Cytotoxic and antimicrobial activities of the Saudi Arabian Marine Algae *Enteromorpha prolifera*

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Abstract

This study aimed to explore the cytotoxicity effect of the green marine Algae Enteromorpha prolifera, collected from the Saudi Arabia coast, against tumor cell HEp-2 (laryngeal epidermoid carcinoma) using the MTT salt reduction assay. Our results showed that the ethyl acetate extract has the highest cytotoxic potentiel against HEp-2 cell and the IC_{50} value (concentration required to inhibit tumor cell proliferation by 50%) was approximately 0.605 mg/mL. When performing the examination effect on the ethanol, ethyl acetate and water extracts to yield the antibacterial, the ethanol extract has showed the highest inhibition effect against Escherichia coli ATCC 35218 and Salmonella typhimurium while, the maximum inhibition effect has been explored with the ethyl acetate extract against the Aeromonas hydrophila ATCC, Salmonella typhimurium and Pseudomonas aeruginosa.

Enteromorpha prolifera extracts have been also valued for their highly nutritious content and have proven to possess high exceptionally content of iron, manganese, magnesium, calcium, potassium, phosphor, zink, cupper and sodium. On the other hand, flavonoids, which may act as antioxidants, have been identified in ethanol and water extracts. Particular n-hydrocarbons, sterols and fatty acids have also been pinpointed through GLC analysis.

Keywords: Marine Algae, Red sea coast, *Enteromorpha prolifera*, Antimicrobial, Cytotoxicity, Nutritional value.

Introduction

Marine algae have abundant possessions and especially novel compounds that have diverse pharmaceuticals effects such as antibacrial, antiviral, antifungal, antidiabetic, anti-hypercholesterolemia, antioxidant and cytotoxicity[1,8].

Different compounds have been isolated from marina algae to develop novel drugs. In recent years, algae has been consumed as a drug and food since it contains polyunsaturated fatty, vitamins, iodine, bromine, essential amino acids and various elements such as sulfur, magnesium, phosphor, potassium and iron[6,7]. In this regard, these bioactive diversities turned algae to be an exceptional source for the development of a novel chemistry for marine algae[3]. Marine biological activities have been identified and could interfere with pathogenesis of many disease[11,13]. In this work, phytochemical study and biological evalutaion are described to highlight the marine algae collected from the Alwajh coast in the Tabuk region in Saudi Arabia.

Material and Methods

Chemical and reagents: All chemicals are of analytical grade. All reagents are purchased from Sigma- Aldrich-Fluka (Saint-Quentin France).

Plant material: *Enteromorpha prolifera* was collected from the red sea of Alwajh (Saudi Arabia) in April 2019 and identified by Professor Amal Fakhry, Botany and Microbiology Department, Faculty of Sciences University of Tabuk-Saudi Arabia. A voucher specimen *Enteromorpha prolifera* has been deposited at the Laboratory of Chemistry, College of Science of Tabuk University. The algae has been kept frozen at –20 ºC and stored in the dark until further use. Dry samples were weighed before the extraction.

Preparation of the extracts: 20 g of dried algae powder were submitted to extraction in 1000 mL of ethyl acetate, ethanol or distilled water during 48 hours and then macerated for 48 h. After filtration, the solvent was removed under vaccum from the resulting organic extracts to drynes. The aqueous filtrate was fractionated using a column chromatography (6 cm x 70 cm, silica gel eo-120 mesh).

Phytochemical screening analysis: The phytochemical screening of the crude algae extract (ethanol 80%) has been studied using reagents [Willstatter, sodium hydroxide (5%), ferric chloride (5%), Borntrager, mercuric chloride (5%), Salkovsky, Dragendoeff and Mayers] for detection of some chemical components.

Material for quantification of elements in samples: Flame Photometer: (Eppendorf, B 700E) was used for assaying Na, K and Ca.
Spectrophotometer: Model Zeiss PM6 used for assaying P.

Atomic absorption spectrophotometer (AAS): Model Zeiss FMD3 used for assaying Mg, Fe, Mn, Zn and Cu.

