PHYTOCHEMICAL STUDIES ON *ULVA LACTUCA* (L.) THURET FROM RED SEA COAST OF JEDDAH, SAUDI ARABIA

Zeinab El Sewafy* and Naseem Ismaeil Radi**

**Botany & Microbiology Dept., Fac. of Science, Al Azhar Univ. (Girl Branch)*
***Biology Department, Faculty of Science, Om El-Qora University, Saudi Arabia*

Abstract

The chemical investigation of *Ulva lactuca* (L.) thuret showed the presence of flavonoids, carbohydrates/glycosides, tannins, steroids, chlorides and sulphates, while free from alkaloids. Also, the free and combined sugars, amino acids, phenolic acids, fatty acids, hydrocarbons, sterols and minerals were analysed quantitatively. The assay of steroidal terpene revealed the presence of 14β-Hpregnan-3one as the main compound.

Keywords: *Ulva lactuca*, Edible seaweeds, Green algae, Saudi Arabia.

Introduction

Marine plants have been used since ancient times as animal and human food, fodder and fertilizer as well as sources of medical drug (Sanchez-Machado *et al*., 2004), because they contain considerable amounts of protein, fatty acids and minerals (Fleurence, 1999; Norziah and Ching, 2000; Wong and Cheung, 2000). Currently, marine plants are attracting increasing interest, in view of their low calorie content and high vitamin, mineral and dietary fiber contents, making them attractive to both consumers and the food industries.

The green algae *Ulva lactuca* has great potential as a commercial product because of its fruitful taste and varying chemical composition and quality. It is consumed as a vegetable in many countries (Indergaard and Minsaas, 1991) and among its nutritional benefits; it is also rich in dietary fiber (Lahaye *et al*., 1995). Currently, it is authorized as vegetable and condiment (Burtin, 2003). With the consumption of any raw ready-to-eat product originating from an environment which may contain fecal and other human clinical pathogens, it is necessary to estimate the potential hazards and associated risks. Therefore, there are various reports on the chemical constituents of *U. lactuca* in different parts of the world. *Gibbons et al*., (1968) idenitified 28-isofucosterol in *U. lactuca* and *Enteromorpha intestinalis*. Meanwhile, Awad (2000) studied the biological active steroids in the green alga *Ulva lactuca*. The metal contents were also investigated by Misheer *et al*., (2006). Further studies on isolation of five norisoprenoids were carried out by Sun *et al*. (2007).

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On the other hand, there are few works concerning the marine algae along the Red Sea coast of Saudi Arabia with regard to their chemical constituents of carbohydrate, protein, lipid, sugars and mineral contents (El- Sayed, 1982; Khalil and El-Tawil, 1982; El-Naggar and Al-Amoudi, 1989; Khafaji *et al*.,1992). The present work is concerned with the chemical composition of the green alga *U. lactu*ca which was collected from Jeddah region along the Red Sea Coast of Saudi Arabia. The aim is to detect and estimate the most active algal constituents to asses their economic and medical valuable importance.

Material and Methods

The *U. lactuca* was collected from Red Sea coast of Saudi Arabia at Shuaba, about 82 km south of Jeddah (longitude 35° 91\\ - 39° 24\\ East and latitude 15° 56\\ - 20° 52\\ North), during summer season, 2007 at depths ranging between 0.30 m and 0.35 m.

The temperature of water ranged between 30.4°C and 31.5 °C, salinity of 33 to 35 gm/L and estimated pH of 9.27 to 9.31. The samples were cleaned from epiphytes and sand, washed with tap water, air dried at room temperature and ground to coarse powder and finally stored in plastic bags in a dry dark place until use.

Ulvan extraction

Five hundred grams of air dried samples were successively extracted with petroleum ether (b.p. 40-60°C), diethyl ether, acetone, chloroform, ethyl acetate, (95%) and (70%) ethyl alcohol respectively. The solvent was removed by distillation and the crude extract obtained from each solvent was dried and weighed.

Phytochemical screening

The preliminary phytochemical screening of the alcohol extract of *U. lactuca* was carried out according to the methods described by (Wall *et al*., 1954; Woo *et al*., 1977; Balbaa, 1986 and A.O.A.C., 1991).

Composition analysis

Free and combined sugars were determined by using the method described by Eaton (1989) and detected by Gas- liquid chromatography GCV Pye-Unicam Sigma 3 B instrument equipped with an GP 3% SP 2330 on 100/120 supelcoport column. Nitrogen was used as the carrier gas with a flow rate of 40 ml/min. The temperature of the column ranged from 100 to 265°C, while the detector and the injector were kept at 300^{o} and 280^{o}C, respectively.

