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CHARACTERIZATION, ANTIOXIDANT POTENTIALITY AND BIOLOGICAL ACTIVITIES OF THE POLYSACCHARIDE ULVAN EXTRACTED FROMTHE MARINE MACROALGA *Ulva* SPP

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ABSTRACT

Marine macroalgae contain many bioactive compounds, which have a wide range of applications.Ulvan (Sulfated heteropolysaccharide) extracted from *Ulvalactuca and Ulvafasciata*,have been characterized, antioxidant capability as well as its biological activity in affecting growth and metabolism of the tested microalga *Chlorella vulgaris.* The polysaccharides yield was about15% and 17 % on dry algal biomass basis respectively. The two *Ulva*sppcontainefour neutral sugars: rhamnose, glucose,galactose and xylose as estimated by HPLC. The FT-IR spectrum of ulvan showed bands corresponding to sulfate esters and uronic acids. Thermogravimetric analysis revieledthatulvan extracted from each*U. lactuca* and *U.fasciata* is thermally stable before degradation at 169^{o}C and 245^{o}C respectively. Viscosity of *U. faciata*ulvanismore viscous than that of *U. lactuca* (15.3 cps and 12.23 cps respectively).Antioxidant activity of ulvan is investigated through estimation of the reducing popwer. The different concentrations of ulvan has significant promoting effect on growth parameters of *C. vulgaris* (culture optical density, dry biomass, photosynthetic pigments, total soluble protein content and carbohydrate content) as well as non enzymatic antioxidants potential (represented by phenolic components content) and enzymatic antioxidants as Guaiacol peroxidase activity (GPX), Ascorbic acid peroxidase (APX) and Catalase activity (CAT). Ulvanenhances the microalgal growth via promoting antioxidants potential, consequentlyulvan can be considered as anpromising candidate material for agricultural, biomedical applications as well as biostimulant for mass production of microalgae.

Keywords: *Ulva*, Ulvan, FT-IR,Antioxidant enzymes, GPX, APX, CAT, Rheology, GravimetricThermal analysisTGA,*Chlorella vulgaris*

INTRODUCTION

Marine green macroalgae belonging to Ulvales are very universal seaweeds dispersed worldwide having various storage materials;mainly carbohydrates, however, they produce larger quantities of polysaccharides (Wood, 1974). These polysaccharides are remarkably different with those of

higher plants, especially theirinvolvement of sulfate groups and unusual sugar residues, high content of ionic groups, as well as water solubility and exceptional rheological properties (Wood, 1974; Jensen, 1993; Michel and Macfarlane, 1996; Popper *et al.*, 2011). *Ulva*sp*ecies o*wn cell walls rich in a characteristic sulphated soluble polysaccharide known as ulvan(Bobin- Dubigeon et al., 1997;Robic et al., 2009;Popper et al., 2011).

Ulvan has been recognized as being a sulphated single polydisperseheteropolysaccharide composed of changeable amounts of uronic acids, containing glucuronic and iduronic acids irregular with neutral sugar, as rhamnose, xylose and glucose residues, connected by α- and β-1→4 bonds (Prosperi, 1994; Lahaye and Ray, 1996; Lahaye *et al.*, 1997; Schaeffer and Krylov, 2000). It yields about 18–29 % of the carbohydrate fraction of green algae (Kaeffer *et al.*, 1999). McKinnell and Percival (1962)illustrated the structure of ulvan demonstrating that sulfate groups connected to rhamnose, maybe in position 2; the branching of the heteropolysaccharide may be suggested, and uronic acid residues wereregarded as possible end groups, being those attached to position 4 in rhamnose.

There are growing attention for marine algae,as sources of unique polysaccharides with novel structures and attractive biological activities for innovative potential applications, is increasing. These include food, pharmaceutical and medical industries as well as biotechnological applications (Bocanegra *et al.*, 2009).

Chlorophyta are composed of ~11 % protein, ~36 % carbohydrate, ~53 % ashe and are rich in minerals like calcium, iron, phosphorous and chloride. Carbohydrates constituted of cell-wall water-soluble sulphatedulvan, alkali-soluble hemicellulosicβ(1,4)-D-glucuronan and β(1,4)-D-glucoxylan and amorphous α-cellulose with xylose residues (Lahaye*et al*. 1997).Ulvancompose about 18–29 % of the carbohydrate portion of green algae(Yu *et al.*, 2010).The antioxidant activity of polysaccharides depends on several structure parameters such as the molecular weight, the type of sugar, the glycosidic branching, and the degree of sulfation and acetylation position as suggested by Wang *et al.*, (2008).

The objective of this study is focused on extraction and characterization of the soluble sulfated polysaccharide ulvanof*Ulvalactuca* and *Ulvafasciata*as a representative substrate of green macroalgal biomass as well as investigating the biological activities of ulvan on growth and metabolism of the tested microalga *Chlorella vulgaris*in addition to the antioxidant systems (antioxidant enzymes plus the non-enzyme components).

MATERIALS AND METHODS

The green macroalgae*Ulvalactuca*and*Ulvafacciata*were collected during the spring of 2011 from the shallow water beside the shore of Mediterranean sea at Abo Quire coast, Alexandria, Egypt.*Ulva*thalliafter washing with tap water dried at 60° C for constant weight.

Ulvan extraction: Ulvan was extracted according to the hot extraction method ofAlves *et al.*, (2010)and precipitated by ethanol.

Chemical characterization of ulvan: Carbohydrate content wasdetermined using phenol sulfuric acid method(Dubois *et al.*, 1956)using rhamnose as a standard. Meta-hydroxydiphenyl method was used in determination of uronic acid content (Filisetti-Cozzi and Carpita, 1991).Sulfate content wasestimated according to Kawai *et al.*(1969).Ulvan hydrolysis and composition analysis was attainedusing a high performance liquid chromatography (HPLC) system and ferric reducing antioxidant power was achieved according to Qiao *et al.*, (2009).

Characterization of ulvan

***Fourier Transform Infrared* (FT-IR) spectroscopy:** FT-IR spectrum was measured on theMattson 5000 FT-IR spectrometerin the frequency range of 400 - 4000 cm^{-1}(Wang *et al.*, 2004).

Thermal gravimetric analysis (TGA): Thiswas measured on a thermoanalyzer of the type D-50 (Shimadzu, Japan). The thermogram was obtained in the range of 25°Cto 800°Cunder nitrogen atmosphere.

Rheological property analysis of ulvan: The rheological measurements of ulvansolutions (10, 20 and 30 mgulvan/ml) were carried out on BROOKFIELDDV-III Ultra Programmable Rheometer(Fernandes *et al.*, 1991).

