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Comparative Phytochemical Investigation of Beneficial Essential Fatty Acids on a Variety of Marine Seaweeds Algae

[1]Gehan A. El-Shoubaky, [2]Amal M. Youssef Moustafa and [3]Essam Abd E. Salem
[1]Department of Biological and Geological Sciences, Faculty of Education, Suez Canal University, BP 41522, Port-Said, Egypt
[2]Department of Chemistry, Faculty of Science, Suez Canal University, BP 41522, Ismailia, Egypt
[3]Department of Botany, Faculty of Science, Suez Canal University, BP 41522, Ismailia, Egypt

Abstract: The methyl esters from the extracts of three green algae (*Enteromorpha intestinales*, *Ulva rigida* and *Ulva fasciata*) and one of red alga *Hypnea cornuta* were qualitatively and quantitatively analysed through GLC technique. A wide variety of saturated and unsaturated fatty acids were detected. Fourteen fatty acids in the four specimens of seaweeds registered namely, capric, lauric, tridecanoic, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, arachidonic and eicosapentanoic. The total sum of the recorded fatty acids increase in the order: *Enteromorpha intestinales* < *Ulva rigida* < *Ulva fasciata* < *Hypnea cornuta*, with percentage; 71.77, 73.05, 74.05 and 88.17%, respectively. *Hypnea cornuta* was characterized by the presence of arachidonic acid (Omega-6 $C_{20:4}$; 1.091%) and eicosapentanoic acid (Omega-3 $C_{20:5}$; 6.264%), while they disappeared from the other selected green algae specimens. The arachidic was existed only in *Ulva rigida* (1.10%) and *Hypnea cornuta* (0.88%). Palmitic content showed its maximum value 25.87% in *Enteromorpha intestinales*. Saturated fatty acids capric, lauric, tridecanoic and unsaturated myristoleic recorded their highest concentrations in *Ulva rigida*, with percentages 3.72, 6.57, 8.57 and 4.5%, respectively. *Ulva fasciata* characterized by containing the highest levels of the most biologically active fatty acids Omega-9 (oleic $C_{18:1}$; 8.03%), Omega-6 (linoleic acid $C_{18:2}$; 12.41%) and Omega-3 (linolenic acid $C_{18:3}$; 3.19%).

Key words: Seaweeds, essential fatty acids, Omega-6 and Omega-3

INTRODUCTION

The marine algae popularly known as seaweeds have attracted attention of many scientists due to their growing importance for various uses (Shehnaz, 2003).

Seaweed has little fat, ranging from 1-5% of dry matter, although seaweed lipids have a higher proportion of Essential Fatty Acids (EFAs) than land plants. Green algae, whose fatty acid make-up is the closest to higher plants, have a much higher oleic and alpha-linoleic acid content. Red algae have a high EFAs content, a substance mostly found in animals, especially fish (Usmanghani and Shameel, 2006).

There are two families of EFAs; Omega-3 and Omega-6. Omega-9 is necessary yet non-essential because the body can manufacture a modest amount on its own, provided EFAs are present. Omega-9 is mainly used when there is an insufficiency of Omega-3, Omega-6 or both. Omega-3 fatty acids are derived from linolenic acid, Omega-6 from linoleic acid and Omega-9 from oleic acid (Phinney *et al*., 1990).

Corresponding Author: Dr. Amal M. Youssef Moustafa, Department of Chemistry, Faculty of Science, Suez Canal University, BP 41522, Ismailia, Egypt
Tel: 002066-3727487, 0020107488306

Seaweeds can be a source of Polyunsaturated Fatty Acids (PUFA); Eicosapentaenoic (EPA), Arachidonic (AA), Gamma Linolenic (GLA, Omega-3) and Linoleic (Omega-6) acids (Ustun *et al*., 2005). Omega-3 fatty acids have recognized anti-inflammatory, antioxidant actions, that may contribute to their beneficial cardiac effects (Huang and Wang, 2004; Mozaffarian *et al*., 2005; Bemelmans *et al*., 2002). Essential fatty acids play an important role in the life and death of cardiac cells, the high percentage of unsaturated fatty acids might protect against Parkinson disease (de Lau *et al*., 2005). Omega-3 PUFAs from both seafood and plant sources may reduce Coronary Heart Disease (CHD) risk (Mozaffarian *et al*., 2005; Bemelmans *et al*., 2002). A higher ratio of Omega-6 to Omega-3 fatty acids is associated with lower bone mineral density at the hip in both sexes.