Determination of phenols and flavonoids: The Folin-Ciocalteu reagent has determined at 760 nm the total phenols by spectrophotometer. Several reports have made systematic effort to investigate flavonoids by means of the colorimetric assay[5,12].

Gas liquid chromatography analysis of the ethyl acetate extract: *n*-hydrocarbons, sterols and fatty acids were identified in the ethyl acetate extract of *Enteromorpha prolifera* by gas liquid chromatograph/Pyeunicam PRO–

GC: OV-17 (methyl phenyl silicone); 70 °C, 10 °C/min, 270–35 °C/min, inject. 250 °C, detect. 300 °C; 70 °C, 8 °C/min. 190–35 °C/min, inject. 250 °C, detect. 300 °C (SP–2300).

Biological effects: The percentage of cell viability of the Algae extracts was examined to form an idea about the amount tumor (*HEp-2*) cells by the MTT salt reduction method. On the other hand, the antimicrobial effect has been described against eight bacteria.

Cytotoxic activity: The cytotoxicity of the algae extracts was carried out *in vitro* against *HEp-2* cell (laryngeal epidermoid carcinoma) using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay method with slight modifications[14]. *HEp-2* cell culture plates were prepared at a concentration of 5 x 10^4 in CO_2 (50%) at 38 °C and kept for 24 h and then the cells were washed in PBS (Phosphate Buffer Saline) with 200 µL of a diluted extract. The inhibition zone was registered in essential medium with Calf Serum (2%) (Two-fold dil. 1 µg – 8 µg). The cells were eroded via PBS and 40 µL of MTT at 5 mg/mL (MTT, Sigma, S. L., MO, USA).

Plates were incubated in CO_2 (5%) for 48 h at 39 °C and the formed formazan crystals were solubilzed in DMSO (Sigma, USA). Only viable cells are effective for reducing of MTT salt to colored formazan. The absorbance (A) of the formazan generated dye was measured with the help of a microplate reader (T. F. S., USA) at 540 nm. This assay was conducted in triplicate and the percentages of cell growth were calculated as follow:

Cell growth (%) = [A (treated cells)/A (control cells)] x 100.

The viability of control cells was set to 100% and the cytotoxicity was expressed as the concentration of sample inhibiting cell growth by 50%.

Antibacterial activity: The isolated extracts were screened for their antibacterial activity by the agar disc diffusion method[4]. The following bacteria were used as indicators (*V. p.* CECT 511, *B. c. ATCC* 11778, *S. a. ATCC* 25923, *A. h.* ATCC 7966^T, *L. m.* ATCC 19115, *P. a.* ATCC 27853, *S. t.* ATCC 1408 and *E. c.* ATCC 35218). The cultivation was carried out for bacteria and yeast (Mueller–Hinton, 9 mL, 10 µL) at 38 °C and the cultures were used for the antimicrobial effect of samples after 48 h of incubation at 33 °C and the photosensitive (0.1 at OD600) for bacteria. The discs (diameter 6 mm, B. L. I) were permeated with 30 µL of the algae extract (47 mg/mL). As positive values were served 15 mg/mL of ampicillin (15 µL/disc) and 15 mg/mL of amphotericin-B (15 µL/disc). The growth of the zone of inhibition was estimated to the antimicrobial effect against the organisms in comparison to a control of reference standards[2].

Results and Discussion

Assessment of chemical components in marine algae extracts

Distribution of elements in samples: As depicted in table 1, the ethanol and the ethyl acetate extracts are rich in calcium (12.72% in ethanol extract and 8.08% in ethyl acetate extcat), while the most abundent element in the water extract is sodium (7.01%). Phosphorus was found the least abundent element in all extracts.

Phytochemical screening analysis: When conducting the phytochemical survey of the ethanolic extract of the algae, it was shown that coumarins, tannins and anthraquinones are in a less percentage than flavonoids, terpenoids, saponins, sterols and alkaloids.