Antioxidant activity of ulvan (Reducing power): The reducing power ability of ulvan solution was quantified according to Kumar *et. al.,*(2011).

Biological activity of ulvan

Chlorella vulgaris* growth conditions:Chlorella vulgaris* Beijerinck MUAC was grown in axenic cultures at 22°C-24°Cunder continuous illumination (72 µmol photon m^{-2} s^{-1}) in 500 ml Erlenmeyer flasks, containing 200 ml BG11media (Rippka *et al.*, 1979). Ulvan was added in two concentrations (5, 10 mg/ 100 ml media) and incubated for 16 days.

Algal growth analysis

Culture optical density: The algal growth was monitored spectrophotometrically (Unico UV-Vis S21000, USA) at 685 nm(Wetherell, 1961).(optical density of culture at 685 nm)

Biomass: Drying cells at 70°C.

Estimation of photosyntheticpigments: According to method recommended by Metzner *et al.*, (1965).

Total soluble proteincontent: It is determined after method ofLowry *et al.*, (1951).

Investigation of antioxidant activities of *C. vulgaris*

Non - enzymatic antioxidants(Estimation of total phenolic components):

Total phenols were determined by Folin-Ciocalteu (F-C) reagent(Kumar *et al.,* 2011)

Enzymatic antioxidants activities

Guaiacol peroxidase activity (GPX, EC: 1.11.1.7):GPX activity was determined as indicated by Upadhyaya *et al.*, (1985).

Ascorbic acid peroxidase activity (APX, EC: 1.11.1.11):APX activity was estimated as stated by Chen and Asada, (1989).

Catalase activity (CAT, EC: 1.11.1.6): CAT activity was assayed following the method of Kang and Saltveit, (2001).

Statistical analysis: Values expressed are means ± *SD* of three replicates

RESULTS

Carbohydrate content of ulvan: carbohydrate fractionsof *U. lactucaulvan* and *U. fasciata*ulvanreached 35.22 % and 37.31% respectively (Table 1).

Yield, chemical analyses and monosaccharide composition of ulvan:

Ulvan yielded about15–17% on basis of algal dry weight as shown in table (1). Monosaccharide composition of*U*. *Lactuca*ulvanis composed mainly of rhamnose, glucose and galactose in addition to xylose, meanwhile concerning *U*. *fasciata*ulvan, itcontains mainly rhamnose and glucose and galactose. Sulfate and uronic acids contents are more or less in the two ulvan samples.

Table 1: Yield, chemical analysis and monosaccharide compositionofulvan extracted from *Ulvalactuca*and*Ulvafasciata*

***Ulva* spp**	**Yield* (%)**	**Sulphate* (%)**	**Uronic Carbohydrate acids* content (%) (%)**	**Monosaccharide amount (%)**			
				Glucose& Galactose	**Rhamnose**	**Arabinose**	**Xylose**
U.Lactuca	14.83 ±0.78	23.84 ±0.95	20.5235.22 ±1.21±1.78	28.78	34.13	13.64	23.64
U.fasciata	16.96 ± 0.86	25.14 ± 0.79	19.1837.31 ± 1.04± 1.98	41.11	47.96	10.88	--

FT-IR characterization of the extracted polysaccharide: The FT-IR absorbing spectra for the two ulvan samples isolated from *U. lactuca*and *U. fasciata*wereanalogous,revealingthecharacteristic functional groups,showing peaks at (3441cm^{-1}, 3443 cm^{-1}), (2853 cm^{-1}–2961cm^{-1}, 2926 cm^{-1}– 2856 cm^{1}), (1636cm^{-1}, 1659cm^{-1}cm^{-1}- 1634cm^{-1}), (1544 cm^{-1}- 1322cm^{-1}, 1440 cm^{-1}-1327cm^{-1}), (1127 cm^{-1} – 1000 cm^{-1}, 1153 cm^{-1}- 1000cm^{-1}), (1000cm^{-1}–500cm^{-1})respectively (Figs.1,2).

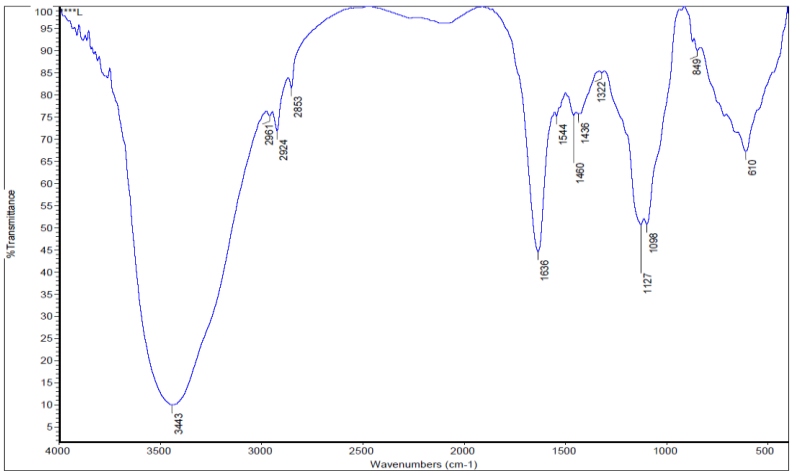

Fig. 1: FT-IR spectra of *U. lactuca*ulvan

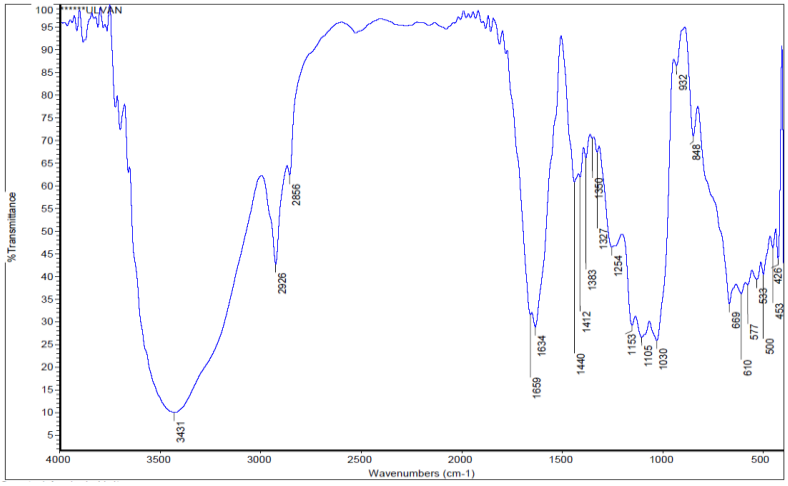

Fig.2: FT-IR spectra of *U. fasciata*ulvan

Thermal characteristics of the extracted polysaccharides: As indicated from thermograms in figures (3 & 4), the first thermal degradation of *U. lactuca* and *U. fasciata*ulvansoccurred at around 93ºC, 113ºC respectively. This disintegrationaccompanied by about11, 13 % of mass loss. The second thermal decomposition took place at about 169ºC,245ºC with a loss of mass about 5, 14% respectively. This followed by the third thermal decomposition which happened at about 221ºC, 329ºC with loss of mass about 13, 10%for both *U. lactuca* and *U. fasciata* polysaccharides respectively. The depolymerization started after this temperature and continued till 666°C, 678 °C resultinginbreakdownwith a further loss of mass about15, 19 %. The next phase of degradation from 666°C or 678 °C to 800°C characterized by very slight weight loss.

