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# STUDIES ON FATTY ACIDS AND STEROLS CONTENT OF SOME CHLOROPHYCEAN MEMBERS.

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## Abstract

In this work some major marine green algal taxa and the fresh water *Chara* species were selected and subjected to sterols and fatty acid analyses. Fatty acid analyses indicated that most of the extracted acid fractions were of the polyunsaturated ones (C16, C18 and C20) with some unusual fractions of C17 and C19. Fatty acids as a taxonomic tool gave a particular picture for each investigated taxa that characterized each member. High similarity matrices were observed between *Dunaliella salina*, *Chlorella salina* and *Enteromorpha compressa*. Sterol data indicated that most of the tested algae contained $\Delta^5$, $\Delta^{5,22}$, $\Delta^7$sterols, peptosterol and the saturated nucleus campestanol. Sterol analyses showed clear variations between genera of the different orders: spinasterol was detected only in Ulvales while 5 β cholestanol and 5 β cholest-7-en- β -ol in Chlorococcales and 5, 22, 24 cholestatrien -3 β -ol was detected only in Charales. In general view, data of sterols and fatty acids indicated that *Chara* species could be placed among green algae.

## *Introduction*

In respect of morphology, green algae include nearly all thallus forms ranging from unicellular to large thalloid forms. The taxonomy of the green algae has been lastely clarified by applying biochemical and physiological characters (Klessler, 1984). Lipid constituents, most frequently studied in green algae are fatty acids (Erwin, 1973, Attavian, *et al*., 1977) and sterols (Volkman, *et al*., 1980 and Mohammady, *et al*. 2000). Chemotaxonomic survey of sterols and fatty acids in six marine raphidophyte algae was investigated by Marshall, *et al*. (2002).

Sterols in plants are considered mainly membrane components (Izzo, *et al*. 1988). Sterols composition of algae has been considered by some authors as potential taxonomic criterion for marine microalgae (Fabregas, *et al*., 1997), for dinoflagellates (Robinson, *et al*., 1987), for chrysophytes (Granwell, *et al*., 1988), for green algae (Belanger, *et al*., 1973, Granwell, *et al*., 1990 and Mohammady, *et al*. 2000), for diatoms (Volkman and Hallegraeff, 1988) and for blue green algae (Kohlhase and Pohl, 1988).

There is much variety in the sterol constituents of Chlorophyta in which a range of $\Delta^5$ -$\Delta^7$ and $\Delta^{5,7}$ sterols with methyl or ethyl constituents at C24 have been detected as major sterols (Goad, 1978 and Akihisa, *et al*. 1991). In order Chlorococcales $\Delta^7$ and $\Delta^{7,22}$ were the dominant sterols detected in *Scenedesmus*

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*quadricauda*, *Ankistrodesmus*, *Oocystis*, *Selenastrum* and *Chlorella* by Granwell, *et al*. (1990). Furthermore, species of *Dunaliella* were characterized by complex distribution of $\Delta^5$, $\Delta^7$,$\Delta^{5,7}$ sterols while *Eudorina* contains both $\Delta^5$ and $\Delta^7$sterols only (Granwell, *et al*. 1990).

Fatty acids are also important constituents of algae where they are located principally in the cell membrane. They have different valuable functions on algae and have value for taxonomic purposes (Stefanov, *et al*., 1988). Systematic variations of fatty acids have been studied by many investigators (Nicholas, 1970, Jamieson and Reid, 1972, Abd El-Salam 1997 and Mohammady, *et al*. 2002). Fatty acids provide more useful information about the classification of Phaeophyta, Rhodophyta and Chlorophyta (Jamieson and Reid, 1972) and in fresh water Chlorophyta (Granwell, *et al*., 1990). As criteria for chemotaxonomy, Volkman, *et al*. (1991) suggests that polyunsaturated fatty acids C18:4, C20:5 and C22:6 comprised 83 – 90 % of the total fatty acids in each of the four species of genus *Pavlova*. However, distribution of unusual polyunsaturated fatty acid fraction C16:4 in some species of green algae analyzed by Dembitsky, *et al*. (1991) deserve special attention and investigation. Also, C20 polyunsaturated fatty acids are the characteristic fatty acids in many members of Rhodophyceae, Phaeophyceae, Chrysophyceae, Haptophyceae, Bacillariophyceae, Xanthophyceae and Chlorophyceae (Wood, 1974 and Arao, *et al*. 1987).

Fatty acid composition of six arctic and fourteen Antractic macroalgae species belonging to Rhodophyta, Phaeophyta and Chlorophyta were investigated by Graeve, *et al*. (2002).

The aim of the present work is concentrated on the analysis of sterols and fatty acid composition in some members belonging to chlorophycean orders as a means for their use as taxonomic tools.

## *Materials and Methods*

### The biological materials

The biological materials (following the classification proposed by Bold and Wynne, 1978) were four species of Ulvales (*Ulva lactuca*, *U. fasciata*, *Enteromorpha intestinalis* and *E. compressa*), two species of Cladophorales (*Cladophora graminea* and *C. albida*), five species of Caulerpales (*Caulerpa prolifera, C. racemosa*, *Codium dichotomum, C. tomentosum* and *C. elongatum*) and two species of Siphonocladales (*Cladophoropsis membranacea* and *Valonia utricales*). They were collected from different localities from Ras-Elten to Abu-Kir along the Mediterranean Sea shore of Alexandria at 2001.