In the marine environment PUFA may provide the degree of desaturation needed to keep cell membranes fluid in cold water. Rather than genetically modifying terrestrial plants to produce eicosapentaenoic and docosahexanoic acid, marine algae can be cultured industrially to provide the fish oil while leaving the fish alone (Wen and Chen, 2003). The original source of the long chain Omega 3 fatty acids found in fish is, however, the chloroplasts of marine algae and phytoplankton at the bottom of the food chain (Nordoy and Dyerberg, 1989).

Lauric acid is a vital in the construction of cellular membranes and act as a source of food under starvation conditions (Siguel, 1996). EPA has been recognized as being effective in preventing arteriosclerosis (Bhaskar *et al*., 2004). While, palmitic and linoleic acids can inhibit insulin-stimulated eNOS activation (Wang *et al*., 2006).

Ulva species is one of the seaweeds consumed as a vegetable in many countries and among its nutritional benefits is richness in dietary fibre. As indigestible food polysaccharides can modulate metabolic and biological processes of the host through their biodegradation products in the colon (Bobin-Dubigeon *et al*., 1997).

The Ulvales (*Ulva lactuca* and *Ulava fasciata*), originated from Mediteranean Sea, Alxeaderia; Egypt, were characterized by highest levels of hexadeca-4, 7, 10, 13-tetraenoic acid (HDTA) and octadeca-6, 9, 12, 15-tetraenoic acid (ODTA) (Mabrouk *et al*., 2001). Also, the green algae *Ulva fasciata* from Japan showed strong algicidal activity, three algicidal compounds were isolated and whose structures were determined to be HDTA, ODTA and alpha-linolenic acid (Alamsjah *et al*., 2005). While, the fatty acid composition of *Ulva lactuca* originated from Marmara Sea, Turkey, presented high proportions of palmitic, palmitoleic, oelic, linoleic and conjugated linolenic acids (Ustun *et al*., 2005). It was reported that, both the green alga; *Ulva pertusa* and the red algae; *Gracilaria incurvata* and *Gracilaria lemaneiformis* contained high levels of palmitic acid (Wen *et al*., 2006).

The objective of this study was to determine the fatty acid composition of four seaweed species, originating from Mediterranean Sea, Egypt, three green algae; *Enteromorpha intestinales* (Liun.) Ness, *Ulva rigida* C. Agardh and *Ulva fasciata* Delile (family Ulvaceae) and one of red alga; *Hypnea cornuta* (Lamouroux) J. Ag. (family Hypneaceae) as a suitable source of PUFA. This study provides a good means for comparison of fatty acids content in the four investigated species, particularly Omega-3, Omega-6 and Omega-9 acids, in the formulation of highly unsaturated diet.

The Omega-9 family is necessary yet non-essential because the body can manufacture a modest amount on its own, provided essential EFAs are present. It does not need to be supplemented.

Sanchez-Machado *et al*. (2004) mentioned that, *Porphyra* contains the essential fatty acids $C_{18:2}$ Omega-3 (linolenic acid), $C_{20:4}$ Omega 6 (arachidonic acid) and $C_{20:5}$ Omega 3 (EPA).

MATERIALS AND METHODS

Plant Material

Three green algae (family Ulvaceae); *Enteromorpha intestinales* (Liun.) Ness, *Ulva rigida* C. Agardh and *Ulva fasciata* Delile and one of red alga; *Hypnea cornuta* (Lamouroux) J. Ag. (family

Hypneaceae) were collected by hand from the submerged marine rocks of El-Tafriaa district of Port Said area, Egypt, Mediterranean sea in low tide, in April-July 2006. Epiphytic and extraneous matter were removed by washing first in sea water and then in fresh water. The algae were transported to the laboratory in polyethylene bags at ice temperature. For convenient use of the samples, the seawater collected were air dried for five days at room temperature and cut into small pieces then ground to powder in a mixer grinder.

