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Phytochemical, antioxidant and anti-inflammatory screening of the Egyptian *Ulva lactuca* methanolic extract

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Abstract

Ulva lactuca is widespread green alga. In some regions, it is used in nutrition. Its nutrients include iron, protein, iodine, vitamins (A, B1 and C) and trace elements. *Ulva* extracts showed potential antibacterial, hypolipidemic, cardioprotective and chemoprotective activities. Phytochemical investigation of the Egyptian *Ulva lactuca* methanolic crude extract/fractions were assessed by measuring total phenolics, flavonoids, alkaloids, carbohydrates, lipids, proteins and amino acids content. Biologically guided fractionation of plants or seaweeds extracts is considered the first step in drug discovery. So, the algal crude extract was fractionated then *in vitro* bioscreened for the determination of diphenylpicrylhydrazyl (DPPH) antioxidant, NO radical scavenging and anti-inflammatory activities. Results showed that water fraction was the most active fraction so it was subjected to further phytochemical investigation. Phenolic content, detected from HPLC, play a role in the antioxidant activity. Finally, it can be concluded that *Ulva* water fraction is effective candidate to be used in the treatment of oxidative stress and related disorders.

Keywords: Green algae, *Ulva lactuca*, phenolic, DPPH, antioxidant activity

1. Introduction:

Green algae are the most diverse group of algae with more than 7000 species growing in a variety of habitats. *Ulva lactuca* is a widespread macro alga occurring at all levels of the intertidal zone. In some regions, it is consumed in nutrition. Its nutrients include iron, protein (15%), iodine, vitamins (A, B1 and C) and trace elements. Tunisian algae powder was characterised by a high content of fibres, minerals, proteins and lipids (Yaich *et al.*, 2011). *Ulva lactuca* showed potential antibacterial activity (Saritha *et al*., 2013); (Kim *et al*., 2007). Because of its antibacterial properties, it has been recommended in treating skin irritations and burns (Apaydın *et al.*, 2010). Ulvan extracted from the algae showed cytotoxic activity (Thanh *et al*., 2016) while the aqueous ethanolic extract showed chemoprotective effect (Delgado-Roche *et al*., 2019). Ethanolic extract also showed hypolipidemic and cardioprotective effects (Kammoun *et al*., 2018). *Ulva lactuca* has a great potential antioxidant activity because it is considered as a potential rich source of natural colorant (El-Baky *et al.*, 2008). Algal extracts eliminated the harmful effects of oxidative stress induced by γ-irradiation (Alam *et al*., 2016). Reactive oxygen species (ROS) are capable of causing damage to biomolecules, such as nucleic acids, lipids, proteins and other cellular

constituents. This is associated with various chronic diseases (Cooke *et al.*, 2002). For these reasons, antioxidant compounds are widely used to minimize oxidative damage. Synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxyltoluene (BHT) should be replaced with natural antioxidants due to their proven toxicity (Li *et al.*, 2007 & Qi *et al.*, 2005).

The aim of this study was to investigate the crude methanolic extract obtained from the Egyptian *Ulva lactuca* and its different fractions using phytochemical and chromatographic methods and evaluate their *in vitro* antioxidant and anti-inflammatory properties in order to identify and utilized effective new sources of antioxidants of natural origin.

2. Experimental

2.1. Chemicals

All reference standards were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). The solvents used for chromatographic assay were of HPLC grade. Other solvents and all chemicals were of analytical reagent (AR) grade.

2.2. Algae collection, extraction and fractionation:

The green algae (*Ulva lactuca*) were collected in October from Abu Kir beach, Alexandria coast, Egypt. The algae were identified by Prof. Dr. Mohamed Saad Abd El -Kareem, Professor of Phycology, Botany and Microbiology Department, Faculty of Science, Alexandria University, Egypt. A voucher specimen (#9012) was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, for future reference. The algae were washed with tap water then left to dry in shade for two days. 1.5 kg of the dried algae were soaked in 5L methanol for one week, then filtrated, and the process was repeated three times till exhaustion. The filtrates were collected together and the solvent was evaporated using rotary evaporator at 50 ^{o}C (Büchi, Switzerland) to yield 120 g dried crude extract. The extract was fractionated using hydroalcoholic method using; light petroleum (0.5L×2), methylene chloride (0.5L×3), ethyl acetate (0.5L×3) and *n*-butanol (0.5L×2) consequently. These fractions were concentrated using rotary evaporated then lyophilized (Dynavac, FD12, Belmont, Australia) to give the dried fractions.

