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Fatty acids composition and profiling of nine abundant marine Macroalgae, Egypt

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Abstract

This study analyzed the fatty acids composition and their profile qualitatively and quantitatively of the nine abundant macroalgal specimens collecting from Egyptian coasts. GC mass analysis identified 23 types, including 13 of saturated fatty acids (SFA) and 10 of monounsaturated fatty acids (MUSFA). SFA dominated with 78%, while MUFAs had 22%, and UFAs were negligible at 0.01%. MUSFA oleic acid (omega-9) was present in all species except green macroalgae *Galaxura rugosa* and *Ulva fasciata,* replaced by MUSFA linoleic acid (omega-6). Oleic acid methyl ester (omega-9) was registered in all the studied species, except red *Hypnea cornuta* & *Jania rubens*, and brown *Hormophysa cuneiformis*. Chlorophyta registered 35% of the fatty acid composition, followed by Rhodophyta (33%) and Phaeophyta (32%). Major SFAs were palmitic acid glycidyl ester, oleic acid glycidyl ester and palmitic methyl ester, comprising over half of total fatty acids. Red and brown macroalgae were richer in palmitic and oleic glycidyl esters, while green macroalgae had more palmitic methyl ester. Linoleic acid, nonadecylic acid, elaidic acid methyl ester, linoleic acid methyl, behenic acid, pentacosylic acid, palmitic acid, and trans-palmitoleic acid were exclusively identified in Chlorophyta. Lacceroic acid was distinguished in Rhodophyta, whereas pelargonic acid just appeared in brown alga Turbinaria turbinata. The maximum values of fatty acids were recorded in the green macroalga Caulerpa racemosa while reed macroalga *Hypnea cornuta*.was the minimum one. The research sheds light on the fatty acid composition and its potential implications for human health and nutrition.

Keywords: Marine Macroalgae; GC mass analysis; Fatty acids profiling; SFA; MUSFA; UFA

1. Introduction

Macroalgae play a vital role in marine ecosystems, serving as essential biological resources. As primary producers, they contribute significantly to the diversity and productivity of marine communities. Moreover, they offer food and shelter to various marine organisms across different life stages [1]. In a study conducted by Sohrabipour *et al*. [2], the significance of macroalgae from three divisions, namely green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta), was evaluated in terms of their fatty acid content and potential therapeutic effects for treating certain human diseases. The research explored how these algae species could potentially serve as valuable resources for medical applications. Furthermore, the ratio between ω-6 and ω-3 and the ratio between PUFAs and SFAs found in red and brown algae are more favorable for human health than those found in green algae [3].

Macroalgae possess specific characteristics in their fatty acid composition. Typically, their fatty acids have linear chains, an even number of carbon atoms, and one or more double bonds [4]. Of particular importance is eicosapentaenoic acid (EPA, C20:5n-3), an essential fatty acid found abundantly in macroalgae. Red and brown algae are notably rich in both eicosapentaenoic acid (EPA) and arachidonic acid (AA). Conversely, green macroalgae, like *Ulva*, predominantly contain hexadecatetraenoic, oleic, and palmitic acids, along with significant levels of polyunsaturated fatty acids (PUFAs), such

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as linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) [5]. Notably, the ratios of ω-6 to ω-3 and PUFAs to SFAs found in red and brown algae are more favorable for human health compared to those present in green algae [3]. These findings suggest that incorporating red and brown macroalgae into the diet might have additional health benefits due to their desirable fatty acid profiles.

The coastlines of both the Mediterranean Sea and the Red Sea, particularly around the Suez Canal, hold significant importance. Migration of marine organisms is a biological necessity, occurring in both spatial and temporal. The Indo-Pacific originated biota has exhibited changes over time and space, emphasizing the importance of conserving and developing these merits for future generations [6]. Researchers have conducted studies on the seasonal and spatial changes in macroalgal vegetation and their nutritional composition in the Red Sea [7-8]. The nutritional composition of seaweed varies with seasonal fluctuations in environmental conditions. El-Manawy *et al*. [8] revealed that the Egyptian Red Sea coast serves as a valuable source of fiber, minerals, carbohydrates, proteins, and fatty acids. The study aims to assess the total fatty acids composition qualitatively and quantitatively as well as their profiles in nine abundant marine macroalgae collected from the Egyptian coasts.

2. Materials and Methods

2.1. Area of study

Marine macroalgae were harvested at the intertidal zone during the low tide on November-December 2022 from three sites. The collection sites, areas and their *coordinates* (Figure 1) were as follows:

Site I: The Hunting Club, Port Said, Mediterranean Sea (31.26941" N& 32.31513" E)

Site II: Aldunfah Beach Club, Ismailia, Suez Canal (*30.58973"*N& *32.30508"*E)

Site III: El Ahyaa District, Hurghada, Red Sea (27.1703"N&33.4618"E)

Figure 1 Map of Egypt showing the studying area and the selected sites

2.2. Macroalgae samples collection and preparation

Nine abundant marine macroalgae samples were manually collected from various sites, representing three macroalgal divisions to showcase the diversity of macroalgae functional groups. The sampled species include:

Chlorophyta: ***Caulerpa racemosa*** (Forsskål) J. Agardh, ***Halimeda tuna*** (J. Ellis & Solander) J.V. Lamouroux from site III and ***Ulva fasciata*** Delile from site I.