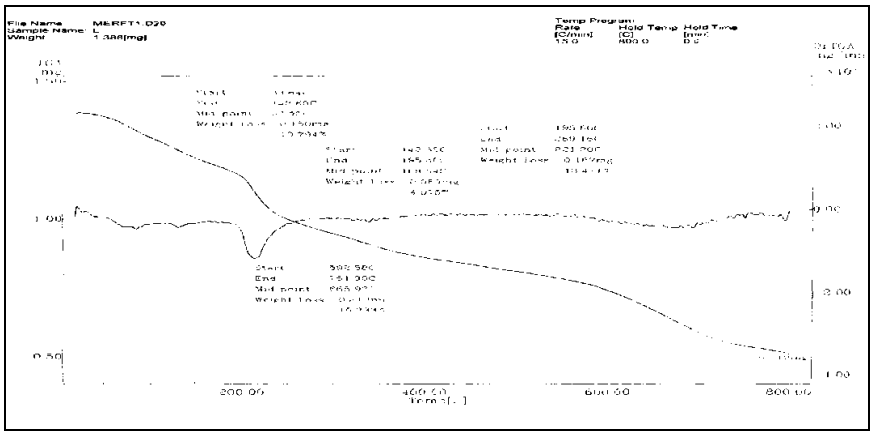

Figure 3: Thermogravimetric analysis (TGA) of*U.lactuca*ulvan

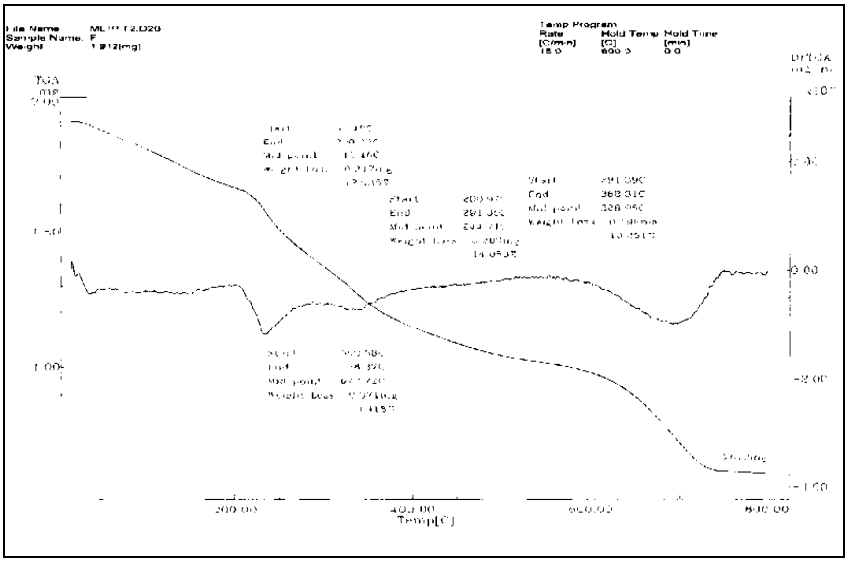

Figure 4: Thermogravimetric analysis (TGA) of*U.fasciata*ulvan

Rhreological properties of the extracted ulvans: Rheograms indicated that with increasing shear rate resulted decreasing viscosity in the two ulvan solutions (figs. 5 & 6). Dynamic viscosity profile (Fig. 7) illustrate increasing viscosity with increasing ulvan concentration recording the highest value to *U. fasciata*ulvan.

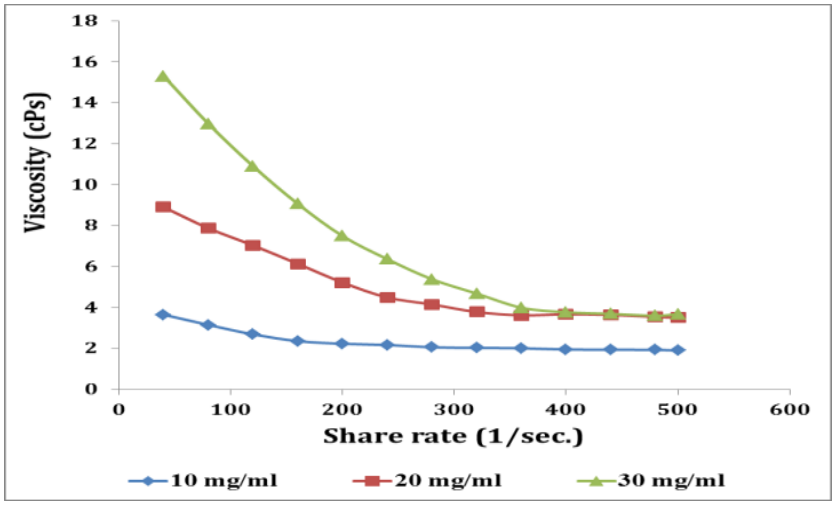

Fig. 5: Viscosity as a function of shear rate of aqueous solutions of *U.lactuca*ulvanat concentrations (10, 20, 30 mgulvan ml^{-1})

