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Evaluation of Photochemical Components, Antioxidant Properties and Cytotoxicity Effects of Some Egyptian Algae Extracts on Some Cancer Cells

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Abstract

In this work, two marine green macro algae are potential (*Enteromorpha intestinalis* and *Ulva lactuca* species) as a source of several critical bioactive substances. Bioactive phenolic and flavonoids compounds were explored. Antioxidant properties and anticancer effects, such as cytotoxicity of different extracts of *Enteromorpha sp* and *Ulva lactuca sp*, have been investigated. The methanolic extracts have the most robust free radical activity (91.89 %) as well as ethyl acetate (86.70%), followed by water extract (82.47 %) and hexane extract (77.25 %) of *Ulva sp* compared to ascorbic acid as standard (98.16%). Percentage inhibition on free radical scavenging generation by the methanolic extract was found to be increasing in *Enteromorpha sp* (83.85 %), followed by water extract (81.14%); ethyl acetate extract (80.56 %) and hexane extract (71.76%). The methanolic extract of *Enteromorpha sp* and *Ulva sp* yielded data demonstrating a high total phenolic content of 66.8 and 40.3 as mg GAE /g sample, respectively. The *Enteromorpha sp* and *Ulva sp* extracted with methanol were significantly higher in their content of total flavonoids (49.4 and 32.1 as quercetin mg/g sample, respectively) than the other extracts. The obtained data reveal that methanol extract had the highest anticancer activity. There was no inhibition of all cancer cells of the samples extracted with water. The results showed that this type of algae contains unique and innovative chemical active antitumor components and can act as an antioxidant and anti-carcinogenic agent of the liver and colon for future applications in the pharmaceutical industries.

Keywords: Marine algae, Enteromorpha intestinalis, Ulva lactuca species, antioxidant activity, anticancer activity

Introduction

Oceans and seas are home to photosynthetic marine algae (also known as seaweeds). They are acknowledged as a source of several significant bioactive chemicals and have a several of advantages. (**Rashad and El-Chaghaby, 2020**). There are many diverse types of marine algae, typically split into two classes, microalgae and macro algae. While macroalgae, often known as seaweed, are plant-like organisms that can grow to be several meters long, microalgae species like phytoplankton thrive suspended in the water column, as reported by **Hernández *et al.*, (2017)** indicated that microalgae could serve as an encouraging source of nutritional and multifunctional compounds because of their high content of essential amino acids, natural colorants, vitamins and minerals that play significant roles in supporting the immune system. Therefore, there is potential for using the studied algae as natural nutraceuticals in preparing functional food products. Various biological activities in seaweeds express the solubility and polarity of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids, making them an excellent source of these substances. **(El-Chaghaby *et al.* , 2019 and Ganesan *et al.*, 2019)**. Classes of marine multicellular algae known as marine algae (Seaweeds), are rich in minerals, vitamins, and polysaccharides. They are considered a source of bioactive compounds, such as proteins, lipids, and polyphenols, which have substantial antibacterial, anticancer, antioxidant, antifungal, and antiviral activities, as reported by **Sundaramurthy *et al.* (2016). Samina *et al.*, (2019)** mentioned that the class of marine multi cellular algae, seaweeds has drawn attention for their potential to treat human diseases due to the presence of antioxidant phytochemical components. **Saadia *et al.* (2021)**

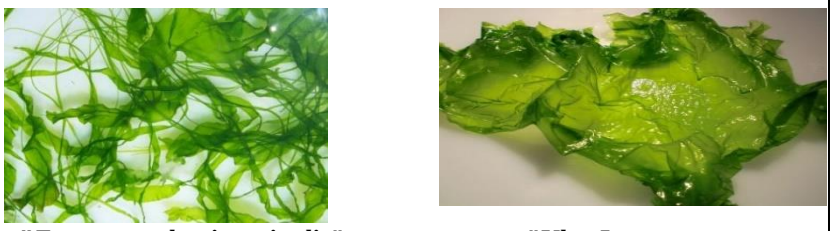
examined algae could be viewed as a reliable supply of potassium (K), calcium (Ca), sodium (Na), iron (Fe) and zinc (Zn). Thus, seaweeds are essential sources of elements vital for metabolic reactions in human health **(Insel *et al*., 2007)**. This work aims to evaluate the potentialities of two Egyptian marine algae collected from the Mediterranean Sea *(Enteromorpha intestinalis* and *Ulva lactuca* species) as a source of bioactive substances. Extract biologically active compounds from different algae species. Fractionation of active compounds and used the most potential extracts onHCT-116 (colonic cancer cell lines), Hep-G2 (hepatic cancer cell lines) and MCF7 (breast Cancer Cell lines) to the improvement of anticancer.

Material and Methods

Materials

Collection of macro algae samples

Two algal species for the proposed study, *Enteromorpha intestinalis* and *Ulva lactuca* were collected (in May 2021) from Abu Qir beaches at Alexandria city from the Mediterranean Sea. All algal samples were washed with seawater and washed several times with fresh water (Fig 1).



"Enteromorpha intestinalis" ***"Ulva Lactuca***

Fig (1) *Enteromorpha intestinalis" and Ulva Lactuca*

Chemicals

Hexane, ethyl acetate, and methanol with a purity of not less than 99% were obtained from Merck, Germany. DPPH (2, 2- diphenyl -1 picrylhydralzyl radical) was purchased from Sigma-Aldrich, Germany. Folin-Ciocalteu reagent was purchased from Fluka, France. All chemicals were purchased from Sigma Chemical Co. (St. Louis, USA). Human tumor carcinoma cell lines (HCT-116, MCF-7, and Hep-G2) used in this study were obtained from the American Type Culture Collection (ATCC, Minnesota, U.S.A).

