distilled water. The soil suspension was agitated at 300 rpm for 30 min, then 10-fold serial dilutions were prepared using Bold’s Basal medium (BBM). Using the spread agar method; Petri-dishes containing solid BBM were inoculated with different soil dilutions and incubated at 26 ± 2.0 ºC. Cultures were illuminated by tubular fluorescent lamps at 50 µmol m^{-2} s^{-1} light intensity. After microalgal growth appeared, the individual colonies were picked up and sub-cultured several times on agar plates until the complete purification of the isolates. Isolated microalgae were examined using a light microscope and subcultured in 50 mL BBM, and incubated under the aforementioned growth conditions. Microalgae were cultivated in batch culture containing 700 mL BBM medium. All flasks were autoclaved, cooled down, and inoculated with exponentially grown culture to get an initial optical density (OD_{680}) of 0.05 ± 0.003. All cultures were aerated using an atmospheric air

pump. Microalgae identification based on the morphological features of these isolates was performed using microscopic examination [19–21].

Growth measurements

Batch cultures were incubated for up to 14 days, and the microalgae growth was monitored by measuring the optical density (OD_{680}) every two days interval. Dry weight was determined at the early exponential and late exponential phases of each microalgal species for biomass productivity determination. To measure the dry weight, algal cells in 10 mL culture were collected by filtration through 0.45 µm pre-weighted filter paper, then oven-dried at 105 ºC until constant weight. Biomass productivity determination using Eq. (1);

$$Biomass\ productivity\ (\text{mg}\ L^{-1}d^{-1}) = (dwt - dwi)/\Delta t \quad (1)$$

Where dwt and dwi represent the cellular dry weight at the measured time (t) and the initial dry weight, repressively, while Δt represents the time interval in days [5].

Lipid content

Microalgae lipid extraction was carried out according to Folch et al modified method [22]. Briefly, 20 mL of microalgal culture were centrifuged at 3000× g for 10 minutes. The supernatant was disposed of, at that point, 10 mL of chloroform/methanol (2/1, v/v) were added to the cell pellet and let at room temperature for two hours on a shaker at 200 rpm. The homogenate was centrifuged at 3000× g for ten minutes to recover the liquid phase, which was transferred to a clean tube. To facilitate the separation of water-soluble components, 2 mL of 0.9% NaCl were added and vortexed vigorously for 30 sec., then the blend was centrifuged at 200× g for 2 min to partition into two phases. The lower phase was pulled to a pre-weighed vial, the solvent was released, dried at 80°C for 30 min, and weighed to determine the lipid content. Microalgae lipid productivity was calculated in the exponential phase of growth according to Eq. (2);

$$Lipid\ productivity\ (mg\ L^{-1}d^{-1}) = (LCt - LCi)/\Delta t \quad (2)$$

Where LCt and LCi represent lipid production (mg L^{-1}) after the time (t) and the initial lipid content, respectively, while Δt represents the time interval in days.

Fatty acids analysis

Biodiesel was prepared from the lipid extract according to Christie's modified method [23]. Lipid extracts were dried under the current of argon gas, then 333 µL of methanol: toluene (1:1, v/v) and 167 µL of 0.5 M sodium methoxide were added and left for 20 min at room temperature. After incubation, 500 µL of 1 M NaCl and 50 µL of 37% HCl were included in the mixture and vortexed vigorously for 30 sec. To extract FAMEs, 1.5 mL of hexane were added and vortexed vigorously. The mixture was centrifuged at 200× g for 2 min to isolate the two phases. The upper hexane phase was pulled to a 2 mL Eppendorf tube, at that point the solvent was released beneath a stream of argon. FAMEs were dissolved in 40 µL of acetonitrile

and analyzed by GC-FID equipped with 30 m column (J&W HP-5, 0.32 mm diameter, and 0.25 µm inner film) using helium as a carrier gas. Injector and detector temperatures were kept at 280°C and 300°C, respectively. The oven temperature program was started at 140°C for 3 min, increased at 5°C min^{-1} until 240°C, then increased at 4°C min^{-1} until 260°C where it was fixed at this temperature for 5 min.

Biodiesel properties based on FAME profiles

Because of the need for large amounts of biodiesel and specialized instrumentation, which are not available. So, Predictive models based on FA composition were used for the calculation of the screened microalgae biodiesel properties. The main biodiesel quality parameters include an average degree of unsaturation (ADU), kinematic viscosity (KV), specific gravity (SG), cloud point (CP), cetane number (CN), iodine value (IV), and higher heating value (HHV) were determined by mathematical models per equations (3–9) [24, 25]. However, cold filter plugging point (CFPP) and long-chain saturation factor (LCSF) were calculated using equations (10–11) [26]. Predictive oxidative stability (OS); was calculated using Eq. (12) [27].

$$ADU = \Sigma M \times Yi \quad (3)$$

Where M is the number of carbon-carbon double bonds in FA constituent and Yi is the mass fraction of each FA constituent, respectively.