Rhodophyta: ***Galaxura rugosa*** (J. Ellis & Solander) J.V. Lamouroux & ***Jania rubens*** (Linnaeus) J.V. Lamouroux from site III and ***Hypnea cornuta*** (Kützing) J. Agardh from site II.

Phaeophyta: ***Turbinaria turbinata*** (Linnaeus) Kuntze from site III, ***Hormophysa cuneiformis*** (J.F. Gmelin) P.C. Silva from site III and ***Polycladia myrica*** (S.G. Gmelin) Draima, Ballesteros, F. Rousseau & T. Thibaut from site III.

The collected samples were firstly washed with seawater to remove epiphytes and other marine organisms. After that, the macroalgal species were transported to the laboratory in sterile polythene bags and identified using references as [9-12]. Next, the samples were rinsed with tap water and then thoroughly washed with distilled water to eliminate salt, epiphytes, and sand particles. The samples were air-dried at room temperature in a shaded area. Once completely dry, the samples were cut into small pieces and further processed into powder using a mixer grinder. This preparation method ensured the removal of impurities and the transformation of the macroalgae into a powdered form for subsequent analyses.

2.3. Extraction of Fatty acids

The extraction of fatty acids from the samples followed the Folch method [13]. For each sample, one gram was extracted using a solvent mixture of chloroform: methanol in a ratio of 2:1 (v/v). To induce phase separation, an equal volume of chloroform and water (1:1 v/v) was added. The lower phase was collected and subsequently dried under nitrogen to achieve dryness [14] for further fatty acid analysis [15]. This method facilitated the efficient extraction of fatty acids from the samples, making them ready for subsequent analysis.

2.4. Gas chromatography–mass spectrometry (GC-MS) analysis

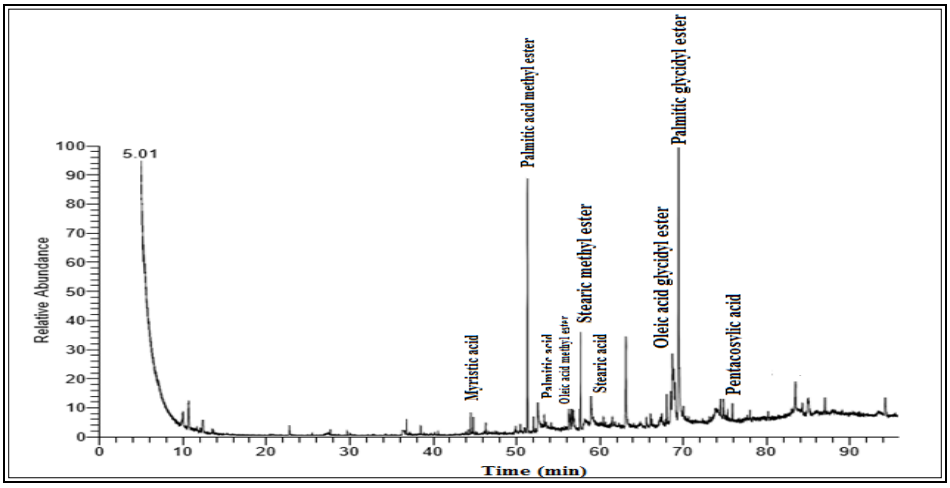
For fatty acid composition analysis, a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness) was used. The column oven temperature was initially set at 35°C and then increased at a rate of 3°C/min until reaching 200°C, where it was held for 3 minutes. Subsequently, the temperature was further increased to the final value of 280°C at a rate of 3°C/min and held for 10 minutes. The injector and MS transfer line temperatures were maintained at 250°C and 260°C, respectively. As a carrier gas, Helium was used at a constant flow rate of 1 ml/min. For analysis, 1 µl of diluted samples was automatically injected using an Autosampler AS1300 coupled with GC in the split mode, with a solvent delay of 3 minutes. Electron impact (EI) mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200°C. Identification of components was performed by comparing their retention times and mass spectra with those from the WILEY 09 and NIST 11 mass spectral databases.