The identification and determination of free and protein amino acids were carried out according to Pellet and Young (1980) using LKB alpha plus high performance Amino Acid Analyzer LKB Biochrom LTD England. Retention times and peak areas were determined using Hewlett Packard 3390 recording integrator. The concentration of each amino acid was calculated as (gm/ 16 gm nitrogen) by a special designed program.

Hydrocarbons, sterols and fatty acids were separated and identified by GLC according to Eaton (1989). The fatty acids were determined as their methyl ester which was obtained by reaction of the acids with an ethereal solution of diazomethane as described by Chistie (1982) and then subjected to GLC analysis. Gas-liquid chromatography runs were performed on a Hewlett Packard HP6890 instrument equipped with an HP INNO wax column (30 m x 0.32 mm x 0.5 µm) and flame ionization detector. Nitrogen was used as the carrier gas with a flow rate of 2.6 ml/min, and the temperature of the column, injector and detector were 300^{o}C, 220^{o}C and 275^{o}C respectively. The relative percentage of each compound was determined using the triangulation method (Nelson *et al*., 1969).

The free phenolic acids were isolated according to the method established by Danny *et al*. (2003) and separated on an HPLC instrument (Knauer, Germany) equipped with a Model 8700 UV detector and a Model 7125 injection valve (Rheodine, Cotati, CA, USA) with a 50 µl sample loop, under computer control (Knauer, HPLC, version 211 a). A gradient mobile phase of water acetonitrile adjusted to pH 2 by phosphoric acid was used on an ODS (5µm) column of 200 x 4.6mm. The flow rate was 1.0 ml/min and detection was carried by UV at 280 nm.

Total ash content was estimated as described by Askar and Treptow (1993) and the minerals were determined according to A.O.A.C. (1991) by using Unicam M.A. 929 atomic absorption spectrophotometer.

Isolation of steroidal hydrocarbon

The steroids were first extracted from the dried pulverized powder of *U. lactuca* with 95% methanol several times. The combined alcohol extracts were concentrated to 50 ml, diluted with 50 ml water and then extracted repeatedly with chloroform (5 x 100 ml) and the chloroform extract was finally evaporated to dryness under reduced pressure. Two grams of the chloroform extract was subjected to silica gel column chromatography and eluted with chloroform - methanol (95: 5 v/v) to give 20 fractions each 10 ml, the elutes were then separated by thin layer chromatography (TLC) using petroleum ether- ethyl acetate (90: 10 v/v) as solvent system. The spots were detected with 1% vanillin / H_2SO_4spraying reagent and heated at 110°C for 10 min. The 'main steroidal

compound was purified by passing through sephadex LH-20 column and eluted with methanol, evaporated till dryness and weighed (Fieser and Fieser, 1959). The IR spectrum of this compound was carried out using Fourier Transform Infrared spectrometer, (FT/ IR- 300 E) between 400- 4000 cm^{-1}, and GC/MS QP 1000 Ex was performed using Varian Mat 711mass spectrometer Finnigan SSQ 7000 and MM 7070 E instrument equipped with a column (30 m x 0.25 mm), the carrier gas was helium, time 20 and temperature 40 - 280°C.

Results and Discussion

The preliminary chemical investigation of *U. lactuca* revealed the presence of flavonoids, carbohydrates and / or glucosides, tannins, sterols, chlorides and sulphates, while free from alkaloids.

The chemical composition of *U. lactuca* is given in table (1), showing that ash content was 25 g % and the dominant mineral components were sodium, potassium, chloride, magnesium, iron and boron as well as minor amounts of manganese, zinc and copper. The present data are in agreement with the finding of Misheer *et al*. (2006) which stated that *U. lactuca* from Kwazulu-Natal Coast South Africa possess an excellent bio-indicator for most of the metal studied. It is worthy to note that Khafaji *et al*. (1992) related the effect of environment on algal composition.

Ribose, rhamnose, glucose and xylose were present as free sugars of which the latter was the predominant free sugar. On the other hand, glucoronic acid had the greatest percentage of the combined sugars present (20%). These results differ from those reported by Khafaji *et al*. (1992), probably due to difference in the environmental conditions of the living algae. However, the data agree with the results found by Salah El Din (1994) for algal species obtained from the Red Sea.

Fifteen amino acids were estimated in the studied *Ulva* species, the amino acid cysteine was not detected among the different amino acids of this alga. Mohsen *et al*. (1979) as well as Munda and Gubensek (1986) also showed that cyteine was present only in brown algae and there were some differences in the amino acids of the different species.

The fatty acid composition of the lipid fraction has been shown to be affected by light, salinity, mineral ions, heavy metals, pollution, herbicides, infection by fungi and bacteria, habitat and environmental conditions (Kim *et al*., 1996). In the present investigation, twelve fatty acids were detected in *Ulva lactuca* obtained from the Red Sea of Jeddah of which, caprylic acid was the major component (30.32%). Yazici *et al*. (2007) also reported that green algal

Table 1. Percentages of different components of *Ulva lactuca* from Jeddah coast.