Preparation of different extracts of algae

Collected algae from different sites were washed several times with fresh water and then left to dry away from sunlight at room temperature and separately to a fine powder using a mechanical grinder. These samples (500g) were extracted and subjected separately to exhaustive continuous successive extraction using the method described. Different extracts were obtained using the following solvents according to their polarities (in ascending order) hexane, ethyl acetate, methanol and distilled water. After filtration, the filtrate was concentrated under reduced pressure using a rotary evaporator Stuart Version Model RE300and RE300DB. The residue in each case was dried under vacuumed and stored at -20̊ C till use in chemical analysis and anticancer activity.

The extraction yield for different extracts of algae *Enteromorpha intestinalis* and *Ulva lactuca* species were calculated according to the following equation:

Yield extract (%) = (total extract/dry weight) x 100 according to **AOAC (2019)**.

Determination of chemical analysis

Moisture, ash, fiber, total protein and total lipids of fine powder from different

Enteromorpha intestinalis and *Ulva lactuca* (dry weight basis) were estimated consistent with the methods of **AOAC (2019)**. Total carbohydrate was calculated by difference as follows:

Total carbohydrate = 100 (dry weight) - (total protein + total lipids+ ash+ fiber) according to **AOAC (2019)**.

Determination of mineral contents

Mineral contents such as sodium(Na), potassium (K), calcium (Ca), phosphorus (P),magnesium (Mg),iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were determined using by Agilent Technologies (Perkin-Elmer, Model 4210 MP-AES), atomic absorption spectrophotometer instrument as described by the **AOAC (2019)** method.

Determination of radical scavenging activity

Based on the scavenging activity of the stable DPPH free radical, the antioxidant activity of extracts was determined by the method described by **Lee *et al*. (2004)**.Inhibition (%) of DPPH free radical was calculated from the equation:

Inhibition % **= (Ac– As / Ac × 100)**

Where: **Ac** is the absorbance of the control and **As** is the absorbance of the sample.

Determination of total phenolic and total flavonoids content

The total phenolic content of different algae extracts was determined using the Folin-Ciocalteu's phenol reagent according to the method described by **Maurya and Singh (2010).** The results were expressed as gallic acid equivalent to mg /g of weight. While, Total flavonoids content of algae samples was determined according to the method described by **Jia *et al.* (1999)**. The results were expressed as mg of quercetin/ g of dry weight basis.

Fractionation and identification of phenolic and flavonoids compounds

Phenolic compounds were fractionated and identified for algae at different extracts by HPLC according to the method described by **Goupy *et al.* (1999)**. While, flavonoid compounds were fractionated and identified of different extracts of algae by HPLC according to the method of **Loon *et al.* (2005).**

Anticancer activity

Determination of potential cytotoxicity of different algae extracts on human cancer cell lines

The cytotoxicity was carried out using sulphorhodamine- B (SRB) assay by the method according to **Vichai and Kirtikara, (2006)**.The percentage of cell survival was calculated as follows:
Surviving fraction= O.D. (treated cells)/O.D. (control cells).
The IC_{50} values (the concentration of resveratrol required to produce 50% inhibition of cell growth) were also calculated.

Statistical analysis

The practical statistical analysis methods were applied to the data. Where, mean and standard deviation were computed. To determine the statistically significant difference between the treated samples, data were analysed using a two-way classification ANOVA, as outlined by **Snedecore and Chochran (1980)**. According to **Waller and Duncan (1969**), the Least Significant Differences (LSD 5%) in Duncan's multiple ring tests indicated that the mean separation was determined.

Results and Discussion

Chemical composition of some Egyptian marine algae

The data in Table 1 demonstrate the preliminary study of some Egyptian marine algae (on dry weight basis). From the obtained results, the moisture content was found to be 13.13± 0.63 and11.79 ± 0.51% in *Ulva sp and Enteromorpha sp*, respectively. However, *Enteromorpha sp* algae cultivar showed a significantly higher ash, fiber and total lipid content than *Ulva sp*. While, *Ulva sp* was significantly higher in total proteins content (18.88± 0.21%) and carbohydrates content (24.77± 0.66%) . These results are in agreement with those reported by **Rashad and El-Chaghaby**, **(2020)** who reported that the numerous microalgae species have high concentrations of nutrients that are beneficial to human nutrition, including polyunsaturated fatty acids, carbohydrates, proteins, essential amino acids, and vitamins A and E. Also, **Salem *et al.* (2018)** reported that compared to brown (Ochrophyta) algae, green (Chlorophyta) algae had higher carbohydrate content. *Ulva sp* is a nutritional component for various fish species and is a good source of protein, pigments, minerals, and vitamins. It is exceptionally high in vitamin C. **(Ortiz *et al.*, 2006; Garcia-Casal *et al.*, 2007)**.