3. Results

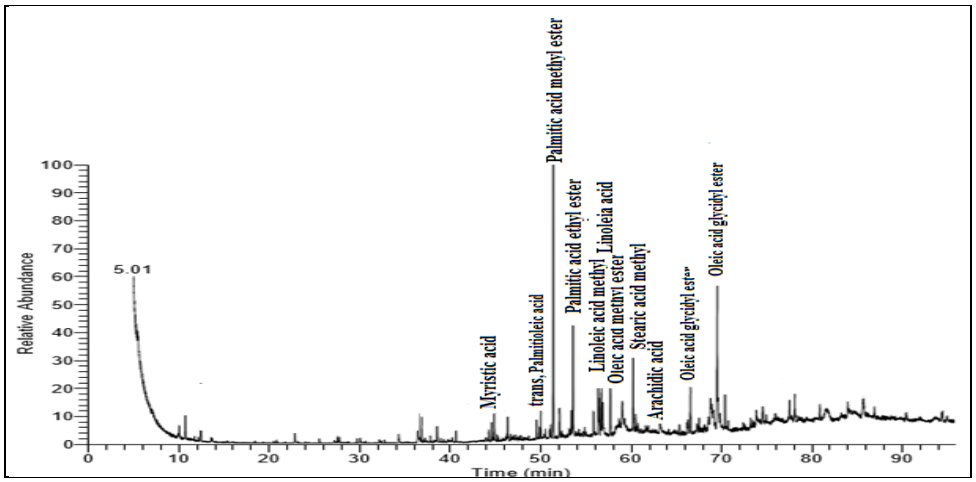
The fatty acids composition of the nine investigated macroalgal samples and their Lipid number (No), Structural formula, Retention time (RT) and Molecular weight (MW) are summarized in Table 1. GC mass analysis recorded 23 types of fatty acids including 13 of SFA and 10 of MUSFA. Lipid number ranged from C7:0 Enanthic acid to Lacceroic acid C21:0. Figure 2 illustrated the fatty acids content profile qualitatively and quantitatively to each of the nine specimens. SFAs consists of enanthic acid, pelargonic acid, myristic acid, palmitic acid, palmitic acid methyl ester, palmitic acid ethyl ester, Stearic acid, palmitic acid glycidylester, glycerol 1-palmitate, arachidic acid, behenic acid, pentacosylic acid and lacceroic acid. The USFAs comprised of one USFA elaidic acid methyl ester and 9 MUSFA namely, trans, palmitioleic acid, palmitoleic acid methyl, oleic acid, oleic acid methyl ester, nonadecylic acid, linoleic acid methyl, stearic acid methyl, linoleic acid and oleic acid glycidyl ester.

Table 1 Area % of the fatty acids in the selected species and their Lipid number (No), Structural formula, Retintion time (RT) and Molecular weight (MW)

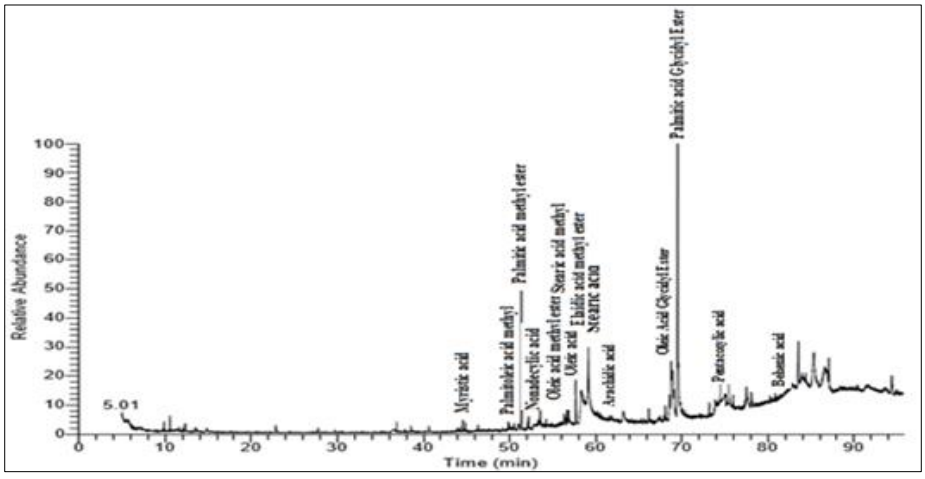
FA Type	Common Name	Lipid No.	Structu-ral Formula	RT	MW	*Caulerpa racemosa*	*Ulva fasciata*	*Halimeda tuna*	*Galaxura rugosa*	*Hypnea cornuta*	*Jania rubens*	*Turbinaria turbinata*	*Hormophysa cuneiformis*	*Polycladia myrica*
SFA	**Enanthic acid**	C7:0	$C_7H_{12}O_3$	9.95	144	-	-	-	0.37	0.35	0.38	0.39	-	0.45
SFA	**Pelargonic acid**	C9:0	$C_9H_{18}O_3$	10.64	174	-	-	-	-	-	-	0.72	-	-
SFA	**Myristic acid**	C14:0	$C_{14}H_{30}O_2$	44.58	242	1.61	0.75	0.56	-	0.27	-	-	-	-
SFA	**Palmitic acid**	C16:0	$C_{16}H_{32}O_2$	52.62	256	2.40	-	-	-	-	-	-	-	-