Component			
gm / l00 gm dry wt.		Combined amino acids	gm / l6 gm N
Ash	25	Aspartic	6.00
Minerals		Threonine	2.10
Na	0.49	Serine	3.10
K	0.74	Glutamic	5.00
Ca	2.45	Glycine	4.00
Mg	2.47	Alanine	3.30
Cl	3.36	Valine	2.10
S	3.95	Methionne	1.30
P	0.12	Isoleucine	1.00
mg/100gm dry wt		Leucine	4.10
Co	0.1	Tyrosine	1.50
Fe	99.7	Ph-alanine	4.00
Cu	1.2	Histidine	3.00
Mn	4.8	Lysine	2.70
Zn	9.2	Arginine	6.50
B	40.0	Fatty acid	% of total lipid
Free Sugars mg %		Caprylic	30.32
		Capric	2.93
Ribose	13.27	Lauric	1.91
Rhamnose	13.51	Myristic	2.57
Glucose	13.01	MyristoUc	0.34
Xylose	26.88	Pentadeconoic	2.10
Galactose	20.1	Palmitic	2.32
Combined sugars		Heptadecylenic	1.83
Rhamnose	8.13	Oleic	2.37
Ribose	6.51	Linolenic	2.63
Galactose	3.16	Arachidic	9.1
Glucose	2.81	Behenic	4.60
Xylose	2.79	Hydrocarbons and sterols	
Glucoronic	20.1	Hexadecane	1.7
Free amino acids gm / 16 gm N		Docosane	1.8
Asparitc	1.09	Tetracosane	1.9
Serine	3.28	Hexacosane	9.5
Glutamic	1.00	Triacosane	16.17
Glycine	0.63	Stigmasterol	2.85
Alanine		Cholesterol	1.80
Valine	2.5	Campesterol	1.80
Isoleucine	1.5	Phenolic acids	mg %
Leucine	1.6	Caffeic	1.6
Tyrosine	1.20	P.coumaric	1.4
Phenylalanine	2.20	Ferulic	1.01
Proline	20.5	Protocatechuic	2.5
Histidine	1.90	Vanillic	0.8
Threonine	0.50	p-hydroxybenzoic	0.83
Lysine	0.80	Chlorogenic	0.2

species had a considerable higher proportion of unsaturated fatty acids and a significantly lower proportion of saturated fatty acids than those in the red and brown algae.

Seven free phenolic acids were identified in the studied Ulvan. The data in table (1) indicates that protocatechuic acid was the greatest component (2.5 mg %). It is noteworthy that the phenolic compounds are known to be highly toxic to certain potozoa and some of them act as antioxidants (Watt and Brandaijk, 1962) or as anticancer (Ross and Brain, 1971).

The substance crystallized from 70 % ethanol (80 mg) was obtained as white needles with melting point 129-130°C, soluble in diethyl ether, chloroform and ethanol. TLC chromatographic investigation of this compound showed a single spot with R_f 0.8 or R_f 0.7 when petroleum ether-ethyl acetate (90: 10 v/v) and chloroform-methanol (95:5v/v) were used as solvent systems respectively. A red color was obtained when the chromatoplate was sprayed with 1% vanillin / H_2SO_4 in visible light.

Infrared spectrum (Fig. l) indicated the presence of a carbonyl group at 1750 cm^{-1} and a relatively weak absorption at1601 cm^{-1} which is due to conjugated C = C. The strong peak at 2870 cm^{-1} is typical for a lactone and the absorption at 2925 cm^{-1} is an indication for the presence of methelene (Piemann, 1983).

