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Evaluation of Marine Alga *Ulva lactuca* L. as A Source of Natural Preservative Ingredient

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Abstract: Total chlorophyll (T-Chl), total carotenoids (TCAR) and total phenolics (TPc) compounds were quantified in the biomasses of *Ulva latutus* grown either in normal (NSW) or artificial sea water (ASW) under indoor condition. The *Ulva* crude organic extracts (*Ulva*-COEs) analyzed by chromatographic method. Antioxidant and antibacterial activities of these extracts were determined. Seven and thirty-four compounds characterized in both *Ulva*-COEs by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Chl *a* (15.60-30.90%), Chl *b* (12.20-14.89%), 9-cis β-carotene (13.12-14.47%), β-carotene (11.44-11.47%) and all-trans β-carotene (6.16-29.70%, of the total area) were recognized as major compounds. The *U*COEs exhibited remarkable antioxidant activity, compared to a synthetic antioxidant: butylated hydroxyanisol (BHA) and butylated hydroxyltoluene (BHT), as evaluated by the rapid TLC screening, β-carotene bleaching, 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical scavenging methods. The positively correlation was apparent between the antioxidant activity (AOE) and present of carotenoids, phenolics and chlorophylls in their extracts. In addition *Ulva*-COEs exhibited great potential antibacterial activities against six bacterial strains, with minimal inhibitory concentration (MIC) values ranged from 400 to 350 µg/ ml. Data indicate that *U. latutus* could be considered as potential rich source of natural colorant with antioxidant and antibacterial properties and could be utilized in as natural preservative ingredient in food and in pharmaceutical industry.

Key words: *Ulva latutus* . antioxidant activity . antibacterial activity

INTRODUCTION

The oxidative damage caused by reactive oxygen species (ROS) includes $O_2^{\cdot-}$, $^{\cdot}OH$ and $LOO^{\cdot}$ on living system are interesting area for research. This is particularly important because the ROS and free radical capable of causing damage to biomolecules, such as nucleic acids, lipids, proteins and other cellular constituents [1, 2]. The degradation and/ or modification of these molecules have been associated with various chronic diseases, such as cancer, coronary heart disease atherosclerosis, cataracts, ageing muscular dystrophy and some neurological dis orders [3, 4]. For these reasons, many products with antioxidant properties are widely used in order to minimize oxidative damage to living cells and to prevent oxidative deterioration of food. Synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxyltoluene (BHT) are commonly used to inhibit lipid peroxidation [5]. Now a day, BHT and BHA need to replaced with natural antioxidant, however they were found to be toxic, responsible for liver damage, promoters of carcinogenesis and alteration the enzyme activities, as well as general consumer rejection of synthetic food additives [6]. Thus, it is important to develop, identify and utilized of new source of safe and effective antioxidants of natural origin [7, 8]. Natural antioxidant such as α-tocopherol, phenols and β-carotene found in higher plants are being use in the food industry to inhibit lipid peroxidation and they can protect the human body from free radicals and retard the progress of many chronic diseases [9, 10]. However, Gey [11] have reported that the synergistic action of wide spectrum of antioxidant is better than the activity of a single antioxidant and that antioxidants from natural source (primarily foods) have a higher bioavailability and therefore higher protective efficacy against oxidative stress than synthetic antioxidants.

Marine algae have been use as a novel food with potential nutritional benefits and in industry and medicine for various purposes [12]. Recently, aquatic habitats have increasing been shown to provide a rich source of natural bioactive compounds with hypocholesterolemia, antinflammatory, antiviral, antineoplastic, antimicrobial and hypertensive properties. According to their chemical structure, most

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of isolated compounds belong to sulfated polysaccharides, phenolics, terpenoids, lactons, sterol and fatty acids [8, 13, 14]. On the other hand, the antioxidant activity is one of the most important active in marine bioactive substances and lots of algal and algae-derived compounds exhibited potent antioxidant such as carotenoids, phenolics, terpenoids and sulphated polysaccharides. The antioxidant activity of these compounds are mainly attributed to scavenging activity against superoxide and hydroxyl radicals, chelating ability, quenching singlet and triplet oxygen and reducing power [15, 16].

In this study, the crude organic extract obtained from *Ulva lactuca* (found in Egypt at the north coast of Mediterranean Sea) grown in natural and artificial sea water medium under laboratory conditions were analyzed with chromatographic methods and evaluated for their activities as antioxidant and antibacterial agents. In addition, the chemical composition in biomasses of *Ulva lactuca* was estimated.

MATERIALS AND METHODS

Algal source: The green algal, *Ulva lactuca* L, of 5-8 cm in height were collected on September 2007 from an exposed rocky site near the edge of beach Shat El-Garam, Marsa-Matroh government, at north coast of Mediterranean Sea, Egypt.

