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Comparative Phycochemical Investigation of Hydrocarbons Content on Some Marine Seaweeds Algae

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Abstract: Isolated hydrocarbons from three green algae (*Enteromorpha intestinales*, *Ulva rigida* and *Ulva fasciata*) and one red alga *Hypnea cornuta* were qualitatively and quantitatively analysed using Gas-Liquid Chromatography (GLC) technique. Eighteen hydrocarbons in the four specimens of seaweeds namely; dodecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, hencosane, docosane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane and triacontane were registered. *Ulva fasciata* was characterized by the presence of triacontane (73.38%) while it disappeared from the other selected algae specimens. Docosane was found in all selected algae with high percentages; *Enteromorpha intestinales* (39.99%), *Hypnea cornuta* (32.17%), *Ulva fasciata* (16.22%) and *Ulva rigida* (10.61%). Octacosane content showed its maximum value 83.03% in *Ulva rigida*. Dodecane recorded its highest concentration in *Enteromorpha intestinales* (31.11%). The data analysis indicates that the selected species are polluted samples and can be used as bioindicators of pollution.

Key words: Seaweeds, hydrocarbons, docosane, triacontane bioindicators

INTRODUCTION

Seaweed is suitable for human and animal feed, as well as for fertilizer, fungicides, herbicides and phycocolloids (algin, carrageenan and agar). *Enteromorpha* as green seaweeds has great potential for commercial exploitation because of its abundant and varied chemical composition, qnality and concentration of basic nutriments for other living organisms (Chapman and Chapman, 1980). This genus is harvested to prepare aonori, which is included in a great variety of dishes, including raw salads, soups, cookies, meals and condiments (Ohono and Critchley, 1993).

The fatty acid content of *Enteromorpha* species (sp.) was high relative to other edible foods like soybeans and beans. EPA (n-3) and DHA (n-3) are greater than other types of seaweed, such as *Porphyra* sp., *Laminaria* sp. and *Ulva* sp. and vegetables, such as spinach and lettuce (El-Shoubaky *et al*., 2007).

Enteromorpha sp. contains 28 times more Ca than spinach, 26 times more than nopal and 13 times more than quelite (Aguilera-Moralesa *et al*., 2005).

The green algae genus *Enteromorpha intestinalis* emits isoprene and a number of other non-methane hydrocarbons. Isoprene is important in controlling the balance of atmospheric oxidants and altering air qnality on both local and global scales (Broadgate *et al*., 2004; Plettner *et al*., 2005).

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During the last decade, our understanding of how marine plants and animals use secondary metabolites as defenses against consumers and competitors has expanded tremendously reviewed by Steinberg (1989). The secondary metabolites diterpenoid alcohols (dictyol E and pachydictyol A) are among the compounds found on the brown seaweed *Dictyota menstrualis* surface. These compounds had a function as chemical defenses against fouling and could select for larvae that avoid hosts producing these metabolites (Tim *et al*., 2004; Greg *et al*., 2004).

Green algae belonging to Ulvales are frequently involved in algal proliferation in eutrophicated coastal and lagoon waters. Ways to use the latter biomass besides compost, methane production or paper making could be based on specific properties of their cell-wall polysaccharides. In edible Ulvales, cell-wall polysaccharides play a nutritional role as dietary fibre and from different genera; they demonstrate biological activities or gelling abilities (Lahaye, 1998). Also, cell-wall polysaccharides act as inclusion complexation agents and retain heavy metals (Gaszo, 2001). Therefore several *Ulva* and *Enteromorpha* species are used as bioindicators of pollution.

Much has been written about hydrocarbons which enter the enviroument due to pollution and rather little is known about biogenic sources of hydrocarbons. The limited existing data indicate great ecological significance of the hydrocarbon compounds of hydrobionts. Marine organisms are capable of accumulating oil hydrocarbons in their body. This fact provides material for studying biogeochemical pathways in the transformation of these compounds and criteria for assessing oil pollution and measures for biomonitoring. Consequently, knowledge of natural hydrocarbon levels in marine organisms is a basic prerequisite for evaluating oil pollution (Mironov *et al*., 1981). According to Farrington and Meyers (1975), the hydrocarbon content varies from 1 to 545 $\mu g\ g^{-1}$ wet weight in polluted samples.