$$\text{Kinematic viscosity} = -0.6313X + 5.2065 \quad (4)$$

$$\text{Specific gravity} = 0.0055X + 0.8726 \quad (5)$$

$$\text{Cloud point} = -13.356X + 19.994 \quad (6)$$

$$\text{Iodine value} = 74.373X + 12.71 \quad (7)$$

$$\text{Cetane number} = -6.6684X + 62.876 \quad (8)$$

$$HHV = 1.7601X + 38.534 \quad (9)$$

Where X is the ADU.

$$LCSF = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (1.5 \times C22) + (2 \times C24) \quad (10)$$
$$CFPP = (3.1417 \times LCSF) - 16.477 \quad (11)$$

$$OS\ (h) = (117.9295/C18p) + 2.5905) \quad (12)$$

C18p represents the content (weight %) of linoleic (C18:2) and linolenic acids (C18:3).

Microalgae selection based on biodiesel properties

The screened microalgae were ranked based on biodiesel properties to select the most suitable microalgae species for biodiesel production using PROMETHEE and GAIA software v1.4.0.0 [28, 29].

Statistical analysis

The results were calculated as the mean ± standard deviation (SD), where each experiment was performed in three replicates. In addition, SPSS software (IBM, v20) was used to perform a one-way (ANOVA) at a probability level ($P) \leq 0.05$.

Results And Discussion

Microalgal identification

The screened microalgae were isolated using the agar plate's technique and sub-culturing in a liquid media. Using microscopic characterization, four green microalgae species were morphologically identified as *Chlorolobion braunii, Tetradesmus obliquus, Monoraphidium miutum*, and *Asterarcys quadricellulare* (Fig. 1).

Microalgae were discussed extensively as a valuable source for third-generation biofuels, especially as a sustainable and environmentally-friendly feedstock for biodiesel production. For this, the study screened four species of soil green microalgae based on their lipid productivity. The screened species showed a lag phase of two days before they began their exponential growth. The growth curves of the four screened microalgae revealed their exponential growth phase in between the fourth and tenth days of incubation time (Fig. 2). *Tetradesmus* significantly recorded the highest biomass productivity 689.2 mg L^{-1} d^{-1}, while *Chlorolobion* was the lowest biomass productive (210.8 mg L^{-1} d^{-1}) compared to the other species (Fig. 3). At the exponential phase of microalgae, lipid content and lipid productivity revealed that *Monoraphidium* was significantly the highest lipid productive species with up to 29.1 mg L^{-1} day^{-1}. Also, significant low lipid content was determined for *Asterarcys* microalga compared to other screened microalgae (Fig. 4).

Microalgae fatty acids profile

Fatty acid profile of the screened green microalgae characterized by GC and their relative percentages are recorded in Table 1. Generally, the detected 26 fatty acids with carbon chains ranging from (C11-C24) and different degrees of unsaturation. The most prevailing fatty acids were palmitic acid (17–26%), palmitolic acid (7–12%), oleic acid (7–16%), linoleic acid (4–17%) and alpha-linolenic acid (7–24%), which set within C16 and C18 fatty acids. Palmitic acid (C16:0) and stearic acid (C18:0) are two of the most commonly observed fatty acids synthesized by microalgae and are suitable for biodiesel production [30]. The degree of unsaturation plays a critical role in biodiesel properties as the higher the degree of unsaturation of the FAMEs, the higher the oxidation stability of the biodiesel [28]. The present results revealed that *A. quadricellulare* recorded the highest percentage of unsaturation fatty acids (75.57%), while *C. braunii* recorded the lowest value (60.27%). In addition, all of our isolates were characterized by a high percentage of PUFAs more than MUFAs (Table 1).

Table 1
Fatty acids composition of the four screened green microalgae species (% of total fatty acids)

Fatty acid		*Chlorolobion braunii*	*Tetradesmus dimorphus*	*Monoraphidium minutum*	*Asterarcys quadricellulare*
C11:0	Undecanoic acid	0.69	0.21	0.3	0.34
C13:0	Tridecanoic acid	1.42	1.37	0.9	1.17
C14:0	Myristic acid	1.21	1.23	1.19	0.87
C15:0	Pentadecanoic acid	2.67	3.88	2.4	2.94
C16:0	Palmitic acid	25.98	23.71	16.75	17.17
C17:0	Heptadecanoic acid	5.26	2.39	4.73	0.95
C18:0	Stearic acid	1.31	1.37	1.05	1.0
C24:0	Lignoceric acid	1.21	0.48	0.7	ND
Total SAF		**39.75**	**34.64**	**28.02**	**24.44**
C16:1 ω9	Palmitolic acid	10.28	11.45	7.04	8.97
C16:1 ω7	Palmitoleic acid	1.99	2.12	1.72	3.55
C18:1 ω9	Oleic acid	12.31	7.21	13.52	16.43
C18:1 ω7	Vaccenic acid	1.29	1.39	1.92	2.43
C20:1 ω9	Gondoic acid	0.34	ND	ND	0.23
C24:1 ω9	Nervonic acid	2.15	1.59	0.7	0.89
Total MUFA		**28.36**	**23.76**	**24.9**	**32.5**
C16:2 ω4	Hexadecadienoic acid	ND	ND	0.29	ND
C16:3 ω4	Hexagonic acid	1.33	1.33	0.96	1.23
C16:4 ω3	Hexadecatetraenoic acid	2.59	6.01	10.4	10.06
C18:2 ω6	Linoleic acid (LA)	16.7	11.67	11.68	3.68