MUSFA	**trans, Palmitioleic acid**	C16:0	$C_{16}H_{30}O_2$	51.16	254	-	0.62	-	-	-	-	-	-	-
SFA	**Palmitic acid methyl ester**	C17:0	$C_{17}H_{34}O_2$	51.38	270	13.22	11.09	6.81	2.19	2.88	1.22	2.36	1.88	2.04
MUSFA	**Palmitoleic acid methyl**	C17:0	$C_{17}H_{32}O_2$	50.57	268	-	0.38	0.33	-	0.28	-	-	-	-
SFA	**Palmitic acid ethyl ester**	C18:0	$C_{18}H_{36}O_2$	53.63	284	-	4.48	-	-	0.56	-	-	-	-
SFA	**Stearic acid**	C18:0	$C_{18}H_{36}O_2$	59.00	284	2.10	0.29	3.30	4.23	-	4.70	4.79	4.60	4.99
MUSFA (ω-9)	**Oleic acid**	C18:0	$C_{18}H_{34}O_2$	58.37	282	-	-	2.51	1.16	0.93	3.55	3.15	2.68	3.75
MUSFA (ω-9)	**Oleic acid methyl ester**	C19:0	$C_{19}H_{34}O_2$	56.86	296	2.03	1.57	0.69	2.64	-	-	1.32		0.6
MUSFA	**Nonadecylic acid**	C19:0	$C_{19}H_{36}O_2$	51.16	296	-	-	0.35	-	-	-	-	-	-
USFA	**Elaidic acid methyl ester**	C19:0	$C_{19}H_{36}O_2$	56.70	296	-	-	0.82	-	-	-	-	-	-
MUSFA (ω-6)	**Linoleic acid methyl**	C19:0	$C_{19}H_{38}O_2$	56.51	292	-	1.42	-	-	-	-	-	-	-
MUSFA	**Stearic acid methyl**	C19:0	$C_{19}H_{38}O_2$	57.74	298	7.84	6.95	2.39	2.02	2.14	1.81	1.87	-	2.01
SFA	**Palmitic acid glycidylester**	C19:0	$C_{19}H_{36}O_3$	69.56	312	19.73	7.47	17.19	24.52	21.89	23.77	22.54	22.07	21.76
SFA	**Glycerol 1-palmitate**	C19:0	$C_{19}H_{38}O_4$	52.80	330	-	-	-	0.42	0.50	0.27	0.69	0.55	0.88
MUSFA (ω-6)	**Linoleic acid**	C19:0	$C_{19}H_{36}O_2$	56.34	294	1.84	2.30	-	-	-	-	-	-	-
SFA	**Arachidic acid**	C20:0	$C_{20}H_{40}O2$	60.27	312	-	3.48	0.47	0.84	0.8	0.75	0.3	0.98	0.45
MUSFA	**Oleic acid glycidyl ester**	C21:0	$C_{21}H_{38}O_3$	68.81	338	4.94	1.8	4.72	6.93	4.91	6.65	6.19	6.03	6.1
SFA	**Behenic acid**	C22:0	$C_{23}H_{46}O_2$	80.30	402	-	-	0.37	-	-	-	-	-	-
SFA	**Pentacosylic acid**	C25:0	$C_{25}H_{50}O_2$	75.9	382	1.03	-	0.70	-	-	-	-	-	-
SFA	**Lacceroic acid**	C32:0	$C_{32}H_{64}O_3$	94.40	496	-	-	-	1.99	1.77	2.03	-	-	-



