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Original Article

Phenolic compound and fatty acid properties of some microalgae species isolated from Erbil City

Propriedades do composto fenólico e dos ácidos graxos de algumas espécies de microalgas isoladas da cidade de Erbil

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Abstract

The total phenolic compound and fatty acid profiles of lipids from microalgae are unique. The present study was designed to investigate aqueous, ethanolic and acetone extracts of several algae (*Spirogyra* sp., *Spirulina* sp.,*Chlorella* sp and *Chara* sp.) for their antioxidant capacities of the crude extracts and fractions by radical scavenging activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl DPPH as well; total phenolic content. The results showed that *Spirulina* sp. indicated significantly higher total phenolic compound and antioxidant activities compared to the other species ($P < 0.05$) and acetone extracts showed higher quantity among three extracts. The fatty acids analysis using High performance liquid chromatography –HPLC showed the presence of palmitic acid, stearic acid, oleic acid, and linoleic acid, palmitic acid showed high quantity than other fatty acid classes in all studied algae. This study concluded that high antioxidant capacity of microalgae could be inspected for different industrial applications.

Keywords: antioxidant, total phenol, fatty acid, algae, DPPH.

Resumo

O composto fenólico total e os perfis de ácidos graxos dos lipídios das microalgas são únicos. O presente estudo foi desenhado para investigar extratos aquosos, etanólicos e acetona de várias algas (*Spirogyra* sp., *Spirulina* sp., *Chlorella* sp. e *Chara* sp.) Quanto às suas capacidades antioxidantes dos extratos brutos e frações por atividade de eliminação de radicais contra o radical estável 1,1-difenil-2-picrilhidrazil DPPH também; fenólico total. Os resultados mostraram que a *Spirulina* sp. indicaram atividade antioxidante e compostos fenólicos totais significativamente maiores em relação às outras espécies ($P < 0,05$), e os extratos de acetona apresentaram maior quantidade entre os três extratos. A análise de ácidos graxos por cromatografia líquida de alta eficiência - HPLC mostrou a presença de ácido palmítico, ácido esteárico, ácido oleico e ácido linoleico; o ácido palmítico apresentou maior quantidade do que outras classes de ácidos graxos em todas as algas estudadas. Este estudo concluiu que a alta capacidade antioxidante pode ser inspecionada para diferentes aplicações industriais.

Palavras-chave: antioxidante, fenol total, ácido graxo, algas, DPPH.

1. Introduction

Algae are a significant natural source of novel compounds with biological activity, Some are living in complex ecosystems which are subject to harsh environmental conditions (Welker et al., 2012). Algae are an enormous biological resource, representing one of the most promising sources for new products and industrial applications (Munir et al., 2013).

As the unavoidable by product of converting food into energy, the body creates free radicals. Through the reaction of these species to several biomolecules like DNA, excessive generation of hydroxyl radical (OH·) and other highly reactive oxygen species (ROS) causes oxidative (Zhang and Tsao, 2016). Many studies on Pharmacological tests have shown that oxidative stress and elevated free radical levels are characteristics of chronic diseases, including cancer, aging (Klaunig and Kamendulis, 2004; Takaichi, 2011), additionally, the neurodegenerative diseases such as Alzheimer's and Parkinson's (Siti et al., 2015). Carotenoids play an important role in reducing reactive oxygen species (ROS) that generated during photosynthesis, especially singlet oxygen. Several studies have demonstrated that carotenoids contribute significantly to the total antioxidant

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capacity of microalgae (Jahnke, 1999; Takaichi, 2011; Martel et al., 2017).

Microalgae are already commercially showed as a source of carotenoid antioxidants such as *Haematococcus* for taxanthin, Dunaliella for beta-carotene that use as additives in food and feed applications, as well as for use in cosmetics and as food supplements (Spolaore et al., 2006). Also different microalgae screened for their radical scavenging activity against the stable radical 1,1-diphenyl-2-picrylhydrazyl by using aqueous and methanolic extracts and showed that that methanol was more efficient to extract selected group of compounds with a higher antioxidant activity (Martel et al., 2017) noticed that algae can biosynthesize, metabolize, accumulate and secrete a great diversity of primary and secondary metabolites including carotenoids, phenolic compounds, phycobilins, sulphated compounds and vitamins (Munir et al., 2013).

