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Bioactivity of Some Egyptian Seaweeds Extract

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ABSTRACT

This research aimed at screening *in vivo* antioxidant and anti-inflammatory activities as well as *in vitro* antimicrobial and cytotoxic activities in *Codium tomentosum*, *Ulva lactuca* and *Hypnea musciformis*, collected form the Suez Canal, Egypt. Samples were cleaned from epiphytes, washed, air dried and powdered. All the methanol/methylene chloride crude extracts showed a marked antioxidant effect compared to the reference drug vitamin E on alloxan induced diabetic rats and *Hypnea musciformis* (red algae) was the most potent (60.18%). Also, it showed the maximum anti-inflammatory activity in carrageenan-induced rat paw edema. Both extracts of *H. musciformis* and *U. lactuca* showed a wide spectrum antibacterial activity, and the highest activity appeared against *Klebsiella pneumoniae* (clinical culture). Moreover, all the crude extracts showed a promising cytotoxic activity (> 70%) against liver (HEPG2) and prostrate (PC3) cancer cell lines using Sulpho-Rhodamine-B (SRB) assay. The ethyl acetate fractions of *U. lactuca*, *C. tomentosum* and *H. musciformis* were significantly enhanced in HEPG2 with IC_{50} (12.8, 24.5 and 17.8 µg/ml), respectively, compared to the control doxorubicin. Such biological activities might be attributed to the presence of phenols, flavonoids, and tannins, as active constituents in all algal extracts. Besides Alkaloids observed in *U. lactuca* and *H. musciformis*, as well as saponins from *U. lactuca.*

Key Words: Seaweeds, Antimicrobial, Anti-inflammatory, Antioxidant, Cytotoxic, Chemical constituents, Suez Canal.

INTRODUCTION

Cancer is one of the most serious threats to human health in the world. Chemotherapy is still the standard treatment method and most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient's recovery (Kumar *et al*., 2011). The discovery and identification of new antitumor drugs with low side-effects on immune system, cheap and effective to combat this dreaded disease, has become an essential goal in many studies of immunopharmacology (Xu *et al*., 2009). On the other hand, reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative damage (Aruoma, 1999). This uncontrolled generation of free radicals is associated with lipid and protein peroxidation, resulting in cell structural damage, tissue injury, or gene mutation and ultimately lead to the development of various health disorders such as z ' diabetes mellitus, hypertension, cancer and ageing (Mantle *et al*., 2000). Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress (Sasikumar *et al*., 2009).

The biodiversity of marine ecosystem provides an important source of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal and anticancer activities (Newman and Cragg, 2004). Seaweeds are the macro benthic (large and attached) forms of marine algae. Together with the seagrasses, mangroves, and phytoplankton, they comprise the most important primary producers in the marine environment. Three major groups are distinguished, based on their dominant photosynthetic pigments. Namely: Chlorophyta, Phaeophyta, and Rhodophyta (South and Whittick, 1987). Since early history seaweeds have been used in traditional and folk medicine throughout the world (Zeng and Tseng, 1984). Compounds derived from macro algae with a broad range of biological activities, such as antibacterial, antifungal, antiviral (Trono, 1999), antitumor and anti-inflammatory activities (Scheuer, 1990) as well as neurotoxins (Kobashi, 1989), were reported. In view of the above, the present work was carried to examine the *in vivo* antioxidant, anti-inflammatory and *in vitro* antimicrobial and cytotoxic activities of three marine seaweeds harvested from the Great Bitter Lake, Suez Canal, Egypt.

MATERIALS AND METHODS

Collection of algae

Ulva lactuca (Linnaeus, 1753), *Codium tomentosum* (Borgesen, 1947) belonging to Chlorophyta and *Hypnea musciformis* (Lamouroux, 1813) belonging to Rhodophyta, were collected in winter from the intertidal zone of the Great Bitter Lake, Suez Canal. Samples were brought to laboratory in plastic bags containing sea water to prevent evaporation. The algae were then cleaned from epiphytes and rock debris then given a quick fresh water rinse to remove surface salts. They were identified by species according to Abbott and Hollenberg (1976) and Taylor (1985).

Preparation of the extracts

Ulva lactuca (Linnaeus, 1753), *Codium tomentosum* (Borgesen, 1947) belonging to Chlorophyta and *Hypnea musciformis* (Lamouroux, 1813) belonging to Rhodophyta, were collected in winter from the intertidal zone of the Great Bitter Lake, Suez Canal. Samples were brought to laboratory in plastic bags containing sea water to prevent evaporation. The algae were then

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cleaned from epiphytes and rock debris then given a quick fresh water rinse to remove surface salts. They were identified by species according to Abbott and Hollenberg (1976) and Taylor (1985).