Cultivation of *Ulva lactuca*: The collected *U. lactuca* algae were transported in sea water to the laboratory, then algal were cleaned, washed with sea water to remove the attached sands and also the rhizoidal portions were removed to avoid microbial contamination in the following cultures. For two weeks (adapted period to aquarium condition), the selected algal thallu were cultivated in aerated an aquarium (30 L) containing natural sea water (NSW), illuminated by ten cool-white fluorescent lamps (40 W, Philips) regularly spaced around the culture and maintained at 25 ^{o}C±3 ^{o}C. The medium was changed weekly. Two weeks after, in the aquarium, healthy thallus segment of 5 cm diameter were cut out and divided into two parts; first one was grown in natural sea water and the second one grown in nutrient enriched artificial sea water descried by Provasoli [17]. All algal cultures were maintained for 30 days under laboratory conditions as the same as described before and stopped 24 h after exponential algal growth phase.

Extraction procedure: The fresh whole-cells of *U. lactuca* algae (30 g) grown either in NSW or ASW medium were homogenized with dichloromethane/ methanol (200 mL; 1:1, v/v), then the homogenate was filtered and the filtrate was evaporated to dryness under vacuum at 40^{o}C. The yield of the extraction was 12.3% and 13.9%, respectively, which was the average of three replicate analyses. The dried extracts were kept at-10 ^{o}C under N_2 gas when not in use.

Determination of chemical composition

Determination of total phenolics content (TPc): Total phenols in *U. lactuca* samples were estimated spectrophotometrically using Folin-Ciocalteu reagent as described by Singleton *et al.* [18]. The absorbance was measured at 760 nm and compared to gallic acid calibration curve.

Determination of total chlorophylls (T-Ch): Three grams of *U. lactuca* samples was homogenized in acetone (30 ml, 80%) and incubated overnight in dark at 4°C for complete extraction followed by centrifugation at 10,000 xg for 5 min. Total chlorophyll (T-Chl), chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) in the supernatant were determined spectrophotometrically at wavelength 664 nm according to Lichtenthaler [19] method.

Determination of total carotenoids (TCAR): The total carotenoids were determined spectrophotometrically at 450 nm according to AOAC [20]. β-carotene was used as a standard.

Chromatographic analysis of *Ulva lactuca*:

TLC analysis: TLC was carried out on 10 × 20 cm silica gel plates (Merck, Germany), 5 µL of *Ulva*-COEs (30 mg/mL) was applied to 1 cm of the base of plat and developed with hexane/acetone (75:25 v/v). The separated compounds were located and identified by visualizing plates with $FeCl_3$-$K(CN)_6$, under UV and observed pigments colors bands. The R_f values of pigments were measured and compared with those reported for some available standard pigments.

HPLC-DAD analysis: The chemical components in the *Ulva*-COEs were identified by the HPLC method already reported by Mendiola *et al*. [21], using Dionex Summit IV HPLC system consisted of a Dionex P680 dual gradient pump, a ASI-100 autosampler equipped with a 20-µL loop and PDA-100 photodiode array detector. A reverse phase column C18 (250 x 4.6 mm, 5 µm partials) was used. The mobile phase was a mixture of solvent A (methanol / ammonium acetate 0.1 N; 7:3, v/v) and solvent B (pure methanol) at 0.9 mL min^{-1} according to a step gradient, lasting 35 min, which started from 25% B, changing at 50% in 1 min, rising up to 100% B at minute 10. Then, the mobile phase composition was kept constant until the end of the

analysis. Total acquisition time was 35 min. The temperature was set at 25^0C. The identification of the peaks was performed, when possible, using some available standard pigments. When no standards were available, tentative identification was done based on UV-Vis spectra characteristics and comparing with data appearing in the literature.

Determination of antioxidant capacity

Rapid screening with TLC method: The TLC plates were used to detect antioxidant activity of *Ulva*-COEs based on spraying the plates with oxidizing reagents. The separated compounds on TLC plates were spraying either with ß-carotene/ linoleic acid reagent as described by Pratt and Miller [22] or with 0.004% DPPH stable radical in methanol [23] to located and detect antioxidant active compounds. The protecting against the bleaching ß-carotene gave orange spots and scavenging DPPH radical gave pale yellow colored spots were taken as positive results.

β-carotene/linoleic acid bleaching method: The procedure is bases on a previously reported method [5] with slight modifications. The reagent mixture was prepared as following: 2 mg ß-carotene dissolved in chloroform (5 ml) were pipeted into flask containing linoleic acid (27 µl) and Tween-20 (0.5 ml), the solvent was evaporated to dryness under vacuum, ultra-pure water (250 ml) was then added and emulsification was achieved by agitation in ultrasonic bath for 15 min. One hundred µL of *Ulva*-COEs (50 mg of the extract in one mL 1% Tween-20) was added to 50 ml reagent mixture and then samples were subjected to thermal auto-oxidation at 50 ^{o}C for 7 h. At intervals time up to 480 min, the absorbance of reaction mixture (4 mL) was measured at 470 nm against blank (10% Tween-20 solution). All samples were done in triplicate. Antioxidant capacities of algal extracts were compared with those of BHT, BHA and a-tocopherol (at 100 ppm) and control. Inhibition of bleaching β-carotene (I%) was calculated in following way : inhibition percentage (I%) = ($A_{Initial}$ -A_{Sample} / $A_{Initial}$) x 100, where A initial and A sample are the absorbance at zero and 2 h, respectively. Also, the antioxidant efficiency percentage (AOE%) depended on the degradation rate (DR) of β-carotene coupled with linoleic acid autoxidation was calculated as following: AOE% = ($DR_{control}$- $DR_{sample\ or\ standard}$) /$DR_{control}$ X 100. Where; DR of sample or standard = ln (a/b) x 1/t, ln is natural log; a and b are absorbance (470 nm) at time 0 and 2 h, respectively.