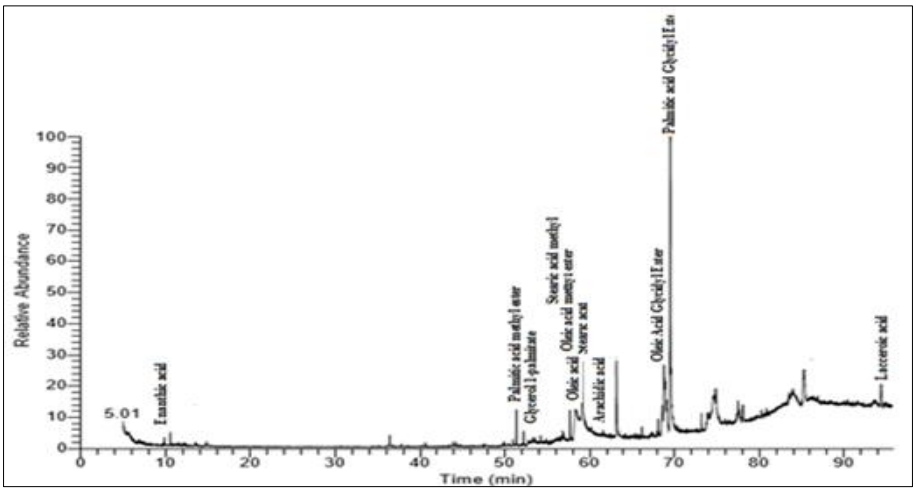
(1) ***Caulerpa racemosa***



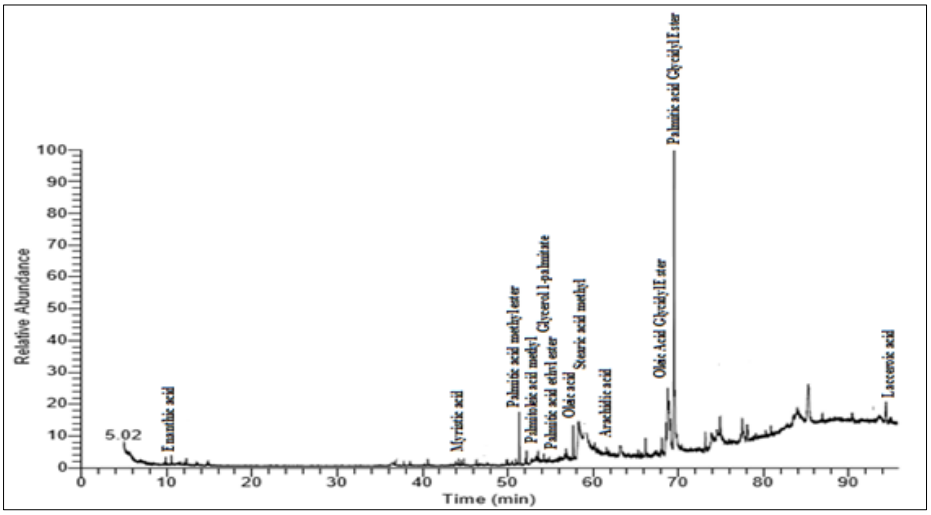
(2) ***Ulva fasciata***



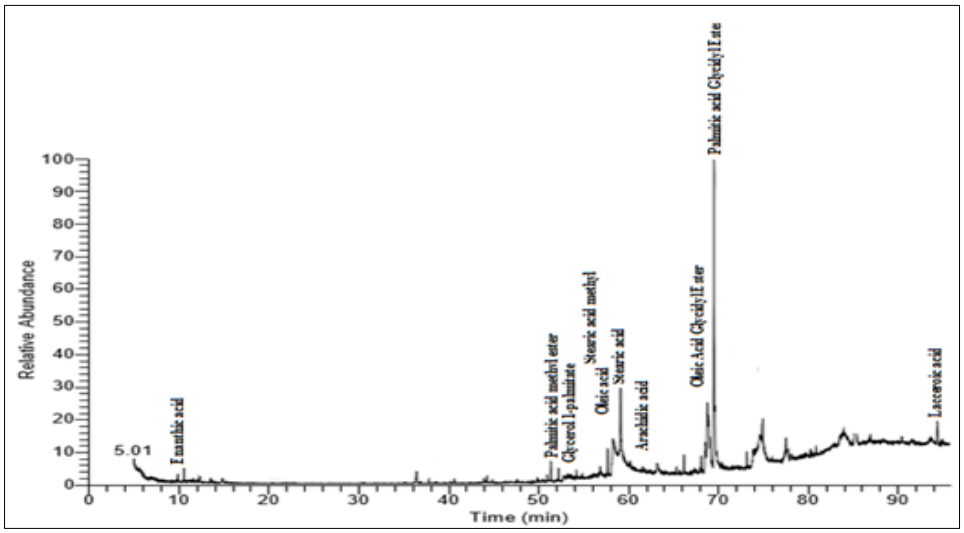
(3) ***Halimeda tuna***



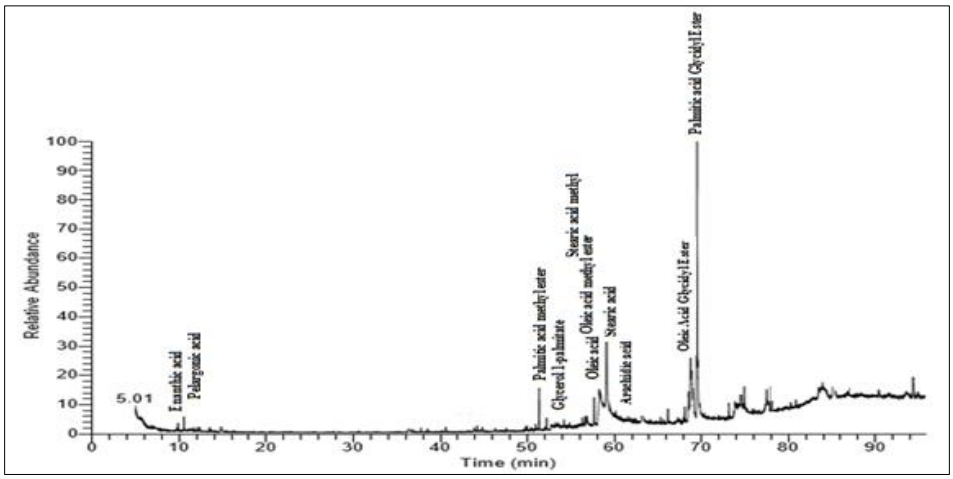
(4) ***Galaxura rugosa***



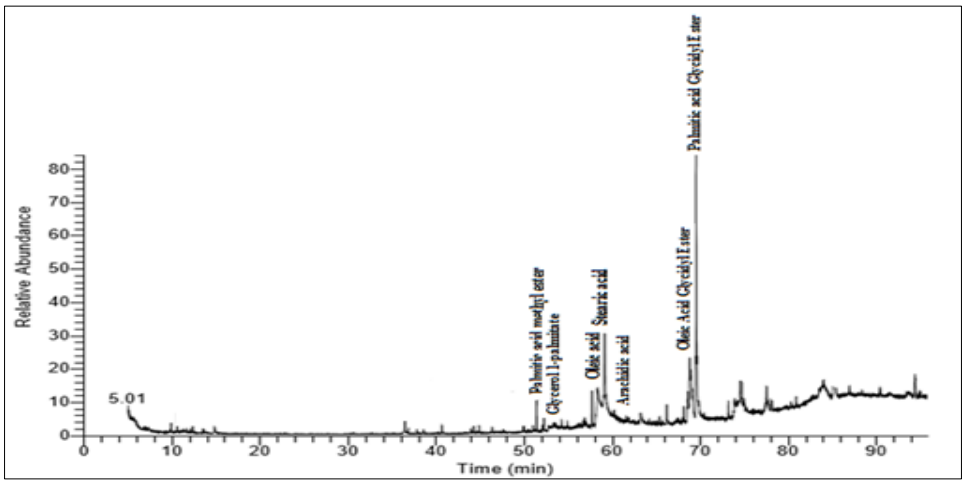
(5) ***Hypnea cornuta***



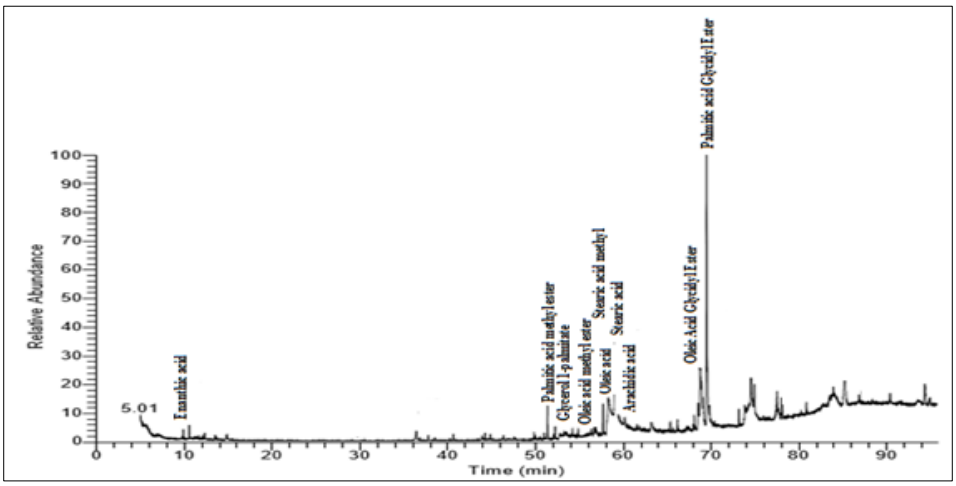
(6) ***Jania rubens***



(7) ***Turbinaria turbinata***



(8) ***Hormophysa cuneiformis***



(9) ***Polycladia myrica***

Figure 2 GC mass analysis of fatty acids in macroalga samples (1-9) showing Relative Abundance and Retention Time

MUSFA oleic acid (ω-9) was recorded in all the selected species except the green macroalgae *C. racemosa* and *U. fasciata*, while oleic acid methyl ester (ω-9) was registered in all studied species not including the red macroalgae *H. cornuta* & *J. rubens* and the brown macroalga *H. cuneiformis*. MUSFA