Preparation of the extracts

Algal samples were air-dried in shade, reduced to small parts, accurately weighed, separately extracted at room temperature three times for seven days with sufficient amount of methylene chloride/methanol, (1:1 v/v) till complete exhaustion then filtered, the solvents were concentrated using rotary evaporator at 30 ºC with reduced pressure.

Fractionation

The crude extract subjected to successive fractionation using vacuum liquid chromatography (VLC). Each crude extract (130 g) was slurred with a small portion of silica gel. The mixture was transferred to a top of a sintered glass Büchner filter funnel (30 X 15 cm) packed with 400 g silica gel and connected to vacuum pump. Step gradient elution with a non-polar solvent *n*-Hexane 100% with increasing the amounts of a polar solvent using ethyl acetate 100% then methanol 100% to give three successive fractions (2 liters each).

Chemical screening

The methanol /methylene chloride crude extracts of the tested seaweeds, were screened for various chemical constituents such as: alkaloids, coumarins, tannins, saponins, flavonoids, anthroquinone, phenols, cardenolides and carbohydrates, using standard procedures (Harborne, 1983; Evans and Trease, 1989).

Experimental models

Animals: adult male albino rats of the Sprague Dawley strain (120-150 g) body weight, utilized for assessment of the antioxidant and anti-inflammatory effects, and were obtained from the animal house colony at the National Research Center, Dokki, Giza, Egypt. The animals were kept under the same hygienic condition and on a standard laboratory diet consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2 %), and casein-95% pure (10.5%) and starch (54.3%). Carcinoma Cell lines: Hepatocellular (HEPG2), breast (MCF7), prostate (PC3), colon (HCT 116) and cervix (HELA) carcinoma human cell lines were kindly provided from the National Cancer Institute (Kasr El Ainy Street, Cairo, Egypt), were used for the assessment of cytotoxic activity. Indicator strains: *Salmonella typhimurium*: (NCMB 74), *Shigella boydii*: (ATCC 9207), *Pseudomonas aeruginosa*: (NCMB 8295), *Staphylococcus aureus*: (NCMB 6571), *Gardnerella vaginitis*: (clinical culture), *Streptomyces antibioticus*: (wild type), *Candida albicans*: (clinical culture), *Aspergillus nigr*: (laboratory culture) were kindly provided from the Suez Canal University Center for Environmental Studies and Consultation, Ismailia, Egypt, were used for the assessment of antimicrobial activity.

Reference drugs and kits for Pharmacological studies

Indomethacin from EIPICO, Egyptian International Pharmaceutical Industries Co, A.R.E., under license of Merck & Co. INC-RAHAWY N.J., U.S.A, was used as positive controls during the evaluation of the anti-inflammatory activity. Vitamin E (dl-α-tocopheryl acetate) from Pharco Pharmaceutical Co., available in the form of gelatinous capsules: each containing 400 mg vitamin E, was used as positive controls during the evaluation of the antioxidant activity. Bio-diagnostic kits were used for the assessment of blood glucose and reduced glutathione (GSH).

Antioxidant activity

It was estimated by determination of blood glutathione in alloxan-induced diabetic rats using vitamin E as a reference drug. Diabetes was induced to normal rats fasted overnight by intraperitoneal injection of alloxan (150 mg/kg body weight) as described by Elisson and Samet (1969). A total of 36 rats were divided into 6 groups (6 animals/group).

Group 1: non-diabetic rats (with a blood glucose of 80-85 mg/dl) served as normal control. Group 2: diabetic rats (with a blood glucose of 200-300 mg/dl) received 1 ml saline, and served as negative control. Group 3: diabetic rats (with a blood glucose of 200-300 mg/dl) received a single oral dose of 7.5 mg / kg body weight (b.wt) of vitamin E. Group 4-6: diabetic rats (with a blood glucose of 200-300 mg/dl) received a single oral dose 100 mg/kg of the crude extract of *H. musciformis*, *U. lactuca*, and *C. tomentosum*, respectively. Fresh heparinized blood samples were collected after 2 days for estimation of blood glutathione level from each group (Beutler *et al*., 1963), using bio-diagnostic kits and measuring the absorbance at 405 nm by Spectrotrophotmeter.

Glutathione (GSH) concentration in blood = A sample X 66.66 mg/dl

The percentage change from diabetic (negative control) observed after dose administration was, in each case, calculated according to the following equation:

$$\text{Percentage change from diabetic control} = (Gt - Gd)/Gd \times 100$$

Where, Gt represented blood glutathione level in drug treated groups, and Gd represented blood glutathione level in negative control group.

Anti-inflammatory activity

The anti-inflammatory activity of the tested organisms was determined following the carrageenan-induced paw edema model suggested by Winter *et al*.