Extraction of Ether Extract

Thirty grams of dry powder of each alga were mixed homiletically with diethylether using a mixer grinder. The powder residue of algae was extracted separately three times with diethylether and the extracts were combined together. Diethyl ether fraction was dried with anhydrous sodium sulphate and then dried under reduced pressure, in rotatory evaporator.

Saponification of Ether Extract

The dry diethylether extract was dissolved in 10% KOH in 70% methanol and refluxed on water bath for four hours until the salts have been converted to acids (Moustafa *et al*., 2007). The saponified extract of each algae was evaporated under reduced pressure, in rotatory evaporator and then partitioned between aqueous and diethyl ether phase. This portioning procedure between diethyl ether and water was repeated for several times. The total combined diethyl ether fraction was acidified over anhydrous sodium sulphate and then concentrated under vaccum to leave a dark green oily residue (diethylether fraction No. 1).

Estimation of Fatty Acids

The alkaline mother liquor extract was acidified by adding sulphuric acid to obtain the free fatty acids. The acidic extract was then extracted with ether several times, to free the extract from any free fatty acids. Concentrate under vaccum to leave a deep green oily residue (diethylether fraction No. 2).

Free fatty acids present in ether fraction No.2 were subjected to the methylation according to Moustafa *et al*. (2007). The concentrated fatty acids were dissolved in methanol containing 3% HCl and refluxed on water bath for 1/2 h. The reaction mixture for each alga was diluted with water and was partioned between water and diethyl ether. The diethyl ether extract was washed several times with distilled water till neutrality. The diethyl ether layer was dried over anhydrous calcium chloride and concentrated to leave deep green oily residue; 0.0679, 0.0788, 0.0820 and 0.1380 g from *Enteromorpha intestinales*, *Ulva rigida*, *Ulva fasciata* and *Hypnea cornuta*, respectively. The obtained fatty acid methyl esters were subjected to gas liquid chromatographic analysis (PYE UNICAM Series 304 Gas Chromatograph equipment with FID and SGE injector split mode, in faculty of Agriculture, Cairo University, using OV-17 column (1.5 m×4 mm I.D., 0.2 μm thickness) packed with methyl phenyl silicone, programmed at 10°C min^{-1} from 70 to 270°C, injector temperature at 250°C, FID detector at 300°C and the flow rate of hydrogen is 30 mL min^{-1}.

The fatty acids fraction of the red alga *Hypnea cornuta* registered the highest weight percent 0.46% dry weight, followed by the three green algae *Ulva fasciata*, *Ulva rigida* and *Enteromorpha intestinales* recording 0.27, 0.26 and 0.23% dry weight, respectively.

RESULTS AND DISCUSSION

During this study, most of the investigated Egyptian seaweeds were phycochemically studied for the first time, except *Ulva fasciata*, which have previously investigated from the Mediteranean Sea, Alexandria, Egypt (Mabrouk *et al*., 2001). Table 1 and Fig. 1a-d showed both qualitative and quantitative analysis of fourteen fatty acids in the four selected specimens of seaweeds, including seven

Table 1: Fatty acids content of the selected seaweeds

Fatty acids	Samples *Enteromorpha intestinales*	*Ulva rigida*	*Ulva fasciata*	*Hypnea cornnta*
	Conc. %			
Capric $C_{10:0}$	1.82	3.72	2.06	1.36
Lauric $C_{12:0}$	2.07	6.57	2.61	2.17
Tridecanoic $C_{13:0}$	1.07	8.57	1.51	2.67
Myristic $C_{14:0}$	1.29	6.64	0.80	9.25
Myristoleic $C_{14:1}$	1.86	4.50	0.62	2.48
Palmitic $C_{16:0}$	25.87	17.98	19.38	22.13
Palmitoleic $C_{16:1}$	15.28	15.61	15.91	16.96
Stearic $C_{18:0}$	7.44	2.42	7.55	15.60
Oleic $C_{18:1}$	5.63	2.67	8.03	2.24
Linoleic $C_{18:2}$	7.99	2.78	12.41	0.82
Linolenic $C_{18:3}$	1.46	0.49	3.19	0.16
Arachidic $C_{20:0}$	-	1.10	-	0.88
Arachidonic $C_{20:4}$	-	-	-	1.09
Eicosapentanoic $C_{20:5}$	-	-	-	6.26
Total sum	71.78	73.05	74.07	84.07