Table 1. The proximate analysis of *Enteromorpha Sp* and *Ulva Sp* (on a dry weight basis)

Algae *sp.*	Contents on dry weight basis					
	Moisture %	Ash %	Fiber %	Total proteins %	Total lipids%	Total carbohydrates
Enteromorpha Sp	11.79^{b} ± 0.51	30.85^{a} ± 0.92	16.85^{a} ± 0.13	14.96^{b}± 0.34	2.26^{a} ± 0.17	23.29^{b} ± 0.36
Ulva Sp	13.13^{a} ± 0.63	26.95^{b} ± 0.62	14.60^{b}± 0.04	18.88^{a} ± 0.21	1.66^{b} ± 0.23	24.78^{a} ± 0.66
LSD 0.05	0.83	1.46	0.73	0.48	0.47	1.28

Each value is mean of three replicates ± SD, number in there isn't much difference between the same column and the same letter. $p < 0.05$

Minerals content of some Egyptian marine algae

Table (2) shows the ash minerals content of *Enteromorpha sp and Ulva sp.* From these data, calcium represents the high content (4088.0 mg/100g) and (3997.0 mg/100g) of respectively, followed by magnesium (3543.0 mg/100g) and (3375.0 mg /100g) of *Ulva sp* and *Enteromorpha sp* respectively. From the obtained results can be concluded that the algae *Enteromorpha Sp* was higher in its content of potassium, sodium, and phosphorous, than the type *Ulva Sp*, At the same time, it was equal in its content of the element manganese with the *Ulva Sp*. For the trace elements (Table 2), Copper (Cu) manganese and zinc contents of the tested seaweeds were found in the 0.01 to 0.03 mg/100g.

Table 2. Minerals content of *Enteromorpha Sp* and *Ulva Sp* as (mg/100g dry weight)

Algae *Sp.*	Minerals content (mg/100g dry weight)								
	Na	K	Ca	P	Mg	Fe	Mn	Zn	Cu
Enteromorpha Sp	650.00	1320.00	4088.00	120.00	3375.00	1.05	0.03	0.03	0.02
Ulva Sp	260.00	710.00	3997.00	100.00	3543.00	0.55	0.03	0.01	0.01

These results are in agreement with **MacArtain *et al.* (2007)** and **Khairy and El-Sheikh, (2015)** indicated that Cu was most plentiful in Chlorophyceae species (*U. lactuca*), especially during the summer, with a concentration of 97.8 mg/100 g. They also discovered that the green alga *U. lactuca* had the highest concentration of Cu throughout the summer. These results are close to **Rashad and El-Chaghaby, (2020)** who reported that the numerous microalgae species are abundant in nutrients including potassium, magnesium, and calcium.

The yield percent for different extracts of some marine algae

Data in Table (3) show the yield extracted percentage of *Enteromorpha sp*, and *Ulva sp*, and the obtained results were found to be 0.08 and 0.25 %, 0.67 and 0.11%, 7.74 and 5.33%, 11.86 and 16.66 % in hexane extract, ethyl acetate extract, methanol extract, and water extract, respectively. These outcomes are consistent with **Saadia *et al.* (2021),** who reported that the extraction yield depends on the solvent polarity.

Table 3. Extract yield percent of *Enteromorpha Sp* and *Ulva Sp* (based on dry weight)

Algae *Sp.*	Yield percentage			
	Hexane extract	Ethyl acetate extract	Methanol extract	Water extract
Enteromorpha Sp	0.08	0.67	7.74	11.86
Ulva Sp	0.25	0.11	5.33	16.66

Radical scavenging activity of different *Enteromorpha Sp* and *Ulva Sp* extracts

Antioxidants protect humans against infections and degenerative diseases, which are crucial in blocking and scavenging free radicals. Many marine algae were said to have antioxidant qualities (**Yasantha *et al.*, 2006**). Table (4) results illustrate the DPPH free radical scavenging activity of different extracts of *Enteromorpha Sp* and *Ulva Sp*. Ascorbic acid was selected as the reference antioxidant, The antioxidant activity of the extracts obtained for this study was assessed using the DPPH method, one of the most efficient ways to evaluate radical scavengers. The methanolic extracts have the most substantial free radical activity (91.89 ±039%), as well as ethyl acetate (86.70 ±0.65%), followed by water extract (82.47±0.76%) and hexane extract (77.25±0.76 %) of *Ulva Sp* compared to ascorbic acid as standard (98.16±3.5%). Percentage inhibition of free radical scavenging generation by the methanolic extract was found to be increasing in the methanolic extract of *Enteromorpha Sp* (83.85 ±0.99%), followed by water extract (81.14±0.75%), ethyl acetate extract (80.56 ±1.35%) and hexane extract (71.76±1.44%).

Table 4. Radical scavenging activity (%) in *Enteromorpha Sp* and *Ulva Sp* extract

Algae *Sp.*	Radical scavenging activity (%)					
	Hexane extract	Ethyl acetate extract	Methanol extract	Water extract	Ascorbic acid(st)	LSD 0.05
Enteromorpha Sp	71.76^{e}±1.44	80.56^{d}±1.35	83.85^{b}±0.99	81.14^{c}±0.75	98.16^{a} ±3.5	2.69
Ulva Sp	77.25^{e}±0.76	86.70^{c}±0.65	91.89^{b}±039	82.47^{d}±0.76	98.16^{a} ±3.5	1.71

Each value is the mean of three replicates ± SD, and there isn't much difference between the same row and the same letter. $p < 0.05$

These results are in agreement with **Khairy and El-Sheikh, (2015)** found that the seaweed (*Ulva lactuca* Linnaeus (Chlorophyta), *Janiarubens* (Linnaeus) J.V. *Lamouroux* and *Pterocladia capillacea* (S.G. Gmelin). The DPPH scavenging capacity of *Bornet* et *Batters* (*Rhodophyta*) extracts dramatically enhanced with increasing their concentration in various species and during various seasons. **Saeed *et al.* (2020)** found that the extracts of *U. Lactuca* and *U. Fasciata* displayed the highest antioxidant activity by using the DPPH scavenging method, and total antioxidant ability was assayed to 2.13 and 1.51 mg ascorbic acid equivalent/g dry weight, respectively.