(1962). Thirty male albino rats were divided into 5 groups (6 animals /group). The rats in groups 2, 3, and 4 were orally treated with 100 mg/kg of *H. musciformis U. lactuca*, and *C. tomentosum*, respectively. The rats of group 1, which served as the control, were treated with 1ml of saline. The rats in the fifth group were treated with 20 mg/kg (b.wt) of the reference drug indomethacin (EIPICO, Egyptian International Pharmaceutical Industries Co).

One hour later, all the animals received a sub plantar injection of 0.1 ml of 1% carrageenan solution in saline, in the right hind paw. The thickness of the right hind paw of each group was measured using a calibre at 1 hour prior to the injection of carrageenan and 1, 2, 3, and 4 hours after the injection. To determine the inhibition percentage of the total edema for each treatment, the following equation was used:

Inhibition percentage of edema = (Vc – Vt)/Vc X 100

Where, Vc= paw volume in control, Vt: volume at time, t: time after which the measurements were made for each treated group.

Antimicrobial activity

The antimicrobial activity of the crude extracts of *H. musciformis, U. lactuca* and *C. tomentosum* were measured at 10 µl against the growth of eight bacterial strains and two fungal strains using disc diffusion method described by Atlas *et al*. (1999). Nutrient agar medium was used for testing all bacteria and fungi. The experiment was performed in triplicate and an average was obtained. Inhibitory activity was recorded by measuring the clear zone diameter in millimetres after incubation at 37º C for 24 hours.

Cytotoxic activity

The potential cytotoxicity of the crude extracts of the seaweeds under investigation was measured by the Sulpho-Rhodamine-B (SRB) assay method adopted from Skehan *et al*. (1990). This was performed on five human cell lines: HEPG2 (liver cancer cell line), MCF7 (breast cancer cell line), PC3 (prostate cancer cell line), HCT 116 (colon cancer cell line) and HELA (cervix cancer cell line). The cells were plated in 96-multiwell plates (104 cells/ well) for 24 hours before treatment with the crude extracts to allow attachment of the cells to the wall of the plate. A single dose (100 µg/ml in DMSO) from the crude extracts of the tested seaweeds were added to the cell monolayer, but in case of fractions different concentrations (5000, 12500, 25000 and 50000 µg/ml in DMSO) were added for each fraction. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extracts for 48 hours, at 37 ºC and in atmosphere of 5% CO_2. After 48 hours, the cells were fixed, washed and stained with Sulpho-Rhodamine-B stain. Excess stain was washed with acetic acid and the attached stain was recovered with Tris-EDTA buffer. The cytotoxicity was determined spectrophtometrically by measuring the developing color at 570 nm by ELISA reader (Tecan Sunrise absorbance reader). The relation between the surviving fraction and the fractions concentration was plotted to get the survival curve of each tumor cell line after being treated with the specified fractions of the most promising organisms which showed promising cytotoxic activity (≥70%) against the tested cancer cell lines and the IC_{50} (Dose of the extracts which reduces survival to 50%) were calculated for the active fractions and the standard drug doxorubicin.

Statistical analysis

Values for antioxidant activity are expressed as mean blood glutathione levels (GSH) in mg/dl ±SE (6 animals / group), S.E. = standard error. The statistical comparison of difference was carried out between the normal control group and other groups using one-way ANOVA by Duncan's multiple range test. Statistically significant different from normal control group at $p < 0.01$. Values for anti-inflammatory activity are expressed as mean increase or decrease in paw volume ±SE (n=6), S.E. = standard error. The statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA by Duncan's multiple range test. Significantly different from control group at $p < 0.05$.

RESULTS

Chemical screening

Results in table (1) clarified the chemical screening of the crude extracts from *H. musciformis, U. lactuca* and *C. tomentosum.* Carbohydrates, phenols, tannins, coumarins and flavonoids presented in all extracts however, cardenolides weren't detected in any of the extracts. Alkaloids were detected in *H. musciformis* and *U. lactuca.* Only *U. lactuca* and *C. tomentosum* showed the presence of anthraquinone while saponins were observed only in *U. lactuca.*

Antioxidant activity

Results presented in table (2) revealed that there was a significant decrease in the blood glutathione (21.6 mg/dl) in the untreated diabetic rats (group, 2) at $p < 0.01$. regarding to the standard drug vitamin E (group, 3), at 7.5 mg/kg (b.wt) dose there was a striking increase in blood glutathione level (35.9 mg/dl) after 2 days of administration to be very close to that of the normal control (group, 1) (36.4 mg/dl), and percentage change from diabetic (negative control) was (66.2%). There was a promising increase in the blood glutathione level (34.6 mg/dl) in group (4) treated with a single dose of 100 mg/kg form the extract of *H. musciformis*, with percentage change from diabetic control of (60.2%), followed by group (6 and 5) received 100 mg/kg of *C. tomentosum* (48.6%) and *U. lactuca* (44.4%) compared to the diabetic control (Table 2).

