International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 06-01-2025, Accepted: 05-02-2025, Published: 20-02-2025
Volume 10, Issue 2, 2025, Page No. 25-29

Biochemical investigation of some Mediterranean seaweeds

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Abstract
The biochemical constituents were investigated for 4 species from Mediterranean Coast at Abou-Kir (*Caulerpa prolifera and Ulva lactuca*) and Baltim (*Ulva rigida and Gracilaria dendroides*). Their constituents of some organic compounds such as tannins, Alkaloids, sterols, saponins and Flavonoids were investigated. Whereas, the crude lipid was averaged from12.3 to 2.7%, 17 *fatty acids* were found *in the tested species; G. dendroides* have the highest number of fatty acids (14), while the lowest number of fatty acid (8) was obtained by *Ulva lactuca*. Total soluble sugars and non-soluble sugars were estimated. *G. dendroides* recorded the highest concentration of total protein (36.25%), and high number of free and bound amino acids. The ash content, major elements (Na, Ca, K, Mg and P), and some heavy metals (Fe, B, Pb, Mn, Zn, Co, Ni, Cu, Cd and Se) were recorded. The biochemical content of these algae makes us look for the best ways to achieve the highest benefit from them in various fields.

Keywords: Seaweed, biochemical constituents, heavy metal, *Ulva lactuca, Gracilaria*

Introduction

Seaweeds considered as an excellent source of bioactive compounds such as polysaccharides, dietary fibers, protein, essential fatty acids, vitamins and minerals, Seaweeds polysaccharides can be hydrolyzed into fermentable sugars using enzymatic or chemical treatments, making seaweed a promising feedstock for bioethanol and other bio products, advantage of seaweed over terrestrial biomass is its lack of lignin, which often complicates the breakdown of plant material in biofuel production. (Olsson *et al*. 2020) [17]. Seaweeds are an economically important group of organisms; they are used as a source in many industrial applications, such as for food, fodders, fertilizers, biofuel, nutraceuticals (Ashour *et al* 2021). The antioxidant activity of seaweed confirms their nutritional, pharmaceutical and medicinal possible applications (Ismail 2017) [11]. Anticancer properties of seaweeds are discussed and this will provide the basic information to develop a novel chemotherapeutic drug to challenge the cancers (Ranahewa *et al* 2019). In line with the need for compounds found in marine algae for the purpose of using them in industry or nutrition, In the present study biochemical composition of 4 seaweeds species was investigated to obtain information about their economic value. Seaweeds had been analyzed to determine major metabolites like total proteins, carbohydrates (soluble, non-soluble,) and lipids (crude and fatty acids), estimation of ash and major elements (N, P, K, Mg, Ca and Na) and minor elements (Fe, Al, Cu, Cd, Pb, Mn and Se). this research was conducted to identify the important compounds found in the algae under study in order to know how to exploit these natural resources in the best possible way in the future

Materials and Methods

The algal collection was made at Mediterranean Coast four species from at Abou-Kir (31°19′00″N 30°04′00″E) (*Caulerpa prolifera* (Forsskal) lamouroux and *Ulva lactuca* Linneus) and Baltim (31.5972°N 31.1086°E) (*Ulva rigida* C. Agardh and *Gracilaria dendroides* Garguilo *et al*.) These species represented the major groups of marine macrophytes; Classification was done according to Aleem (1993) [1]. The algal species (were collected in a considerable amount for assay in plastic bags and transferred to the laboratory. At the laboratory, the algal materials were sorted to the species level, cleaned from epiphytes and were successively rinsed with sea water, fresh water and distilled water. After these operation seaweeds were air dried, weighed to the nearest gram and powdered using electric mill in preparation for the extractions and different experiments.

1-Preliminary determination of some organic compounds

One hundred grams of each of the ten-air dried algal species powder were extracted with 80% ethyl alcohol for 48 hours, using soxhlet apparatus. The extracts were concentrated to 30 ml then used for the following qualitative tests:
Test for tannins (Claus, 1967) [3], alkaloids (Jenkins *et al*., 1957) [13], sterols (Fieser and Fieser, 1959) [7], flavonoids (Wall *et al*., 1954) [24] and saponins (Wall *et al*., 1954) [24].