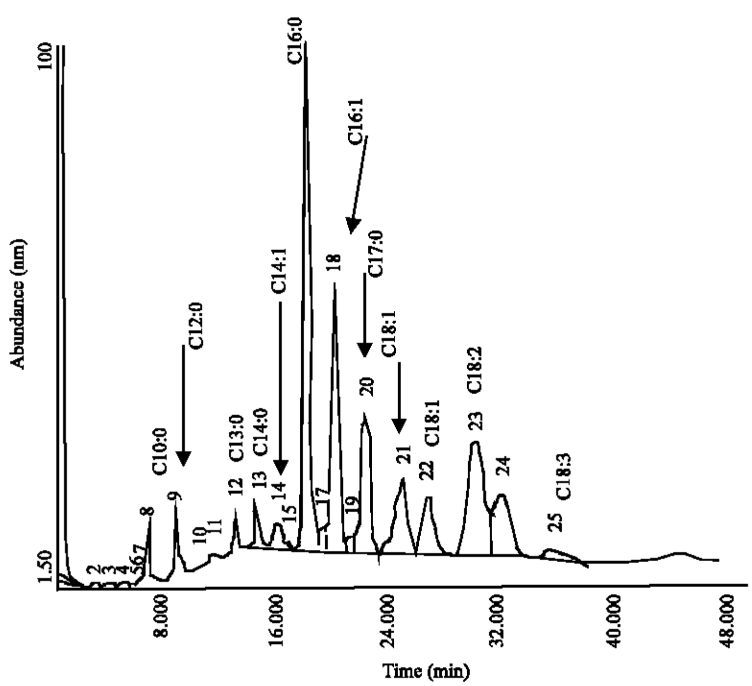

Fig. 1a: GLC results of methyl esters of fatty acids of *Enteromorpha intestinales*

Saturated Fatty Acids (SFA) and seven Unsaturated Fatty Acids (UFA). The UFAs, comprised of three monounsaturated; myristoleic, palmitoleic and oleic, one diunsaturated; linoleic, one triunsaturated; linolenic and two (PUFA); arachidonic and eicosapentanoic. Saturated fatty acids among the studied species recording the highest quantity, especially in *Hypnea cornuta* (54.06%), *Ulva rigida* (47.00%) and *Enteromorpha intestinalis* (39.56%) of total saturated fatty acids. These results were confirmed by Wen *et al*. (2006).

Almost the SFAs of the investigated species were found in a larger proportion (0.88-25.87%) than the USFAs (0.62-16.96%).