The total phenols and total flavonoids contents of *Enteromorpha sp* and *Ulva sp*

Klejdus *et al*, (2010) reported that the phenolic compounds are secondary metabolites typically present in all terrestrial plants and microalgae. Data in Table (5) illustrate the total phenol contents of different extracts of *Enteromorpha sp* and *Ulva sp*. All total phenol results are expressed as Gallic equivalents (GAE). The results of the methanolic extracts of *Enteromorpha Sp* and *Ulva Sp*, showed a

significantly higher total phenolic content of 66.8±0.05 and 40.3±0.15 as mg gallic acid/g sample, respectively. The samples extracted with hexane were significantly lower in their phenols content. *Ulva sp* was lower in phenols (1.6±0.02 mg gallic acid/g sample) than *Enteromorpha Sp* (2.10±0.03 mg gallic acid /g sample). The total phenols were equal in *Ulva sp* and *Enteromorpha Sp* extracted with water (27.1±0.03 mg gallic acid /g sample). These findings contradict those made public by **Delfan *et al.* (2018)**, who said that *Spirulina platensis* had 26.64-31.90.16 mg GAE/g.

Table 5. Total phenolic contents (gallic acid mg/g sample) of *Enteromorpha Sp* and *Ulva Sp*

Algae *Sp.*	Total phenolic contents (gallic acid mg/g sample)				
	Hexane extract	Ethyl acetate extract	Methanol extract	Water extract	LSD 0.05
Enteromorpha Sp	2.1^{d}±0.03	16.5^{c}±0.09	66.8^{a}±0.05	27.1^{b}±0.02	0.13
Ulva Sp	1.6^{d}±0.02	20.1^{c}±0.09	40.3^{a}±0.15	27.1^{b}±0.03	0.32

Each value is the mean of three replicates ± SD, and there isn't much difference between the same row and the same letter. $p < 0.05$

Total phenolic concentrations vary depending on various influencing factors, including the kind of solvent used for the extraction procedure, algal species, geographic origin, culture season, and environmental fluctuations **(Marinho-Soriano *et al.*, 2006)**. This has been attributed to some variables, including the kind and quantity of phenolic compounds. When using various polar and non-polar organic solvents, different strains of microalgae were reported to contain between 0.54 and 5.8 mg Gallic acid/g dry weight (**Hajimahmoodi *et al.*, 2010**). However, flavonoids results are expressed as quercetin equivalents (QE), and the obtained data are presented in Table (6). The *Enteromorpha Sp* and *Ulva Sp* extracted with methanol were significantly higher in their content of total flavonoids (49.4 ±0.32and 32.1±0.12 as quercetin (mg/g sample), respectively, than the other extracts. Also, the results from Table (6) showed that the hexane extract was significantly lower in the extraction rate of flavonoids in the mentioned algae species. The difference in flavonoids content may be due to the difference in physical and chemical compositions, such as salinity between the selected species (*Enteromorpha Sp and Ulva Sp*) as reported by **Farasat *et al.* (2014).**

Table 6. Total flavonoid contents as quercetin (mg/g sample) of different extracts of dried marine algae

Algae *Sp.*	Total flavonoids contents as quercetin mg/g sample				
	Hexane extract	Ethyl acetate extract	Methanol Extract	Water extract	LSD 0.05
Enteromorpha Sp	1.8^{d}±0.01	6.6^{c}±0.02	49.4^{a}±0.32	17.4^{b}±0.02	0.37
Ulva Sp	0.9^{d}±0.00	12.1^{c}±0.22	32.1^{a}±0.12	17.1^{b}±0.05	0.30

Each value is the mean of three replicates ± SD, and there isn't much difference between the same row and the same letter. $p < 0.05$

Fractionation and identification of phenolic and flavonoids compounds

Plants and seaweed frequently contain phenolic chemicals. like other plants, seaweeds contain a various organic and inorganic compounds that are beneficial to human health **(Kuda *et al.*, 2007)**. The results of phenols compounds (mg/100g) by HPLC are shown in Table (7). Class *Enteromorpha sp* increased in its content of phenolic compounds, where the compound was ellagic (6.15 mg/100g), The compound salicylic (4.96mg/100g), catechein (2.79mg/100g), pyrogallol (2.72mg/100g), caffeine (2.34mg/100g) and vanillic (2.28mg/100g) while, the compound alpha-coumaric recorded the lowest percentage (0.04mg/100g). Then, the results of Table (7) showed that *Ulva sp* was high in benzoic (4.36mg/100g) compound, followed by pyrogallol (3.98mg/100g) and salicylic (3.78mg/100g) while, the alpha-coumaric compound recorded the lowest value (0.02 mg/100g). From the obtained results it can be concluded that the methanol extract of *Enteromorpha Sp* and *Ulva Sp* was higher in its content of phenolic compounds compared to ethyl acetate extract and water extract. According to the study's findings, phenolic content and antioxidant activity are strongly correlated. The ability of phenolics to function as reducing agents, hydrogen donors, and free radical quenchers, as well as their capacity to function as metal chelator, which prevents the catalytic function of metal in the process of initiating radicals, is thought to be the cause of their antioxidant properties **(Wu and Hansen, 2008)**. According to **Ganesan *et al.*, (2008)**, phenolic compounds are frequently found in edible brown, green, and red seaweeds; the anti- oxidative characteristic of these seaweeds has been associated with their phenolic concentration.