The Volvocales member was the unicellular green alga *Dunaliella salina*, isolated from brine water of salty lagoons at El-Max district (Alexandria). It was purified and identified using the key of Masjuk (1972). The Chlorococcales member *Chlorella salina* was obtained from the Institute of Oceanography and

Fisheries at Alexandria (ARE), while *Chara fragilis* was collected from Mariut Lake.

For collection of marine algae visits were made to each locality of the Mediterranean Sea shore and healthy specimens were picked out, washed several times by sea water to remove sand particles, epiphytes and then taken to the laboratory in plastic bags immersed in water. The herbarium specimens of late professor Nasr (late Professor of Phycology, Faculty of Science, Alexandria University) were mainly used for identification of the collected taxa.

**Lipid extraction and identification of fatty acids**

Total lipid was extracted from algal cells with chloroform methanol (2:1). The chloroform layer containing the total lipid was evaporated to dryness and saponified by boiling for 2 hours in 50 ml of 2M NaOH in 50% ethanol (Dembitsky, *et al*., 1991). The saponifiable fractions were converted into fatty acid methyl esters (FAME) using the procedure applied by Radwan (1991). FAME were identified on a Shimadzu gas liquid chromatography (GLC), equipped with a flame ionization detector with packing column material Hp-5. The carrier gas was nitrogen and the short speed was 5mm/min. Identification of each fatty acid was carried out by comparing its retention times with those of standards. The percentage of concentration was based on the internal standard method.

**Isolation, purification and identification of sterols**

The unsaponifiable matter was extracted according to Ramadan and Morsel (2002). The unsaponifiable matter was extracted three times with 10ml of petroleum ether; the extracts were combined and washed three times with 10ml of ethanol/water (1:1, v/v) mixture, then dried over anhydrous sodium sulphate. The combined extract was evaporated in a rotary evaporator at 25 ˚C under reduced pressure; the residual ether was then completely evaporated under nitrogen.

Sterols were isolated according to Nadal (1971) and purified using thin layer chromatography (TLC) plastic sheet of precoated silica gel. The gel layer is 250 m thick. The sterols were analyzed by direct injection into GLC-4 cm Schimadzu coated with 30% SE-30. The carrier gas was nitrogen with the flow rate 30ml/min. Identification of sterol fractions were carried out by comparing their retention times with those of standard. Quantification was based on the internal standard method.

**Estimation of similarity coefficient**:

$$\text{Similarity coefficient} = \frac{2a}{2a + b + c} \quad (0 \longrightarrow 1)$$

Where a number of similar fatty acid or sterol in both species.

b number of marker fatty acid or sterol in first species.

c number of marker fatty acid or sterol in second species.

It assumes values from zero for nil similarity or complete dissimilarity to unity for complete similarity. The coefficient (0 $\longrightarrow$ 1) has been expressed as a percentage (0% - 100%). The coincidences of Czekanowski's coefficient actually represent the extent of similarity between the two operational taxonomic units (OUT's) rather than others (Czekanowski, 1913).

## *Results and Discussion*

The sterol constituents of the studied green algal taxa that belong to different orders and families have been investigated. Table (1) shows the 17 sterol fractions and their concentrations as a percentage of total sterols that were found among the investigated taxa. The results obtained show that beside the saturated nucleus fraction campestanol ($\Delta^0$), most of the investigated algal members contained also the common $\Delta^5$ unsaturated sterols: Cholesterol, β- sitosterol and Isofucosterol. These compounds are characterized by the presence of methyl or ethyl substituents at C-24. The most sterols commonly distributed in higher plants have a methyl or ethyl group at C-24 on the side chain (Uomori, 1992) which runs in parallel with our results for green algae. Some other algal members might contain $\Delta^{5,22}$ i.e. stigmasterol while still others have a variety of sterol constituents of $\Delta^7$ as avenasterol and isoavenasterol. These results go in harmony with those obtained by Akihisa, *et al*. (1991) who reported that both $\Delta^5$ and $\Delta^7$ sterols represent the main constituents among green algae.

However, 5,22,24- cholesta-trien – 3 β -ol was detected only in *Chara fragilis* but *Chlorella salina* was the only investigated alga that contained both 5 β - cholestanol and α 5 β cholest – 7- en β - ol. While 14 α - methyl 5 α - cholest (14) en – 3 β - ol was detected in *Enteromorpha intestinalis*, but 5 α stigmast – 7- en – 3 β - ol was detected only in both *Ulva fasciata* and *Codium tomentosum.* This fraction was previously detected in some algae by Thompson, *et al*. (1980) and in Acacia sp. by Abd El- Salam (1997). Cycloartenol was detected only in both *Valonia utricales* and *Cladophoropsis membranacea*. *Ulva lactuca* was the only species among green algae that contained spinasterol, an isomer of stigmasterol found in *Spinache*, *Alfalfa* and *Senega* root (Harborne, 1973). The remaining sterol individuals were distributed among all the investigated members as shown in table (1).