DPPH• free radical-scavenging assay: The method described by Tagashira and Ohtake [24] was used in order to assess the DPPH radical scavenging of *Ulva*-COEs. The different concentrations of the *Ulva*-COEs (ranged 5-100 µg/mL) were added to 25 ml of 100 mmol L^{-1} DPPH radical. The reaction mixture were vortexed and incubated in dark at 30±1ºC. The absorbance of mixtures was measured at 517 nm for 90 min, at 15 min intervals, against blank (methanol absolute). The differences in absorbance between a test sample and a control (DPPH· in methanol) was considered as activity. The BHT, BHA and a-tocopherol (200 ppm) were used as reference standard. The radical scavenging activity of *Ulva*-COEs in the mixture was calculated from a calibration curve. All tests were run in triplicate and averaged. Extracts concentration providing 50% inhibition (IC_{50}) was calculate from graph plotted of inhibition percentage against extract concentration.

Determination of antibacterial activity

Microorganisms: For the antibacterial evaluation, strains from the Fisher Scientific Co. (Texas, USA) were used: *Bacillus subtilis, Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus, Klebsiella pneumoniae, Serralia marcescencs*. Bacteria were grown on Difco nutrient agar medium.

Preparation of inoculums: The bacteria strains were inoculated on nutrient broth (Difco) and incubated for 24 h at 30°±1 °C. Adequate amounts of autoclaved Difco Agar medium were dispensed into sterile plates and allowed to solidify under aseptic conditions. Then, bacteria strains were inoculated with a sterile swab on the surface of plates and incubated at 27°C for 1 h.

Antibacterial bioassay: The antibacterial activity was evaluated with the paper disk diffusion method [25]. Briefly, 6 mm paper disks (Whatman No. 1) were impregnated with 2-8 µL of three different concentrations (1, 2 and 4 mg $disk^{-1}$) of the respective *Ulva*-COEs (50 mg / 0.1ml of Dimethylsulfoxide, DMSO) and allow to dried. Then dicks were applied to the agar plates that were inoculated previously with the test organisms and incubated for 1 h. The bacterial plates were incubated at 30°±1 °C for 24-48 h. Also, chloramphenicol (Sigma Chemical) were used as positive control (10 and 20 µg/ disk). After incubation, all plates were observed for zones of growth inhibition and the diameters of these zones were measured. Inhibitory activity of DMSO was also tested. All tests were carried out under sterile conditions in duplicate and repeated three times.

Minimal Inhibitory Concentration (MIC): The minimal inhibitory concentration tests were carried out

according the methods of the European Pharmacopoeia [26]. The MIC was defined as the lowest concentration of tested samples showing no visible bacterial growth after incubation time for 24 h at 37 ºC.

Statistical analyses: Data obtained from measurements and biochemical analyses for each variable were subjected to analysis of variance using the COSTAT computer package (Cohort Software, CA, USA). The mean values were compared with LSD.

RESULTS AND DISCUSSION

As shows in Table 1, the amounts of total phenolics compounds (TPC), total chlorophyll (T-Chl), Chl *a*, Chl *b* and total carotenoids (TCAR) in *Ulva lactuca* grown in NSW and AWS (in parentheses) were 4.6 (4.8), 21.27 (34.13), 17.64 (28.16), 3.62 (5.97) and 12.73 mg/g DW (23.91 mg/g DW), respectively. The values revealed that the significant quantities of T-Chl, Chl *a*, Chl *b* and TCAR were detected in *Ulva* grown in ASW medium than that occurred in NSW. Whereas, algal did not induce any significantly differences for production of TPC when grown in either NSW or ASW medium. Thus, nutrient composition of growth medium has an important influence on the production of photosynthetic pigments and phenolics compounds by *Ulva lactuca*.

Identification of photosyntetic pigments by chromatographic methods

TLC: TLC chromatogram of separated *Ulva*-COE grown in NSW and ASW showed the predominance of phenolic compounds and photosynthetic pigments including carotenoids and chlorophyll-derived (Fig. 1). Green, grey-greenish, blue, red, orange-yellowish, orange and yellow bands were separated on TLC plates. These observations are in agreement with colors of main photosynthetic pigments detected in many algae [23, 27]. These pigment bands were located and identified at different hR_f values ($hR_f = R_f x\ 100$). The bands at hR_f = 7, 15, 24, 81 and 94 corresponded to lycopen, myxoxanthin, violaxanthin, α-carotene and β-carotene, respectively. In additional, bands at hR_f = 35 and 43 were chl *a* and Chl *b*. Many phenolic bands were detected on TLC plate at hR_f ranged from 0-10, that gave a blue colored after spraying with $FeCl_3$-$K_3Fe(CN)_6$ reagent. The R_f values obtained in study are in agree with that reported by Santoyo *et al*. [27]. They found that bands at R_f = 0.94, 0.84, 0.72, 0.24 and 0.15 corresponded to α-carotene, β-carotene, zeaxanthin and myoxyanthophyll like compounds, respectively.