Table 7. Phenols compounds fractionation (mg/100g) of different extracts from *Enteromorpha Sp and Ulva Sp.*

Phenols mg/100g	*Enteromorpha Sp*				*Ulva Sp*			
	Raw	Methanol extract	Ethyl acetate extract	Water extract	Raw	Methanol extract	Ethyl acetate extract	Water extract
Gallic	0.61	9.79	2.95	1.25	0.12	1.55	1.38	0.78
Pyrogallol	2.72	29.76	74.93	10.84	3.98	58.43	12.30	4.75
4-Amino-benzoic	0.23	3.64	2.55	0.13	0.12	1.16	0.65	0.18
Protocatchuic	1.73	20.74	7.69	0.46	0.85	8.51	1.82	1.71
Catechein	2.79	57.66	14.61	0.57	1.66	22.32	4.89	1.61
Chlorogenic	0.74	16.89	3.63	0.14	0.24	9.65	1.40	0.33
Catechol	0.41	24.52	17.26	0.50	0.78	5.79	9.45	1.16
Caffeine	2.34	70.13	9.88	1.06	0.18	20.51	4.62	1.61
P-OH- benzoic	0.84	28.46	7.09	0.40	0.51	4.54	3.79	0.23
Caffeic	0.24	5.35	3.04	0.07	0.06	2.92	0.72	0.10
Vanillic	2.28	57.15	6.18	0.28	0.30	9.64	0.80	0.05
P-Coumaric	0.14	3.51	0.92	0.09	0.09	1.52	0.19	0.08
Ferulic	0.50	7.25	1.59	0.14	0.34	2.07	0.24	0.20
Iso-Ferulic	0.26	12.49	2.21	0.17	0.10	3.19	0.73	0.19
Ellagic	6.15	51.72	49.49	0.45	3.31	35.73	11.21	1.92
Benzoic	2.26	238.07	27.31	2.02	4.36	113.29	40.15	3.72
Alpha-Coumaric	0.04	5.56	0.57	0.03	0.02	2.57	0.67	0.07
3,4,5-methoxy-cinnamic	0.33	19.23	5.41	0.53	0.90	19.86	4.65	0.84
Coumarin	0.31	15.04	4.29	0.25	0.22	35.77	7.74	1.72
Salycilic	4.96	181.86	35.93	0.35	3.78	178.42	18.02	4.73
Cinnamic	0.08	0.83	0.63	0.03	0.18	1.80	0.20	0.10

On the other hand, the results in Table (8) show the flavonoids compounds fractionation as (mg/100g) of different extracts from the tested algae. The *Enteromorpha Sp* of the raw algae and their extracts were higher in its content of flavonoids than the *Ulva Sp* (raw and their extracts). The compound of hesperidine recorded the highest value in raw and all extracts of *Enteromorpha Sp*. whereas; methanol extract was higher in its content of flavonoids compounds in both types of algae. These results agree with those reported by **El-Chaghaby *et al.*, 2019; Ganesan, *et al.*, 2019 ; Saadia *et al.*, 2021**).

Table 8. Flavonoid compounds fractionation (mg/100g) of different extracts from *Enteromorpha Sp and Ulva Sp.*

Flavonoids mg/100g	*Enteromorpha Sp*				*Ulva Sp*			
	Raw	Methanol Extract	Ethyl acetate Extract	Water Extract	Raw	Methanol Extract	Ethyl acetate Extract	Water Extract
Naringenin	3.95	35.30	25.22	0.56	3.39	12.14	7.87	1.37
Rutin	0.57	13.04	5.64	0.06	0.32	7.99	3.32	0.35
Hesperidine	10.41	79.35	65.28	1.23	1.43	45.82	22.98	3.79
Qurectrin	1.16	16.31	7.19	0.07	0.96	29.89	5.62	2.91
Qurectin	0.29	11.72	2.33	0.05	0.19	1.38	2.67	0.16
Narengenin	0.14	3.83	1.95	0.01	0.10	2.89	1.56	0.05
Hespertin	0.77	8.04	8.93	0.09	0.58	23.14	4.41	0.19
Kampferol	0.31	17.65	6.52	0.10	0.33	23.23	11.09	0.51
Apiginenin	1.31	12.34	7.76	0.61	0.23	13.43	5.38	0.22

Cytotoxic effects of different extracts of *Enteromorpha SP.* on colonic cancer cell lines, hepatic cancer cell lines and breast cancer cell lines

The human body naturally produces reactive oxygen species (ROS) through metabolic processes. Exogenous sources of ROS include smoking, air pollution, radiation, ozone, and industrial chemicals. The stabilization of ROS results in cellular damage, the creation of carcinogenic DNA adducts, and the development of several malignancies, among other human disorders. **Omar *et al.* (2018)** reported that antioxidant consumption has been demonstrated to lower the chances of contracting certain disorders. Because they have minimal to no side effects, natural and plant-based anticancer agents are an effective

weapon in the fight against cancer cells. Marine algae are Currently used as an antioxidant and nutritional supplement, **Samina *et al.* (2019)**. Seaweeds offer a new source of bioactive chemicals for the manufacturing pharmaceutical sector **(Hamed *et al.*, 2018)**. Cytoxicity of algae *(Enteromorpha. Sp)* extract with various solvents (*i.e*., methanol and water extracts) were investigated on the selected cell lines of colonic cancer (HCT-116), hepatic cancer cells lines (Hep- G2) and MCF7 (breast Cancer Cell lines). Several aspects of cytotoxic effects are observed, as shown in Table (9). The obtained data reveal that methanol extract had the highest anticancer activity. The IC_{50} of methanolic extract of *Enteromorph.SP*. It were 22 µg/ mL on hepatic cancer cells lines (Hep- G2), followed by colonic cancer (HCT-116) IC_{50} 39.21 µg/mL and 44.0 µg/mL of breast cancer cell lines (MCF7). These extract concentrations can inhibit, kill and controll cancer cells. The least of them affected inhibiting cancer cells in breast cancer and there was no inhibition of all cancer cells of the samples extracted with water.