2- Biochemical Analysis

The extraction and determination of crude lipid were carried out according to the method used by Rao *et al*. (1986) [19], while the determination of fatty acids was determined according to Daniel (1979) [4]. Extraction and determination of total soluble sugars were determined as glucose in the ethanolic extract as described according to the method of Dubois *et al*. (1956) [5]. Also, the determined of non-soluble sugar was through the phenol – sulfuric acid method as described by Dubois *et al.* (1956) [5]. The extraction and determination of total soluble protein were done as mentioned by Lowery *et al*. (1951), with slight modification suggested by Ansell and Trevallion (1967) [2]. Meanwhile, the determination of free and bound amino acids was done using thin layer chromatography (TLC) was carried out according to Impellizzeri *et al*. (1975) [9]. Estimation of total nitrogen was carried out by Microkjeldahl method Pirie (1955) [18]. Total protein percentage was obtained by multiplying total nitrogen by 6.25 (Indergaard and Minsaas, 1991) [10].

Estimation of phosphorus, potassium and sodium by flame photometer was carried out according to Williams and Twine (1960) [25]. Estimation of calcium and magnesium depends upon the formation of a complex by titration with EDTA in the presence of indicators (Jackson, 1973) [12]. Estimation of Fe, Cu, Zn, B, Cd, Se, Mn, Co, Ni and Pb was carried out by using Atomic Absorption (Varian Spectra AA.20).

Results

The present study evaluates some important organic compounds for the studied algal species as mentioned in Table1, all the tested species contain tannins, alkaloids are detected in all the species with exception for *U. lactuca all* algal species contain sterols Saponins are found by high amount in *C. prolifera* and *U. rigida*, flavonoids are not detected in the tested species except *C. prolifera*

Table 1: The organic compounds present in the seaweed species

	Tannins	Alkaloids	Sterols	Flavonoids	Saponins
Ulva lactuca	+ ve	- ve	+ ve	- ve	- ve
Ulva rigida	+ ve	++ ve	+ ve	- ve	+++ ve
Caulerpa prolifera	+ ve	++ ve	+ ve	+ ve	+++ ve
Gracilaria dendroides	+ ve	++ ve	+ ve	- ve	- ve

The crude lipid percentage was different in between the tested species as showed in figure 1 The highest value (12.5 ± 0.44%) was obtained by *G. dendroides* followed by *C. prolifera* (11.8 ± 0.35%), on the other hand, the lowest one (3.1 ± 0.1%) was obtained by *U. lactuca*

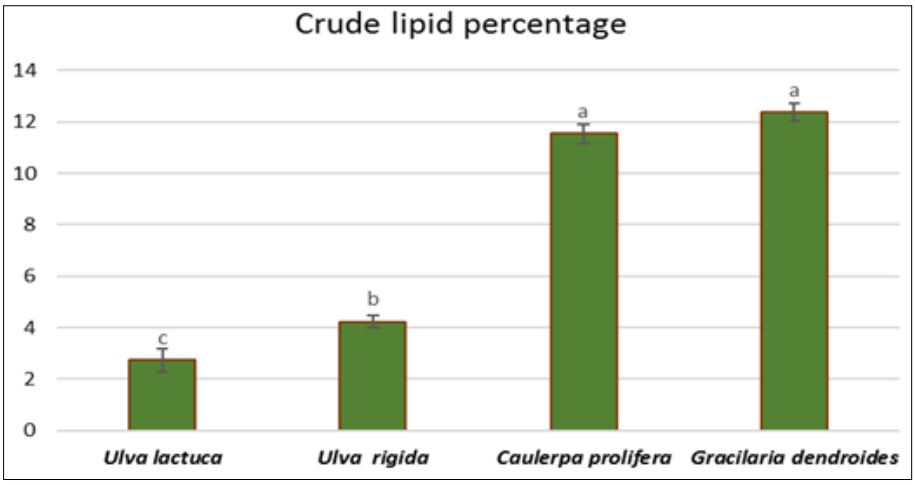

Fig 1: Crude lipid results are means of triplicate determination ± standard deviation

Results showed that the total number of fatty acids were 17 fatty acids. The studied species varied in their fatty acids composition, where, *G. dendroides* have the highest number of fatty acids (14), while the lowest number of fatty acid (8) was obtained by *U. lactuca* (Table 2). Valeric acid (C_5) has the highest level in *C. prolifera, U. lactuca and U. rigida* (45.7, 28.9 and 28.1%). Linoeleic acid ($C_{18:2}$) occurred in large percent among the acids isolated (36 and 20.3) for *U. lactuca and U. rigida*, respectively, while, it occurred in small percent (1.5) in *C. prolifera*,