HPLC: The data obtained from HPLC-DAD profile of photosynthetic pigments of *Ulva* was relatively in compatible with that obtained from TLC analysis (Table 2). The *Ulva* grown in NSW media characterized by the presence of Chl *a* (30.90%), Chl *b* (14.89%), 9-cis β-carotene (14.47%) and α-carotene (11.47%) as major compounds (> 10% of total area). In addition, all-trans β-carotene (6.16%), carotene-isomers (5.62%), violaxanthin (4.87%), astazanthein (4.12%) and antheraxanthin (1.30%) were identified as minor compounds (> 1.0-<10%) and traces compounds of lutein (0.51%), zeaxanthin (0.16%) and cryptoxanthin (0.16%) were detected. In contrast, the presence of all-trans β-carotene (29.70%), Chl *a* (15.60%), Chl *b* (12.20%), α-carotene (11.44%) and 9-cis β-carotene (13.12%) as major constituents, as well as violaxanthin (3.68%), astazanthein (1.27%), lutein (1.18%), zeaxanthin (2.32%) and cryptoxanthin (1.24%) as minor compounds were characteristic of the photosynthetic pigments of *Ulva* grown in ASW media. Thus, the relative% of many compounds such as Chl *a* and all-trans β-carotene in *U. lactuca* cells was significant differences as a result of composition of nutrient media. HPLC can be identified 12 components in organic extracts of *Ulva* grown in either NSW or ASW medium, representing 94.63% and 92.51% of the total area. Chemical constituents of pigment produced by *U. lactuca* were differing quantitatively and qualitatively as a resulting to nutrient composition of growth medium. In conclusion, *U. lactuca* grown in artificial sea water, has ability to accumulated high amounts of all-trans β-carotene and coupled with

Table 1: Chlorophylls (Chl), carotenoids and phenolic compounds contents and yield of organic extracts of *Ulva lactuca* grown in Natural Sea Water (NSW) and Artificial Sea Water (ASW)

	Concentrations mg/g D.W.					
Sample	Chl a	Chl b	Total Chl.	Total carotenoids	Total phenolic	Extracts yield (%)
U. lactuca grown in NSW	17.64±1.65	3.62±0.45	21.27±1.67	12.73±1.32	4.60±0.58	12.30±0.36
U. lactuca grow in ASW	28.16±2.69	5.97±0.85	34.13±2.57	23.91±1.55	4.80±0.54	13.90±0.42
LSD at level (P< 0.01)	1.50	0.47	2.45	1.80	0.15	0.51

All values show mean of three replicates, ± standard deviation, Values are significant at (P=≤ 0.01), ±SD