**Table (1): Distribution of the identified (known) sterol fractions among the investigated members (from 1 to 16) and their relative concentrations expressed as percentage of total sterol fractions.**

| Sterol | Investigated taxa | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| 5β cholestanol | | 7.9 | | | | | | | | | | | | | | |
| 5β- cholest-7-en-β-ol | | 5.3 | | | | | | | | | | | | | | |
| Z-22-Dehydro-cholesterol | | 15.9 | 23.73 | | | 51.00 | | 4.29 | | | 20.96 | 4.24 | | 0.55 | | 1.86 |
| 5,22,24-cholesta- trien 3 β- ol | | | | | | | | | | | | | | | | 1.59 |
| Cholesterol | 42.3 | 36.8 | | | | | | 10.27 | 3.65 | 3.64 | | 4.30 | | 0.97 | 1.55 | 2.55 |
| 14 α – Methyl – 5 α – cholest (14) en-3 β- ol | | | | | 3.11 | | | | | | | | | | | |
| 14 α – Methyl – 5 α – cholest 7-en-3 β- ol | | | | | | | | | 5.60 | 2.29 | | 6.98 | | | | |
| 24-Methylene chlorosterol | | | | | | | 0.90 | 1.75 | | | | | | 2.42 | | |
| Campestanol | | | | | | | 14.60 | 2.02 | | 2.71 | | 3.45 | | | | 3.50 |
| Stigmasterol | 16.5 | | | | | | | | 4.83 | | | | 48.13 | 3.65 | 0.91 | 6.81 |
| Spinasterol | | | 8.73 | | | | | | | | | | | | | |
| B- sitosterol | 13.4 | | | 38.60 | 52.70 | 8.30 | | | | 22.57 | | | | | | 13.81 |
| Isofucosterol | | | 22.72 | | 5.88 | | 10.13 | 9.45 | | | | | 1.04 | | 0.06 | |
| 5α-Stigmast-7-en-3 β – ol | | | | 13.16 | | | | | | | | 36.81 | | | | |
| Cycloartenol | | | | | | | | | | | | | | 16.54 | 0.80 | |
| Isoavenasterol | | | | | | | | | | | 22.03 | | | | | |
| Avenasterol | | | 16.37 | | | 10.40 | | | 13.73 | | | | 8.60 | | | |
| 24-Methylcyclo- artanol | 9.6 | | | | 6.38 | | | 1.30 | | 16.50 | | | | 12.30 | | |
| Peposterol | | | | | | | 22.02 | 5.45 | 31.92 | 13.00 | | | | 19.61 | | 2.39 |

1- *Dunaliella salina*; 2- *Chlorella salina*; 3- *Ulva lactuca*; 4- *U. fascista*; 5- *Enteromorpha intestinales*; 6- *E. compressa*; 7- *Cladophora graminea*; 8- *C. albida*; 9- *Caulerpa prolifera*; 10- *C. racemosa*; 11- *Codium dichotomum*; 12- *C. tomentosum*; 13- *C. elongatum*; 14- *Valonia utricales*; 15- *Cladophoropsis membranacea*; 16- *Chara fragilis*.

From a comparative point of view β - sitosterol, was the predominant fraction in both *Ulva fasciata* and *Enteromorpha intestinalis*. It constituted nearly 38.6 % and 52.7 % of the total sterol fractions respectively. Members of order Caulerpales i.e. *Caulerpa prolifera, Caulerpa racemosa* and *Codium tomentosum* contained 14 α methyl – 5 α cholest – 7 en – 3 β - ol.

The results of sterol analyses of *Dunaliella salina* indicated the presence of four unsaturated sterol fractions: cholesterol 42.3 %, stigmasterol 16.5 %, β sitosterol 13.4 % and 24 methyl cycloartanol 9.6 % of the total sterols. All these sterols are of the advanced type except 24 methylcycloartanol which is a primitive one. These results for *Dunaliella salina* coincides with those obtained by Mohammady (2002).

The similarity matrices and dendrogram of the studied algal taxa based on the distribution of their sterol fractions were recorded in Table (2) and Figure (1). It is obvious from this table and figure, that highly significant similarity was found between *Cladophora graminea* and *C. albida* (72.7 %), followed by *Cladophora albida* and *Valonia utricales* (71.4 %). The similarity matrices between *Cladophora albida* and *Caulerpa racemosa* was 66.7 % while between *Chara fragilis* and *Caulerpa racemosa* it was 61.51 %. At the same time the similarity matrices between both *Caulerpa racemosa* and *Dunaliella salina* was 60 %; while, this correlation reached about 57.1 % between each pair of the following taxa: *Cladophoropsis membranacea* and *Codium elongatum, Chara fragilis* and *Valonia utricales, Enteromorpha compressa* and *Ulva lactuca* and finally between *Codium elongatum* and *Ulva lactuca*. Lastly, the similarity matrices between *Caulerpa racemosa* and *Caulerpa prolifera, Codium tomentosum* and *Caulerpa racemosa* and between *Valonia utricales* and *Dunaliella salina* were 54.5 %. It is clear from the above mentioned results that similarity matrices between *Chara fragilis* and some representative genera in descending order could be arranged as follows: 61.5 % for *Caulerpa racemosa* followed by 57.1 % for *Valonia utricales*, 54.5 % for *Dunaliella salina* and 50 % for both *Caulerpa prolifera* and *Codium tomentosum*. These results encourage the authors to place *Chara* species at the top rank among members of Chlorophyta.