Table 2: The fatty acid picture of the studied algal species data expressed as relative percentage

Type	Systematic name	Common name	*Ulva lactuca*	*Ulva rigida*	*Caulerpa. prolifera*	*Gracilaria dendroides*
C_6	Hexanoic acid	Caproic acid	-	-	-	4.5
C_8	Octanoic acid	Caprilic acid	1.6	6.9	1.6	-
C_9	Nonanoic acid	Pelargonic acid	8.1	8.6	6.5	31.8
C_{10}	Decanoic acid	Capric acid	6.1	8.6	-	8.8
C_{12}	Dodecanoic acid	Lauric acid	4.6	6.6	14.0	-
C_{14}	Tetradecanoic acid	Myristic acid	-	13.3	9.8	7.8
$C_{14:1}$	Tetradecenoic acid	Myristoleic acid	-	-	-	5.7
C_{16}	Hexadecanoic acid	Palmitic acid	6.3	2.1	-	4.5
C_{18}	Octadecanoic acid	Stearic acid	-	1.3	5.0	10.9
$C_{18:1}$	Octadecenoic acid	Cis-Vaccenic acid	-	0.2	2.9	0.4
$C_{18:2}$	Octadecadienoic acid	Linoeleic acid	36.0	20.3	1.5	0.9
$C_{18:3}$	Octadecatrinoic acid	Linolenic acid	8.5	4.0	8.2	9.0
C_{20}	Eicosanoic acid	Arachidic acid	-	-	5.1	0.8
C_{22}	Docosanoic acid	Behenic acid	-	-	-	0.4
$C_{22:1}$	Docosenoic acid	Erucic acid	-	-	-	0.7

The only red species tested *G. dendroides* has pelargonic acid (31.8%) as the high percentage of fatty acid followed by butyric acid present also by moderate percentage (13.8), while, Cis- vaccenic acid and behenic acid were the lowest fatty acids found with percentage (0.4), respectively (Table 2).

All over the tested species *G. dendroides* gave the highest percentage of total protein (36.25 ± 2), followed by *U. rigida* (26.44 ± 1.25) then *C. prolifera* (22.5 ± 1.56).

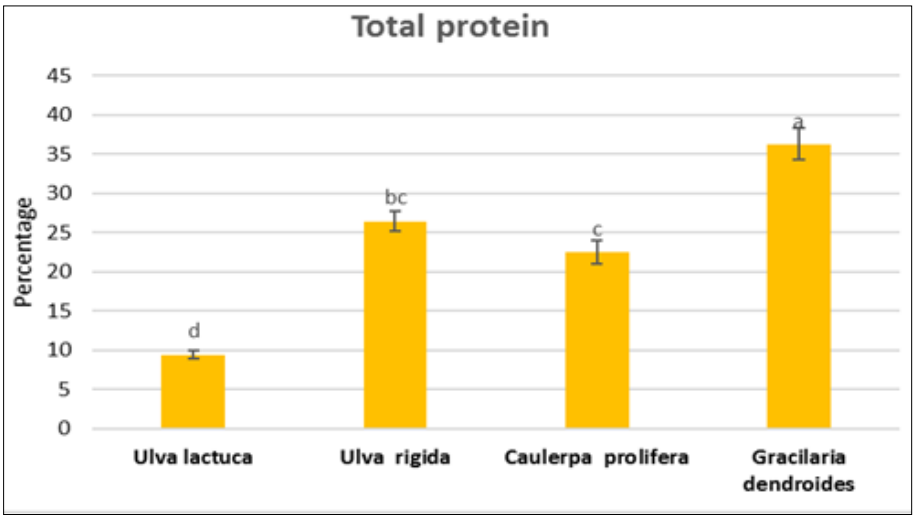

Fig 2: Total protein represented as means of triplicate determination ± standard deviation

The maximum total soluble protein amounts of 0.25 ± 0.01 was detected in *U. lactuca*, while the minimum values (0.11 ± 0.004 mg/g) were determined in *U. rigida*.