Table (1): Chemical screening of the crude extracts from the investigated seaweeds

Seaweeds	***H. musciformis***	***U. lactuca***	***C. tomentosum***
Chemical groups			
Alkaloids	+	+	-
Carbohydrates	+	+	+
Cardenolides	-	-	-
Anthraquinones	-	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Coumarins	+	+	+
Saponins	-	+	-
Phenols	+	+	+

Legend: (+) present and (-) absent

Table (2): Antioxidant activity of the crude extracts of seaweeds and vitamin E drug in diabetic male albino rats.

Groups	**Dose of treatment**	**(GSH) Blood glutathione mg/dl**	**% change from diabetic**
1. Normal control	1 ml saline	36.4±1.5	68.5
2. Diabetic (negative control)	1 ml saline	21.6±0.4*	-------
3. Diabetic + Vitamin E	7.5 mg / kg (b.wt)	35.9±1.3	66.2
4. Diabetic + *H. musciformis*	100 mg/kg	34.6±1.2	60.2
5. Diabetic + *U. lactuca*	100 mg/kg	31.2±1.1	44.4
6. Diabetic + *C. tomentosum*	100 mg/kg	32.1±1.2	48.6

Blood glutathione levels (GSH) were expressed in mg/dl as mean ±SE (6 animals / group), S.E. = standard error. * Statistically significant different from normal control group at $p < 0.01$.

Anti-inflammatory activity

The anti-inflammatory properties of the crude extracts of seaweeds were investigated using the carrageenan induced rat paw oedema model. As shown in table (3) a sub plantar injection of 0.1 ml of 1% carrageenan (group 1) into right hind paw, induced a time-dependent increase in the paw thickness compared with the baseline (zero time, before carrageenan injection). The oedema reached its peak after 4 h of carrageenan injection. The increase in paw thickness in group 5 was highly suppressed by oral pre-treatment with the reference drug indomethacin at dose 20 mg/kg b. wt., and a significant mean decrease in paw thickness at $p < 0.05$ presented during the four hours of observation with inhibition percentage (31, 34.4, 37.6 and 40.4%). The inhibitory activity of the crude extract of *H. musciformis* (group 2) was significant as early as the second hour (21.7%), and the percentage inhibition was very high after four hours (26.9%). There was a marked reduction in paw thickness in group 3 and 4, received 100 mg/kg of the crude extract of *U. lactuca*, and *C. tomentosum*, but, it wasn't significant except after 4 hours of injection.

Table (3): Anti-inflammatory activity of the crude extracts of seaweeds and indomethacin drug after 1, 2, 3 and 4 hours of carrageenan injection in male albino rats.

Groups	**Paw diameter (mm)**				
	0h	**1h**	**2h**	**3h**	**4h**
Control	3.61±0.09	4.93±0.07	5.03±0.13	5.13±0.13	5.27±0.8
H. musciformis	3.42±0.09	4.01±0.09 (18.7%)	3.94±0.08* (21.7%)	3.9±0.09* (24%)	3.85±0.09* (26.9%)
U. lactuca	3.61±0.1	4.39±0.14 (11%)	4.23±0.14 (15.9%)	4.14±0.14 (19.3%)	4.07±0.11* (22.77%)
C. tomentosum	3.45±0.08	4.28±0.1 (13.2%)	4.17±0.13 (17.1%)	4.14±0.12 (19.3%)	4.11±0.08* (22%)
Indomethacin	3.0±0.09	3.4±0.04* (31%)	3.3±0.06* (34.4%)	3.2±0.01* (37.6%)	3.14±0.01* (40.4%)

* Significantly different from control group at $p < 0.05$. - The percentage edema inhibition is shown in parenthesis.

Antimicrobial activity

The antimicrobial activity of the crude extracts from *H. musciformis*, *U. lactuca* and *C. tomentosum* was evaluated against eight bacterial and two fungal strains using disc diffusion method, tables (4). All the tested extracts possessed no effect on both fungal pathogens (*Candida albicans*, and *Aspergillus niger*). *H. musciformis* extract showed antibacterial activity at concentration of 10 µl against *Salmonella typhimurium, Klebsiella pneumonia Escherichia coli, Shigella boydii*

and *Streptomyces antibioticus*, and the maximum inhibition zone presented on *K. pneumonia* and *E. coli* (12 and 10.5 mm), receptively. However, it didn't display any activity against, *Gardnerella vaginalis*, and *P. aeruginosa*. *U. lactuca* showed inhibition activity only on *S. typhimurium, K. pneumonia, E. coli, S. boydii,* and *Staphylococcus aureus,* and size of inhibition zone ranging from 6-9 mm. *C. tomentosum,* didn't inhibit the tested bacterial strains except for *S. typhimurium,* and *S. boydii,* with inhibition zone of 6 and 5 mm, respectively.