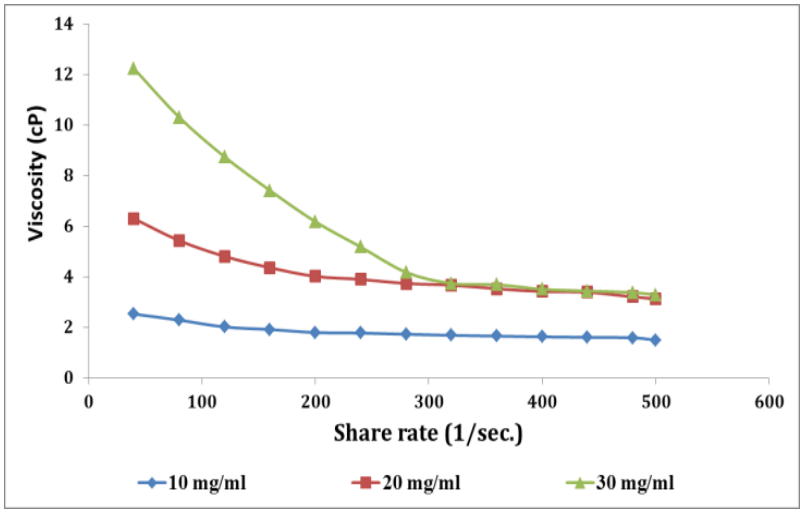

Fig. 6: Viscosity as a function of shear rate of aqueous solutions of *U.fasciata*ulvanat concentrations (10, 20, 30 mgulvan ml^{-1})

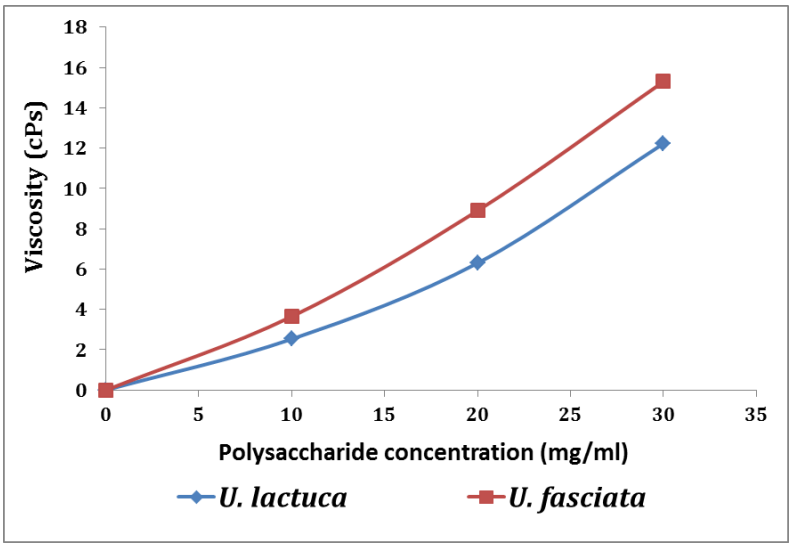

Fig. 7: Rheogram of viscosity dependence onulvanconcentrations (10, 20 & 30 mg ml^{-1})

Antioxidant potential of ulvan (reducing capacity): Prominent absorbance value indicates stronger reducing potential.The reducing power of ulvan samples increased with increasing concentration in dose - responding manner (Fig. 8).

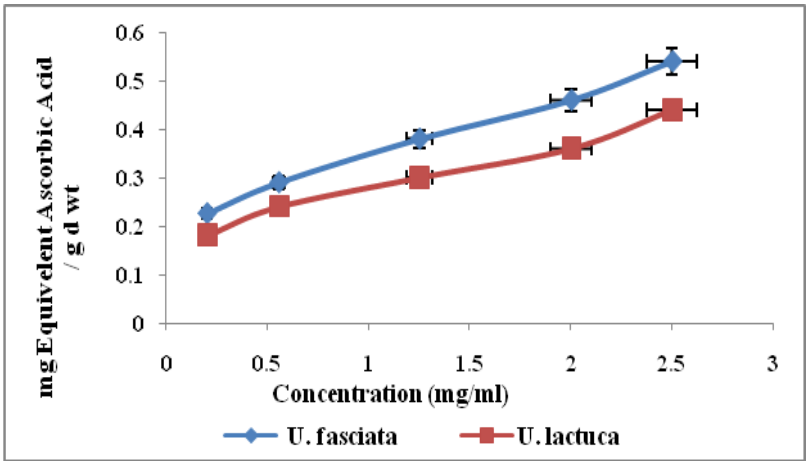

Fig. 8: Reducing capacity of ulvan (mg Equeivelent Ascorbic Acid g^{-1} d wt).

Effect ofulvan supplementation on growth of *Chlorella vulgaris*

Changes in culture optical density: Data (Fig. 8) showed a progressive increase in*C. vulgaris*growth throughout the experimental period. and all ulvan treatments significantly stimulategrowth over control value.

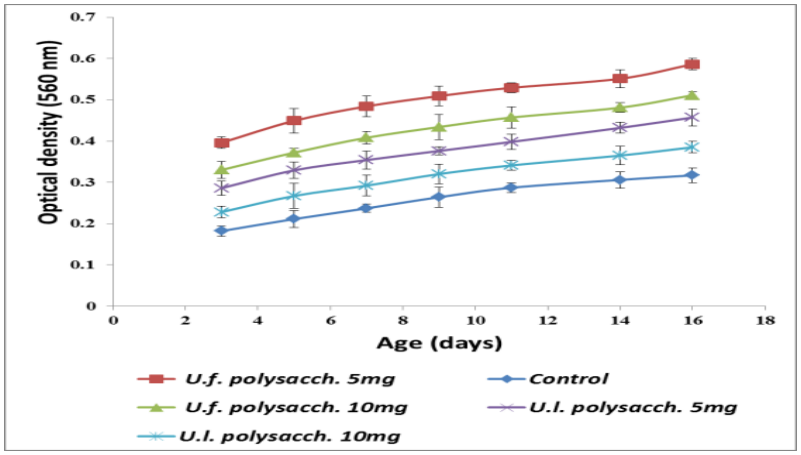

Fig.9: Changes in *C. vulgaris* culture optical density as affected with ulvan concentrations (5& 10 mg/100 ml medium).

Changes in *Chorellavulgaris*biomass: Allulvan treatments induced significant progressive increase in *C. vulgaris*biomass (gdwt/L culture) throughout the experimentalperiod(Fig.10)givingthe maximum value in the culture supplemented with*U. fasciata*ulvan(5mg/100 ml medium).