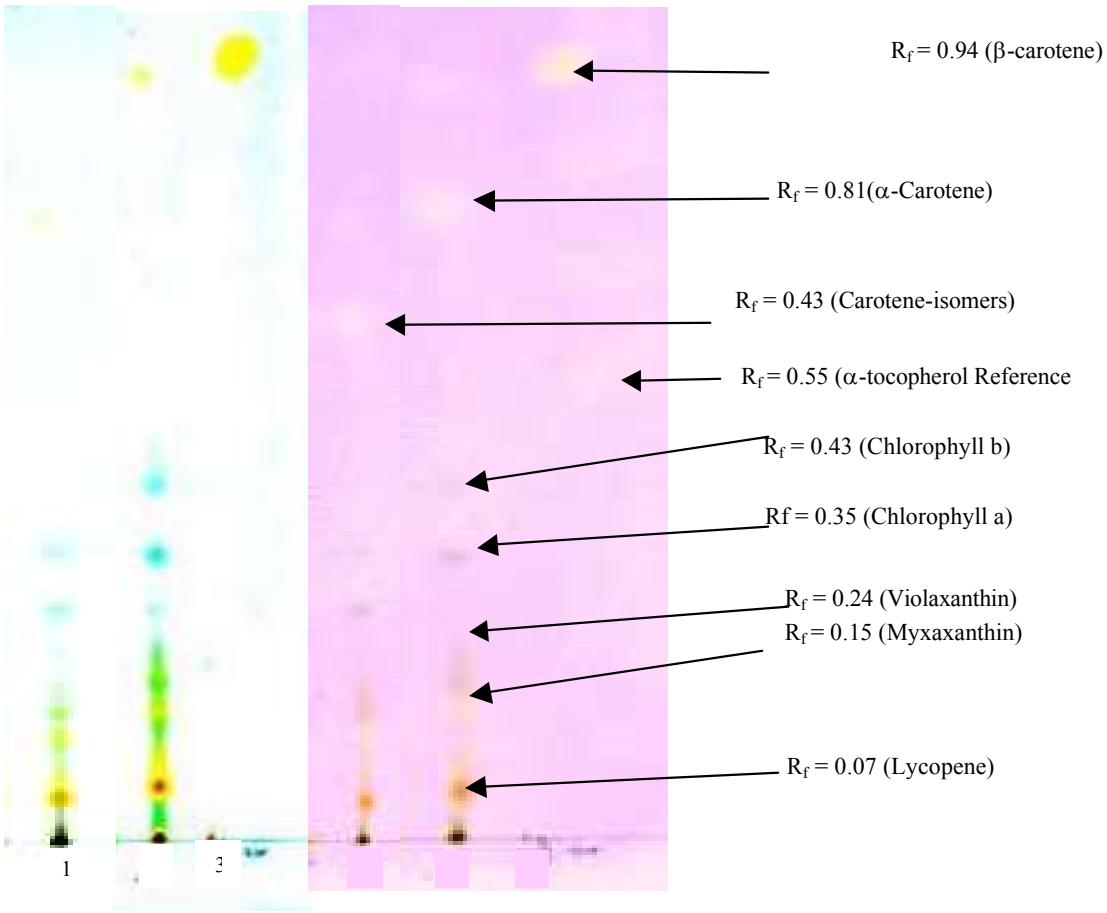

Fig. 1: (a) TLC chromatogram of *Ulva lactuca* organic extracts, (b) TLC chromatogram of *Ulva lactuca* organic extracts stained with DPPH·, stained with 0.5 mmol/L DPPH· methanolic solution
1: *U. lactuca* extract of cells grown in NSW
2: *U. lactuc*a extract of cells grown in ASW
3: β-carotene and α-tocopherol reference

depletion in Chl *a* content, as compared with that in algae grown in natural sea water. As emphasized before, the pigments contents in algae can be affect with the influence of several environmental factors. For example, the severe hyper salinity stress was reported to alter the photosynthetic pigment contents in green algal [28].

Antioxidant capacity of crude organic extracts *Ulva lactuca*

Rapid TLC-screening assay: Rapid TLC screening assay based on decolorization of methanolic DPPH· radical or β-carotene coupled with linoleic acid, that spraying into TLC plates, was choice with many scientists as a rapid test to evaluate the antioxidant activity of natural compounds [29].

Bleaching β-carotene/ linoleic acid-TLC (BC-TLC) assay: In this assay, the *Ulva*-COEs separated on TLC plats was sprayed with β-carotene/linoleic acid mixture and then the chromatogram was exposed to day-sun light lamp until the background was bleached (>one hr after spraying), zones in which a yellow color appeared possessed antioxidant activity. Among all separated bands of *Ulva* organic extracts, bands at hR_f = 94, 81, 43, 35 and >7 showed an excellent antioxidant activity and those bands were identified by HPLC and TLC technique as carotenoids and phenolic compounds. However, due to the orange and red colours of the main compounds in *Ulva*-COEs, differences in colours between the background and the bands themselves were not easy to examine. The similar noticed was reported by Santoyo *et al*. [27].

DPPH· Decolorization-TLC (DPPH-TLC) assay: The DPPH-TLC assay was used as a rapid method to detect the *Ulva*-COEs antioxidant capacity. The spots with antioxidant activity were able to reduce the stable

Table 2: HPLC profile of photosynthetic pigments in *Ulva lactuca* organic extracts grown in natural sea water (NSW) and artificial sea water ASW)

		Relative area peak (%)	
Rf	Compounds	*Ulva* grown in NSW extract	*Ulva* grown in ASW extract
3.46	Unknown	0.63	0.28
4.5	Violaxaxanthin	4.87	3.68
4.92	Unknown	0.02	0.07
5.2	Antheraxanthin	1.30	0.11
5.7	Unknown	0.75	-
6.23	Unknown	2.35	-
6.46	Unknown	-	3.47
7.27	Astazanthein	4.12	1.27
7.98	Lutein	0.51	1.18
8.62	Unknown	0.27	-
8.81	Zeaxanthin	0.16	2.32
9.57	Cryptoxanthin	0.16	1.24
10.57	Chlorophyll b	14.89	12.20
11.22	Chlorophyll a	30.90	15.60
12.67	α-carotene	11.47	11.44
13.68	Carotene-isomers	5.62	0.68
14.99	All transβ-carotene	6.16	29.70
15.18	9-cis β-carotene	14.47	13.12
15.63	Unknown	1.80	-
16.55	Unknown	1.13	-
17.66	Unknown	0.34	-
18.55	Unknown	0.14	-
Total identified compounds		94.63	92.51
Unknown		5.36	7.49

Rf refers to retention time

Table 3: Antioxidant efficiency (%)[a] and inhibation (%)[b] of *Ulva lactuca* organic extracts as measured by ß-carotene-linoleic acid coupled oxidation

Treatments	Antioxidant efficiency (%)[a]	Inhibition (%)[b]
Control	0.00	0.00
BHA	83.49±1.22	73.20
BHT	79.27±0.95	69.40
Ulva grow in NSW extract	71.39±1.31	47.77
Ulva grow in ASW extract	77.19±1.12	54.92

[a:] Antioxidant efficiency (%) = $(DR_{control}-DR_{Sample\ or\ Standard})/DR_{control}$ X 100, $DR_{Sample\ or\ Standard} = \ln(a/b)x1/t$, Where DR =Degradation Rate, Where ln is natural log, a: is initial absorbance (470nm) at time 0, b: is the absorbance at 2 h,

[b:] Inhibition (%) = $(A_{t=0} - A_{t=2\ hr}) / A_{t=0}$ X 100, Where $A_{t=0}$ = absorbance of β-carotene linoleic acid solution at zero time, A t=2 h = absorbance of β-carotene after 2 h

Concentration sample was 100 µg/ml, BHT and BHA at 200 ppm was used as the synthetic antioxidant standard.