ND = not detected

Fatty acid		*Chlorolobion braunii*	*Tetradesmus dimorphus*	*Monoraphidium minutum*	*Asterarcys quadricellulare*
C18:3 ω6	γ-linolenic acid	0.43	0.9	0.45	0.18
C18:3 ω4	Octadecadienoic acid	ND	0.86	ND	0.32
C18:3 ω3	α-Linolenic acid (ALA)	6.5	14.79	18.34	24.32
C18:4 ω1	Octadecatetraenoic acid	1.11	2.45	3.78	2.87
C20:4 ω6	Arachidonic acid (AA)	ND	1.38	ND	0.41
C20:5 ω3	Eicosapentaenoic acid (EPA)	0.19	0.22	ND	ND
C22:5 ω6	Docosapentaenoic acid	2.33	1.46	0.3	ND
C22:6 ω3	Docosahexaenoic acid (DHA)	0.73	0.51	0.19	ND
Total PUFA		**31.91**	**41.58**	**46.39**	**43.07**
ND = not detected					

Microalgal biodiesel properties

Biodiesel properties analysis of a biodiesel sample requires a long time and a lot of money. In some cases, it may be impossible to obtain a sufficiently large sample of biodiesel from an arising feedstock oil for detailed analyses, such as algal biodiesel. Mathematical models were applied to predict the properties of biodiesel depending on the fatty acid profile. Subsequently, it may be utilized as a bioprospecting tool for rapidly estimating the potential utility of a new feedstock [31]. The most important properties of biodiesel as a substitute for diesel fuel are the cetane number, viscosity, and density, cold filter plugging point, oxidative stability, ignition quality, combustion heat and cold flow [32]. Calculated biodiesel characteristics of the screened microalgae were compared to the international standards, where it showed ideal cetane number, viscosity, specific gravity, and oxidation stability (Table 2). Results of the present study also revealed that biodiesel characteristics are in agreement with some other related studies [28, 5, 33].

The present study recorded that the content of saturated fatty acids ranged from 24–40% (Table 1). It was reported that the more saturated fatty acids supply biodiesel with a higher cetane number, high oxidative stability, decreased emission of nitrous oxide, and improved ignition [34]. High cetane number led to better combustion, low emission of nitrous oxide and easier engine start-up [35]. Cetane numbers

for screened microalgae ranged from 51.45–54.99, where it falls within the limits of the international standards.

High iodine value is always accomplished with a high unsaturation degree and high susceptibility to oxidation and it can be improved by antioxidants addition. While low iodine value offers appropriate oxidation stability [28]. The present results recorded that *Chlorolobion* species has the lowest iodine value and high oxidative stability in comparison with the other screened microalgae. Specific gravity and viscosity are important parameters because they affect the efficiency of fuel atomization. Specific gravity results recorded 0.88 while kinematic viscosity values (4.12–4.46) comply with the international standards. One of the main troubles associated with the use of biodiesel is the bad flow properties in cold weather. The present study recorded a relatively high PUFAs content, especially linoleic acid (3.7–16.7%), and lower CFPP (-16.4), which agree with standards limits and are in favor of colder environments. Within the present research, the levels of Stearic acid C 18:0 (Table 1) were generally very low (below 1.37%). The low content of stearic acid contributes to lowering the temperatures of CFPP [36]. The long-chain PUFAs, especially linoleic acid, resulted in improvement for biodiesel liquefaction like CFPP which is in desire for cold weather [37, 33]. In agreement with Santhakumaran et al [38], a comparison for the biodiesel properties of the four screened microalgae species (Table 2) revealed that *Chlorolobion* lipid extract was the most suitable for biodiesel production. Where it has the highest characteristic biodiesel properties such as the ratio of both saturated fatty acids and monounsaturated fatty acids to the polyunsaturated fatty acids (2.13), the highest CN value (54.99), the highest score in LCSF (0.06%), and the lowest IV value (100.57).