Antioxidant activities were identified in different types of marine algae such as red, green, and brown algae species (Kelman et al., 2012; Marinho et al., 2021). The Ethanol extracts of species of red algae *Callophyllis japonica* (Kang et al., 2005) and *Gracilaria tenuistipitata* (Yang et al., 2012) shows antioxidant effects. Studies of an aqueous extract were examined with cell line which showed the recovery and enhanced of these cells from H_2O_2 - induced DNA damage, counteracts cellular proliferation, and induced G2/M arrest that treatment of *G. tenuistipitata* (Yang et al., 2012; Lazzarotto-Figueiró et al., 2021). The discovery of novel fatty acids (FAs) with a wide range of new functions groups the study of fatty acid profiles as well as the presence of (FAs) in various lipid classes in microalgae is a new topic that promises to reveal a lot specially during their stationary phase in time producing and accumulate oil as carbon source, changes in fatty acid profiles under certain circumstances occur when microalgae try to acclimatize under unfavorable conditions (Wan Afifudeen et al., 2021).

Numerous studies have been carried out on the fatty acid, total phenol, and antioxidant activity of several algal species obtained from different geographical locations, but no such report was found on our selected site. The aim of this study were to determine the antioxidant total phenols and fatty acid activity of extracts obtained from several microalgae strains with regard to their relation to different type of extraction.

2. Material and Methods

2.1. Material and chemicals

(18.2 M ohm.cm Milli-pore, Bedford, MA, USA) used to obtain ultra-pure water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Garlic acid, sodium carbonate (Na_2CO_3) ethanol, acetone, Folin Ciocalteu‟s phenol reagent were taken from Merck (Darmstadt, Germany).

BG11 medium used to culture algal isolates.

2.2. Methods

2.2.1. Isolation and identification microalgae

Microalgae samples were isolated from different sites of Erbil City. Algae were identified using compound microscope based on morphological characterization such as Filament or Unicellular, Akinet, Heterocyst, Hormogonia, Colour, Chloroplast and cell shape depending on the following references (Krammer, 2002, 2003; Sant'anna et al; 2004; Al-Naqishbandy, 2020).

The pure microalga was cultured in a 250 ml conical flask containing 100ml of BG11 and the pure cultures were scaled up to 5-L flasks, under controlled conditions, at a light intensity of 2000 lux. with a photoperiod of 16: 8 (Light: Dark), temperature at 28 ± 2◦C, and continuous aeration supplied with CO_2 pulse addition at a rate of one minute every hour. Biomass samples were harvested, Dried and stored for further use.

2.2.2. Determination of total phenolic compound

The total phenolic content in the extracts was determined by the method described by Viuda-Martos et al. (2010) by using (100 µl) of extract and 0.5 ml of Folin-Ciocalteu reagent was mixed and position for 5 min at room temperature. Then added Sodium carbonate (7.5%, 0.4 ml) to the mixture and stand for 2 h at room temperature. The absorbance was measured at 760 nm. The content of phenolic compounds was stated as mg Gallic acid equivalent (GAE) per 100g dry weight of sample.

2.2.3. DPPH(2,2'-diphenyl-1 picrylhydrazyl) assay

The assay was performed according to the method reported by Blois (1958). Briefly, 1 ml of 6 x 10-5 M of ethanolic solution of DPPH was added to 25 µl sample. The mixture was mixed well, and allowed to stand in the dark at room temperature for 1 hour. The absorbance measurements were taken at 517 nm using a (spectrophotometer), with ethanol as a blank. A control was measured without added antioxidant. Ascorbic acid (0-10) mM was used as a reference. Each measurement was performed in triplicate.

2.2.4. Extraction of fatty acid

Fatty acid extracted on the base of method that described by Matyash et al. (2008), which is a altering of the Folch/Bligh and Dyer method. The solvent that used for extraction included Methyl-tert-butyl ether (MTBE), 0.75 ml of methanol was added to 100 ml sample and mixed rigorously using vortex followed by the adding of 3 ml of MTBE and the mixture was incubated for 1 h at room temperature. To develop the phase separation a volume of 75 ml of water was added to the mixture and left in room temperature for 10 min. The upper organic phase was collected after centrifugation.

2.2.5. Isolation and characterization of fatty acids by HPLC

The extracted fatty acids used to diagnose and determine the class of each taxon by using HPLC. The analysis was conducted in the college central lab of college of Agriculture/ University of Salahaddin-Erbil.