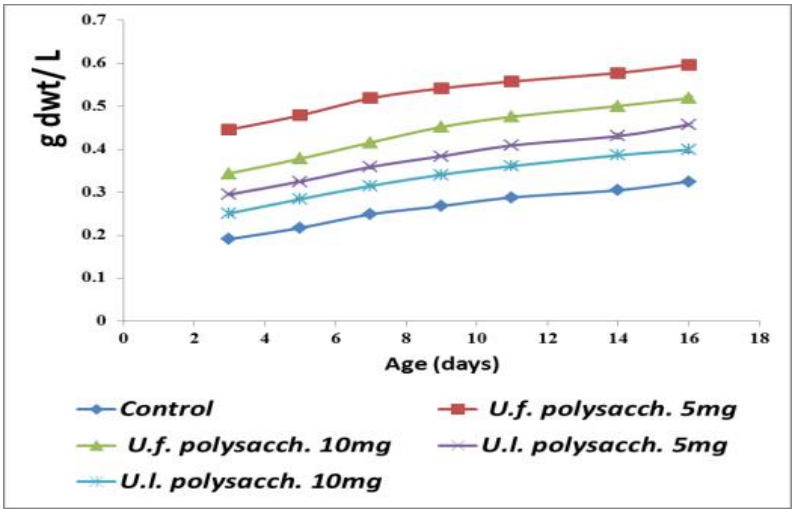

Fig.10: Effectofulvanonbiomass (g dwt/L culture) of*C. vulgaris*cultures

Photosynthetic pigments contents: Ulvantreatmentssignificantly stimulated biosynthesis of the photosynthetic pigments figures (11, 12& 13). The maximum value of chlorophyll a .b, caroteniods and consequently total pigments are recorded to *C. vulgaris*culture treated with*U. fasciata*ulvan (5mg/100 ml medium).

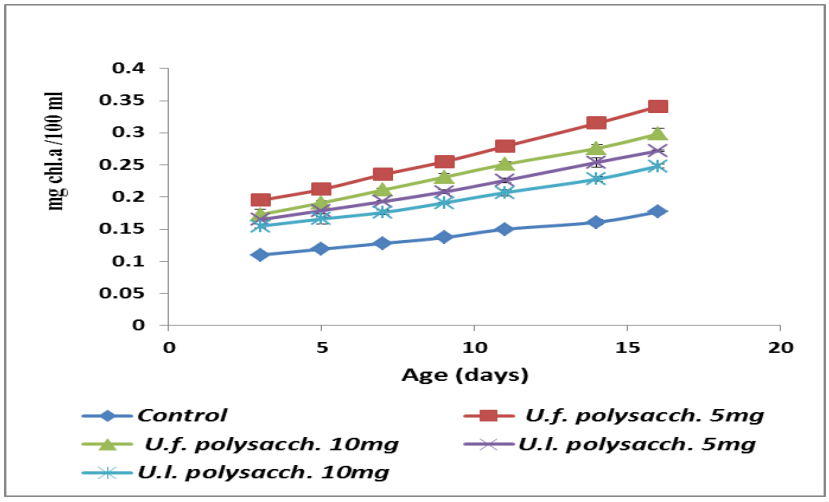

Fig. 11: EffectofulvanonChl. acontent of *C. vulgaris*

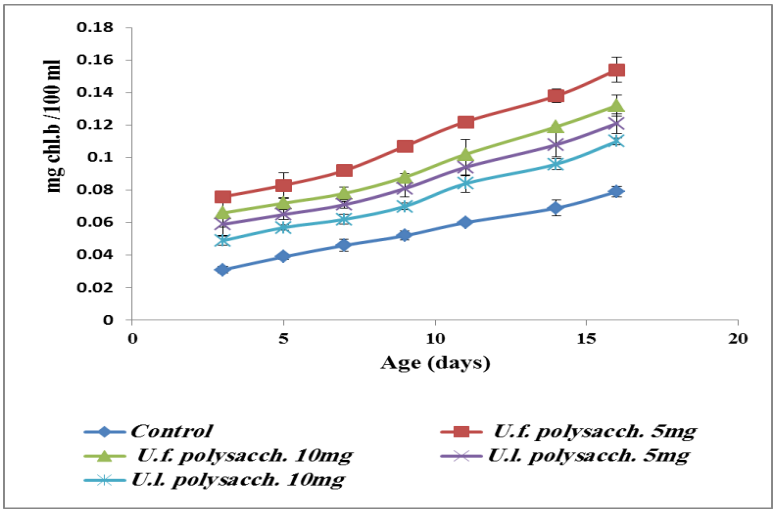

Fig. 12: EffectofulvanonChl. b content of *C. vulgaris*

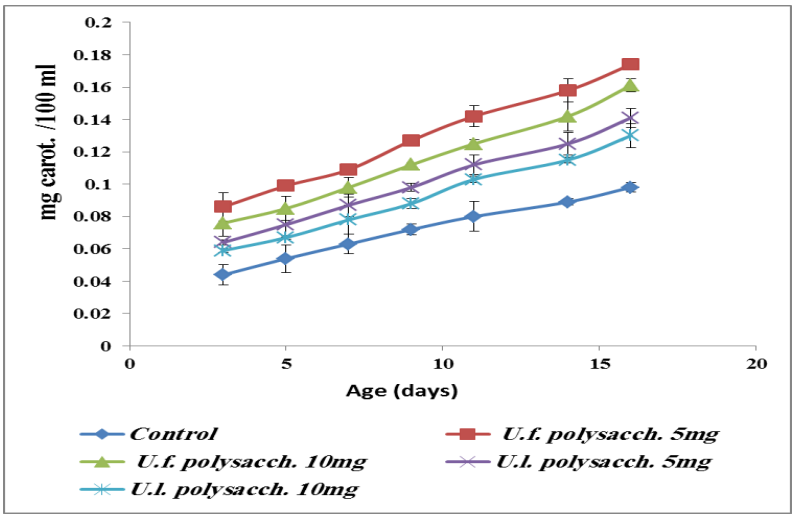

Fig. 13: Effect ofulvanoncaroteniods content of *C. vulgaris*

Carbohydrates content: Data (Fig.14) demonstrate the presence of significant increases in carbohydrate fractions of *C. vulgaris* with all treatments compared to control values. The high response is recorted*U.fasciata*ulvan (5 mg) supplementation.

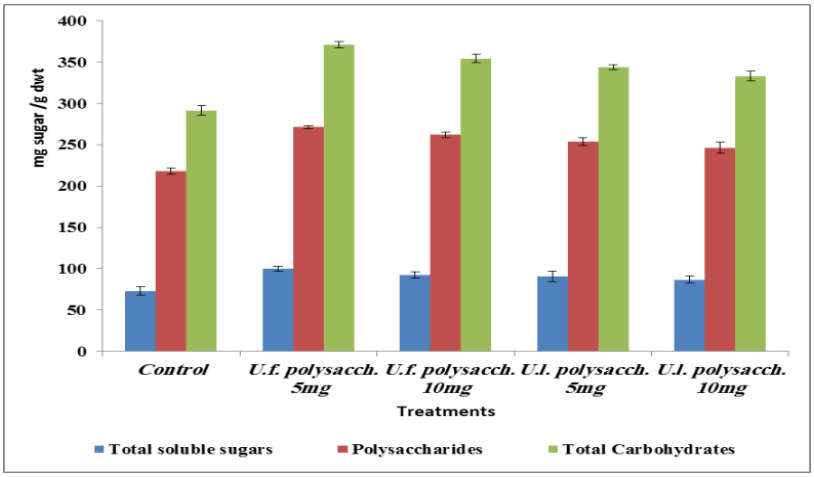

Fig.14: Effect of ulvan on carbohydrates contents of *C. vulgaris* (mg sugar/ g dwt).