Anent, GLC data was concerning the fatty acids distribution (Table 3). The results obtained indicated variations in carbon chain lengths that ranged between C8 and C24 with differences in the degree of saturation. Fourteen saturated fatty acids and twenty-one unsaturated ones were detected. The distribution of fatty acid fractions and their relative concentrations differed according to the types of algal taxa. C18:2 demonstrated the mostly wide distributed fraction between the operational taxonomic units (OUT's), while C12:0 shows a minimum representation among fatty acid fractions. The last one was detected only in *Dunaliella salina*. *Codium elongatum* was the only investigated alga that contained C21:0, while *Ulva fasciata* contained only C17:4. The remaining detected fatty acid fractions were unevenly distributed among all

**Table (2): The similarity matrices of the investigated members (from 1 to 16) in relation to their sterols composition.**

| Operational taxonomic unit | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | 25.0 | 0.0 | 333 | 50.0 | 28.6 | 0.0 | 36.4 | 44.4 | 60.0 | 0.0 | 22.2 | 28.6 | 54.5 | 50.0 | 54.5 |
| 2 | | -- | 25.0 | 0.0 | 0.0 | 28.6 | 0.0 | 36.4 | 22.2 | 20.0 | 33.3 | 44.4 | 0.0 | 36.4 | 25.0 | 36.4 |
| 3 | | | -- | 0.0 | 25.0 | 57.1 | 25.0 | 36.4 | 22.2 | 0.0 | 33.3 | 22.2 | 57.1 | 18.2 | 25.0 | 18.2 |
| 4 | | | | -- | 33.3 | 40.0 | 0.0 | 0.0 | 0.0 | 25.0 | 0.0 | 28.6 | 0.0 | 0.0 | 0.0 | 22.2 |
| 5 | | | | | -- | 28.6 | 25.0 | 36.4 | 0.0 | 40.0 | 0.0 | 0.0 | 28.6 | 18.2 | 25.0 | 18.2 |
| 6 | | | | | | -- | 0.0 | 20.0 | 25.0 | 22.2 | 40.0 | 25.0 | 33.3 | 20.0 | 0.0 | 40.0 |
| 7 | | | | | | | -- | 72.7 | 22.2 | 40.0 | 0.0 | 22.2 | 28.6 | 36.4 | 25.0 | 36.4 |
| 8 | | | | | | | | -- | 33.3 | 66.7 | 22.2 | 50.0 | 20.0 | 71.4 | 36.4 | 42.9 |
| 9 | | | | | | | | | -- | 54.5 | 0.0 | 40.0 | 50.0 | 50.0 | 44.4 | 50.0 |
| 10 | | | | | | | | | | -- | 0.0 | 54.5 | 0.0 | 46.1 | 20.0 | 61.5 |
| 11 | | | | | | | | | | | -- | 28.6 | 0.0 | 33.3 | 0.0 | 22.2 |
| 12 | | | | | | | | | | | | -- | 0.0 | 33.3 | 22.2 | 50.0 |
| 13 | | | | | | | | | | | | | -- | 20.0 | 57.1 | 20.0 |
| 14 | | | | | | | | | | | | | | -- | 54.5 | 57.1 |
| 15 | | | | | | | | | | | | | | | -- | 36.4 |
| 16 | | | | | | | | | | | | | | | | -- |

**1- *Dunaliella salina*; 2- *Chlorella salina*; 3- *Ulva lactuca*; 4- *U. fascista*;**
**5- *Enteromorpha intestinales*; 6- *E. compressa*; 7- *Cladophora graminea*; 8- *C. albida*;**
**9- *Caulerpa prolifera*; 10- *C. racemosa*; 11- *Codium dichotomum*; 12- *C. tomentosum*;**
**13- *C. elongatum*; 14- *Valonia utricales*; 15- *Cladophoropsis membranacea*;**
**16- *Chara fragilis*.**