Ben-Amotz *et al*. (1985) mentioned that the green colonial alga *Botryococcus braunii* has been widely studied because of its high hydrocarbon content of 15 to 75%, which is conspicuously higher than that commouly observed in other unicellular algae (ca. 0.1-10%). The halophilic species *Dunaliella salina* besides *Botryococcus braunii* was the ouly other hydrocarbon rich algae known at the present time (Tornabene *et al*., 1980). Sheehan *et al*. (1998) reported the existence of numerous algal forms that can be suitably used for bio-diesel production. The biomass can be efficiently converted to bio-diesel and utilized for internal combustion engines. One attractive feature of algal biomass over conventional higher plant biomass is that algal production rates can be more than 5 times those of land plants.

In *Ulva* sp. and two species of *Enterornorpha*, n-pentadecane was most typical. Unbranched alkanes were identified in *Ulva rigida* from C_{14} to C_{22} with n-C_{17} predominance. *Ulva thallomes* from polluted areas contained low-boiling hydrocarbons C_{11} to C_{13} as well as the isoprenoides i-C_{18}, i-C_{19} (Mironov *et al*., 1981).

In *Ulva rigida*, hydrocarbon content of alga is contained paraffins n-C_{13} to n-C_{24} with n-C_{15} predominance. The hydrocarbons in *Enteromorpha intestinales* are represented by n-alkanes C_{17} and C_{19}. Polluted *Enteromorpha intestinales* contained paraffins n-C_{14} to n-C_{24} with n-C_{15} predominance (Mironov *et al*., 1981).

The aim of this study was to compare the hydrocarbons content qualitatively and quantitatively of the selected algal species, namely *Enteromorpha intestinales*, *Ulva rigida*, *Ulva fasciata* and *Hypnea cornuta* and detect the polluted species contradiction.

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, filtered and then dried under reduced pressure, by rotatory evaporator.

Saponification of Ether Extract

The dry diethylether extract was dissolved in 10% alcoholic KOH 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, by rotatory evaporator and then partitioned between aqueous and diethyl ether phases. 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 vacuum to leave a dark green oily residue of hydrocarbon fraction for the selected species.

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 vacuum to leave a deep green oily residue (fatty acids fraction).

The obtained hydrocarbon fractions were subjected in duplicate 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 identification of the chemical constituents was based on comparisons of their relative retention times with those obtained from authentic samples.

RESULTS AND DISCUSSION

Through this study, most of the selected Egyptian seaweeds were phycochemically studied for the first time. Table 1 and Fig. 1a-d represented both qualitative and quantitative GLC aualysis of eighteen hydrocarbons in four selected specimens of seaweeds.

By comparing the data obtained from the GLC analysis of hydrocarbons, we find that, docosane was the most commouly occurring hydrocarbons. It was detected in all the investigated species and found in predominant quantity, ranged from 10.61 to 39.99%. These data are not detected before.

Also, heptadecane was observed to be one of the most common hydrocarbons; it was detected in appreciable amount in *Hypnea cornuta* (26.38%) as mentioned in Mironov *et al*. (1981) and it was represented in small amount in the other selected species (0.23-3.94%), this is in contradiction with Mironov *et al*. (1981).

It also noticed that, dodecane, tetradecane and pentadecane were characterized by their existence in *Enteromorpha intestinales* and *Hypnea cornuta*, this is in accordance to the polluted samples of *Enteromorpha intestinales* while they disappeared from the selected *Ulva* species. The red alga *Hypnea cornuta* and the green alga *Enteromorpha intestinales* recorded high content of eicosane C_{20} (diterpenes) (4.14 and 3.96%) than the other *Ulva* sp., this detection not mentioned before in