Table 9. Cytotoxic effects of different extracts of *Enteromorpha Sp*. on HCT-116 (colonic cancer cell lines), Hep-G2 (hepatic cancer cell lines), and MCF7 (breast cancer cell lines)

Extracts Conc.	*Enteromorpha Sp*					
	HCT-116		Hep-G2		MCF7	
	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
0	1.00	1.00	1.00	1.00	1.00	1.00
12.5	0.875	0.991	0.810	0.985	0.880	0.984
25	0.691	0.970	0.525	0.957	0.666	0.998
50	0.365	0.914	0.222	0.934	0.449	0.967
100	0.257	0.961	0.211	0.899	0.427	0.957
IC_{50} µg/ml	39.2l	0.00	22.0	0.00	44.0	0.00

Moreover, Table (10) shows the percentage of inhibition of extracts of *U. lactuca* against HCT-116 cells (colonic cancer cell lines), Hep-G2 (hepatic cancer cell lines) and MCF7 (breast cancer cell lines) tumor cell lines. From these data the MeOH extract of algae exhibited the highest cytotoxicity towards HCT-116 with IC_{50} 42 µg/mL of *Ulva Lactuca*, followed by Hep- G2 was 48.6 µg/mL. Meanwhile, the extracted with water did not affect on the inhibition of tumor cells of the tested types. There was no effect of methanolic and water extract of this kind of algae (*Ulva Lactuca*) on inhibiting breast cancer cells. This result is nearly similar to those of **Saeed *et al*. (2020)** who checking for cytotoxicity using MTT assay, revealed *U. lactuca* extract had excellent activity against cell lines MCF-7 and Hela (IC_{50} 10.83±1.0, 12.43±1.3µg/mL, respectively), and *U. Fasciata* displayed great activity against cell lines PC3 and HepG2 (IC_{50}12.99±1.2, 16.75±1.5µg/mL, respectively). **De Alencar *et al.* (2016)** found that antioxidant compounds such as alkaloids, flavonoids, phenols, tannins, phlorotannin, terpenoids, pigments, glycosides, and steroids were believed to operate as a defensive mechanism, and shielding algae from reactive oxygen species (ROS) brought on by harsh environmental circumstances.

Table 10. Cytotoxic effects of different extracts of *Ulva Lactuca* on HCT-116 (colonic cancer cell lines), Hep-G2 (hepatic cancer cell lines) and MCF7 (breast Cancer Cell lines)

Extracts Conc.	*Ulva Lactuca*					
	HCT-116		Hep-G2		MCF7	
	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
0	1.00	1.00	1.00	1.00	1.00	1.00
12.5	0.928	0.964	0.891	0.957	0.972	0.994
25	0.745	0.983	0.652	0.971	0.865	0.972
50	0.383	0.952	0.489	0.935	0.655	0.955
100	0.302	0.965	0.307	0.908	0.516	0.903
IC_{50} µg/ml	42.0	0.00	48.6.0	0.00	0.00	0.00

Antioxidants in macro algae shielded the structural elements of the species from oxidative environmental degradation. Due to the demand for substances with bioactivity for future medicinal uses or other potential economic qualities, several studies on marine algae have increased recently. It is reasonable to infer that the secondary metabolites of marine organisms differ considerably from those of terrestrial species, since they live in an ecosystem that is very different from that of terrestrial species. The antioxidant and anticancer activity of the seaweed extract (*Enteromorpha. Sp and U. lactuca)* may be the result of their high phenolic component content **(Yuan and Walsh 2006, Senguttuvan *et al.*, 2014 and Morsy *et al.*, 2020)**.

From the abovementioned results, it can be concluded that the preliminary investigation of the antioxidants in some Egyptian seaweeds, where the

use of these natural and plant-based anti-cancer products is a valuable tool to fight cancer cells as they leave little or no side effects. Compared to aqueous substance extract, and this investigation demonstrated the methanol extract of *Enteromorpha .Sp* and *U. lactuca* had more vital antioxidant and anti-carcinogenic action. Additionally, different doses of methanol extracts from *Enteromorpha. Sp* and *U. lactuca* significantly influenced breast, liver, and colon cancer cell lines when the extracts were tested for anticancer activity. As a result, this species has the potential to be a source of nutritious food for human diets as well as a source of food for the pharmaceutical business.

Reference

AOAC (2019). Official Methods of Analysis, AOAC International 21st edition Association of Official Analytical Chemists. Washington, D.C. Available from: https://www.aoac.org/official-methods-of analysis-21st edition-2019.

De Alencar, D. B.; Teles, D. C.; Helena, R.; dos Santos, D.R.; dos Santos, K.M.; Cavalcante, P.; De Lima,R.L.; Baracho,B.M.; Bezerra, R.M.; Viana, F.A.; Vieira,R.H.; Sampaio,A.H.; De Sousa,O.V. and Sampaio, S.S. (2016). Bioactive extracts of red seaweeds *Pterocladiella capillacea* and *Osmundaria obtusiloba* (*Floridophyceae: Rhodophyta*) with antioxidant and bacterial agglutination potential. *Asian Pac. J. Trop. Med.*, **9**:372–379.