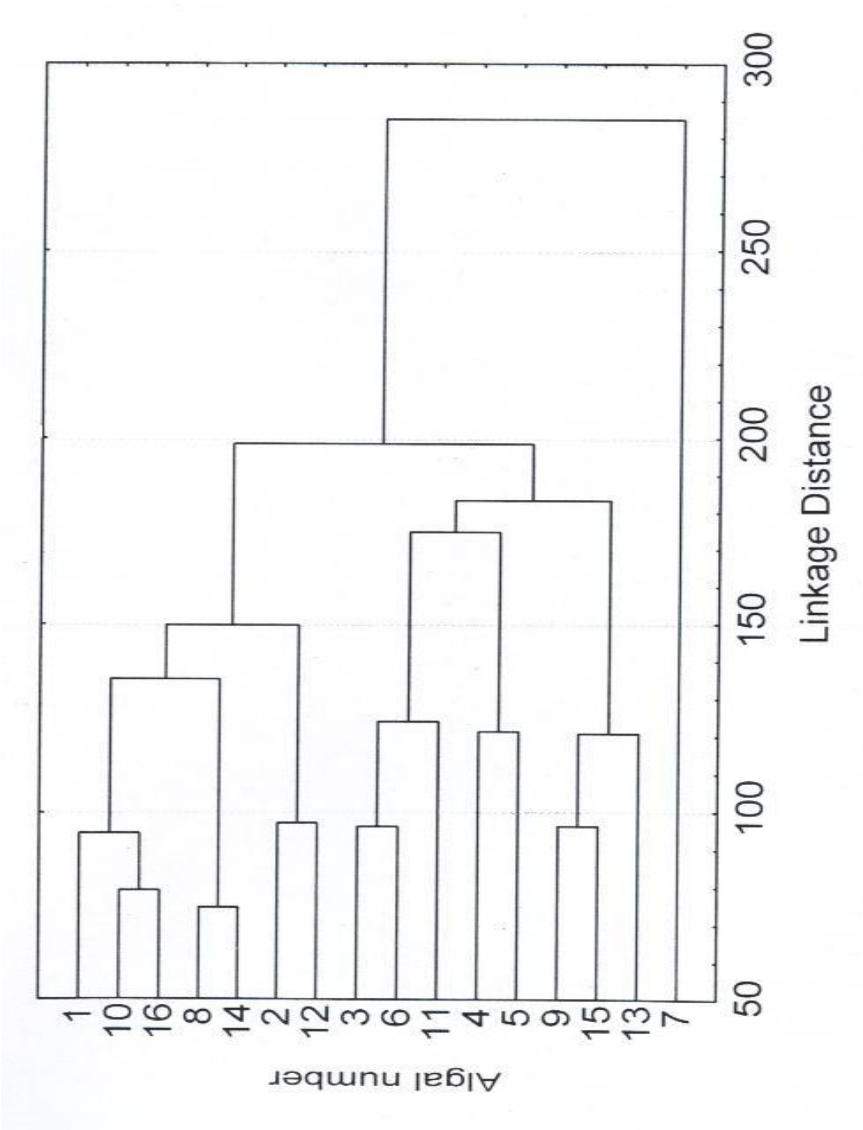


**Figure (1): The dendrogram of the investigated members (from 1 to 16) in relation to their sterols composition.**

**1-** ***Dunaliella salina*****; 2-** ***Chlorella salina*****; 3-** ***Ulva lactuca*****; 4-** ***U. fascista*****; 5-** ***Enteromorpha intestinales*****; 6-** ***E. compressa*****; 7-** ***Cladophora graminea*****; 8-** ***C. albida*****; 9-** ***Caulerpa prolifera*****; 10-** ***C. racemosa*****; 11-** ***Codium dichotomum*****; 12-** ***C. tomentosum*****; 13-** ***C. elongatum*****; 14-** ***Valonia utricales*****; 15-** ***Cladophoropsis membranacea*****; 16-** ***Chara fragilis*****.**

**Table (3): Distribution of the identified (known) fatty acids among the investigated members (from 1 to 16) and their relative concentrations expressed as percentage of total fatty acid fractions.**

| Fatty acids | | Investigated taxa | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Saturated fatty acids | C 8:0 | | | | | | | 32.78 | | | | | | | 18.83 | | |
| | C 10:0 | 4.4 | | | | | | 3.12 | | | | | | | 12.49 | | |
| | C 11:0 | | | | | | | 3.14 | | | | | | | 7.13 | | |
| | C 12:0 | 5.7 | | | | | | | | | | | | | | | |
| | C 14:0 | 1.9 | 1.5 | | | | | | 2.30 | | | | | | | | 20.5 |
| | C 15:0 | | | | | | | | | | | | | | 6.26 | | |
| | C 16:0 | 26.3 | 19.9 | | | | 6.40 | | 1.70 | | | | | | | | 6.83 |
| | C 18:0 | 25.2 | 7.3 | 11.39 | | | 1.28 | | 1.50 | 20.5 | 5.50 | | | | 3.37 | 5.17 | |
| | C 19:0 | | | | 0.24 | | | | | | | 4.50 | | 1.38 | | | |
| | C 20:0 | | | | 0.31 | | | 10.46 | | 2.77 | 0.05 | 0.89 | 0.60 | | 2.85 | 21.6 | 14.35 |
| | C 21:0 | | | | | | | | | | | | | 2.14 | | | |
| | C 22:0 | | | 9.94 | 70.96 | | | | 16.27 | | | | | | | | |
| | C 23:0 | | | | | | | 18.13 | | | | | | | 22.00 | | 21.19 |
| | C 24:0 | | | | | | | | 37.7 | | | | | | | | |
| Monounsaturated f.a. | C 16:1 | 3.2 | 2.4 | | | 8.26 | 4.25 | 9.0 | 1.60 | | | | | | 1.99 | 3.19 | |
| | C 17:1 | | | 6.23 | | | 13.00 | | | 8.30 | | 13.40 | | 13.97 | | 2.52 | |
| | C 18:1 | 25.2 | 48.6 | | 2.18 | | 0.67 | | | 5.60 | 1.50 | 12.2 | 10.50 | 10.98 | | | |
| | C 19:1 | | | | | 3.45 | | | | 14.8 | 0.9 | | | 8.29 | | | |
| | C 20:1 | | | 3.8 | 0.21 | 0.55 | | | | 1.98 | | | 0.90 | | 6.42 | | |
| | C 21:1 | | | 1.13 | 4.30 | 13.99 | | | | | | 1.5 | 2.68 | 29.69 | | | |
| | C 22:1 | | | | | | | | 1.80 | | | | | | | | |
| Polyunsaturated f.a. | C 16:2 | | | 4.25 | | 2.70 | | | | 7.14 | | | 2.34 | | | 2.84 | |
| | C 18:2 | 4.6 | 15.5 | 12.75 | 1.5 | 1.06 | 27.20 | | 22.1 | 16.7 | | | 3.35 | 11.11 | | 13.10 | |
| | C 20:2 | | | | 0.50 | 1.76 | | 11.77 | | 0.86 | 2.56 | | | 0.79 | 20.70 | 4.27 | |
| | C 21:2 | | | | | | 12.30 | | | | | | | | | | |
| | C 14:3 | | | | | | | | | 3.55 | | | | | | | |
| | C 15:3 | | | 2.06 | | 5.56 | | | | 4.90 | 2.70 | | 7.29 | 11.40 | | | |
| | C 16:3 | | | 6.75 | | 4.84 | | | | | 5.60 | | 4.47 | | | 3.49 | |
| | C 17:3 | | | | | | | | | | 4.00 | | | | | | |
| | C 18:3 | 1.7 | 1.9 | 4.13 | | | 7.76 | | 3.3 | 1.93 | | | 11.70 | | 2.24 | 3.80 | |
| | C 19:3 | | | | 0.25 | | | | | | | 1.03 | | | | | |
| | C 20:3 | | | | 2.07 | | | | | | | 3.20 | 46.90 | | | | |
| | C 16:4 | | | 16.00 | 6.3 | 10.20 | | | | | | | | | | 5.59 | |
| | C 17:4 | | | | 0.55 | | | | | | | | | | | | |
| | C 20:4 | | | | 2.11 | | | | | | | | | | | 26.40 | |