Total soluble protein content: Data (Fig.15) indicate thepresence of significantincreases in total soluble protein content of *C. vulgaris*cultures supplementations. The high response is recorted*U.fasciata*ulvan (5 mg) supplementation.

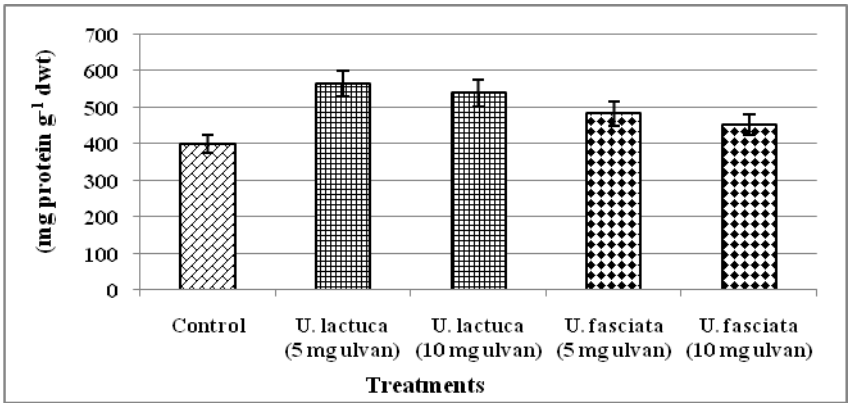

Fig. 15: Effect of ulvan on total soluble protein content of *C. vulgaris* (mg protein / g dwt).

Antioxidant activities

Estimation of total phenolic components: Total phenol content of treatedcultures showed significant increases above the control level in response toulvan treatment showingthemaximum value to *U. fasciata*ulvan (5mg) supplementation(Fig. 16).

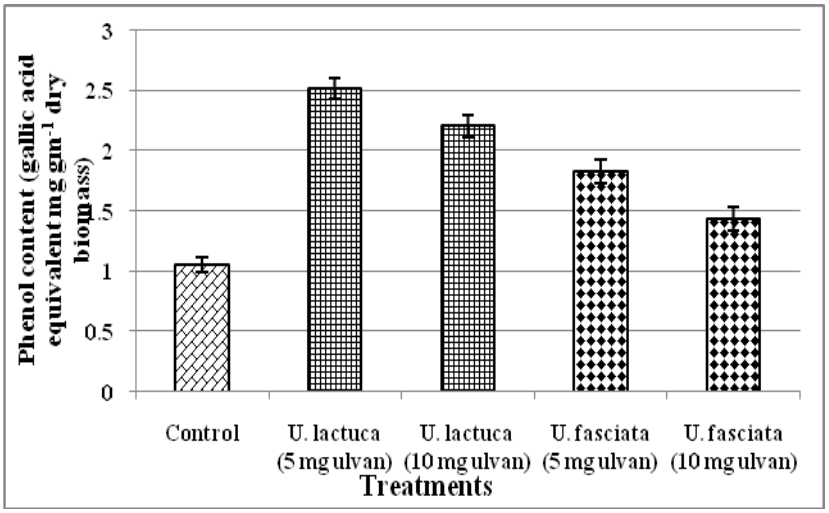

Fig. 16: Effect of ulvanon phenol content of *C. vulgaris* (gallic acid equivalent mg g^{-1} dry biomass).

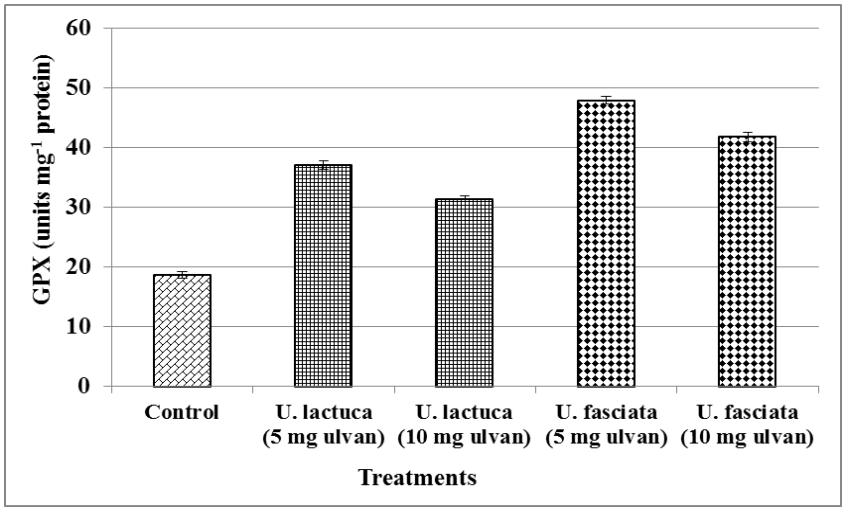

Fig. 17: Effect of ulvanonGuaiacol Peroxidase Activity (GPX units mg^{-1} protein) of *Chlorella vulgaris*.

Enzymatic antioxidants activity

Guaiacol peroxidase activity (GPX, EC: 1.11.1.7): Peroxidase activity induced significant increases in response to all ulvan amended cultures giving the highest vaslue in case of 5 mg*U.fasciata*/100 ml medium supplementation (Fig17).

Ascorbic acid peroxidase activity (APX, EC: 1.11.1.11): Results has pointed out that ulvan addition facilitatesincreases of ascorbic acid peroxidase (APX) activity(Fig. 18), giving the highest response to ulvanexogenous treatment with 5, mg/ml medium *U. fasciata*supplementation (15.83 APX units mg^{-1} protein).