Delfan, P.; Mortazavi, A.; Rad, A. H. E. and Zenoozian, M. S. (2018). Measurement of phenolic content and antioxidant capacity of pennyroyal (*Mentha pulegiumL.*) and microalgae *Spirulina platensis* extracted by steeping, ultrasonic and microwave methods. *J. of Food Pro. and Technol.*, 9:1-7.

El-Chaghaby, G.A.; Rashad, S.; Abdel-Kader, S.F.; Rawash, E.S.A. and Moneem, M.A., (2019). Assessment of phytochemical components, proximate composition and antioxidant properties of Scenedes musobliquus, *Chlorella vulgaris and Spirulina platensis* algae extracts. *Egy. J. of Aq. Bio. and Fish*, 23(4):521–526.

Farasat, M.; Ramazan, A.; Khavari, N.; Seyed, M.; Bagher, N. and Foroogh, N. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from Northern coasts of the persian gulf. *Iran. J. of Pharma. Res*., 13(1):163-170.

Ganesan, A.R.; Tiwari, U. and Rajauria, G. (2019). Seaweed nutraceuticals and their therapeutic role in disease prevention. *Food Sci. Hum. Wellness* 8:252–263.

Ganesan, P.; Kumar C.S. and Bhaskar, N. (2008). Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour Technol.*, 99 (8):2717-2723.

Garcia-Casal, M.N.; Pereira, A.C, Leets, I.; Ramirez, J. and Quiroga, M.E. (2007). High iron content and bioavailability in humans from four species of marine algae. *J. Nutr*., 137:2691-2695.

Goupy, P.; Hugues, M.; Biovin, P. and Amiot, M.J. (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and isolated phenolic compounds. *J. Sci. Food Agric*., 79:1625-1634.

Hajimahmoodi, M.; Faramarzi, M.A.; Mohammadi, N.; Soltani, N.; Oveisi, M.R. and Nafissi-Varcheh, N. (2010). Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. *J. Appl. Phycol*., 22:43–50.

Hamed, S.M.; Abd El-Rhman, A.A.; Abdel-Raouf, N. and Ibraheem, I.B.M. (2018). Role of marine macroalgae in plant protection & improvement for sustainable agriculture technology. *Beni-Suef, Univ. J. Basic Appl. Sci*., 7:104–110.

Hernández. Fariñas, T.; Ribeiro, L.; Soudant, D.; Belin, C.; Bacher, C.; Lampert, L. and Barillé, L. (2017). Contribution of benthic microalgae to the temporal variation in phytoplankton assemblages in a macrotidal system. *J. Phycol*., 53:1020–1034.

Insel, P., Ross, D., McMahon, K. and Bernstein, M., (2007). Nutrition, 3ed. Jones and Bartlett Publishers, Sudbury, Canada.

Jia, Z.; Tang, M.and Wu, J. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*., 64:555–559.

Khairy, H. M and El-Sheikh, M.A. (2015). Antioxidant activity and mineral composition of three Mediterranean common seaweeds from Abu-Qir Bay, Egypt. King Saud Univ. *Saudi J. of Biol. Sci*., 623-630.

Klejdus, B.; Lojková, L.; Plaza, M.; Šnóblová, M.; and Štˇerbová, D. (2010). Hyphenated technique for the extraction and determination of isoflavones in algae: Ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. *J. Chromatogr.A.*, 1217:7956–7965.

Kuda T, Kunii T, Goto H, Suzuki T and Yano T. (2007). Varieties of antioxidant and anti-bacterial properties of *Ecklonia stolonifera* and *Ecklonia kurome* harvested and processed in the Noto peninsula, Japan. *Food Chem.*, 103:900-905

Lee, J.Y., Hwang, W.I and Lim, S.T. (2004). Antioxidant and anticancer activities of organic extracts from *Platycodon grandiflorum* A. De

Candolle roots. *J. of Ethnopharmacol*., 93:409–415.

Loon, Y.; Wong, J.; Yap, S. and Yuen, K. (2005). Determination of flavanoids from *ortho siphonstamineus* in plasma using a simple HPLC method with ultraviolet detection. *J. Chromatogr. B*., 816:161– 166.

MacArtain, P., Gill, C.I.R., Brooks, M., Campbell, R. and Rowland, I.R. (2007). Nutritional value of edible seaweeds. *Nutr. Rev*., 65:535–543.

Marinho-Soriano, E., Fonseca, P.C, Carneiro, M. A. A. and **Moreira, W.S.C. (2006)**. Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technol.*, **97**:2402-2406.

Maurya, S. and Singh, D. (2010). Quantitative analysis of total phenolic content in Adhatoda vasica Nees extracts. *Int J. Pharm Tech Res*., 2(4):2403-2406.

Morsy, G.M.T.; Bekhet, E.K. and Mohamed, E.A. (2020). Phytochemical screening for antibacterial compounds of some seaweed from coastal area of Abu-qir, Alexandria. *Egyptian J. of Phycol*., 19:47-57.

Omar, H., Al-Judaibiand, A. and El-Gendy, A. (2018). Antimicrobial, Antioxidant, Anticancer Activity and Phytochemical Analysis of the Red Alga, *Laurencia papillosa*. *Int. J. Pharmacol.*, **14**:572–583.

Ortiz, J.; Romero, N.; Robert, P.; Araya J. and Lopez-Hernandez, J. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds (*Ulva lactuca)* and (*Durvillaea antarctica*). *Food Chem*., 99:98-104.

Rashad,S. and El-Chaghaby,G.A.(2020). Marine Algae in Egypt: distribution, phytochemical composition and biological use (a review). *Egyptian J. of Aquatic Biol.and Fisheries*. 24(5):147–160.