1- *Dunaliella salina*; 2- *Chlorella salina*; 3- *Ulva lactuca*; 4- *U. fascista*; 5- *Enteromorpha intestinales*; 6- *E. compressa*; 7- *Cladophora graminea*; 8- *C. albida*; 9- *Caulerpa prolifera*; 10- *C. racemosa*; 11- *Codium dichotomum*; 12- *C. tomentosum*; 13- *C. elongatum*; 14- *Valonia utricales*; 15- *Cladophoropsis membranacea*; 16- *Chara fragilis*.

the investigated taxa. Different *Dunaliella* species were previously analyzed by some authors for their fatty acid composition and was found to be identical to most green algae. Fried *et al*. (1982) found that *Dunaliella bardawil* contained C16:0, C16:1, C16:4, C18:0, C18:2 and C18:3 acids, a distribution pattern which in parallel with the results obtained for *Dunaliella salina* except that C16:4 fraction was not detected. The same author found that C16:1 which occurs in the phosphatidyl glycerol of most micro-algae is thought to be important in plant photosynthesis. Graeve *et al*. (2002) reported that Chlorophyta comprise the most modern group and this is supported by primarily C18 unsaturated fatty acid typical of the vegetative tissue of higher plants. This agrees with the results obtained that C18:1, C18:2 and C18:3 demonstrated the mostly wide distributed fractions between the investigated green algae. C18:2 were detected in *Enteromprpha compressa*, *Caulerpa prolifera*, *Chlorella salina*, *Ulva lactuca* and C18:1 in *Chlorella salina*, *Dunaliella salina*, *Codium dichotomum*, *Codium tomentosum* and in *Codium elongatum*. However, C18:0 and C18:3 were detected in nine species of the green algal members but at lower concentrations. *Cladophora albida* is the only investigated alga that contained the long chain saturated fatty acid C24. However, the results obtained for *C. albida* appear to be similar to those obtained by Jamieson and Reid (1972) for *C. albida*. All investigated *Codium* species contained C18:1 and this is consistent with results obtained by Aknin *et al*. (1992).

The fatty acid fractions obtained in *Cladophoropsis membranacea* (siphonales) indicated the frequent distribution of C16 fractions (nearly ½ of the total fractions). This coincides with the results obtained by Aknin *et al*. (1992) who proved the frequently detected C16 within siphonales taxa. However an appreciable amount of C20 was detected which nearly reached 21.6% of the total fatty acids. Our results indicated that most fatty acids of the investigated algal taxa are of the polyunsaturated ones. The highly dominant fractions are C16, C18 and C20 while C17 and C19 fractions are the least. Finally, the results indicated also that four ulvacean members can be distinguished by C18:2 fatty acids, the cladophoracean members by C16:1 fatty acid and the siphonous members and characean members by C20:0 fatty acids.

The similarity matrices and dendrogram of the studied algal materials based on their fatty acids distribution were represented in table (4) and figure (2). These data reflected considerable relation between the investigated algal members. The highest similarity value (87.8 %) existed between *Dunaliella salina* and *Chlorella salina* followed by *Chlorella salina* and *Enteromorpha compressa* (80 %) followed by *Cladophora greaminea* and *Valonia utricales* (77.8 %). The similarity value between *Caulerpa prolifera* and *Caulerpa racemosa* as well as between *Enteromorpha intestinalis* and *Codium tomentosum* as well as between *Ulva fasciata* and *Codium dichotomum* reached 60 %. It is obvious therefore that low similarity value was observed between the two members of Ulvales and between the two investigated members of Caulerpaceae.