linoleic acid methyl (ω-6) only found in *U. fasciata*. MUSFA linoleic acid (ω-6) characterized only in the green macroalgae *C. racemosa* and *U. fasciata* than the other species.

Percentage of fatty acids composition in the studied macroalgae divisions (Figure 3) was represented in Chlorophyta (35%), followed by Rhodophyta (33%), and finally Phaeophyta (32%). In general, the amounts of fatty acids types varied notably among the tested species. SFAs were constructed the most abundant fatty acids composition in the studied sample species representing 78%, while MUFAs were detected low values (22%). UFAs registered very low content considering neglected 0.01% (Figure 4).

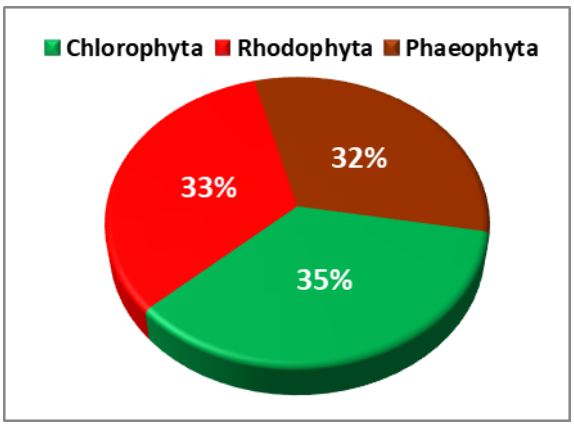


Figure 3 Percentage of fatty acids in the studied macroalgae divisions

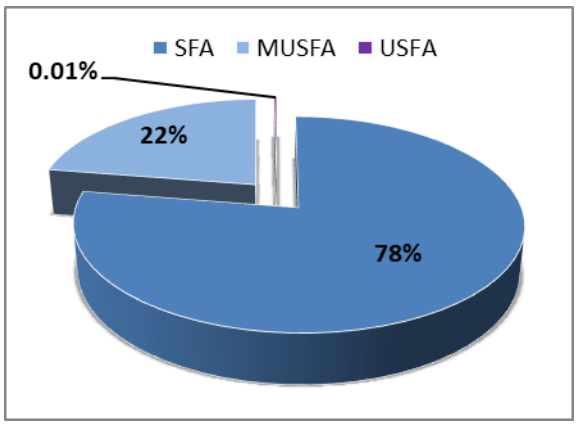


Figure 4 The amounts of fatty acids types (SFA, MUSFA and USFA among the tested species