Values are expressed as mean ±SD (n=3)

radical DPPH to the yellow or light yellow colored on a violet background, within few min (≤5 min) were considered as positive results. As shown in Fig. 1, bands at hR_f = 95, 81, 43, 15 and 7 showed a good DPPH radical scavenging, the both *Ulva* extracts was found to have the same antioxidant properties. Therefore, antioxidant activity coincides with carotenoids and phenolic components containing in both *Ulv*-COEs grown in either NSW or ASW medium.

Antioxidant activity of *Ulva*-COE in aqueous model system: The antioxidant activity was examined, by the determination of the rate of β-carotene bleaching and time required for the disappearance of β-carotene colors in aqueous model system (Table 3 and Fig. 2). As compared with that in control (free-antioxidant), the both organic extracts of *Ulv* grown in NSW and ASW and BHT and BHA were able to increase the values of antioxidant activity (AA%) with 71.39, 77.19, 79.27 and 83.49%, respectively. Therefore, both *Ulva*-COEs exhibited higher antioxidant activity by protecting β-carotene from auto-oxidation, due to free radical chain generated from linoleic acid peroxidation. In order to compare the antioxdiative behavior of the *Ulva*-COEs in aqueous media, values of the relative inhibition percentage of β-carotene oxidation was also calculated. These values were 47.77, 54.92, 69.40 and 73.20%, for *Ulva* extract of cells grown in NSW and ASW, BHT and BHA (positive antioxidant, at 200 µg/ ml), respectively. Thus, *Ulva*-COEs seem to be efficient antioxidant when compared to commonly synthetic antioxidant, BHT and BHA.

Free radical scavenging activity (FRSA): Table 4 shows the DPPH free radical scavenging activity (FRSA) and the values IC_{50} for *Ulva-COE*s and BHA, BHT and α-tecopherol. The *Ulva*-COEs of cells grown in ASW and NSW medium showed a good FRSA, with IC_{50} of 16.5 and 18.7 µg/ml. While, IC_{50} values for α-tecopherol, BHT and BHA were 14.4, 12.2 and 13.1µg/ml, respectively. Thus, *Ulva*-COEs showed high great potential DPPH radical scavenging activity and their radical scavenging activity was found to be not far from those of commercial antioxidant such as α-TOC efficient. However, the protecting β-carotene from oxidation with chain reaction products generated from linoleic acid peroxidation and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of test samples was in increasing order *Ulva*-COE from NSW< *Ulva*-COE from ASW<α-TOC< BHT< BHA.

Findings in this study, decolonization of DPPH radical suggested the presence of electron and hydrogen donors' constituent in both *Ulva* organic extracts. Thus,

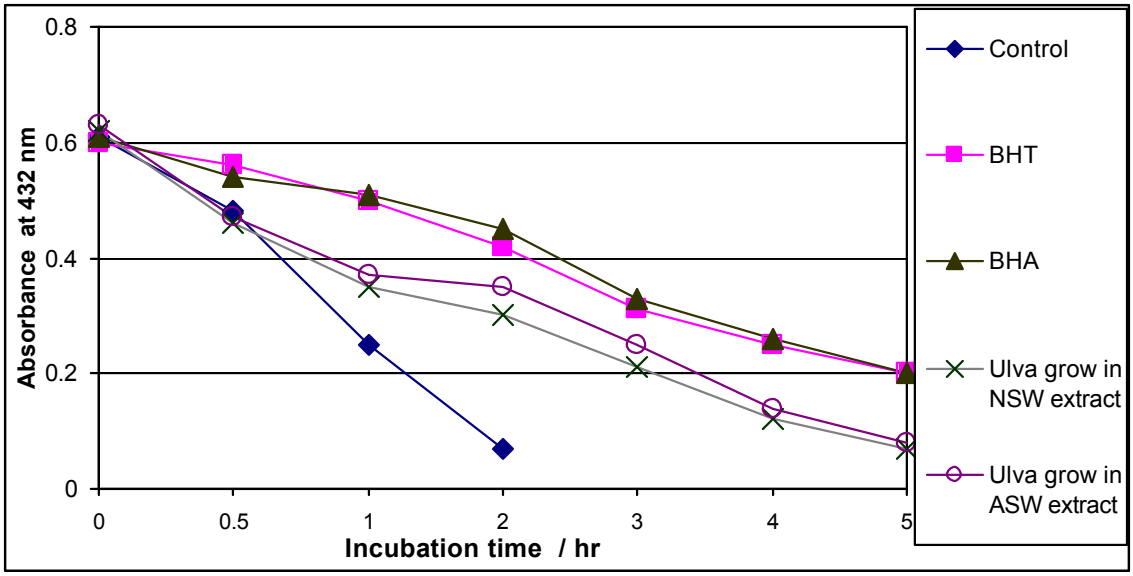

Fig. 2: Effect of *Ulva lactuca* organic extracts obtained from cells grown in natural sea water (NSW) and artificial sea water (ASW) on bleaching ß-carotene coupled with autoxidized linoleic acid. Values are average of three treatments