Cytotoxic activity of the crude extracts

Figure (2) clarified the results of Sulpho-Rhodamine-B (SRB) assay using a single dose (100 µg/ml) of the crude extracts of *H. musciformis*, *U. lactuca* or *C. tomentosum* on five human cancer cell lines: HEPG2 (liver cancer cell line), MCF7 (breast cancer cell line), PC3 (prostate cancer cell line), HCT 116 (colon cancer cell line) and HELA (cervix cancer cell line). Results of cytotoxic screening on HEPG2 showed that the crude extracts of *H. musciformis, U. lactuca,* and *C. tomentosum* could inhibit HEPG2 and inhibition percentage was 76.2, 76.9 and 78.2%, respectively. On PC3 cancer cell line, the highest induction was represented by *C. tomentosum* extract (78.1 %) followed by *U. lactuca* and *H. musciformis* (72 and 68.6%), respectively. In case of HELA and MCF-7 cell lines, the crude extracts of *H. musciformis, U. lactuca,* and *C. tomentosum* showed week activity and inhibition percentage was < 50% except for the extract of *Ulva lactuca* (51.5%). Nevertheless, on HCT cell line, extracts of *U. lactuca* and *H. musciformis* showed inhibition (53.7 and 54.7%).

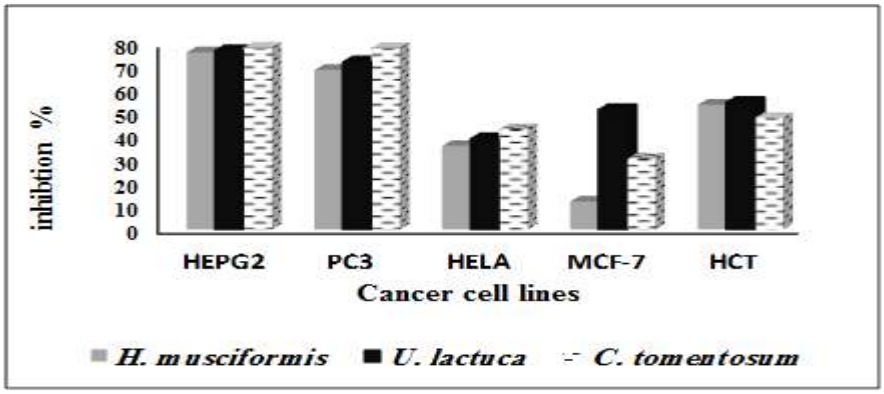

Figure (2): Cytotoxic activity of the crude extracts seaweeds on HEPG2, MCF7, PC3, HCT 116 and HELA cancer cell lines at (100 µg/ml).

Cytotoxic activity of the fractions

The three fractions (hexane, ethyl acetate and methanol) from each algal crude extract were applied to the same test (SRB), to determine the active anticancer fraction. IC_{50} (Dose of the extract which reduces survival to 50%) was calculated for all fractions from the crude extracts which showed cytotoxic activity against the tested cancer cell lines (≥70%) at the single dose (100 µg/ml).

Results indicated that the ethyl acetate fraction from *H. musciformis, C. tomentosum* and *U. lactuca* had a dose-dependent inhibitory effect at concentrations (5000, 12500, 25000 and 50000 µg/ml) on HEPG2 cell line, with IC_{50} of (17.8, 12.8 and 24.5 µg/ml), respectively, compared to doxorubicin with IC_{50} (3.83 µg/ml), (Figure 3). The ethyl acetate fraction of *C. tomentosum* inhibit PC3 cell lines with IC_{50} (48.7 µg/ml) compared to the standard drug doxorubicin with IC_{50} (4.13 µg/ml), (Figure 4). The hexane and methanol fractions in all algal species weren't active.

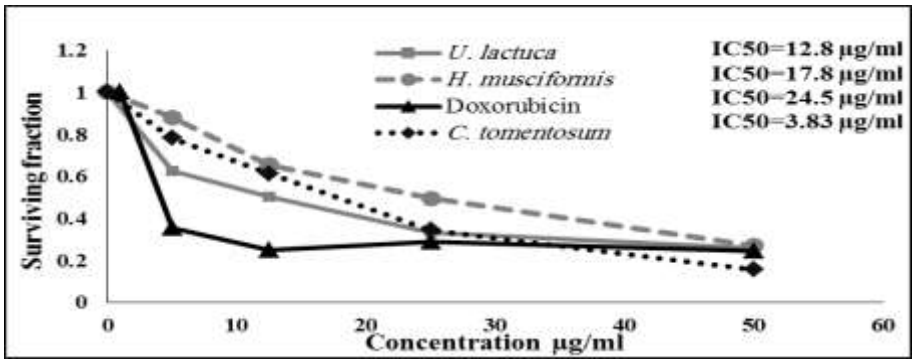

Figure (3): Effect of the ethyl acetate fractions of *H. musciformis, C. tomentosum, U. lactuca,* and the standard drug doxorubicin on (HEPG2) cancer cells