Table 1: Isolated hydrocarbons from selected seaweeds using GLC analysis

		Enteromorpha intestinales		*Ulva rigida*		*Ulva fasciata*		*Hypnea cornuta*	
Components	Carbon No.	RT (min)	RA (%)	RT (min)	RA (%)	RT (min)	RA (%)	RT (min)	RA (%)
Dodecane	$C_{12:0}$	4.600	31.11	-	-	-	-	5.550	6.04
Tetradecane	$C_{14:0}$	8.950	0.58	-	-	-	-	9.217	0.13
Pentadecane	$C_{15:0}$	10.283	2.36	-	-	-	-	10.467	0.40
Hexadecane	$C_{16:0}$	12.933	4.33	-	-	-	-	12.117	0.30
Heptadecane	$C_{17:0}$	13.433	3.94	13.867	0.23	13.017	1.77	13.083	26.38
Octadecane	$C_{18:0}$	14.950	5.11	14.567	0.52	14.950	1.77	14.983	3.76
Nonadecane	$C_{19:0}$	15.517	6.57	15.150	0.37	15.533	2.79	15.550	2.73
Eicosane	$C_{20:0}$	16.617	3.86	16.233	1.36	16.650	2.61	16.600	4.14
Hencosane	$C_{21:0}$	17.583	0.27	17.200	0.25	17.600	0.09	17.600	0.40
Docosane	$C_{22:0}$	19.083	39.99	18.450	10.61	18.850	16.22	18.917	32.17
Tricosane	$C_{23:0}$	-	-	19.867	0.38	-	-	19.733	2.03
Tetracosane	$C_{24:0}$	20.583	0.25	20.467	1.47	20.583	0.57	20.517	0.79
Pentacosane	$C_{25:0}$	-	-	21.200	1.26	21.600	0.32	21.783	0.34
Hexacosane	$C_{26:0}$	-	-	23.850	0.52	23.733	0.28	23.417	0.06
Heptacosane	$C_{27:0}$	-	-	-	-	24.583	0.30	24.517	0.21
Octacosane	$C_{28:0}$	25.600	0.92	25.417	83.03	-	-	25.733	20.12
Nonacosane	$C_{29:0}$	29.300	0.71	-	-	-	-	-	-
Triacontane	$C_{30:0}$	-	-	-	-	26.217	73.38	-	-

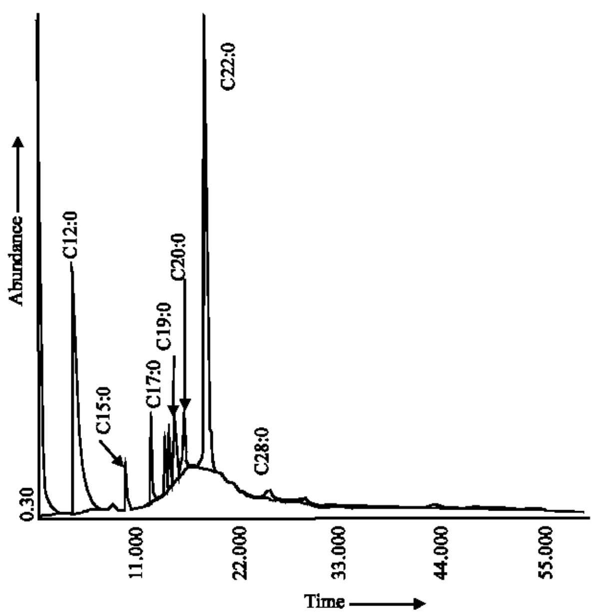

Fig. 1a: GLC results of hydrocarbons of *Enteromorpha intestinales*

literature. *Enteromorpha intestinales* contained the highest content of pentadecane C_{15} (2.36%) than *Hypnea cornuta* (0.40%) while the other two *Ulva* sp. devoided of C_{15}. These data are in accordance with Mironov *et al.* (1981). Seaweeds are known to contain appreciable quantities of plant growth regulators (Mooney and Van Staden, 1985), IAA C_{15} (Abe *et al.*, 1972), gibberllins and gibberllin like substance C_{20} (Sekar *et al.*, 1995).

Hydrocarbons content of *Enteromorpha intestinales* recorded high proportion of $C_{12:0}$, $C_{16:0}$, $C_{18:0}$, $C_{19:0}$ and $C_{22:0}$ represented 31.11, 4.33, 5.11, 6.57 and 39.99%, respectively. Low proportions of $C_{14:0}$ to $C_{28:0}$ (0.25 to 3.94%) were recorded in comparison with the other specimens.