2.3. Statistical analysis

The result obtained from the experiment was statistically analyzed. All the experiments were carried out in triplicate and the data were subjected to analysis of variance by using SPSS version (28) and Microsoft excel office 2010. Compared by the significant correlation difference test, a probability value of P = 0.05 was considered significant and one way ANOVA independent-Samples Kruskal-Wallis test used to define the hypothesis on the base of Null Hypothesis with 0.05 significant levels.

3. Results and Discussion

3.1. Isolation and identification of algal samples

Five strains of algae from studying sites were isolated (Figure 1), purified and identified on the base of morphological features, the isolates included two isolates of *Spirogyra* sp.; unbranched filamentous, chloroplast spiral shaped, one genera of green algae (*Spirulina* sp. the filaments were unbranched, spiral shapes, trichrome width, cell length, pointed calyptras and three genus of green algae *Chlorella* sp.; unicellular spherical shaped cell with cup shaped chloroplast, *Chara,* The plant body consists of long, cylinder, jointed, green main axis with regular's succession of node and inter node. The central axis is branched, at each node arises a whorl of lateral branches) Abdulkareem and Anwer (2020) isolated Spirulina from Koya city that showed the same characters, on the base of morphological characteristics Sdiq et al. (2020) isolated Spirogyra, Chara and Chlorella in Erbil City showed similar features.

3.2. Total phenolic compound

The total phenolic content extracting by solvents acetone, ethanol, and water, varied and based on the absorbance values of extracts reacted with the Folin–Ciocalteu reagent, assessed (Figure 2), as Gallic acid equivalents by reference to standard curve. The highest content recorded with acetone, and followed by ethanol, and water extracts. The acetone extracts of *Spirulina* sp. showed high total phenolic content than other species with acetone, water, and ethanol solvents and followed by *Chlorella, Chara*, and *Spyrogera*

respectively. To identify differences between two antioxidant analysis methods (DPPH & total Phenolic content) the Kruskal-wallis used for non-parametric. As shown in Figures 3 *Spirulina, Chara* and *Chlorella* shows a highly significant result at level 0.05 for all three extracts acetone, ethanol, and water in both DPPH and total Phenol. All shows a significant result between the treatments only while *Spirogyra* sp. was significant in total phenol (Figure 3a, b, c, d). However, other researchers report have been agreed with our results (Assis et al., 2014).

Although several species of Chlorophyceae, Phaeophyceae, and Rhodophyceae exhibit secondary metabolites with broad ecological significance (Peria,