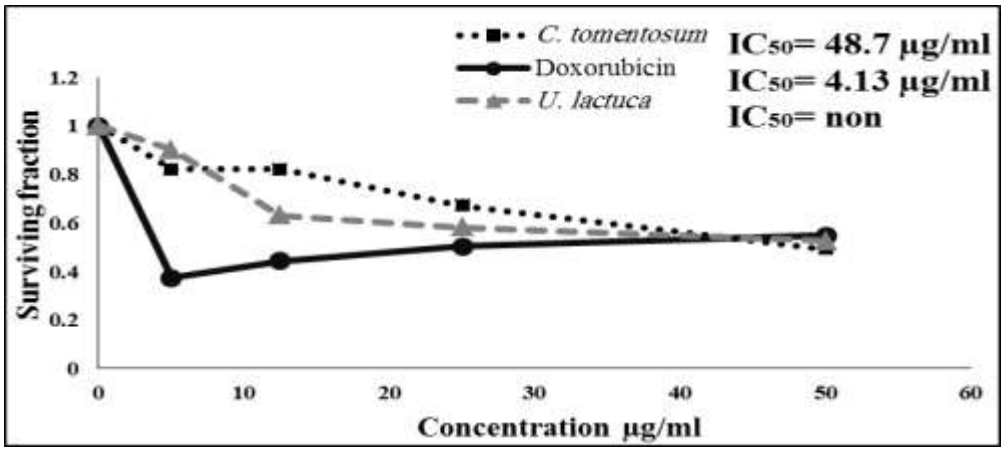

Figure (4): Effect of the ethyl acetate fraction of *C. tomentosum, U. lactuca,* and the standard drug doxorubicin on (PC3) cancer cells.

DISSCUSION

Chemical screening

Phenols, flavonoids, tannins, coumarins and carbohydrates were detected in methanol/methylene chloride crude extract of *U. lactuca, H. musciformis* and *C. tomentosum.* However, alkaloids were detected in *U. lactuca* and *H. musciformis* only *U. lactuca* showed the presence of saponins. These results are, to some extent, in agreement with the data obtained by Alghazeer *et al*. (2013) who indicated that crude methanolic extracts of *U. lactuca, C. tomentosum* and *H. musciformis* showed the presence of alkaloids, tannins, saponins, flavonoids, while anthraquinones, and coumarins were absent. Also, Domettila *et al*. (2013) observed that the petroleum ether extracts of *U. lactuca* and the red seaweeds *Gracilaria debilis,* and *G. idinthakaraiensis* were rich in phenols, tannins and saponins. However, Abdel-Khaliq *et al*. (2014) found that saponins, cardiac glycosides, anthraquinones, and alkaloids were absent in the ethanol extracts from *Ulva fasciata*, *U. intestinalis*, and *U. lactuca.* These variable findings may be attributed to the methods and solvents used in extraction.

Antioxidant activity

Alloxan, induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves way for the decreased utilization of glucose by the tissues and inducing hyperglycaemia (Grover *et al*., 2000). Hyperglycemia significantly diminishes glutathione levels lowering defenses against oxidative stress (Baynes, 1991). A single dose (100 mg/kg) of the methanol/methylene chloride crude extracts of *U. lactuca, C. tomentosum* and *H. musciformis* that was administered separately to the alloxan induced diabetic rats was able to increase blood glutathione level indicating that they possessed antioxidant properties . The red seaweed *H. musciformis* showed higher activity than the green algae *C. tomentosum*, and *U. lactuca*. The obtained results are in harmony with that detected by Dotulong *et al*. (2013) who found that among the tested seaweeds *Caulerpa. sertularoides* (Chlorophyta), and *Padina australis* (Phaeophyta), the hexane fraction (the fraction of non-polar) of *Laurencia tronoi* (Rhodophyta) showed that the highest free radical preventive activity DPPH. Moreover, the crude ethanolic extract of *Codium tomentosum* had a strong anti-oxidative effect (in soluble lipid and water) (Celikler *et al*., 2009). In this study, the antioxidant capacity of the crude extracts of *U. lactuca, C. tomentosum and H. musciformis* may be due to the presence of phenols, flavonoids and tannins. Our observation confirms the finding of Farasat *et al*. (2014) who founded that there was a strong positive and significant correlations between DPPH radical scavenging and phenolic and flavonoids contents in the methanolic extracts of four *Ulva* species (*U. clathrata, U. linza, U. flexuosa*and *U. intestinalis*).