Table 1: Weight of each fraction from *Ulva lactuca*

Fraction type/Extract	Extract weight [g /500 g dried algae]
Total methanolic extract	120
Light petroleum fraction	17.6
CH_2Cl_2 fraction	6.98
Ethyl acetate fraction	0.1
n-butanol fraction	27.5
Aqueous fraction	55.36

2.3. Phytochemical investigation of *Ulva lactuca* extract:

Total phenolics were assessed by Folin–Ciocalteu method (Alhakmani *et al*., 2013). Total flavonoid was analyzed by aluminum chloride colorimetric method (Pourmorad *et al.*, 2006). Total alkaloid was determined by Bromocresol green method (John *et al*., 2014). Total carbohydrates and total lipids were measured by anthrone method (Ludwig and Goldberg, 1956) and sulphuric acid and phosphovanilin (SPVA) method (McMahon *et al.*, 2014). Finally, Amino acid content and total protein were assayed using ninhydrin and modified Folin-Lowary methods (Nobel and Bailey, 2009), respectively.

2.4. *In vitro* biological screening

2.4.1. Determination of DPPH antioxidant activity:

Ulva extracts, 100 uL each, were added to 100 uL methanolic solution of diphenyl picryl hydrazyl; DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C was used as a positive control. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals (Sharma and Bhat, 2009).

2.4.2. Determination of NO radical scavenging activity:

Sodium nitroprusside (10 mM, 100 uL), in phosphate-buffered saline, was mixed with 100 uL of each extract/fraction, dissolved in water, and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control (Marcocci *et al.*, 1994).

2.4.3. Determination of anti-Inflammatory activity

Anti-inflammatory activity of each extract/fractions was assessed by Human Red Blood Corpuscles (HRBCs) membrane stabilizing method (Oyedapo *et al.*, 2010) with slight modifications. The blood was collected from healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks prior to the experiment and transferred to the heparinized centrifuge tubes and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension in normal saline was made. Diclofenac potassium (50 mcg/ml) was used as a standard. The reaction mixture (4-5 ml) consisted of 2 ml hypotonic saline (0.25% w/v NaCl), 1 ml 0.15 M phosphate buffer (pH 7.4), 1 ml of test solution (1 mg/ml) in normal saline and 0.5 ml 10% HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of the test solution. The mixtures were incubated at 56ºC for 30 min and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm using visible Spectrophotometer (UV-1201 Shimadzu spectrometer). The experiment was performed in triplicates. The control represents 100% lyses. The Percentage membrane stabilization was calculated using the following formula;

% Inhibition of haemolysis = 100 x [Absorbance of control – Absorbance of test]/ Absorbance of control.

2.5. Experimental statistical analyses

All data were expressed as the mean ± standard deviation (SD). The differences were considered to be statistically significant at $p < 0.05$. Statistical analysis was performed using the unpaired Student's *t*-test and one-way analysis of variance (ANOVA) using primer of Biostatistics (Version 5) software program.

2.6. Quantitative determination of *Ulva lactuca* water fraction contents by high performance liquid chromatography (HPLC)

Analysis of the water fraction of *Ulva Lactuca* extract was carried out on an Agilent-1100 HPLC system equipped with a quaternary gradient pump unit, ultra violet (UV) detector at a wave length of 320 nm and the analytical column was Zorbax Eclipse XDB-C18 (150 x 406 mm, 5 µm particle size). Column (Agilent, Santa Clara, CA, USA) was used. Elution was carried out at a flow rate of 0.075 ml/ min at 23°C. The mobile phase used was 8 % acetonitrile, 22 % isopropyl alchohol and 70 % formic acid solution (1%). All reference standards and sample were dissolved in distilled water (1 mg/ml) and were filtered through 0.22 µm syringe filter prior to HPLC analysis. The injection volume was 20 µl. The results were calculated by measuring the chromatographic peak area. *Ulva* water fraction identification was made by comparing the relative retention times of sample peaks with those of the reference standards.

3. Result and Discussion

Bioactivity of *Ulva Lactuca* methanolic extract and its fractions were tested to select the most active one. Table1 & Figure 1showed the yield that obtained from each fraction. The phytochemical investigation quantify lipid, protein, amino acid, carbohydrate, total phenolic, alkaloid and flavonoid content (Table 2).

Table 2: Quantitative phytochemical Screening of *Ulva lactuca*.