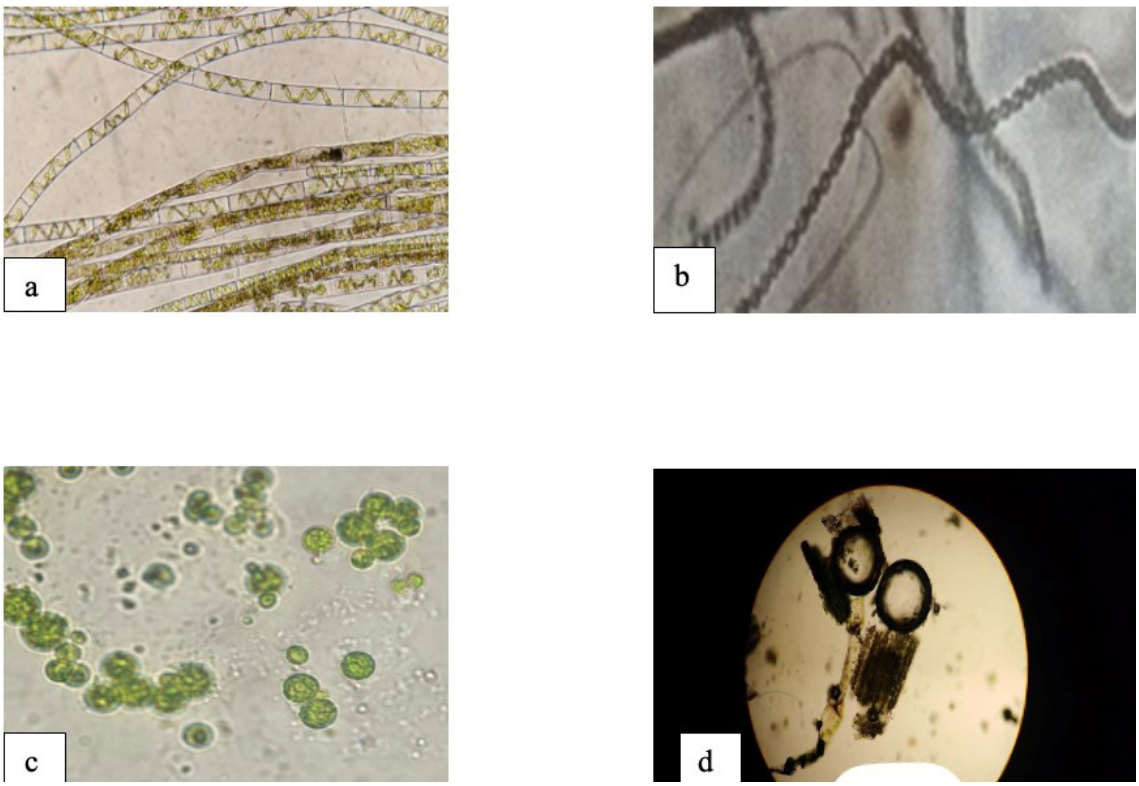

Figure 1. Morphology of Algal genera isolated from Erbil City (a-Spirogyra, b-Spirulina, C-Chlorella d- Chara).

2003). Effects of extraction solvent and harvest period on phytochemical analysis and phenolic compounds, antioxidants and antimicrobial activities of extracts evaluated, specially from *Spirogyra* sp. were confirmed to have strong contribution of flavonoids and phenols (Belyagoubi et al., 2021). Besides, Jerez-Martel et al. (2017) studied Phenolic profile and antioxidant activity using aqueous and methanolic extracts of several microalgae and they found that the greatest antioxidant activity showed in green microalgae *Euglena cantabrica*, as result of presence of high phenolic content.

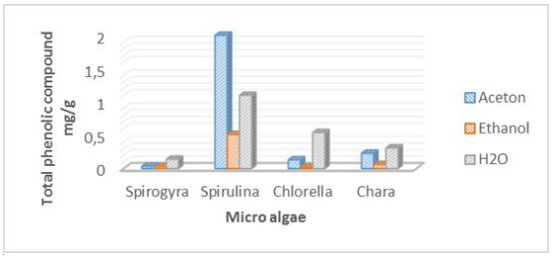

Figure 2. Total phenolic content (mg/g) extracted from algal isolates.

Microorganisms are excellent resources for the bioactive secondary metabolites investigated has been made on *Spirulina* sp. due to their potential for industrial application of antioxidants. Meanwhile the variability in the chemical composition of algae is dependent on many factors, including the growth medium nutrients, which reflect their natural habitat conditions (Ismaiel et al.,