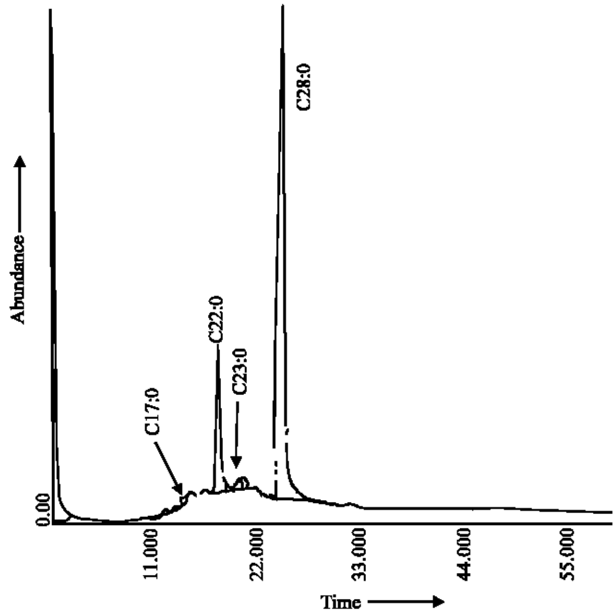

Fig. 1b: GLC results of hydrocarbons of *Ulva rigida*

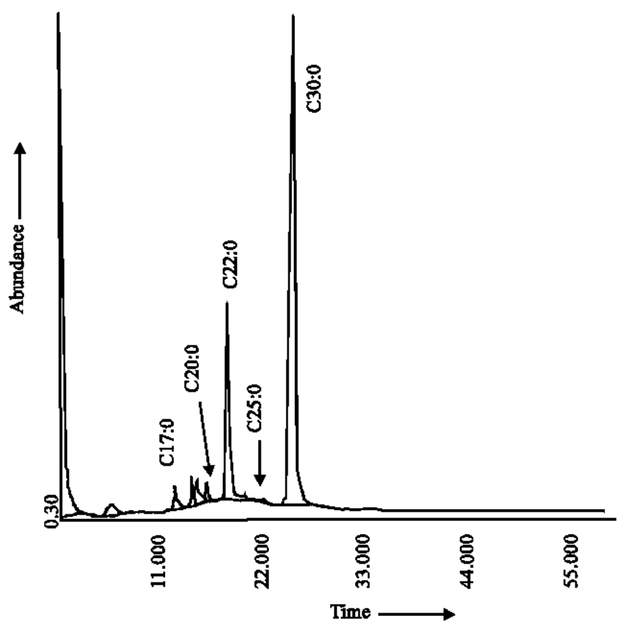

Fig. 1c: GLC results of hydrocarbons of *Ulva fasciata*

The hydrocarbons content of red algae in *Hypnea cornuta* was characterized by the highest concentration percentage of $C_{22:0}$, $C_{17:0}$ and $C_{28:0}$ represented 32.17, 26.38 and 20.12%, respectively; these data are detected for the first time. Also contained different proportions of $C_{12:0}$ to $C_{27:0}$ ranged from 0.06 to 6.04%. *Hypnea cornuta* contained the highest concentration percentage of heptadecane C_{17} (26.38%) in compared with the other studied species and high concentration percentage of

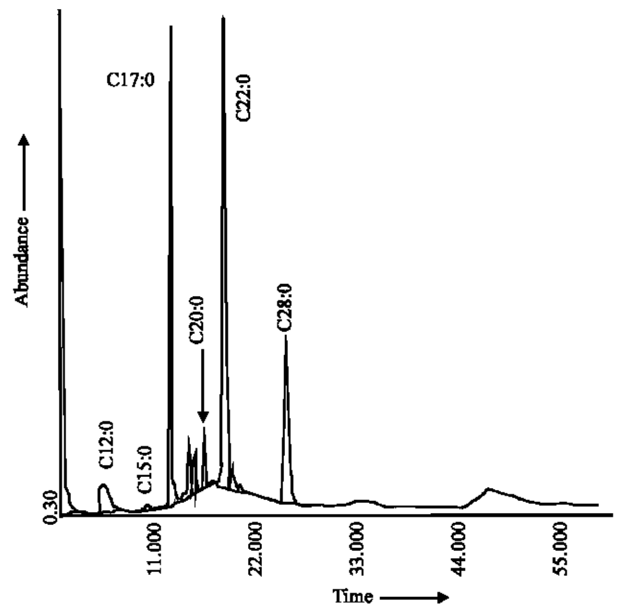

Fig. 1d: GLC results of methyl esters of fatty acids of *Hypnea cornuta*

docosane (32.17%). As Clark and Blumer (1967) stated, n-heptadecane was most typical for red algae. Normal alkanes of carbon number less than C_{14} and more than C_{28} are rarely present to any appreciable extent in blue green algae and green algae. In this concern, Jerry and Melvin (1969) mentioned that the normal heptadecane is the predominant compound in the hydrocarbon fraction of all the photosynthetic microorganisms.

Ulva fasciata characterized by containing the highest levels of triacontane recording 73.38%. While the percentages of the other hydrocarbons; $C_{17:0}$ to $C_{28:0}$ ranged from 0.09 to16.22% (Table 1). These data were detected for the first time.