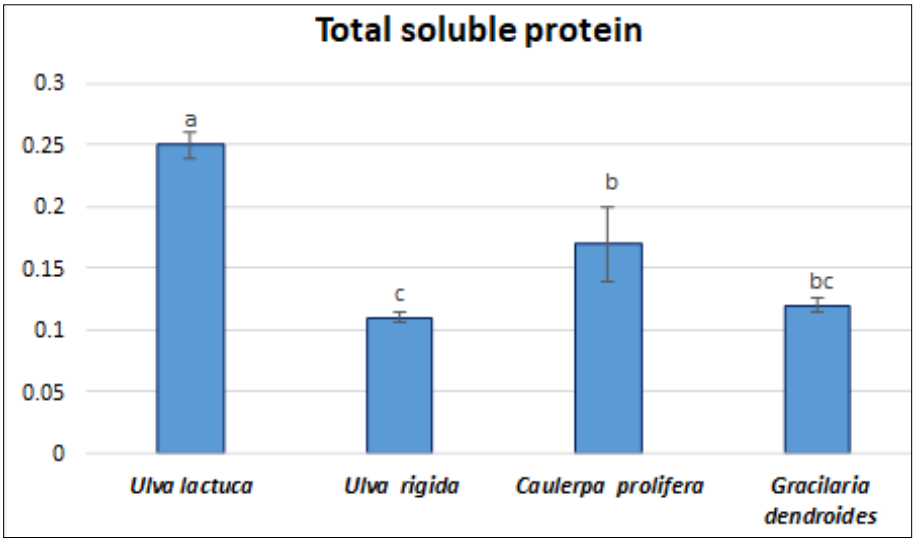

Fig 3: Total soluble protein represented as means of triplicate determination ± standard deviation

G. dendroides containing 6 free amino acids (Asparagine, Glutamine, Glycine, Tyrosine, Phenyl alanine and Typtophan) and 9 bound amino acids.

Table 3: Amino Acids composition in the studied algal species

	Ulva lactuca	*Ulva rigida*	*Caulerpa. prolifera*	*Gracilaria dendroides*
Free amino acids composition				
Arginine	-	+	-	-
Asparagine	-	-	-	+
Threonine	-	-	+	-
Valine	-	-	+	-
Phenylalanine	-	-	-	+
Glutamic acid	+	+	-	-
Methionine	+	-	-	-
Glycine	-	-	-	+
Cysteine	-	-	+	-
Tryptophan	-	-	-	+
Glutamine	-	+	-	+
Tyrosine	-	-	+	+
Bound amino acids composition				
Arginine	+	+	+	+

Asparagine	+	+	+	+
Threonine	+	-	-	+
Valine	+	+	+	-
Phenyl alanine	+	+	+	+
Glutamic acid	+	-	+	+
Methionine	-	+	+	+
Tyrosine	+	+	+	+
Leucine	+	+	+	+
Tryptophan	+	-	-	+
Alanine	-	+	-	-

U. lactuca have the highest number of bound amino acids, it has 9 types. Meanwhile, eight different amino acids were found in both of *U. rigida* and *C. prolifera*, the total soluble carbohydrates were varied in their amount between the tested algal species the values ranged from (0.012 ± 0.003 mg/g) in *C. prolifera* to (2.46 ± 0.05 mg/g) in *G. dendroides*. *U. rigida* and *U. lactuca* have moderate amounts of total soluble carbohydrates. The non-soluble sugar had a maximum amount of (1.55 ± 0.06 mg/g) which, was obtained by *C. prolifera*. Followed by *G. dendroides*, 1.12 ± 0.04

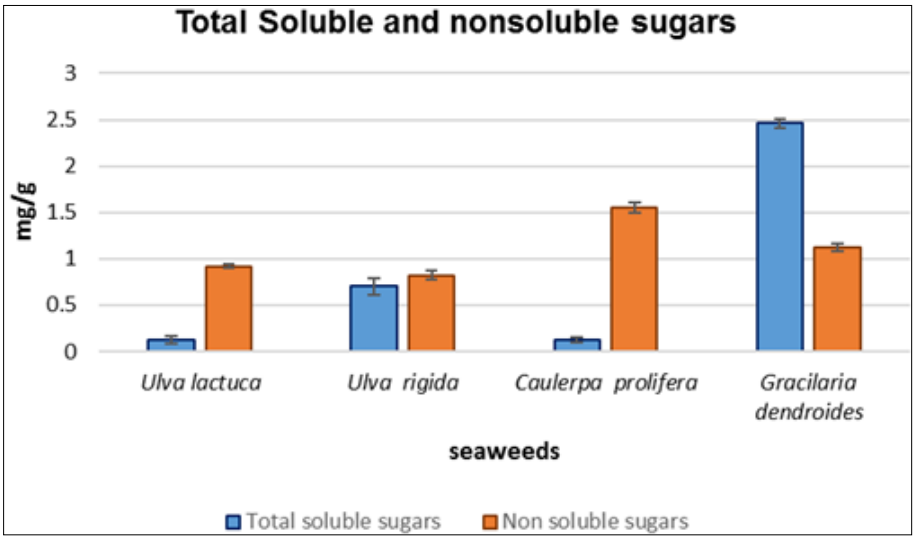

Fig 4: Total soluble and non-soluble sugars represented as means ± standard deviation