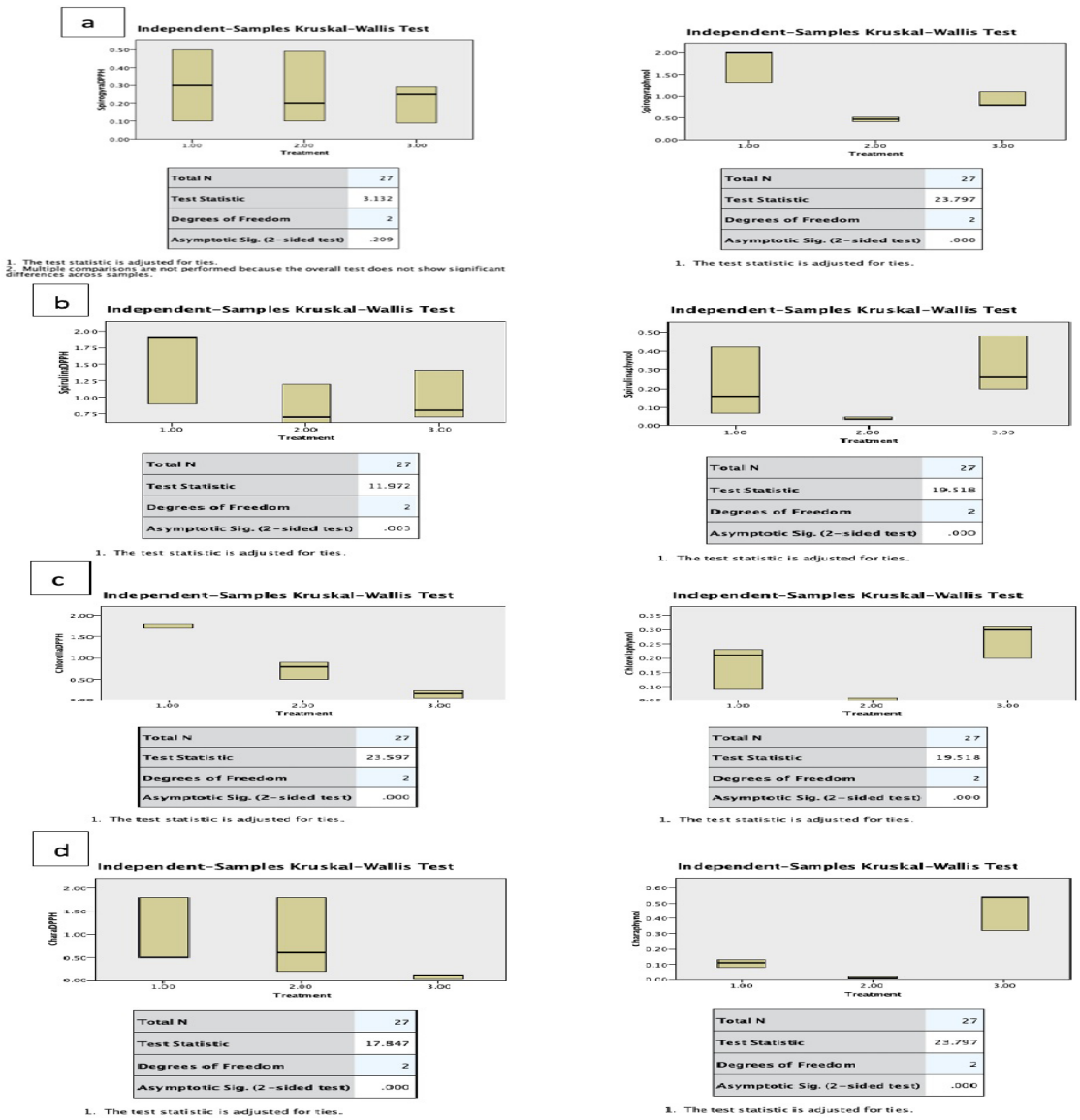

Figure 3. The distribution of DPPH and total phenol shows the same across categories of Treatment, Independent-Samples Kruskal-Wallis Test and rejects the hypothesis on the base of Null Hypothesis with highly significant levels. A- *Spirogyra* sp., b-*Spirulina* sp. c- *Chlorell* sp. *a* d- *Chara* sp.

2016) *Spirulina* sp. produce some antioxidant in both in vitro and in vivo systems (Miranda et al., 1998).

The most well-known microalgae genus *Chlorella* and *Spirulina* these have a significant content of proteins, vitamins, pigments, fatty acids, sterols, among others, which make their production by the food industry quite interesting (Andrade et al., 2018). Although the different extractions of *C. vulgaris* cells with solvents like methanol, ethanol, chloroform and diethyl ether, show their antibacterial activity against gram negative and gram positive human pathogenic bacteria (Dineshkumar et al., 2017). Biological treatment of phenol solution using the green macroalga Chara sp and Chlorella vulgaris used in wastewater treatment reveal to their ability to remove phenol from aqueous solution beside they have strongest antimicrobial properties and may be considered as alternative source for synthetic substances, these substances are potential source of bioactive compounds (Dwaish et al., 2018).