Table 2
Biodiesel properties for the four screened microalgae compared to American Society for Testing and Materials [39] and European standards [40]

	Microalgae of this study				Biodiesel standards	
	Chlorolobion	*Tetradesmus*	*Monoraphidium*	*Asterarcys*	ASTM D6751-08	CEN 14214
Unsaturation degree	1.18	1.51	1.67	1.71	-	-
Kinematic viscosity (mm^2s^{-1})	4.46	4.25	4.15	4.12	1.9-6.0	3.5-5.0
Specific gravity (kg^{-1})	0.88	0.88	0.88	0.88	0.88	0.86–0.9
Cloud point (°C)	4.22	-0.22	-2.37	-2.89	– 3 to 12	-
Iodine value (g I_2100g^{-1})	100.57	125.27	137.25	140.16	-	Max. 120
Cetane number	54.99	52.78	51.71	51.45	Min. 47	51–120
HHV ($MJkg^{-1}$)	40.61	41.20	41.48	41.55	-	42
Oxidation stability (h)	7.58	6.77	6.46	6.73	Min. 3	Min. 6
LCSF (wt%)	0.06	0.04	0.02	0.02	-	-
CFPP (°C)	-16.30	-16.35	-16.41	-16.41	-13 to -5	-20 to 5

Selection of suitable microalgae for biodiesel

Lipid content may vary from organism to organism, where a particular species may have high biomass with a low lipid content and vice versa. So, screening and selection of suitable microalgal species based on various biodiesel properties require a multi-criterion decision analysis (MCDA). (PROMETHEE) and (GAIA) have significant advantages compared to other MCDA methods because it is a promising method, i.e. the decision vector stretch toward the preferred solution [41]. In this study, based on phi value, the calculated outranking flows of the tested microalgae pointed out that the most appropriate microalgae for biodiesel production in descending order are *Chlorolobion braunii, Tetradesmus dimorphus, Monoraphidium minutum, Asterarcys quadricellulare* (Table 3). GAIA plane is a descriptive supplement to the PROMETHEE rankings. The blue lines and diamonds represent the biodiesel quality parameters, the green circles represent the microalgae samples analyzed, i.e. actions; and the red line represents the

decision vector. The species along with the decision axis, i.e. *Chlorolobion braunii* microalga is the most suitable candidate among the other species (Fig. 5). Most of the biodiesel properties lie adjacent to the decision axis (Du, KV, SG, CP, CN, HHV, LCSF, and CFPP), while some lie opposite to the decision axis and have less influence on the decision axis.

Table 3
PROMETHEE ranking the four screened green microalgae species based on the Phi scores

Rank	Microalgae	Phi
1	*Chlorolobion braunii*,	0.0466
2	*Tetradesmus dimorphus*,	-0.0020
3	*Monoraphidium minutum*,	-0.0211
4	*Asterarcys quadricellulare*	-0.0235

Omega fatty acids from microalgae

Microalgae have been demonstrated as a prospective source of value-added bioactive compounds with the high commercial value used in the pharmaceutical, healthcare and food industries [42]. The nutritional value of microalgae is highly related to their essential fatty acid contents [43]. Among value-added products of microalgae, long-chain PUFAs are broadly known for their useful impacts on human health [44], as they have an important biological role in maintaining a healthy brain function and coronary artery [45].

Fatty acids of the screened microalgae revealed that oleic acid has the greatest values within the group of omega-9 fatty acids, which ranged from 7.2–16.4% (Table 1). Oleic acid is associated with the prevention of brain-related disorders [46]. Palmitoleic acid an omega-7 MUFA fatty acid which ranged from 1.7–3.6% plays a significant role in human metabolism. Researchers have correlated dietary palmitoleic acid with decreased risks of cardiovascular diseases, diabetes, and inflammation [47]. Results presented in Table 4 elucidated that fatty acids profiles of the screened microalgae are characterized by a high level of omega-9 fatty acids (20.3–26.3%), while omega-7 fatty acids percentage (3.3-6%), which does not exceed 1.9% in many edible plant sources. MUFA fatty acid utilization is appeared to lower blood glucose in individuals with type II diabetes [48].

PUFAs are divided into two major groups i.e., Omega-3 and omega-6, which play a principal role in the formation of the structure and function of nervous and visual systems of humans and have a protective impact against inflammatory and cardiovascular diseases [49]. Interestingly, most of our microalgae contained a high percentage of omega-3 fatty acids, which exceeds that of many oil sources (Table 4).

For example, their percentage was in *A. quadricellulare* recorded 34.38%, while it was 25.14% in fish oil [50]. In general, omega-3 fatty acids represent the largest proportion of unsaturated fatty acids in most isolates, especially *A. quadricellulare* and *M. minutum* Moreover, the predominant active omega-3 fatty acid in most isolates is α-linolenic acid, which constitutes about a quarter of fatty acids content in *A. quadricellulare* compared to other sources of lipids, Similar results in which the soil algae *Protosiphon botryoides* produced fatty acids with high PUFAs and high omega-3 fatty acids [33]. Microalgae are the richest source of PUFAs particularly, EPA and DHA, which are the foremost vital PUFA's due to their metabolic functions [51]. DHA and EPA are synthesized by microalgae under various culture conditions [52]. Supplementation with omega-3 PUFAs as fish oil or microalgae is more useful compared to supplementation with ALA through flaxseed oil due to the generally low transformation of ALA to EPA and DHA [53].