In *Ulva rigida*, octacosane registered the highest concentration; 83.03%. The rest of the detected hydrocarbons ($C_{17:0}$ to $C_{26:0}$) recorded the proportions 0.23 to 10.61%. These data were supported with Mironov *et al*. (1981). This indicates that, different species of the same genus may behave variably in their hydrocarbons composition and percentage. Similarly, the investigated two species of *Ulva* also exhibited differences in the percentages of hydrocarbons composition.

As recorded by El-Shoubaky *et al*. (2007), the fatty acids fractions weight of the selected species represented; 2263.3 μg g^{-1} (0.23%), 2626.7 μg g^{-1} (0.26%), 2733.3 μg g^{-1} (0.27%) and 4600 μg g^{-1} (0.46%) from *Enteromorpha intestinales*, *Ulva rigida*, *Ulva fasciata* and *Hypnea cornuta*, respectively.

The hydrocarbon fraction of the *Hypnea cornuta* registered the highest weight 3010 μg g^{-1} (0.30%) dry weight, followed by the three green algae *Ulva fasciata*, *Enteromorpha intestinales* and *Ulva rigida* recording 1240 μg g^{-1} (0.124%), 1203 μg g^{-1} (0.120%) and 826.7 μg g^{-1} (0.083%) dry weight, respectively. According to Farrington and Meyers (1975) Lahaye (1998) and Gaszo (2001), the selected species *Hypnea cornuta*, *Enteromorpha intestinales* and *Ulva* species are polluted samples.

CONCLUSIONS

We can conclude that, algal dry matter shows interesting hydrocarbons composition particularly regarding with $C_{12:0}$, $C_{17:0}$, $C_{18:0}$, $C_{22:0}$, $C_{28:0}$ and $C_{30:0}$ which play an integral role in over all health. The detected hydrocarbons were mostly with 12-30 carbon atoms. Their compositions varied not only from genus to genus but also from species to species, the predominant hydrocarbons also varied from species to species. *Hypnea cornuta* contained the highest number of hydrocarbons (16 compounds), whereas the other selected three species contained 11 to 13 compounds for each of the other selected species. *Ulva* species were characterized by absence of hydrocarbons below C_{17}.

The following data were recorded for the first time; the high concentrations of triacontane were recorded in *Ulva fasciata*. *Hypnea cornuta* characterised by $C_{17:0}$ and $C_{22:0}$ as compared of these hydrocarbons with the other two studied species of green algae. *Hypnea cornuta* and *Enteromorpha intestinales* recorded high content of eicosane Docosane was detected in all the investigated species in predominant quantity. *Enteromorpha intestinales* registered the highest concentration percentage of docosane (39.99 %). Dodecane, tetradecane and pentadecane were represented only in *Enteromorpha intestinales* and *Hypnea cornuta* with different concentration percentage levels.

From present data and according to marine organisms are capable of accumnlating oil hydrocarbons in their body. So El-Tafriaa district of Port Said area, Egypt, Mediterranean Sea need further investigation of studying biogeochemical pathways in the transformation of these compounds and criteria for assessing oil pollution and measures for biomonitoring. El-Tafriaa district of Port Said port is located in a distinguished spot on the east of the northern entrance of Suez Canal branch in the meeting point of three continents and at the crossroad of the most important world sea trade route between the East and the west. Its northern boundary is the Mediterranean Sea, its southern boundary is the Industrial Zone, its eastern boundary is the Malaha Lake and its western boundary is the Suez Canal branch, within the geographical boundaries of Port Said Governorate. A lot of Ships arrive to East Port Said Port daily leaving oil waste. During a typical visit, a cruise ship can spew as many diesel particles as a few thousand heavy-duty trucks, according to port calculations. Even when docked, cruise ships typically run diesel engines to power air conditioners, washing machines and refrigerators. Cruise ships, however, are just one contributor to the long-overlooked pollution of east Coast port. Consequently, knowledge of natural hydrocarbon levels in marine organisms is a basic prerequisite for evaluating oil pollution (Mironov *et al*., 1981). According to Zhu *et al*. (2007), the oil hydrocarbons were adsorbed onto the surface of the seaweeds algae. So, the selected species *Hypnea cornuta*, *Enteromorpha intestinales* and *Ulva* species are polluted samples and can be used as bioindicators of pollution.

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