Anti-inflammatory activity

The crude extracts from *H. musciformis*, *U. lactuca*, and *C. tomentosum* produced significant and marked inhibition activity in carrageenan-induced rat paw edema, after four hours of carrageenan injection. The red alga *H. musciformis* exhibit the highest anti-inflammatory activity followed by *U. lactuca,* and *C. tomentosum* which showed a convergent percentage inhibition. On the contrary, Khan *et al*. (2008) screened thirty-seven species of seaweeds collected from the coast of Korea for anti-inflammatory activity. At 40 mg/ml of methanol extracts, *Ulva linza*, *U. compressa* and *U. pertusa* strongly inhibited edema with inhibition activity being higher than *Codium fragile* and *Hypnea charoides.* However, some other tested red seaweeds *Gracilariaverrucosa, Pachymeniopsis elliptica* and *Porphyra yezoensis* showed good inhibition activity (Khan *et al*., 2008).

It is known that the edema induced by carrageenan involves different phases, with the participation of

different chemical mediators, such as histamine, serotonine, kinine, prostagalndins and leucotriens (Ward, 1994). Therefore, anti-inflammatory effects presented in the current study suggested that the properties of anti-inflammatory components in these species could possibly interfere with some of the mediators of inflammation, by either inhibiting their production or antagonizing their actions (Olajide *et al*., 1999). Indeed, it was reported that seaweed extracts decrease the production of inflammatory prostagalndins and leucotriens (James *et al*., 2000).

The observed anti-inflammatory activity in our work may be attributed to several anti-inflammatory compounds have been reported from macro algal species, which include polyunsaturated fatty acids (Docosahexaenoic acid, Eicosapentaenoic acid, Stearidonic acid, and Eicosatrienoic acid), alkaloids and carotenoids (Jaswir and Monsur, 2011).

Antimicrobial activity

The crude extracts of *H. musciformis* and *U. lactuca* showed antibacterial activity each against five of the tested bacterial strains. While, *C. tomentosum* didn't exhibit any antibacterial activity except for *S. typhimurium* and *S. boydii*. Both *H. musciformis,* and *U. lactuca* showed the highest activity against *K. pneumoniae* while the minimum activity presented by *U. lactuca* against *S.aureus* and by *H. musciformis* against *S. typhimurium*. On the other hand, they were not effective against any of the tested fungal strains.

Several authors obtained similar results, supporting the hypothesis those algae extracts are active mainly against bacteria, according to Abd El-Baky*et al*., (2009) who revealed that the crude extracts of *Ulva lactuca* collected from Egyptian Mediterranean Sea, exhibited great potential antibacterial activities against *Bacillus cereus, Bacillus subtilis, S. aureus* and *K. pneumoniae*. As well, Shareef *et al*. (2012) reported that *Hypnea musciformis* had a promising antibacterial activity against *E. coli, S. typhimurium, P. aeruginosa, S. aureus* and *Proteus mirabilis.* Zheng *et al*. (2001) stated that no antifungal activity was found in all solvents extracts of *Gloiopeltis furcate, Ulva pertusa, Enteromorpha prolifera* and *Surgassum sunpergi.*

Cytotoxic activity of the crude extracts and fractions

The methanol/ methylene chloride crude extracts of *H. musciformis, U. lactuca* and *C. tomentosum*, showed a promising cytotoxic activity against HEPG2 and PC3 cancer cell lines and *C. tomentosum* had the highest cytotoxic activity. However, their effect on MCF7, HCT 116 and HELA didn't exceed 55%. The current results are consistent with Ibrahim *et al*. (2005) who declared that ethanolic extracts of *Codium tomentosum, Ulva lactuca,* and *Hypnea musciformis* presented antitumor activity. Likewise, Guedes *et al*. (2013) showed that a promising cytotoxicity presented by dichloromethane, chloroform and ethanol extracts of *H. musciformis* against NCI-H292 (human lung cancer), Hep-2 (human larynx epidermoid cancer) and K562 (chronic myelocytic leukemia). However, the methanolic extract of *H. musciformis* showed no cytotoxic activity. Moreover, *Ulva fasciata* extract was able to inhibit the growth of HCT116 human colon cancer cells by 50% at a 200 µg/ml (Ryu *et al*., 2013).