Table 4
Omega fatty acids percentage of the four tested microalgae in comparison with some other lipid sources

lipid source	ω-3	ω-6	ω-7	ω-9	Total	References
Asterarcys quadricellulare	34.4	4.3	6.0	26.3	70.9	This study
Monoraphidium minutum	28.9	12.1	3.6	21.3	66.0	
Tetradesmus dimorphus	21.5	15.4	3.5	20.3	60.7	
Chlorolobion braunii	10.0	19.46	3.3	25.1	57.8	
Fish oil	25.1	19.4	10.1	23.3	77.8	[50]
Palm oil	0.3	11.2	0.3	43.4	55.2	[54]
Soybean oil	-	6.1	-	46.6	52.6	[50]
Ghee (Clarified butter)	0.6	2.0	1.9	23.2	27.6	[54]
Coconut oil	0.3	10.5	0.3	4.3	15.2	[50]

Conclusion

The present study evaluated the four locally isolated green microalgae based on their fatty acids profile for biodiesel and nutraceutical production. Generally, the results confirmed that lipids of these microalgae are suitable as biodiesel feedstock to be performed in a cold climate. Also, it pointed out that *Chlorolobion braunii* microalga is the best suitable candidate among the three other species. In addition, the results concluded that lipids of these microalgae are a convenient nutraceutical source because of their high percentage of essential PUFAs (omega fatty acids), where they could be applied in the nutraceutical industry.

Declarations

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Author contribution: Yasmine Ibrahim, Hamed Eladel, and Soha.Mohammed, conducted the experiments and analyzed data. Hamed Eladel and Soha Mohammed wrote, edited and reviewed the manuscript. Mohamed Battah reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

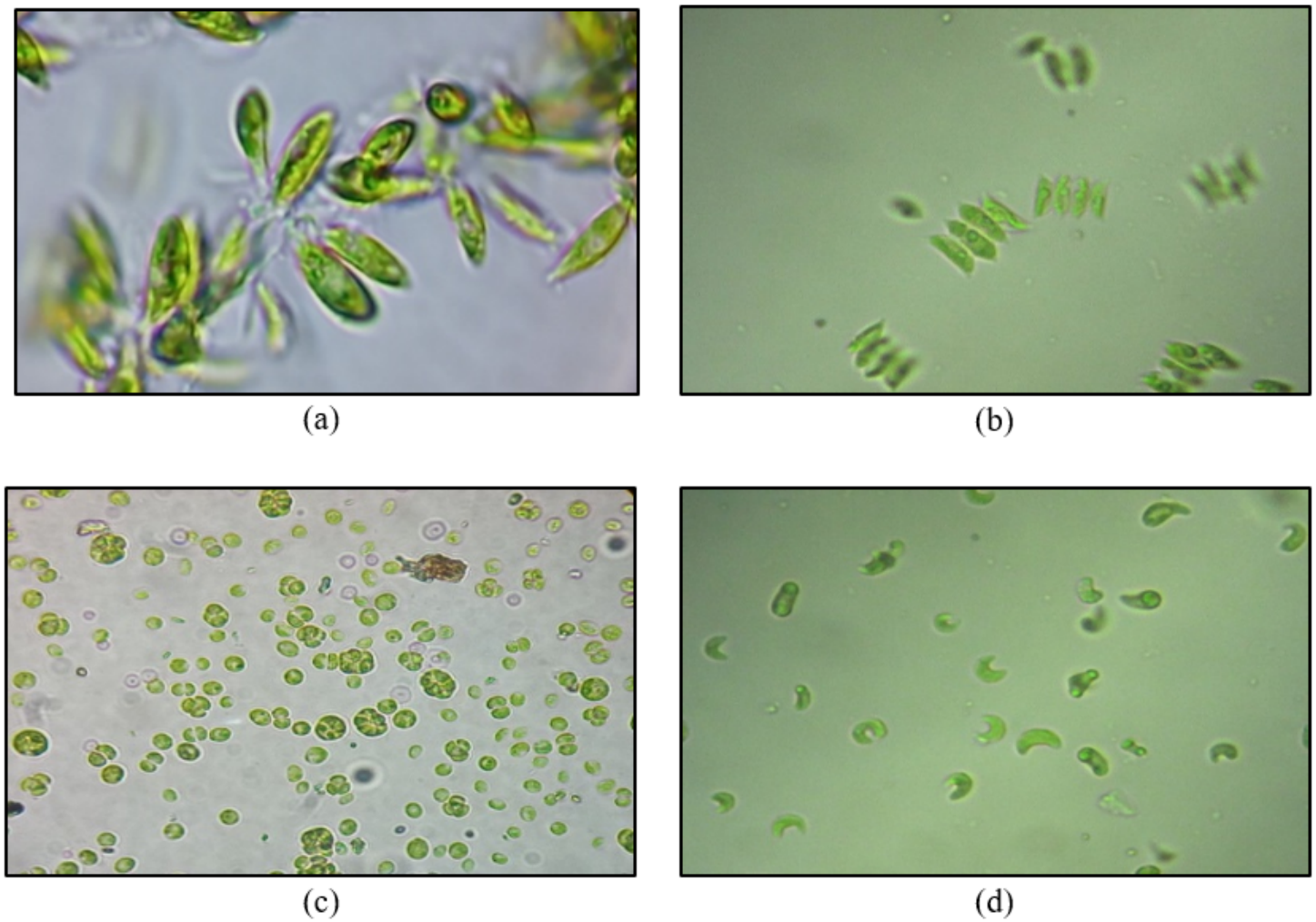

Figure 1

Photomicrographs of the four isolated microalgae from. Scale represents 10 µm. (a) *Chlorolobion braunii,* (b) *Tetradesmus obliquus,* (c) *Asterarcys quadricellulare,* (d) *Monoraphidium minutum*