The first major of SFAs found in all the selected species were palmitic acid glycidyl ester (180.94%)> oleic acid glycidyl ester (48.27%) > palimitic methyl ester (43.69 %) as shown in Figure 5. It constituted more than a half of the total fatty acids content. Palmitic acid glycidyl ester and oleic glycidyl ester mostly increased in red and brown macroalgae, while palimitic methyl ester was concentrated in green macroalgae as recoding in Table (1). The second major fatty acids fluctuated between the studied species namely, SFA stearic acid and MUSFA stearic acid methyl accounting 29% and 27.03% of total fatty acids composition respectively. These two fatty acids found in all samples except the red macroalga *H. cornuta* with stearic acid and the brown alga *H. cuneiformis* with stearic acid methyl. The Third major fatty acids were established as follows: oleic acid (17.73%) > oleic acid methyl ester (8.85%) > arachidic acid (8.07%) lacceroic acid (5.79%) > palmitic acid ethyl ester (5.04%) > glycerol 1-palmitate (3.31%). In addition to the major fatty acids in the different samples, linoleic acid, nonadecylic acid, elaidic acid methyl ester, linoleic acid methyl, behenic acid, pentacosylic acid, palmitic acid and trans, palmitioleic acid were characterized only in Chlorophyta. Lacceroic acid C32:0 was distinguished in Rhodophyta while pelargonic acid C9:0 just appeared in Phaeophyta in *T. turbinata*.

Figure 6 showed the total values of fatty acid*s* (concentration %) recording in the selected macroalgae divisions. In Chlorophytes group, the total fatty acid*s* was *C. racemosa* (56.74%) > *U. fasciata* (42.6%)> *H. tuna* (41.31%). Within Rhodophytes group, the values was *G. rugosa* (47.31%) > *J. rubens* (45.13%) > *H. cornuta* (37.28%). In Phaeophytes group, the total fatty acid*s* composed of *T. turbinata* (44.32%) > *P. myrica* (43.03%) > *H. cuneiformis* (38.79%). In general, the maximum values of fatty acid*s* were recorded in the green macroalga *C. racemosa* while the minimum one was the red macroalga *H. cornuta*.