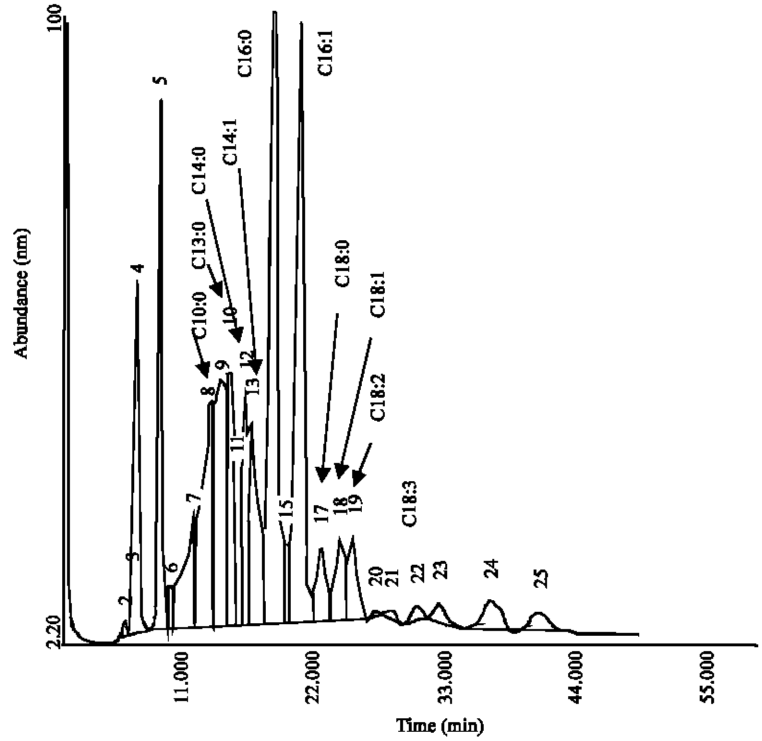

Fig. 1b: GLC results of methyl esters of fatty acids of *Ulva rigida*

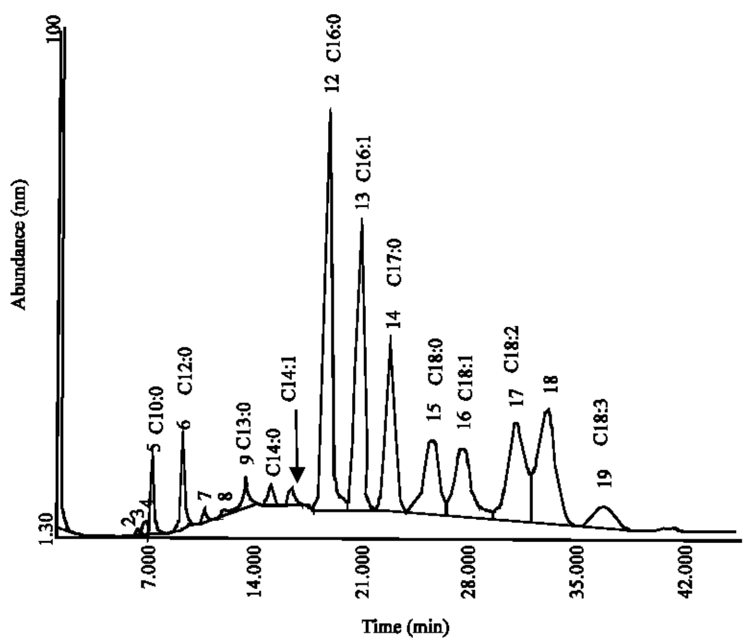

Fig. 1c: GLC results of methyl esters of fatty acids of *Ulva fasciata*

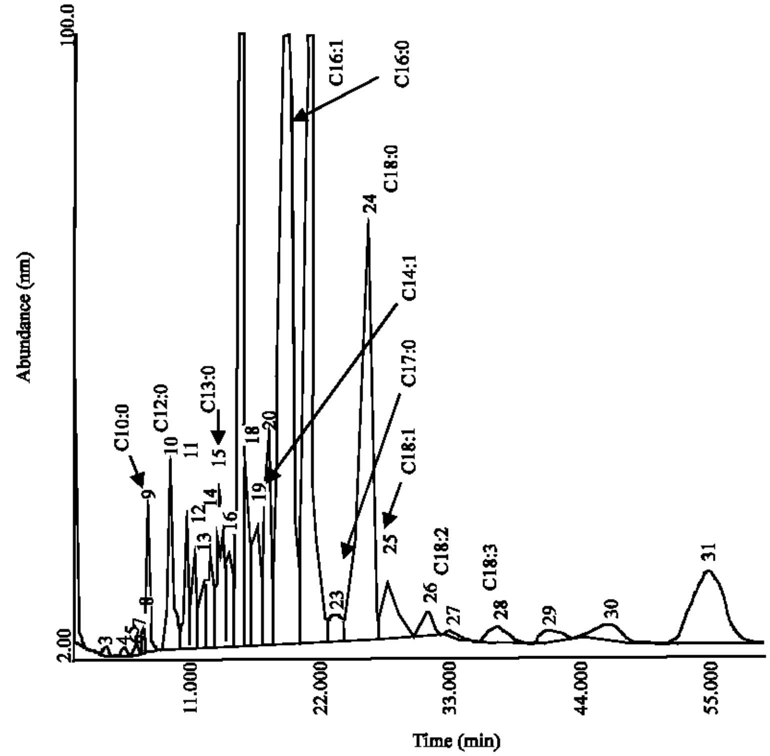

Fig. 1d: GLC results of methyl esters of fatty acids of *Hypnea cornuta*

Ulva fasciata characterized by containing the highest levels of the most biologically active fatty acids Oleic $C_{18:1}$, Omega-6 (linoleic Acid) and Omega-3 (linolenic acid) recording 8.03, 12.41 and 3.19%, respectively. While the other fatty acids; $C_{10:0}$, $C_{12:0}$, $C_{13:0}$, $C_{14:0}$ and $C_{14:1}$ recorded 2.057, 2.61, 1.51, 0.80 and 0.620%, respectively, saturated $C_{16:0}$; 19.375% and $C_{18:0}$; 7.550% and unsaturated $C_{16:1}$ and $C_{18:1}$ recorded 15.91 and 8.030%, Omega-6 (linoleic acid $C_{18:2}$; 12.41% and Omega-3 (linolenic $C_{18:3}$; 3.190%). These results are in accordance with that previously obtained from *Ulva fasciata* originated in Japan (Alamsjah *et al*., 2005) and almost similar results were obtained from the previously studies conducted on the marine benthic algae *Ulva lactuca* originated from Marmara Sea, Turkey (Ustun *et al*., 2005). While, *Ulva fasciata*, Mediteranean Sea, Alexandria, Egypt was previously detected and showed two unsaturated fatty acids hexadeca-4, 7, 10, 13-tetraenoic acid (HDTA) and octadeca-6, 9, 12, 15-tetraenoic acid (Mabrouk *et al*., 2001).