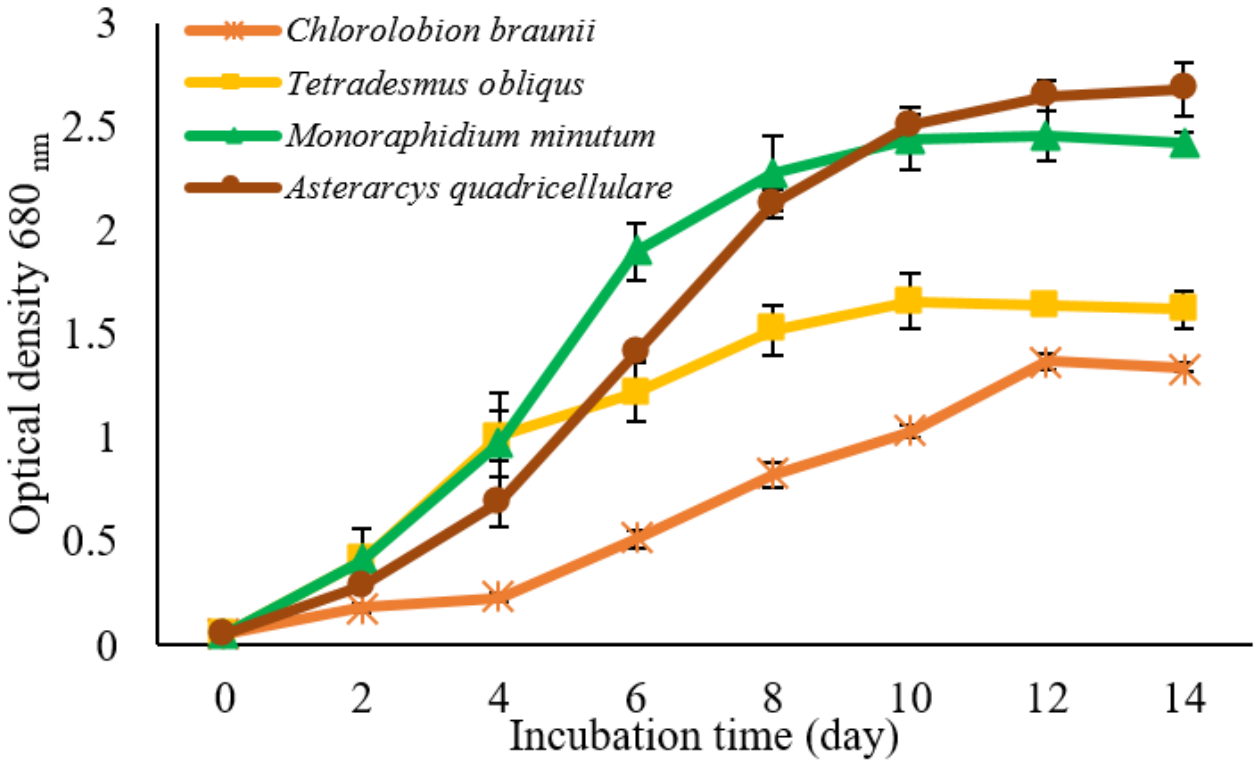

Figure 2

Growth curves of the four screened microalgae grown in liquid Bold's Basal medium for 14 days. Error bars show the standard deviation of three replicates