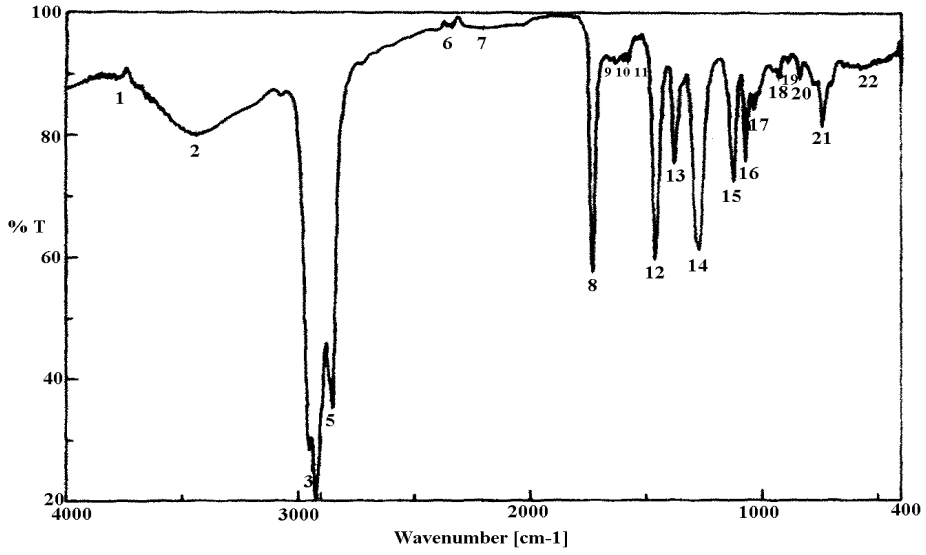

Figure (1): The IR spectrum of the isolated steroidal compound from Ulva lactuca

The mass spectrum (Fig. 2) showed molecular ion peak at m/z 149 which was likely for molecular formula $C_{21}H_{36}O$ (M. Wt. 288). Further investigation of the fragments by the MS revealed the presence of ion peaks at 125, 111, 97, 83, 71, 57 and 43 such as (M- Me) and [(M- (Me- H_2O)] which are characteristic for steroid compound. Mass spectra of this compound shows a close resemblance to the published data for 14 β-H pregnan-3 one (William, 2001). It is noteworthy that this steroidal compound was isolated for the first time from *U. lactuca* species obtained from the Red Sea coast of Jeddah, Saudi Arabia.

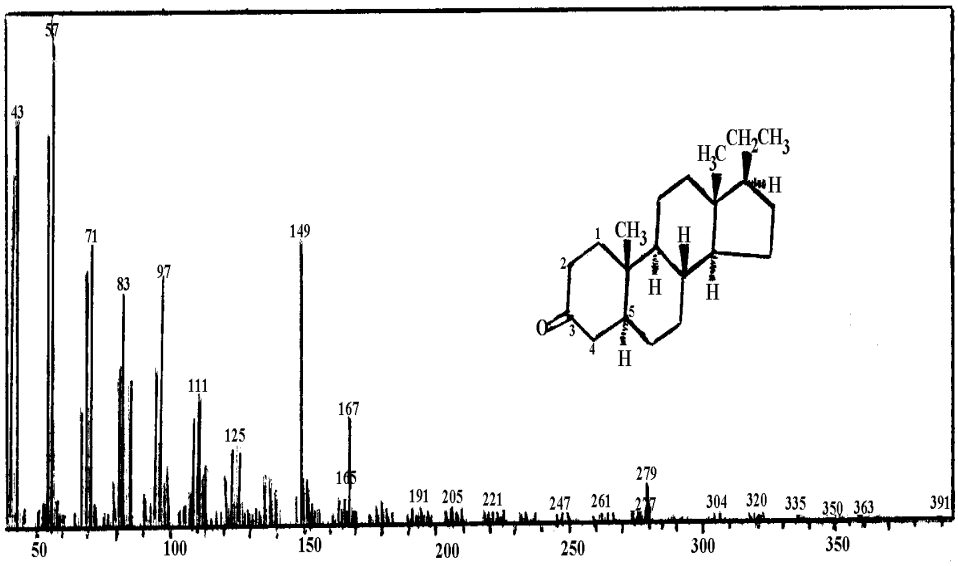

Figure (2): The Mass spectrum of the isolated steroidal compound

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دراسات كيميائية على طحلب أولفا لاكتيوكا من ساحل البحر الاحمر، بمنطقة جدة، المملكة العربية السعودية

*** زينب السويفى و** نسيم إسماعيل راضى**

** قسم النبات والميكروبيولوجى، كلية العلوم، جامعة الأزهر (فرع البنات)*

*** قسم الأحياء، كلية العلوم التطبيقية،جامعة أم القرى، المملكة العربية السعودية*

أثبتت التحاليل الكيميائيه أن طحلب أولفا لاكتيوكا يحتوى على مواد فلافونيديه و كربوهيدرات وتانينات واستيرويدات وكبريتات وكلوريدات، كما انه يخلو تماما من القلويات.كما شملت التحاليل الكيميائيه النسب المؤيه لكل من الكربوهيدرات، الأحماض الأمينية، الليبيدات و الأحماض الفينولية و الرماد الكلى وبعض العناصر مثل الصوديوم، البوتاسيوم، الكالسيوم، الماغنسيوم، الكبريت و الفوسفور. و تم عمل مسح للمركبات التربينيه الستيرويديه للطحلب حيث ظهر أنه يحتوى على المركب الأستيرويدى 14- بيتاهيدروجين بيرجنان-3 واحد (14β-Hpregnan-3one) كمركب اساسى.