In *Ulva rigida*, saturated lipids registered the highest concentrations from $C_{10:0}$; 3.72%, $C_{12:0}$; 6.57% and $C_{13:0}$; 8.57% and unsaturated fatty acid $C_{14:1}$; 4.50%. The rest of the detected fatty acids recorded the following proportions: saturated $C_{14:0}$; 6.64%, $C_{16:0}$; 17.98% and $C_{18:0}$; 2.42% and $C_{20:0}$; 1.098%. While the unsaturated FA represented $C_{16:1}$; 15.61%, $C_{18:1}$; 2.67%, Omega-6 ($C_{18:2}$; 2.78%) and Omega-3 ($C_{18:3}$; 0.49%).

This indicates that, different species of the same genus may behave variably in their fatty acid composition and percentage. Similarly, the investigated two species of *Ulva* also exhibited differences in the percentages of FA composition.

Palmitic acid ($C_{16:0}$) was the most commouly occurring FA. It was detected in all the investigated species. It was found in predominant qnantity (17.98-25.87%).

Palmitoleic $C_{16:1}$ was observed to be the most common UFAs, it was detected in appreciable amount (15.28-16.96%), in the four species.

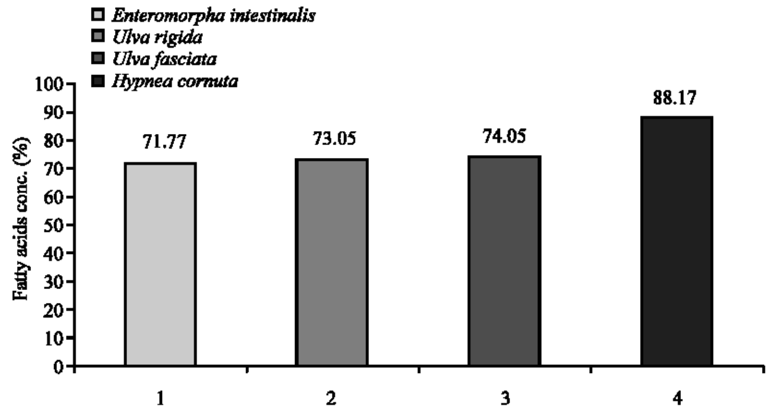

Fig. 2: The concentrations percentage of the total fatty acids of the selected species

The arachidic $C_{20:0}$ characterized by their existence in *Ulva rigida* and *Hypnea cornuta*, while disappeared from *Enteromorpha intestinales* and *Ulva fasciata*.