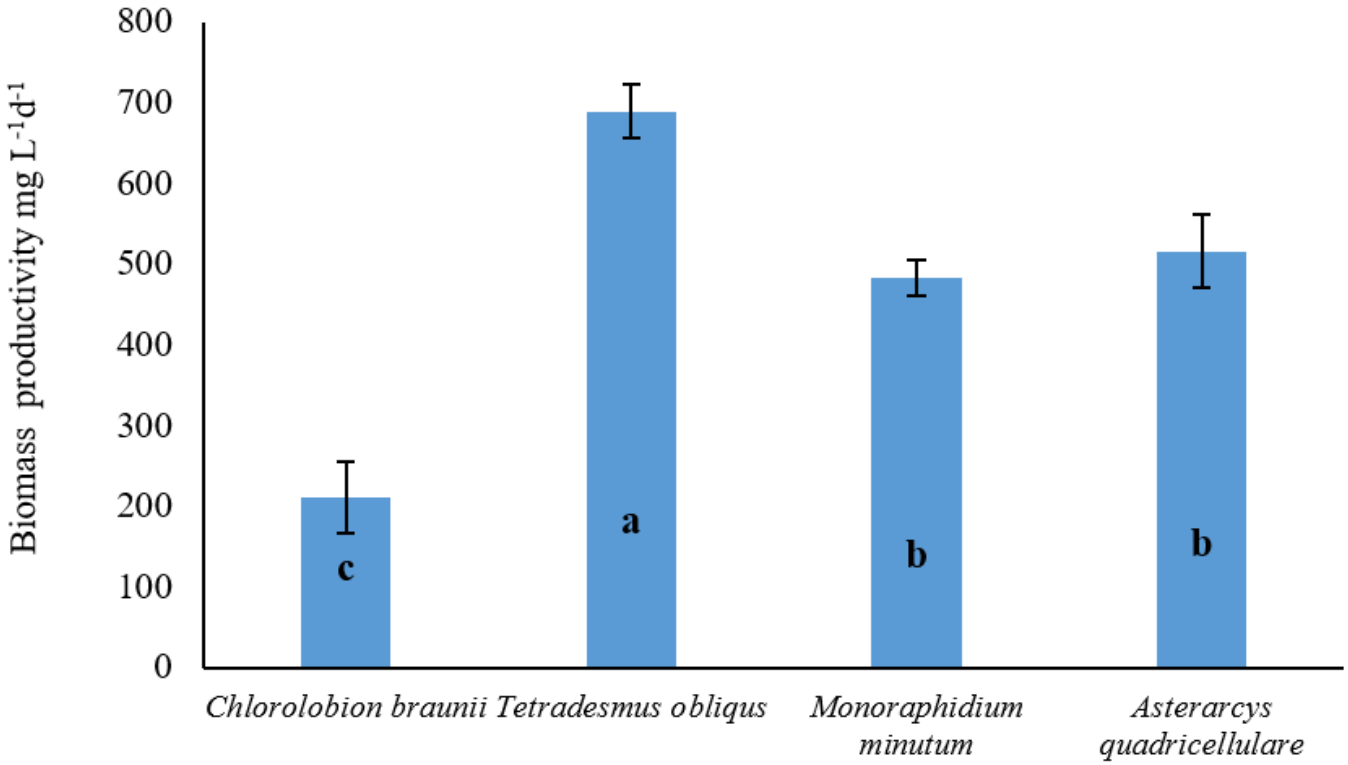

Figure 3

Biomass productivity of the four screened microalgae at exponential phase. Error bars show the SD for three replicates. Columns of the same series with the same letter showed an insignificant difference at $P \leq 0.05$