**Table (4): The similarity matrices of the investigated members (from 1 to 16) in relation to their fatty acids composition**

| Operational taxonomic unit | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | 87.5 | 30.0 | 18.2 | 21.1 | 70.6 | 25.0 | 66.7 | 38.1 | 23.5 | 12.5 | 31.6 | 18.2 | 40.0 | 40.0 | 30.8 |
| 2 | | -- | 33.3 | 20.0 | 23.5 | 80.0 | 14.3 | 75.0 | 42.1 | 26.7 | 14.3 | 35.3 | 25.0 | 33.3 | 44.4 | 36.4 |
| 3 | | | -- | 33.3 | 66.7 | 42.1 | 0.0 | 40.0 | 60.9 | 31.6 | 22.2 | 66.7 | 40.0 | 27.3 | 63.6 | 0.0 |
| 4 | | | | -- | 43.5 | 19.0 | 20.0 | 9.1 | 40.0 | 28.6 | 60.0 | 52.2 | 45.5 | 25.0 | 41.7 | 11.8 |
| 5 | | | | | -- | 22.2 | 23.5 | 21.1 | 54.5 | 44.4 | 11.8 | 60.0 | 52.6 | 28.6 | 57.1 | 0.0 |
| 6 | | | | | | -- | 13.3 | 58.8 | 50.0 | 25.0 | 26.7 | 33.3 | 35.3 | 31.6 | 52.6 | 16.7 |
| 7 | | | | | | | -- | 12.5 | 21.1 | 26.7 | 14.3 | 11.8 | 12.5 | 77.8 | 33.3 | 36.4 |
| 8 | | | | | | | | -- | 28.6 | 11.8 | 0.0 | 21.1 | 11.1 | 30.0 | 40.0 | 30.8 |
| 9 | | | | | | | | | -- | 60.0 | 31.6 | 63.6 | 57.1 | 52.2 | 60.9 | 25.0 |
| 10 | | | | | | | | | | -- | 26.7 | 44.4 | 47.1 | 31.6 | 42.1 | 16.7 |
| 11 | | | | | | | | | | | -- | 47.1 | 50.0 | 11.1 | 22.2 | 18.2 |
| 12 | | | | | | | | | | | | -- | 42.1 | 28.6 | 47.6 | 14.3 |
| 13 | | | | | | | | | | | | | -- | 30.0 | 30.0 | 0.0 |
| 14 | | | | | | | | | | | | | | -- | 45.5 | 26.7 |
| 15 | | | | | | | | | | | | | | | -- | 13.3 |
| 16 | | | | | | | | | | | | | | | | -- |

**1-** ***Dunaliella salina*****; 2-** ***Chlorella salina*****; 3-** ***Ulva lactuca*****; 4-** ***U. fascista*****;**
**5-** ***Enteromorpha intestinales*****; 6-** ***E. compressa*****; 7-** ***Cladophora graminea*****; 8-** ***C. albida*****;**
**9-** ***Caulerpa prolifera*****; 10-** ***C. racemosa*****; 11-** ***Codium dichotomum*****; 12-** ***C. tomentosum*****;**
**13-** ***C. elongatum*****; 14-** ***Valonia utricales*****; 15-** ***Cladophoropsis membranacea*****;**
**16-** ***Chara fragilis*****.**