3.3. Fatty acid profile

The fatty acid form of microalgal lipids are well-defined and the majority of fatty acids consist of saturated and monounsaturated fatty acids (Yusof et al., 2011; Shen et al., 2016). Fatty acid composition of ethanol extracts from algal isolates *Spirogyra, Spirulina, Chlorella and Chara* were observed by High performance liquid chromatography. The distinguished fatty acids were categorized into (palmitic acid and stearic acid) saturated fatty acids, monounsaturated oleic acid and polyunsaturated fatty acids linoleic acid. The higher constituents showed in Palmitic acid 23.1%, 22.3%, 36.3% and 46.07% respectively, followed by oleic acid, stearic acid and linoleic acid (Figure 4). The relation between fatty acids in Algae resolute by using SPSS 28, from the Tables (1-4) which illustrate steric acid correlated positively with oleic acid and negatively with linoleic acid only in *Spirulina* sp. at level 0.05 and, for more reassure the scatter plot matrix used, (Figure 5). The scatter plot matrix shows additional results, Oleic acid positively correlated with linoleic acid in *Spirogyra* while negatively in *Chara* and *Spirulina* on the other hand oleic acid positively correlated in *Spirulina* and negatively with stearic acid in *Spirogyra* and *Chara.* whereas Stearic acid positively correlated with oleic acid in *Spirulina* and negatively with palmitic acid in *Spirogyra* and *Chara* specie High number of microalgal species produce a wide range of antioxidants, including carotenoids, polyunsaturated fatty acids (Jerez-Martel et al., 2017). The *Spirogyra* sp and *Chara* sp. is valuable to extract polyunsaturated fatty acids and convert triglycerides and alternative fatty acid as a potential source to oil (Trifa et al., 2013). *Chara* sp most abundant for fatty acids and there were small amounts only of saturated and C_{20}, contained a high proportion of fatty acids a component not normally associated with green photosynthetic tissues (Stefanov et al., 1986).

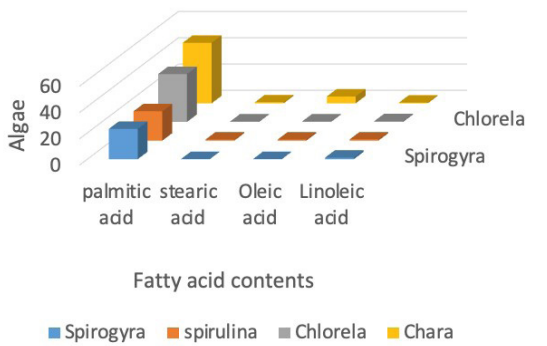

Figure 4. Chemical composition of fatty acid in algae obtained by HPLC.

Table 1. Correlation between different fatty acids detected in Spirogyra.

	Palmitic	Stearic	Oleic	linoleic
Palmitic	1			
Stearic	-0.563	1		
Oleic	0.12	-0.225	1	
linoleic	-0.29	-0.046	0.377	1

Note: No significant correlation

Table 2. Correlation between different fatty acids detected in Spirulina.

	Palmitic	Stearic	Oleic	linoleic
Palmitic	1			
Stearic	-0.174	1		
Oleic	-0.058	.746*	1	
linoleic	0.046	-.668*	-0.355	1

*Correlation is significant at the 0.05 level.

Table 3. Correlation between different fatty acids detected in Chlorella.

	Palmitic	Stearic	Oleic	linoleic
Palmatic	1			
Staric	0.24	1		
Olic	0.125	-0.144	1	
linoleic	-0.036	-0.146	-0.081	1

Note: No significant correlation

Table 4. Correlation between different fatty acids detected in Chara.

	Palmitic	Stearic	Oleic	linoleic
Palmitic	1			
Stearic	-0.347	1		
Oleic	-0.03	-0.317	1	
linoleic	-0.136	0.389	-0.512	1