The ash percentage of green algae gave the highest value ***22.7*** ± ***0.52*** in *U. rigida*, followed by *U. lactuca* (15.8± ***0***.35) and *C. prolifera* 13.7 ± 0.58 respectively. Whereas, for the only Rhodophyta species (*G. dendroides*) the ash content falls near the lowest percentage of the Chlorophyta group with being 14.6 ± 0.4.

Table 4: The ash content and some metals percentage

	Ash	**Na**	**K**	**P**	**Ca**	**Mg**
Ulva lactuca	15.8 ± 0.35	4.79 ± 0.15	0.6 ± 0.02	0.07 ± 0.02	2.53 ± 0.25	4.34 ± 0.15
Ulva rigida	22.7 ± 0.52	4.13 ± 0.47	0.27 ± 0.01	0.047 ± 0.006	2.03 ± 0.2	4.63± 0.15
Caulerpa prolifera	13.7 ± 0.58	11.27 ± 0.7	3.03 ± 0.25	0.16± 0.05	3.8 ± 0.26	4.38 ± 0.16
Gracilaria dendroides	14.6 ± 0.4	7.4 ± 0.35	3.75 ± 0.18	0.24 ± 0.05	2.63 ± 0.05	2.8 ± 0.17

Table 5: The concentration of the minor cations in the studied algal species (the data expressed as ppm)

	B	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Se**	**Cd**	**Pb**
Ulva lactuca	34.6	74	510	14	7	16.2	31.8	1.48	6.8	41.2
Ulva rigida	29.6	16	520	42	6.3	5.2	15.6	1.98	8	55
Caulerpa prolifera	52.6	68	400	19	0	11.8	61.8	3.2	9	50
Gracilaria dendroides	32	28	1000	21	13	11.4	19.8	2.6	7.2	40

Data presented in Table (5) indicated that in all the tested algal species the ten minor elements and heavy metals were accumulated inside the studied species with one exception for *C. prolifera* in which Ni was not detected. On the basis of the element concentrations, iron and boron were the most abundant metals in the studied algae, followed by lead, manganese, zinc, cobalt, nickel, copper, cadmium, and selenium.

Discussion

The studied species contains secondary metabolites such as saponins, alkaloids, tannins, and flavonoids which recognized for their diverse biological activities, such as anti-inflammatory anticancer, antioxidant and antimicrobial effects flavonoids also showed neuroprotective and cardio-protective effects as mentioned by Ullah *et al* 2020 [23]. *U. rigida* contain high phenolic content this finding is consistent previous study (Elbilawy *et al* 2022).

Fatty acids profile showed that *Gracillaria* contain higher concentrations of Pelargonic acid which can be used in the preparation of plasticizers, Esters of pelargonic acid are precursors to lubricants, *Gracillaria* also have 14 fatty acids saturated and unsaturated, this finding similar with that illustrated by Thomas *et al* 2019 [22], and Salem *et al* 2020 [20].

Ulva species and *Caulerpa* contain highest amount of valeric acid, the Volatile esters of valeric acid tend to have pleasant odors and are used in perfumes and cosmetics,

The presence of total protein in tested species by apercentage more than 20 % suggest the property of using these species specially *Gracilaria* in feeding of different animals, protein content was higher in Rhodophyta, than chlorophyte, this is consistent with what was confirmed by Kumar *et al* 2017 [14].

The amino acid compositions of *Gracillaria* suggest their possible use as sources of essential amino acids according to high content of free and bound amino acids, the most abundant amino acid in the two species of Ulva Glutamic acid and aspartic acid as mentioned by Shuuluka, *et al* 2013 [21].

The red seaweed of *Gracilaria* sp. had the highest K, and Fe, these results are consistent with what Kustantinah *et al* 2022 [15] found, while the percentage of other major elements was lower for us than for them

Our results showed the important compounds found in the algae under study in order to know how to exploit these natural resources in the best possible way in the future

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