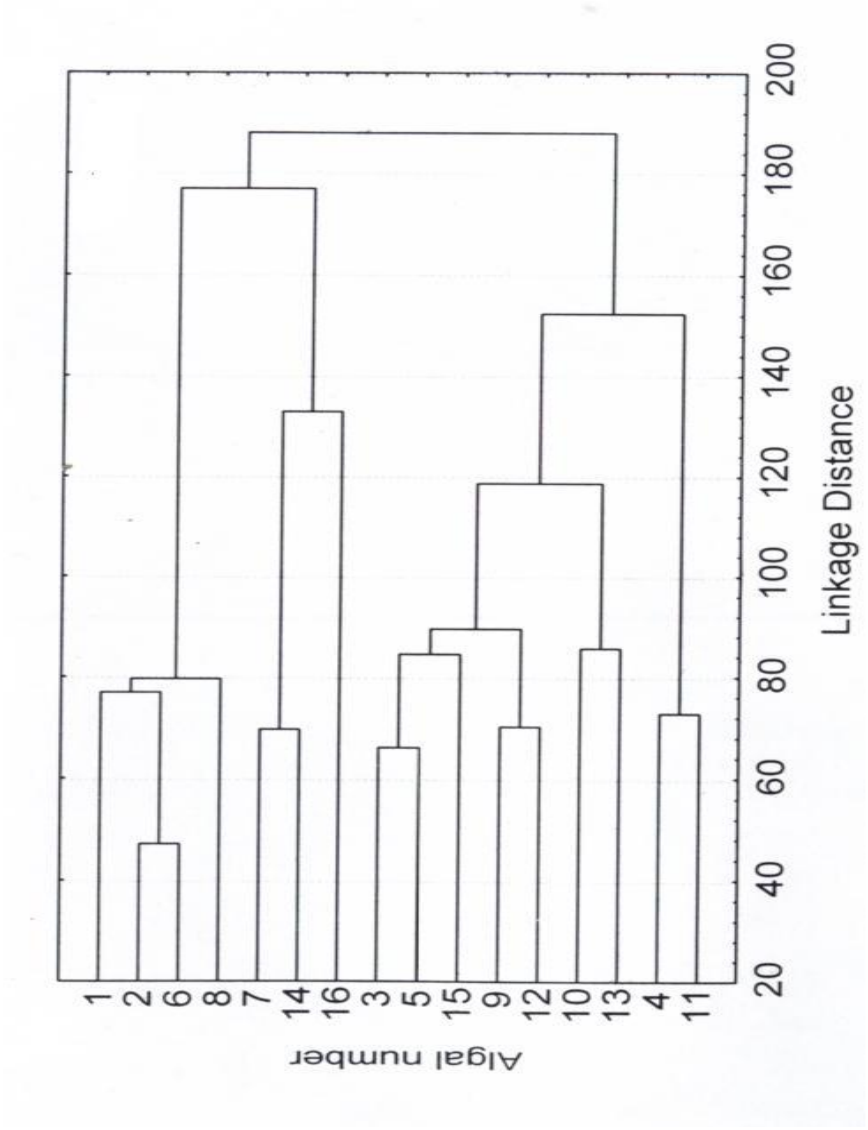


**Figure (2): The dendrogram of the investigated members (from 1 to 16) in relation to their fatty acids composition.**

**1- *Dunaliella salina*; 2- *Chlorella salina*; 3- *Ulva lactuca*; 4- *U. fascista*; 5- *Enteromorpha intestinales*; 6- *E. compressa*; 7- *Cladophora graminea*; 8- *C. albida*; 9- *Caulerpa prolifera*; 10- *C. racemosa*; 11- *Codium dichotomum*; 12- *C. tomentosum*; 13- *C. elongatum*; 14- *Valonia utricales*; 15- *Cladophoropsis membranacea*; 16- *Chara fragilis*.**

Also a similarity value was observed between the studied members of Ulvales and Caulerpales. A high similarity value was observed between *Dunaliella salina*, *Chlorella salina* and the two investigated *Enteromorpha* species.

The lowest similarity values (9.1 %) was recorded between *Ulva fasciata* and *Cladophora albida* followed by (11.1 %) between *Cladophora albida* and *Codium elongatum* as well as between *Codium dichotomum* and *Valonia utricales*. A degree of similarity between *Cladophoropsis membranacea* and *Ulva lactuca* reached up to 63.6 % while this similarity between *Cladophoropsis membranacea* and *E. intestinalis* reached to 57.1 %. This may indicate a similarity between *C. membranacea* and ulvacean members. All fatty acids detected in chladophoracean individuals were also observed in *Valonia utricales*. This result was supported by the obviously high similarity matrices between them (77.8 %).

In conclusion, the results obtained in this work concerning the distribution pattern of fatty acids and sterols in the experimental algal orders could be useful in solving some taxonomic problems among the investigated species and other related taxa.

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# دراسات على محتوى الأحماض الدهنية والأستيرولات لبعض أفراد الطحالب الخضراء.

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في هذا البحث تم اختيار بعض من الأفراد الأساسية للطحالب البحرية الخضراء بجانب طحلب المياه العذبة كارا فراجيل لدراسة محتوى هذه الطحالب من الأستيرولات والأحماض الدهنية وقد أظهر محتوى هذه الطحالب من الأحماض الدهنية أن معظمها من الأنواع غير المشبعة طويلة السلسلة (ك16 ، ك18 ، ك20) بالإضافة إلى بعض الأحماض الدهنية الغير معتادة مثل ك17 ، ك19.

وقد أثبتت هذه التحليلات أيضا أن استخدام الأحماض الدهنية كأداة تقسيمية قد تعطي صورة مميزة لكل جنس وبذلك يمكن بواسطتها التمييز بين الأجناس. كما أثبتت النتائج وجود تشابه كبير بين كل من دوناليلا سالينا وكلوريللا سالينا وانتيرومورفا كامبريسا.

أما نتائج الأستيرولات فقد وجد أن معظم الأستيرولات المستخلصة من الأجناس المختبرة من الأنواع $\Delta^5$، $\Delta^{5,22}$، $\Delta^7$، بيبوستيرول والكامبيستانول ذات النواة المشبعة. كما أظهرت نتائج تحليل الأستيرولات وجود اختلافات واضحة بين الرتب المختلفة، فقد وجد سبيناستيرول فقط في رتبة الألفات و5 بيتاكوليستانول و5 بيتا-7-اين-بيتا-اول في رتبة الكلوريلات و 5،22،24 كوليستاترايين 3بيتا-اول في رتبة الكارات.

وعلى وجه العموم أظهرت نتائج تحليل كل من الأستيرولات والأحماض الدهنية أنه يمكن وضع الطحالب الكارية ضمن الطحالب الخضراء.