The total sum of fatty acids recorded increase in the order: *Enteromorpha intestinales* < *Ulva rigida* < *Ulva fasciata* < *Hypnea cornuta*, recording concentration percentage; 71.77, 73.05, 74.05, 88.17%, respectively (Fig. 2).

Fatty acid contents of *Enteromorpha intestinales* recorded low proportions of saturated $C_{10:0}$; 1.815%, $C_{12:0}$; 2.070%, $C_{13:0}$; 1.069%, $C_{14:0}$; 1.288% and low unsaturated $C_{14:1}$; 1.858%. High proportion of saturated $C_{16:0}$; 25.87% was recorded in comparison with the other specimens. Also contained $C_{18:0}$; 7.436% and unsaturated $C_{16:1}$; 15.28%, $C_{18:1}$; 5.63%, Omega-6 (linoleic $C_{18:2}$; 7.99%), Omega-3 (linolenic $C_{18:3}$; 1.46%).

The fatty acids content of red algae in *Hypnea cornuta* was characterized by the highest concentration percentage of $C_{14:0}$; 9.25%, unsaturated $C_{16:1}$; 16.96% and $C_{18:0}$; 15.60%. Also contained different proportions of $C_{10:0}$; 1.36%, $C_{12:0}$; 2.17%, $C_{13:0}$; 2.67%, $C_{14:0}$; 9.25%, $C_{14:1}$; 2.48%, saturated $C_{16:0}$; 22.13%, $C_{20:0}$; 0.88%. Omega-6 $C_{18:2}$; 0.82%, Omega-3 ($C_{18:3}$; 0.16%) and $C_{18:1}$; 2.24%. It also noticed that, *Hypnea cornuta* was characterized by the presence of Arachidomic acid (Omega-6 $C_{20:4}$; 1.09%) and Eicosapentanoic acid (Omega-3 $C_{20:5}$; 6.26%), while they disappeared from the other selected green algae specimens.

Low proportions of saturated fatty acids were obtained in *Enteromorpha intestinales*, *Ulva rigida* and *Hypnea cornuta*, while *Ulva rigida* recorded moderate increase of saturated fatty acids especially lauric acid in comparison with the other species.

In the present study, unsaturated fatty acids were found in larger proportion (46.65-74.3%) than the saturated ones (25.64-53.32%). The acids detected were mostly with 9-29 C atoms. Their compositions varied not only from genus to genus but also from species to species, the predominant acids also varied from species to species (Aliya, 1994).

The results of this study demonstrate that, three families of essential fatty acids were recorded: Omega-3 (linolenic $C_{18:3}$ and eicosapentanoic $C_{20:5}$), Omega-6 (linoleic $C_{18:2}$ and arachidonic $C_{20:4}$) and Omega-9 (oleic $C_{18:1}$). *Ulva fasciata* contained significantly higher amounts Omega -6 $C_{18:2}$ and Omega-3 $C_{18:3}$ than the other species.

This study recorded high concentrations of Omega-9 in *Ulva fasciata* and *Enteromorpha intestinales*.

Hypnea cornuta characterised only by eicosapentanoic acid and arachidonic acid as compared of these fatty acids in the other species of green algae. Similar conclusion was reported by Sanchez-Machado *et al*. (2004) and Bhaskar *et al*. (2004). So, we can conclude that, algal dry matter shows an interesting polyunsaturated fatty acid composition particnlarly regarding with Omega-3 and Omega-6 acids which play an integral role in over all health.

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