Table 4: Scavenging activity of effect of *Ulva lactuca* organic extracts on DPPH•

Sample	Scavenging activity (%)[a]	IC_{50}[b] (µg ml^{-1})
Ulva lactuca grown in NSW	74.50	16.50
Ulva lactuca grown in ASW	69.10	18.70
TBA	92.10	13.10
BHT	94.00	12.20
α-Tocopherol	90.20	14.40

[a]: Percentage of antioxidant inhibition was calculated from following equation: $\%^{a} = (A_{blank} - A_{sample} / A_{blank}) \times 100$

Where A_{blank} = absorbance of methanolic DPPH·

A_{sample} = absorbance of DPPH· radical + samples

IC50[b]: Concentration (µg/ml) for a 50% inhibition was calculated from the plot of inhibition (%) against *Ulva* extracts concentration

Tests were carried out in triplicate

potential of antioxidant properties of *Ulva* extracts could be due to there extracts containing some substances such as carotenoids and phenolic compounds. However, the spots exhibit antioxidant activity on TLC chromatogram, had been confirmed and identified as carotenoids, chlorophyll-derived and phenolic compounds. As reported elsewhere, the positive correlations between phenolic, phycocyanin and carotenoids containing in algal extracts and its antioxidant activity is well documented [16, 28, 30]. Here again, from the all antioxidant results of inhibition of ß-carotene bleaching in aqueous media, decolonization of DPPH· and radical scavenging activity, it may be possible to find effective antioxidants among the green algae such as *Ulva*. The total phenoilc, total carotenoids and chlorophyll-derived containing in the algal cells might explain their high antioxidant activity. For instance, algal phenolic compounds are effective antioxidant to delay of peroxidation, that phenols easily transfer a hydrogen atom to lipid peroxyl cycle and form the aryloxyl, which being incapable of acting as a chain carrier, couples with another radical, thus, quenching the radical process [15]. In the other words, *Ulva* containing phenolic compounds are able to donate hydrogen atom to the free radical thus stopping the propagation chain reaction during lipid oxidation process [31, 32]. On the other hand, carotenoid compound is capable of scavenging of free radicals and quench singlet oxygen primarily by physical mechanism, in which the excess energy of singlet oxygen is transferred to the electron rich structure [33, 34].

Finally, crude organic-extract of *Ulv latutus* showed potential activities as antioxidant agents compared to synthetic antioxidants (a-Tocopherol, BHA and BHT). The natural antioxidant is preferred over synthetic antioxidant to minimize adverse effects on humankind. Therefore, it could be suggested that the *Ulva-COEs* may exert a better function in free radical scavenging and may be a promising alternative to synthetic substances as natural compound with high

Table 5: Antibacterial activities (inhibition zone in diameter (mm) around the disks and MIC [b]) of organic extracts *Ulva lactuca* grown in natural sea water (NSW) and artificial sea water (ASW)

	Reference antibiotic			*U. lactuca* extract of cells grown in NSW				*U. lactuca* extract of cells grown in ASW			
	(µg/disk)		(µg/ml)	(mg/disk)			(µg/ml)	(mg/disk)			µg/ml
Organisms	10	20	MIC	1	2	4	MIC	1	2	4	MIC
Bacillus cereus (+)	15	20	20	8	12	15	400	9	13	16	350
Bacillus substilis (+)	16	21	20	9	13	16	400	9	14	17	350
Staphyloccus aureus (+)	14	19	20	8	12	15	400	9	13	16	350
Micrococcus luteus (+)	18	23	20	9	12	15	400	9	13	16	350
Serralia marcescens (-)	13	18	20	10	14	17	400	11	14	17	350
Klebsiella pneumoniae (-)	17	22	20	11	14	17	400	12	14	17	350

[a]: Values represent the mean of three replicates and rebated three times, [b]: MIC: Minimum inhibition concentration, values given as µg/ml for samples and µg/ml for chloramphenicol