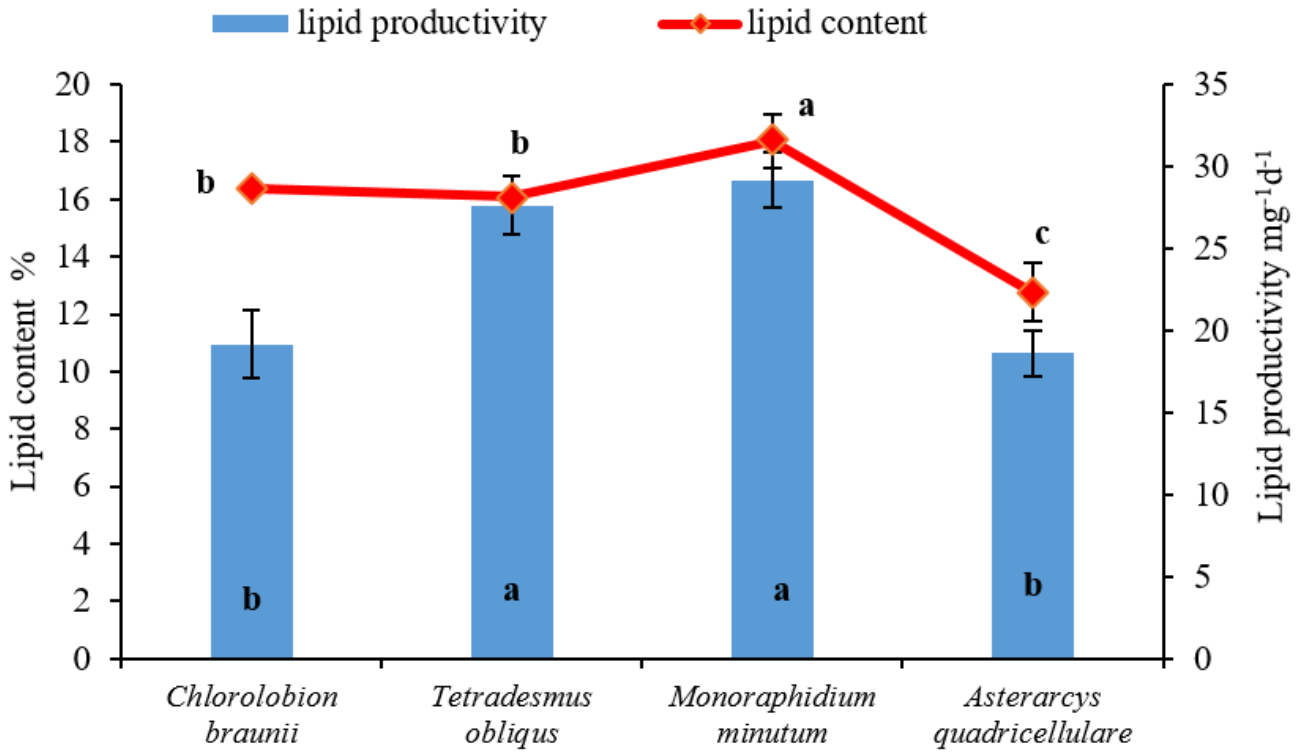

Figure 4

Lipid content and Lipid productivity of the four screened microalgae at the exponential phase. Error bars show the SD for the three replicates. The same series with the same letter showed an insignificant difference at $P \leq 0.05$

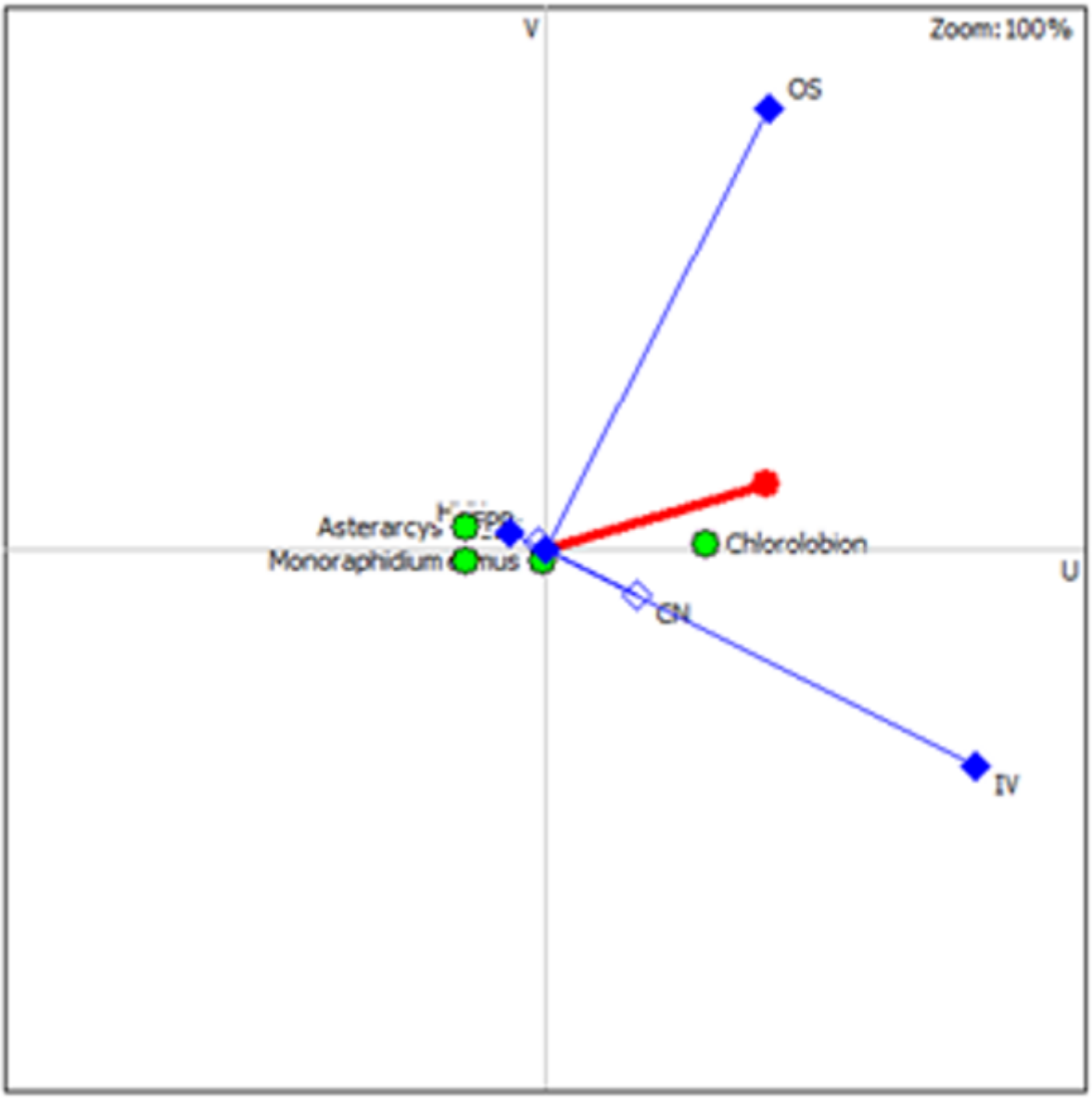

Figure 5

Graphical Analysis for Interactive Assistance (GAIA) plot of the four screened green microalgae species from the present study showing decision vector