Extract	Lipids (g/100g extract)	Proteins (g/100 g extract)	Amino-acids (mg/100g extract)	Carbohydrates (g/100 g extract)	Phenolics (g/100 g extract)	Alkaloids (g/100 g extract)	Flavonoids (mg/ 100 g extract)
Light Petroleum	56.8±16	3±0.016	0	0.4±0.02	11.8±0.2	6±1.3	2.4±0.05
CH_2Cl_2	30±11	3.3±0.022	0	0.93±0.15	11.6±0.3	8.4±0.7	0
Ethyl Acetate	94.7±26	3.2±0.036	84.1±12	0.47±0.03	11.9±0.5	4.3±1.6	0.03±0.003
Butanol	0	2.9±0.03	120±10	0.52±0.09	9.8±0.4	0	0.05±0.001
Water	0	2.6±0.18	400±23	0.44±0.06	9.5±0.9	0	0

Each value is the mean ± SD of triple determinations.

Within each column, values with the same letter are significantly different at $P < 0.05$.

Table 3: The antioxidants, radical scavenging and anti-inflammatory activities of different algae fractions at concentration of 2 mg/ml:

Extract/Fractions	DPPH scavenging %	NO scavenging %	Haemolysis-Prevention %
Crude extract	60	33	19
Light Petroleum	33	33	29
CH_2Cl_2	36	37	22
Ethyl acetate	36	23	12
Butanol	37	30	13
Water fraction	44	38	15

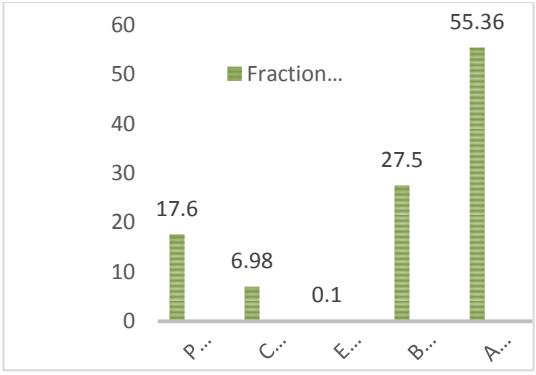

Figure 1: Percentage weight of each fraction

Table 4: Quantitative determination of phenolic content of *Ulva lactuca* aqueous extract using HPLC data

Compounds	Aqueous extract content
Chlorogenic acid (mg/l)	64.355
Caffeic acid	50.52
3,4-Dicaffeoyl quinic acid	34.75
3,5-Dicaffeoyl quinic acid	110.12
4,5 dicaffeoyl quinic acid	42.922
Galic acid (mg/ml)	13.354
Rutin	3.557
Tanic acid (mg/ml)	4.63
Quercetin	1.1
Retinol ug/l	258.62331

Proteins, carbohydrates and phenolic compounds were found in all fractions. Other phytochemicals were varied in their presence or absence from one fraction to another.

All green alga fractions showed antioxidants activity where the highest one was the water fraction (Table 3). The antioxidant and anti-inflammatory activities are concentration dependent where 2mg/ml of each one showed the maximum activity peak. DPPH scavenging activity revealed that the most powerful antioxidant was the crude extract followed by its water fraction. NO scavenging test revealed that water fraction was the most potent fraction. Overall, among the tested fractions, water fraction is the most active one so it was chosen as a candidate for further chromatographic study.

Water fraction HPLC analysis (Table 4) revealed the presence of phenolic active constituents in high content acting to increase its antioxidant bioactivity.

4. Conclusion

Seaweeds can be excellent sources that could provide the basis of a new therapeutic agent. The results of this study suggest that *Ulva lactuca* water fraction could be a promising source for the treatment of infectious diseases and oxidative stress related disorders because they are rich in phenolic constituents, offering a potential use as antioxidant candidate.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Not applicable.

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5. References

Alam, S. S., Abd El-Kader, H. A. M., Abd El-Rahim, A.H., Hamed, S. M., Saber, A. A. 2016. The Protective Role of *Ulva lactuca* Against Genotoxic and Biochemical Effects Induced by γ-Irradiation in Rats. Int. J. Pharm. Sci. Rev. Res. 37(2), 40-48

Alhakmani F., Kumar, S. and Khan, S. A. 2013. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of Moringaoleifera. Asian Pac J Trop Biomed. 3(8), 623–627.

Apaydın, G., Aylıkcı, V., Cengiz, E., Saydam, M., Küp, N., and Tıraşoğlu, E. 2010. Analysis of Metal Contents of Seaweed (*Ulva lactuca*) from Istanbul, Turkey by EDXRF. Turkish Journal of Fisheries and Aquatic Sciences. 10, 215-220.