Note: No significant correlation

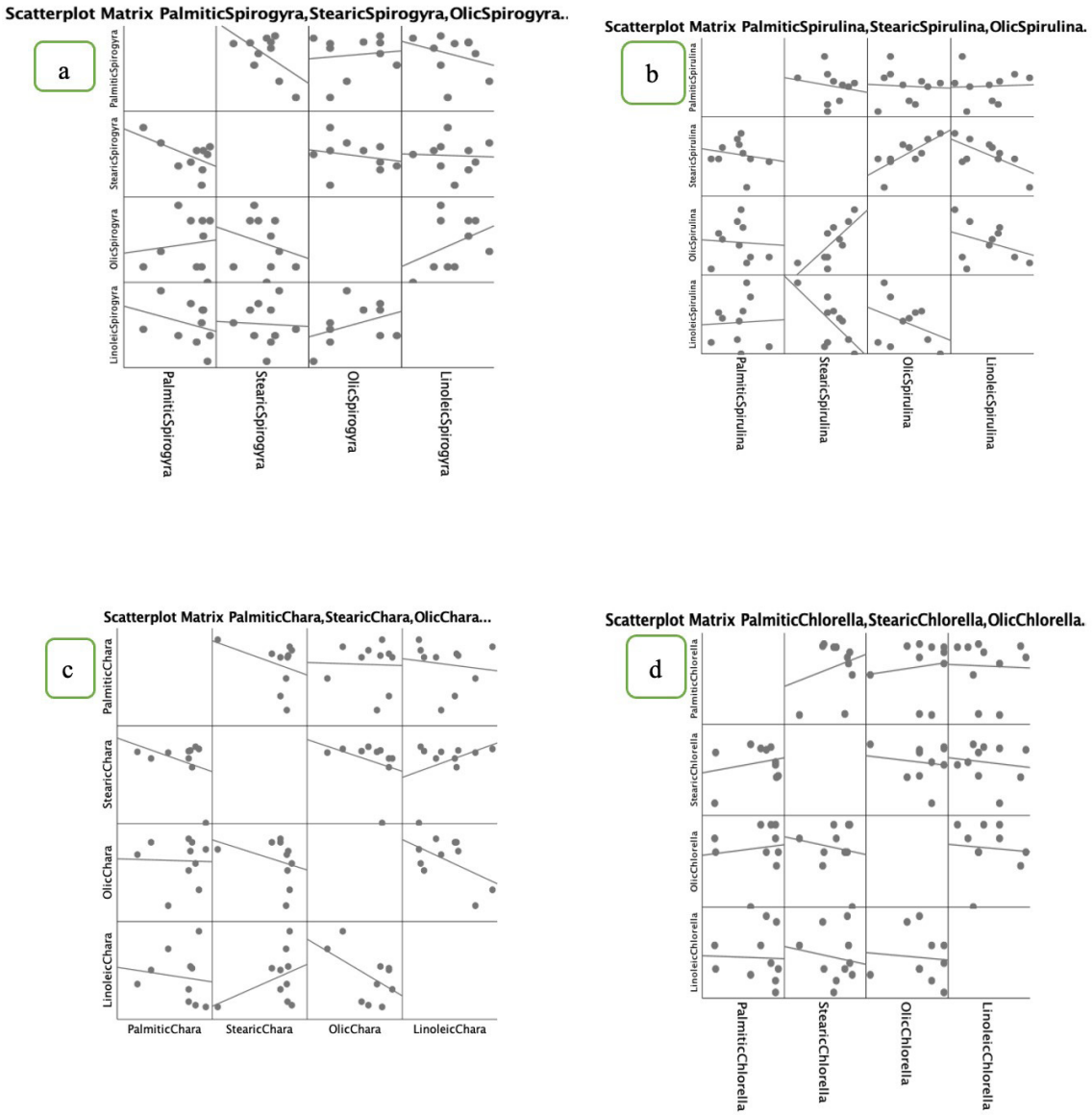

Figure 5. Scatterplot matrix shows the correlation between palmitic acid, stearic acid, oleic acid and linoleic acid in a- *Spirogyra* sp. b- *Spirulina* sp. c- *Chara* sp. d- *Chlorella* sp.

On the other hand, The *Spirulina* and the fresh water algae *Chlorella* contain about 50% to 70% protein, vitamins, fiber, minerals and fatty acids at high concentrations, the *Spirulina* microalgae higher phenolic content than *Chlorella* because the phenolic compounds of microalgae were satisfactorily adhered to lipid (Assis et al., 2014). Antibacterial substance, named 'Chlorellin', was firstly isolated from Chlorella consist of the mixture of fatty acids was found to exhibit inhibitory activity against microbes (Dineshkumar et al., 2017).

Spirulina and *Chlorella* displayed different characteristics in fatty acid composition. Total amounts of polyunsaturated fatty acids in *Spirulina* and *Chlorella* were almost similar, but the total level of unsaturated fatty acids in Chlorella was higher than that in Spirulina (Ötleş and Pire, 2001).

4. Conclusion

In conclusion, the results distinctly showed the antioxidant activity of four algae (*Spirogyra* sp. , *Spirulina* sp. *Chlorella* sp. and *Chara* sp.) were screened for their co-responsibility of Total phenols and fatty acids. The present algae extracted by different solvents (`acetone, ethanol, water).The highest total phenol recorded by *Spirulina* sp. in acetone extract while the highest fatty acid was Palmitic acid and a significant relation found between Steric acid and Oleic acid in *Spirulina sp.* although this was the first study on the antioxidant effects of algae from north of Iraq further research is needed in order to increase the lipid concentration for the enhancement and production of high nutritional value added products and for the

production of a better and cheaper, biproducts, and their further potential using in nutritional, pharmaceutical, and medicinal implications due to their easy (cultivation) and economy.

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