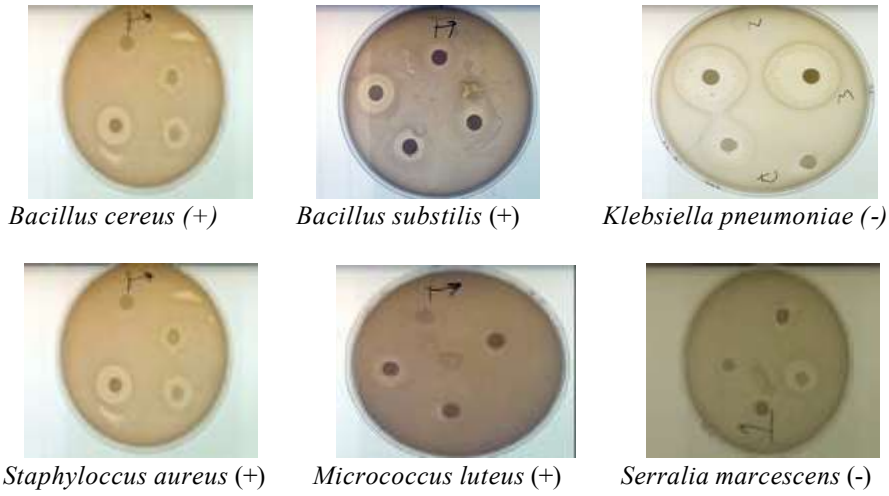

Fig: 3: Diffusion disk antibacterial test of crude organic extracts grown in natural sea water (NSW) and artificial sea water (ASW) against the six tested strains of bacteria

antioxidant activity and as food colorant. These properties can be improving by change the culture conditions and/ or the production of these agents could be promising.

Antibacterial activity of *Ulva lactuca* organic extracts: The potential antibacterial activity of crude organic-extracts of *U. lactuts* grown on NSW or ASW medium as well as chloramphenicol (reference of antibiotic) was shows in Table 5 and Fig 3. The *Ulva*-COEs showed a narrow antibacterial activity and was found in a dose depended manner. The most susceptible bacterium was *K. pneumoniae* and *S. marcescence* towered organic-extracts of *U. lactus,* grown in either NSW or ASW medium, with highest inhibition zone values ranged 8-17 mm at concentration was 1-4 mg/disk. In contrast, antibacterial activity of both extracts of *Ulva* (grown in NSW and ASW) seemed to be lower than that of chloramphenicol (inhibition zone ranged from 13-23 mm) the standard antibiotic, towered to all bacterium strains. The antibacterial activity of *Ulva* organic extracts are apparently related to their lipophelic and phenolic contents, in particularly steroids fatty substances.

The antibacterial activity quantitatively assessed by the determination of the minimum inhibitory concentration (MIC) for all the bacteria tested in Table 5. The *Ulva* organic extracts of showed potential antimicrobial activity against all tested microorganisms with MIC value ranged from 350 to 400 µg/ml (ppm). When compared with standard antibiotic chloramphinicol (MIC was 20 µg/ml), the antibacterial

activity of *Ulva* organic extracts were not as effective as commercial drugs. However, microorganisms do acquire resistance to antibiotics after some time. In general, the *Ulva*-COE grown in ASW medium was higher active (MIC 350 mg/ml) to that extract of *Ulva* grown in NSW medium (MIC 400 mg/ml). Compared to crude extract of *Ulva* grown in ASW, extracts of *Ulva* grown in NSW exhibited low antibacterial activity. Its can be explained as they have less phenolic contents (Table 1). These data seemed to indicate that the extraction of the compounds responsible for the antimicrobial activity was related to the concentration of lipophilic and phenolic compounds containing in *Ulva* extracts. A large number of algal extracts products have been found to have antimicrobial activity, many of these the structure identified as fatty acids and hydroxyl unsaturated fatty acids, glycolipid, steroid, phenolics and terpenoids [35, 36]. Mundt *et al*. [37] have attributed the antimicrobial activity of an n-hexane extracts of some macroalgae to presence of unsaturated-fatty acids and hydroxylated unsaturated-fatty acids. The organic solvents used in this work (dichloromethane/methanol; 1:1, v/v) is able to extract a large quantity of lipophilic compounds such as glycolipid, phenolic-terpeniods and unsaturated-fatty acids, thus explaining the higher antimicrobial activity found in those extracts. Prindle and Wright [38] mentioned that the antibacterial effect of phenolic compounds could be due to inhibited the enzyme activity, especially of those enzymes associated with energy production and it cause protein denaturation. In addition, Nychas, [39] reported that phenolic compounds could denature the enzymes responsible for spore germination or interfere with the amino acid involved in germination, in which, once these compounds have crossed the cellular membrane, interactions with membrane enzymes and proteins would cause an opposite flow of protons, affecting cellular activity or disturb genetic. On the other hand, phenolics and fatty acids exhibited higher antimicrobial effect on microbial growth and toxin production. These effect could be the result of the ability of these compounds to alter microbial cell permeability, permitting the loss of macromolecules from the interior and could also interact with membrane proteins, causing a deformation in there structure and functionality [37, 40].

In conclusion, *Ulva lactuca* grow under laboratory conditions are rich in non-and moderate-polar compounds including: carotenoids, chlorophyll derived and phenolic compounds and possesses antioxidant and antibacterial activity. The production of these components might have promoted in algae by changing the culture conditions to overproduce the targeted molecules. Consequently, they are valuable increasing shelf life of foodstuffs replacing synthetic antioxidant such as BHT and BHA as well as for preventing cellular damage, cause of aging and human diseases. In addition, antibacterial activity and food colorant properties can therefore be used as natural preservative ingredient in food and in pharmaceutical industry.

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