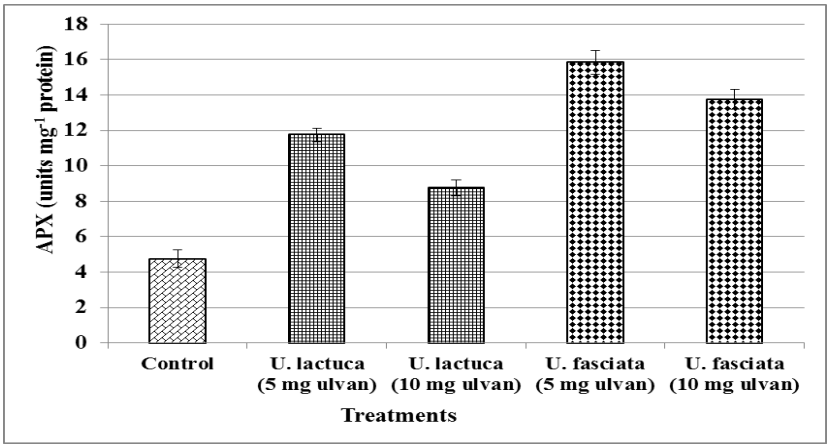

Fig. 18: Effect of UlvanonAscorbatePeroxidaseActivity (APX units mg^{-1} protein) of *Chlorella vulgaris*.

Catalase activity (CAT, EC: 1.11.1.6): Ctalase is a key enzyme that participates in antioxidant processes of microalgae.Ulvantreatments induced significant positive responses in all treated *C. vulgaris* cultures with maximum value (33.615units g^{-1}protein) recorded to*U.fasciata*5mg ulvan addition/100 ml medium (Figure 19).

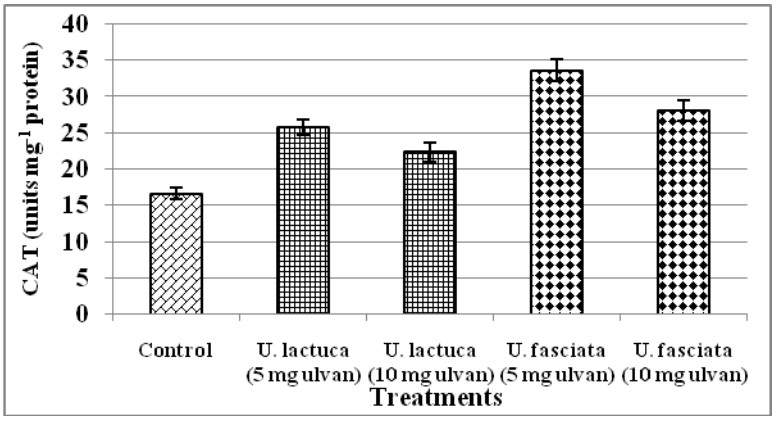

Figure 19: Effect of ulvanon Catalase Activity (CATunits mg^{-1} protein) of *Chlorella vulgaris*.

DISCUSSION

Yield and chemical composition of ulvan:The present results are in agreement with De Vuyst and Degeest, (1999)who found that yield of extraction of ulvan varies between 1.2 to 27.5 % and the cell wall carbohydrate content of green macroalgae ranges from 38 to 54 %, and the ulvan content may vary between 8 and 29 % (Gordillo et al., 1998; De Vuyst and Degeest, 1999; Yu*et al*., 2010; Rossi *et al.,* 2012;Chakraborty and Pal, 2014).Ulvan isolated from *Ulva reticulate* was composed of rhamnose, galactose, xylose, mannose, and glucose, uronic acid content of 19.3%, and sulfate content of 14.3% as reported by Quach*et al*. (2015).

FT-IR characterization of ulvan:The FT-IR spectrum of ulvan in both *Ulva* species demonstrated a broad strong and intense signal within the range (3500 cm^{-1} – 3300 cm^{-1}),this was attributed to the stretching vibration of the hydroxyl group (O-H) characteristic to saccharide structures that existed in the H-bonds of the molecules(Jensen *et al.*, 2001)as well as the medium stretching vibration of secondary amines (N-H). Bands at the absorption range (3000 cm^{-1} – 2850 cm^{-1}) are attributed to the strong stretching of alkanes (-CH_2 groups) and alkenes (= C-H or C=C groups). The absorption peaks at 1636 cm^{-1}, 1659 cm^{-1}, 1634 cm^{-1} indicate the strong bending asymmetricvibration of the carboxylate group (-C=O) of uronic acids in ulvan(Jensen *et al.*, 2001).Bands of absorption spectra at 1254 cm^{-1} and 1322 cm^{-1} are attributed stretching of sulfate ester (S=O), the strong signals at 1153 cm^{-1}, 1105 cm^{-1}, 1030 cm^{-1}, 1098cm^{-1} is familiar to all sulfated polysaccharides as glycosidic bond vibration (Lama *et al.*, 1996), while peaks at 849 cm^{-1} , 848 cm^{-1}might correspond to the bending vibration of C-O-S of sulfate in equatorial position and are related to sugar cycles (Lama *et al.*, 1996)and that bands around 868 cm^{-1} indicate the presence of a β-anomeric configuration for arabinopyranose(Robic *et al.*, 2009).The signal at 1049-1216 cm^{-1} is attributed to S=O stretching. The minor absorptionpeaks around800 cm^{-1} may be considered as the fingerprinting region, definite of each polysaccharide(Mao *et al.*, 2006, Alves*et. al.*, 2010 ; Jaulneau *et al.*, 2010).

Thermal characteristics of the extracted polysaccharides:

Thermogravimetric analysis measures the weight loss of a material with dehydration, decomposition and oxidation of a sample as a function of temperature.The obtained thermograms of *U. lactuca* and *U. fasciata*can be explained as follow:the first thermal decomposition of the ulvan occurred at around 93^{o}C, 113^{o}C which can be attributed to the loss of bound water present in ulvan and it was accompanied by 11, 13 % mass loss.

The second stage at about 169^{o}C,245^{o}C with mass loss of 5, 14% owing to removal of structural water (dehydration reactions). The third stage at221^{o}C, 329^{o}C with mass loss of13, 10%. This decomposition continued with a further loss of mass about 15, 19 % at temperature of about 666^{o}C, 678^{o}Crespectively and finally the remaining mass corresponded to the ash content in the sample as explained byBruhn *et al.*, (2011).

This residual mass is probably constituted by sulfates, phosphates and carbonates, which are minerals usually found in polysaccharides structures(Parikh and Madamwar, 2006; Alves *et al.*, 2010; Mota *et al.*, 2013).