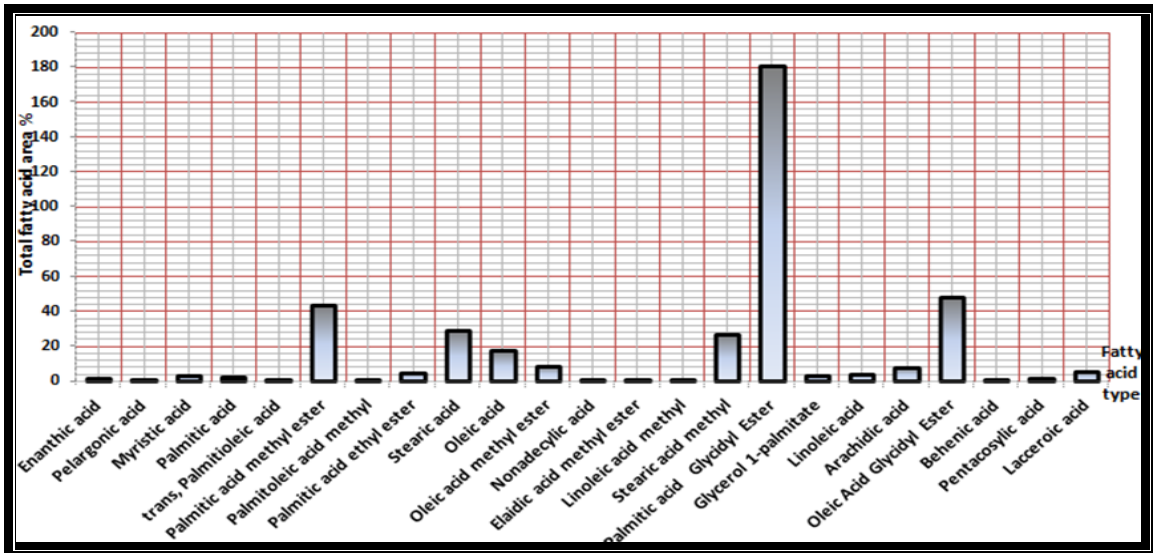


Figure 5 The area % of each fatty acid type in all the selected species

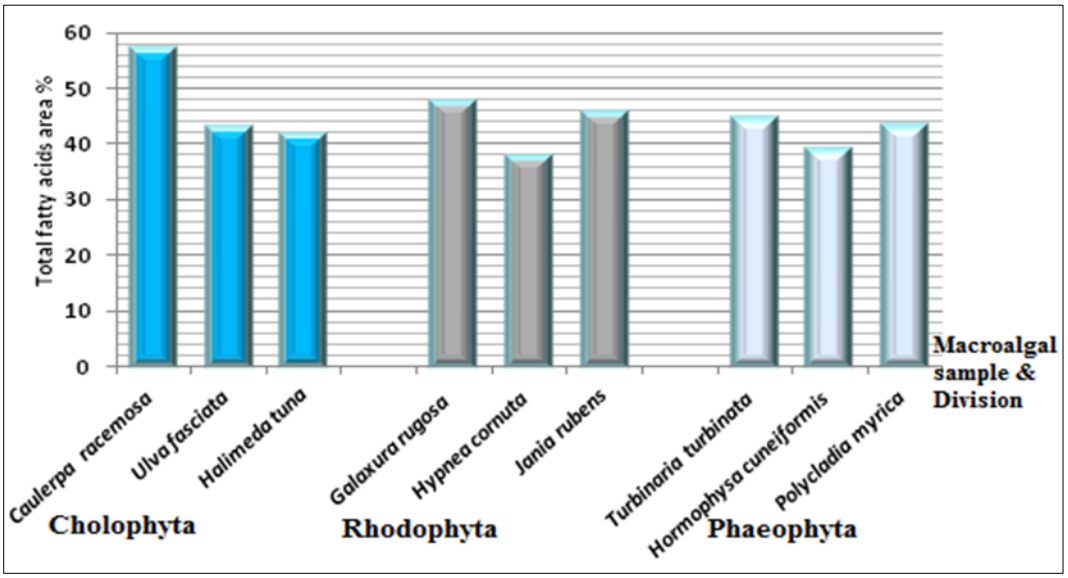


Figure 6 Total of Fatty acids Concentration (area %) in each macroalgal group

4. Discussion

The fatty acids content in the studied macroalgae divisions was almost the same but Chlorophyta was a substantial level, followed by Rhodophyta, and finally Phaeophyta. The selection of these species aims to encompass a wide range of macroalgal functional groups and provide valuable insights into their ecological diversity, distribution and importance along the Egyptian coasts. In general, the amounts of fatty acids types varied remarkably among the tested species. Fatty acids composition of algal lipids varies widely with species, habitat, light, salinity, pollution and environmental conditions [16].

In this study, SFAs were constructed the most abundant fatty acids composition in the studied samples representing 78%, than the MUFAs, whereas UFAs registered very low content considering neglected. The total values of fatty acid*s* were recorded as follows: *C. racemosa* > *G. rugosa* > *J. rubens* > *T. turbinata* > *P. myrica* > *U. fasciata* > *H. tuna* > *H. cuneiformis* > *H. cornuta*.

The present data reflect clearly distinguishable fatty acid profiles with high levels of SFA palmitic acid glycidyl ester, MUSFA oleic acid glycidyl ester and SFA palimitic methyl ester which found in all the selected species. It accounted more than a half of the total fatty acids content species. Palmitic acid glycidyl ester represented the first major SFA.

Palmitic acid glycidyl ester and oleic glycidyl ester mostly increased in red and browm macroalgae, while palimitic methyl ester was concentrated in green macroalgae. In most previous studies, palmitic acid is predominant in seaweeds [17- [18].

SFA Stearic acid and MUSFA Stearic acid methyl was considered the second major fatty acids composition found in the nine species except the red macroalga H. cornuta and the brown alga H. cuneiformis respectively. The Third major fatty acids were established in this way: oleic acid > oleic acid methyl ester > arachidic acid lacceroic acid > palmitic acid ethyl ester > glycerol 1-palmitate. Linoleic acid, nonadecylic acid, elaidic acid methyl ester, linoleic acid methyl, behenic acid, pentacosylic acid, palmitic acid and trans, palmitioleic acid were characterized only in Chlorophyta. Lacceroic acid was distinguished in Rhodophyta while pelargonic acid just appeared in Phaeophyta in T. turbinata. This is agreement with many studies which have demonstrated that fatty acid profiles were specific to taxonomic groups [19-22].

Oleic acid and Oleic acid methyl ester in the studied species were regarded as biosource to omega–9 (ω-9). Linoleic acid (MUSFA) is considered a particular potential for green *U. fasciata* as a source for the omega-6 (ω-9) [23]. El Shoubaky *et al*. [18] mentioned that *Ulva fasciata* characterized by containing high levels of the most biologically active fatty acids as Oleic acid and Linoleic acid.

The palmitic acid, palmitoleic acid and oleic acid, as well as their esters in macroalgae samples might represent a useful source as food or food supplement. Moreover, they exhibit strong antimicrobial activity against oral microorganisms as *Streptococcus mutans*, *Candida albicans*, *Aggregati bacter actinomycetem comitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. MUFAs derivatives, of C18 and C16 FAs, may aid in resisting many pathological conditions such as cardio-diseases and cancer [24-25]. Moustafa and Batran [26] mentioned that C18-PUFA acquire special importance in human nutrition and other vertebrates which are not able to synthesis them [27-28]. Erkkila *et al*. [29] stated that several studies have created inverse correlation between the PUFA/SFA ratios and cardiovascular diseases and suggested that replacement of SFA with PUFA in the human diet will decrease similar health problems. In this study, the ratio of SFA to MUSFA was found higher in all analyzed species and to be useful in cardiovascular diseases. So, these findings highlight the importance of fatty acid composition in macroalgae and its potential implications for human health and nutrition.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to disclosed.

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