Cooke, M. S., Evans, M. D., Mistry, N. and Lunec, J. 2002. Role of dietary antioxidants in the prevention of *in vivo* oxidative DNA damage. Nut. Res. Rev. 15,19-41.

Delgado-Roche, L., Rodeiro, I., Riera, M., Herrera, J. A., Venturi, I., Hernández, Y., … Paz, M. T. 2019). Chemoprotective effects of *Ulva lactuca* (green seaweed) aqueous-ethanolic extract against subchronic exposure to benzo(a)pyrene by CYP1A1 inhibition in mice. Phytotherapy Research.33(4), 958–967. https://doi.org/10.1002/ptr.6289

El-Baky, H., Farouk, K., Gamal, S. 2008. Evaluation of Marine Alga *Ulva lactuca* L. as A Source of Natural Preservative Ingredient. American-Eurasian J. Agric. and Environ. Sci., 3(3), 434-444.

John, B., Sulaiman, C. T., George, S. and Reddy, V. R. K. 2014. Spectrophotometric estimation of total alkaloids in selected *Justicia* species. International Journal of Pharmacy and Pharmaceutical Sciences. 6(5), 647-648.

Kammoun, I., Ben Salah, H., Ben Saad, H., Cherif, B., Droguet, M., Magné, C., Kallel, C., Boudawara, O., Hakim, A., Gharsallah, N., Ben Amara, I. 2018. Hypolipidemic and cardioprotective effects of *Ulva lactuca* ethanolic extract in hypercholesterolemic mice. Archives of Physiology and Biochemistry. 124 (4), 313-325.

Kim, I. H., Lee, D.G., Lee, S. H., Ha, J.M., Ha, B.J., Kim, S.K., Lee, J.H. 2007. Antibacterial Activity of *Ulva lactuca* against Methicillin-Resistant Staphylococcus aureus (MRSA). Biotechnology and Bioprocess Engineering.12(5),579–582.

https://doi.org/10.1007/BF02931358

Li, A. H., Cheng, K., Wong, C., Fan, K. W., Chen, F., Jiang, Y. 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 102, 771-776.

Ludwig, T.G. and Goldberg H.J.V. 1956. The anthrone method for the determination of carbohydrates in food and in oral rinsing. Journal of dental research. 35(1), 90-94.

Marcocci, L., Maguire, J., Droylefaix, M., Packert, L. 1994. The Nitric Oxide-Scavenging Properties of *Ginkgo Biloba* Extract EGb 761. Biochemical and Biophysical Research Communications. 201(2), 748-755.https://doi.org/10.1006/bbrc.1994.1764

McMahon, A., Lu, H., Butovich, I.A. 2014. The Spectrophotometric Sulfo-Phospho-Vanillin Assessment of Total Lipids in Human Meibomian Gland Secretions. Lipids. 48(5), 513–525

Noble,J. E. and Bailey, M. J. 2009. Quantitation of protein. Methods in enzymology . 463,73-95

Oyedapo, O., Akinpelu, B. A., Akinwunmi, K. F., AdeyinkaM. O., Sipeolu F. O. 2010. Red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. International Journal of Plant Physiology and Biochemistry. 2(4), 46-51.

Pourmorad, F., Hosseinimehr, J., Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology. 5, 1142-1145.

Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z., Xing, R. 2005. Antioxidant activity of different molecular weight sulfated polysaccharides from

Ulva pertusa Kjellm (Chlorophyta). Appl. Phycol. 17,527-534.

Saritha, K., Mani, A. E., Priyalaxmi, M., Patterson, J. 2013. Antibacterial activity and biochemical constituents of seaweed *ulva lactuca*. Global Journal of Pharmacology. 7(3),276–282.

https://doi.org/10.5829/idosi.gjp.2013.7.3.75156

Sharma, O. and Bhat,T. 2009. DPPH antioxidant assay revisited. Food Chemistry. 113(4),1202-1205.

https://doi.org/10.1016/j.foodchem.2008.08.008

Thanh, T. T. T., Quach, T. M. T., Nguyen, T. N., Vu Luong, D., Bui, M. L., Tran, T. T. Van. 2016. Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. International Journal of Biological Macromolecules. 93, 695–702.

https://doi.org/10.1016/j.ijbiomac.2016.09.040

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., Attia, H. 2011. Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. Food Chemistry.*128*(4), 895–901.

https://doi.org/10.1016/j.foodchem.2011.03.114