Viscosity measurements of the extracted polysaccharide: The present results indicated that increasing the concentration of polysaccharide solutions lead to an increase in viscosity. These results are analogousto that obtained by with Yaich *et al.*(2014) who demonstrated thatulvan hydrocolloidsolutionreviled a shear-thinning fluid demonstratingpseudoplastic behavior.Robic *et al.*, (2009)found that ulvantends to display in a bead-like structure, partially connected by filaments.High sulfate incorboration in ulvan, commonlyinsertedwithrhamnose, may compose crosslinking(Prosperi, 1994).This depends on intra- and inter-molecular crosslinks, which are obstructed by densely negative groups, as carboxylic acids in uronic acids, sulphate groups and/or methyl groups especially inrhamnose (Gordillo *et al.*, 1998).

Antioxidant potential of ulvan (reducing capacity):

Wang *et al.*(2008) indicated that antioxidant activity have a direct, positive relationship with the reducing potential which depends on molecular weight, the type of sugar, the glycosidic branching, and the degree of sulfation and acetylation position,consequently, the strong antioxidant potential of ulvan sample extracted from *U. fasciata* may be attributed to the high sulfate content of *U. fasciata* polysaccharide.

Biological activity of ulvan

Algal growth analysis: The observed positive growth responses in *C. vulgaris*arein accordance with Fábregas *et al.*, (1996)who suggested that agricultural amendements, could be used as organic substrates for the growth of microalgaewhich converted into biomass in mixotrophic growth conditions.The present results are in accordance with that of Xu *et al.*, (2001)who demonstrated thatelevatedcontent of carotenoids in *Caulerparacemosa* may be actas an alternativeantioxidant for scavenging the reactiveoxygen species. The higher antioxidant capacity is positively correlated with the presence of phenolic components, chlorophylls as well as carotenoids, lutein and pheophytin in *Chlorella* vulgaris extracts as reported by Mager and Thomas, (2011).Organic substrates play an important role in promoting biomass accumulation of *Chlorella vulgaris* during microalgae cultivation (Abreu *et al.*, 2012and El-sheekh *et al.* 2012)).

Antioxidants activities of *C. vulgaris*:

Non-enzymatic antioxidants potential (Phenol components):

Phenolic components play a significant role as an antioxidant substance according to their capability to contribute a hydrogen atom (Deng *et al.*, 2013).

Enzymatic antioxidants potential

Guaiacol peroxidase activity (GPX): Peroxidases are the proteins that involved in maintaining cell redox potential. The accumulation of H_2O_2 in the cell is prevented by guaiacol peroxidase and catalase (GPX & CAT), reducing it to H_2O (Jaki *et al.*, 2000).

Ascorbic acid peroxidase activity (APX): Jaulneau *et al*., (2010) suggested thatreactive oxygen species (ROS) in addition to antioxidant enzymes, mainly plant peroxidases (APX and GPX), dynamically contribute in the metabolic regulation.

Catalase activity (CAT): Andersen, (2005)indicated that superoxide plays a significant role in the development of other reactive oxygen species, for instance hydrogen peroxide, hydroxyl radical, or singlet oxygen in living cells. In the present study, all samples effectively inhibit superoxide. Freitas*et al*. (2009)suggested that antioxidant activity may have originated from their hydrogenatom donating capacity. High sulphate content polysaccharide isolated from *Ulvapertusa* induced strong antioxidant activity (catalase) *in vitro* (Qi and Sun 2015) The observed growth promotion of *Chlorella vulgaris* may be attributed to the stimulatory action of the supplemented ulvan that induce the antioxidant machinery represented in total phenol content, , as well as high activities of guaiacol peroxidase, ascorbic acid peroxidase and catalase giving rise to the recorded enhanced growth pattern.

It is concluded that ulvanas a sulfated soluble polysaccharide of green macroalgaehavehigh potential use in technological and industrial-related applications and biostimulant for mass production of microalgae.

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التوصيف و الإمكانيات المضادة للأكسدة و الأنشطة البيولوجية لسكاريد أولفان المستخلص من جنس الطحلب البحرى أولفا

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أجريت هذه الدراسة بهدف إستخلاص و توصيف سكاريد أولفان من طحلب أولف بنوعيه Ulvalactucaو Ulvafasciata و قد تم تجميع العينات موضع الدراسة من الطحلب من على الشواطئ المصرية الشمالية بالبحر المتوسط حيث إنتشار الطحلب بكميات وفيرة و حتى يكون هناك إستفادة من هذا الطحلب فقد أجريت دراسة للأنشطة الحيوية و الإمكانيات المضادة للأكسده و أظهرت نتائج إستخلاص سكاريد أولفان إحتواء طحلبlactucaUlvaعلى 15% ألفان فى حين إحتوى طحلب Ulvafasciata على 17% من الوزن الجاف. تم التعرف على مكونات الأولفان بإستخدام جهاز HPLC فوجد أن الجلوكوز و الجالاكتوز و الرامنوز تحتل الجانب الأكبر فى تكوين الأولفان يليه شق الكبريتات و حمض اليورونك. و قد كشفت التحاليل البيانية الناتجة من التحليل بالاشعة تحت الحمراء أطيافا متشابهة مع ظهور قمم مميزة تؤكد وجود الأولفان بصورة نقية. و لقد وجد ثباتا حراريا لأولفان طحلب U. lactuca حتى درجة حرارة 169 درجة مئوية بينما هى 245 درجة مئوية لأولفان طحلب Ulvafasciata . كما إتضح من دراسة اللزوجة أن محاليل الأولفان تمثل محاليل غروية لها shear thinning and pseudoplastic behavior و زيادة اللزوجة مع زيادة التركيز. تم دراسة النشاط الحيوى لسكاريد أولفان حيث وجد أن له قدرة إختزالية أيضا تم دراسة تأثيره على النمو و الأنشطة الأيضية للطحلب الدقيق Chlorella vulgaris تحت تاثير التركيزات 5 و 10 ميللجرام أولفان / 100 ميلليلتر وسط غذائى.و قد أسفرت النتائج أن المعاملة 5 ميللجرام من طحلب U. fasciata أعلى تأثير معنوى على كل المعايير المقاسة لطحلب Chlorellaمثل النمو ممثلا فى قياس الكثافة الضوئية للمزارع و الوزن الجاف و الأصباغ التمثيلية. و قد أظهر تحليل الكربوهيدرات الكلى و عديدات التسكر و البروتين و مضادات التأكسد (المكونات الفينولية)و أيضا الإنزيمية (إنزيمات البيروكسيديز GXP و APX الكاتاليز CAT) ذات التأثير السابق. تخلص هذه الدراسة إلى إمكانية إستخدام أولفان كمحفز حيوى للإنتاج الكتلى للطحالب الدقيقة.