Assessment of cytotoxic activity was conducted in accordance with the protocol of the American Cancer Institute (NCI), which recommended that IC_{50} values < 30µg/ml should be considered significant for crude extracts (Geran *et al*., 1972). Our work revealed that the ethyl acetate (intermediate polar) fractions from *H. musciformis, U. lactuca* and *C. tomentosum* exhibited cytotoxic activity against HEPG2 and PC3, whereas the hexane (non-polar) and methanol fractions (polar) weren't active.

The ethyl acetate fraction of *U. lactuca* presented potent cytotoxic activity, with IC_{50} (12.8 µg/mL) on the HEPG2 cancer cells compared to the standard drug doxorubicin, while there was some inhibition but didn't reach (IC_{50}) on PC3 cancer cells. On the other hand, the ethyl acetate fraction of *C. tomentosum* showed more significant cytotoxic effect on HEPG2 with IC_{50} (24.8 µg/ml) than on PC3 as IC_{50} was (48.7 µg/ml). These findings are in agreement with El-Baroty *et al*. (2011) who reported that glycolipids in *Laurencia popillose, Galaxoura cylindriea* (Rhodophyceae) and *Ulva fasciata* (Chlorophyceae) collected from the Egyptian Red and Mediterranean Sea, exhibited remarkable anticancer activities against HEPG2 cancer cell line with IC_{50} ranging from 0.67 to 2.89 µg/ml.

Numerous compounds such as sulfated polysaccharides, polyunsaturated fatty acids, glycolipids and polyphenolic compounds were isolated from marine seaweeds proved to have anticancer and cytotoxic activities (Gill and Valivety, 1997; Boopathy and Wijesekara *et al*., 2011; El-Baroty *et al*., 2011). Thus, the possibility of existence of theses cited compounds in the ethyl acetate fractions of *H. musciformis, U. lactuca* and *C. tomentosum* are responsible, at least in part, for their cytotoxic properties. In conclusion, seaweeds namely (*H. musciformis, U. lactuca* and *C. tomentosum*) collected from the Great Bitter lakes, Suez Canal possessed antioxidant, anti-inflammatory, antimicrobial and cytotoxic properties.

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الأنشطة الحيوية لمستخلص بعض الطحالب البحرية المصرية

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الملخص العربي

تهدف هذه الدراسه الى تقييم بعض الأنشطة الحيوية مثل مضادات الاكسدة ومضادات الإلتهابات ومضادات الميكروبات ومضادات السرطان ببعض الطحالب البحرية التى تم تجميعها خلال فصل الشتاء (٢٠١٣) من (منطقة البحيرات المرة) قناة السويس مثل *H. musciformis, U. lactuca, C. tomentosum* . تم تنظيف العينات من النباتات والحيوانات المتطفلة، وغسلها بالماء الجارى، وتجفيفها فى الهواء ثم سحقها. وقد تم استخلاص كل طحلب على حده بإستخدام المثيليين كلوريد والميثانول (١: ١). وأظهرت جميع المستخلصات الخام نشاط ملحوظ كمضادات للأكسدة مقارنة مع الدواء المستخدم (فيتامين E) فى الجرذان المصابة بداء السكري باستخدام الآلوكسان. وأظهر الطحلب الاحمر *H. musciformis* أعلى نشاط مضاد للأكسدة (٦٠.١٨ %)، كما كان له ايضا أقوى نشاط كمضاد للألتهابات المستحثه بالكاراجينان فى مخلب الجرذان بمعدل تثبيط (٧,١٨- ٩,٢٦ ٪) مقارنه بالاندوميثاسين. بالاضافة إلى ان كلا المستخلصات من *U. lactuca, H. musciformis* كان لهما نشاط ضد بكتيري لمدى واسع من البكتيريا. وسجل افضل نشاط لهم كمضاد حيوى ضد *Klebsiella pneumoniae*. وعلاوة على ذلك، أظهرت كل المستخلصات الخام نشاط ملحوظ فى القضاء على خلايا الكبد والبروستاتا السرطانية (< ٧٠ ٪) باستخدام تحليل Sulpho-Rhodamine-B (SRB). وأظهر الايثيل اسيتات كجزء من المستخلص الخام للطحالب *H. musciformis, U. lactuca, C. tomentosum* نشاط سمى قوية ضد خلايا الكبد السرطانية وكان التركيز اللازم لتثبيط ٥٠% من الخلايا (١٢.٨ ، ٢٤.٥ ، ١٧.٨ ميكروجرام / مل)، بالترتيب مقارنة مع الدوكسوروبيسين. وقد يعزى وجود مثل هذه الأنشطة البيولوجية في هذه الانواع من الطحالب الى ظهور بعض المجموعات الكيمائيه مثل الفينولات ، الفلافونويد ، و االتانين في جميع مستخلصات الطحالب الخام ، إلى جانب القلويات التي وجدت في *U. lactuca* و *H. musciformis* وكذلك الصابونين من *U. lactuca* .