Saadia, M.; Hashem, Abd El-Lahot, M. S.; Amr, M. H. and Mona I. M. (2021). Evaluation of the phytochemicals and nutritional characteristics of some microalgae grown in Egypt as healthy food supplements. *Egyptian J. of Food Sci*., Vol. 49**,** (1)**:** 173-185.

Saeed, A.M.; Abotaleb, S.I.; Alam, N.G.; Elmehalawy, A.A. and Gheda, S.F. (2020). In vitro assessment of antimicrobial, antioxidant and anticancer activities of some marine macroalgae. *Egypt. J. Bot*., 60:81–96.

Salem, D. M. S. A.; El Sikaily, A. and Abou-taleb, A. E. A. (2018). Nutritional value and health quotient of algae collected from Egyptian coast, Alexandria. *Egyptian J. of Aquatic Biol. and Fisheries***,** 22:419-429.

Samina, H. H.; Ghaida, A.R.; Moudhi, A.; Al-Mutlaq, S. A.; Maha, Naji1, A.M.; Sarah A.R, Qura T.A.; Abdullah A.A. and Adnan A.M. (2019). Antioxidant, anticancer activity and phytochemical analysis of green algae, *Chaetomorpha* collected from the Arabian Gulf. www.nature.com/scientificreports https://doi.org/10.1038/s41598-019-55309-1

Senguttuvan, J.; Paulsamy, S. and Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *hypochaeris radicata L*. for *in vitro* antioxidant activities. *Asian Pac. J. Trop. Biomed.,* **4**:S359–S367.

Snedecore,G.W. and Chochran,W.G. (1980). Shortcut and non-parametric methods. In: Statistical methods. In: Statistical methods. Oxford and J.B.H., publishing Com, Ames, Lowa Univ. Press, 7:143-146.

Sundaramurthy, A., Suresh Babut, V. S. and Shantaram, M. S. (2016). Seaweed extracts exhibit anticancer activity against hela cell lines. *Int. J. Curr. Pharm. Res.,* 9:114 -120

Vichai,V. and Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*, 1(3):1112-1116.

Waller.R.A. and Duncan, D.B. (1969). A bayes rule for symmetric multiple comparison problems. *J. Amer. Stat. Ass*., 64:1484-1503

Wu, X.J. and Hansen, C. (2008). Antioxidant capacity, phenolic content, polysaccharide content of *Lentinus edodes* grown in Whey permeate-based submerged culture. *J Food Sci*., 73(1): M1-M8.

Yasantha, A.; Kim, K.N. and Jeon, Y.J. (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown algae *Ecklonia cava*. *Food Chem. Toxicol*. 44:1065–1074.

Yuan, Y. V. and Walsh, N. A.(2006). Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.,* 44:1144–1150 .

تقييم المكونات الكيموضوئيه وخصائص مضادات الاكسدة وتأثير السميه لمستخلصات بعض الطحالب المصرية على بعض الخلايا السرطانية

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تهدف هذه الدراسة الى الاستفادة من صنفين من الطحالب البحرية الخضراء المصرية (الانتيرومورفا واليولفا) كمصدر للعديد من المركبات الحيوية النشطة. ومركبات الفينول والفلافونيدات النشطة بيولوجيا و الخصائص المضادة للأكسدة والتأثيرات المضادة للسرطان مثل السمية الخلوية لمستخلصات مختلفة من الانتيرومورفا واليولفا. بينما احتوت المستخلصات الميثانولية على أقوى نشاط للجذور الحرة (91.89٪) وكذلك أسيتات الإيثيل (86.70٪) ، يليها المستخلص المائي (82.47٪) وخلاصة الهكسان (77.25٪) من اليولفا مقارنة بحمض الأسكوربيك كمعيار قياسى (98.16٪) .كما وجد أن النسبة المئوية للتثبيط على إنتاج الجذور الحرة بواسطة المستخلص الميثانولي تزداد في الانتيرومورفا (83.85٪) ، يليه المستخلص المائي (81.14٪) ، مستخلص أسيتات الإيثيل (80.56٪) وخلاصة الهكسان (71.76٪). و أظهرت نتائج المستخلص الميثانولي لكل من الانتيرومورفا و اليولفا محتوى الفينولات الكلية مرتفع حيث بلغ 66.8 و 40.3 ملليحم حمض جاليك / جم عينة على التوالي. وكان كل من الانتيرومورفا و اليولفا المستخلصين بالميثانول أعلى بكثير في محتواهما من إجمالي مركبات الفلافونيدات (49.4 و 32.1 كعينة كيرسيتين مجم / جم ، على التوالي) من المستخلصات الأخرى. وأظهرت البيانات التي تم الحصول عليها أن مستخلص الميثانول كان له أعلى نشاط مضاد للسرطان. وكانت تركيزات المستخلصات هذه قادرة على تثبيط الخلايا السرطانية وقتلها والسيطرة عليها. ولم يكن هناك أي تثبيط لجميع الخلايا السرطانية للعينات المستخرجة بالماء. كما أظهرت النتائج أن هذا النوع من الطحالب يحتوي على مكونات كيميائية فعالة ومضادة للأورام .ويمكن أن تعمل كعامل مضاد للأكسدة ومضاد للسرطان في الكبد والقولون للتطبيقات المستقبلية في الصناعات الدوائية.

الكلمات الافتتاحية :-

الطحالب البحرية – الانتيرومورفا – اليولفا – المركبات النشطة بيولوجيا – النشاط المضاد للاكسدة – النشاط المضاد للسرطان