Total phenolic compounds content: The ethanolic extract (125 µL) was mixed with 3 mL of Folin-Ciocalteu, 375 µL of distilled water and 2 mL of an aqueous solution of sodium carbonate (10%). The resulting mixture was incubated for about 5 min in a thermostatic bath at 55 ºC and then cooled to 25 ºC. A spectrophotometer was used to measure the photosensitivity of the samples. Diffrents concentrations of gallic acid (12, 28, 40 and 56 µg) were used to organize the standard curves.

Table 1
Distribution of elements in the extracts obtained from the marine algae

Element	Extract		
	Ethyl acetate	Ethanol	Water
K %	1.01	2.04	0.31
Na %	3.41	4.99	7.01
Ca %	8.08	12.72	4.43
Mg %	1.52	3.37	0.59
P ppm	19	32	44
Fe ppm	451	570	213
Mn ppm	22	117	121
Zn ppm	79	234	174
Cu ppm	33	50	211

Total flavonoids content: To 0.5 mL of an aqueous solution of $NaNO_2$ (10%) were added 0.5 mL of $AlCl_3$ solution (15%) and 5 mL of an aqueous NaOH solution (1 M). Amounts of the phenolic compounds that have been identied in ethanol and water extracts are presented in table 2.

Gas liquid chromatography of the ethyl acetate extract: As depicted in table 3, *n*-octacosane showed the highest rate (17.47%) from the identified *n*-hydrocarbons. While in sterols, stigmasterol appears as the major component (18.84%) and from the fatty acids, myristic is the most abundent one (11.19%).

Cytotoxic effect: We have evaluated the cytotoxicity in *HEp-2* cells in order to investigate the cytotoxic effects of the diffrent algae extracts. Fig. 1 shows the cell viability percentage of the three algae extracts at various concentrations.

According to data (Fig. 1), the cell viability decreased as a function of the concentration and the sensitivity of *HEp-2* to the ethyl acetate extract was found considerably important and the IC_{50} value was approximately 0.605 mg/mL.

Table 2
Evaluation of the amount of flavonoids and phenols in the marine Algae extracts

No. mg CE (CE g^{-1} FM)	Abs1	Abs 2	Phenolic compound (Ethanol extract)	Phenolic compound (Water extract)
Rep 1	0.018	0.046	0.00110633	0.00394828
Rep 2	0.028	0.041	0.00212131	0.00344079
Rep 3	0.023	0.032	0.00161382	0.00252730
		mean	0.00161382	0.00330546
		ect.	0.00050749	0.00072009
	Abs 1	Abs 2	Flavonoid (Ethanol extract)	Flavonoid (Water extract)
Rep 1	0.166	0.268	43.05555556	71.38888889
Rep 2	0.139	0.262	35.55555556	69.72222222
Rep 3	0.146	0.175	37.54444444	45.55555556
		mean	38.70370370	62.22222222
		ect	3.892194362	14.45779298

Table 3
Analysis of *n*-hydrocarbons, sterols and fatty acids of the green marine Algae

No. of C	*n*-Hydrocarbons and sterols	%	No. of C	Fatty acids	%
C_8	*n*-octane	2.30	C4:0	buturic	5.24
C_{10}	*n*-decane	3.75	C6:0	caproic	3.55
C_{11}	*n*-henedecane	2.12	C8:0	coprylic	0.11
C_{12}	*n*-dodecane	0.32	C10:0	capric acid	1.99
C_{13}	*n*-tridecane	3.67	C12:0	lauric acid	3.11
C_{14}	*n*-tetradecane	2.51	C14:0	myristic	11.19
C_{15}	*n*-pentadecane	0.25	C14:1	myristoleic	1.29
C_{16}	*n*-hexadecane	6.34	C16:0	palmitic	2.33
C_{17}	*n*-heptadecane	2.08	C16:1	palmitoleic	0.87
C_{18}	*n*-octadecane	2.19	C17:0	margaric	9.85
C_{20}	*n*-eicosane	1.01	C18:0	stearic	0.54
C_{21}	*n*-eneicosane	6.12	C18:1	oleic	0.32
C_{22}	*n*-docosane	3.39	C18:2	linoleic	7.26
C_{23}	*n*-tricosane	5.37	C18:3	linolenic	3.41
C_{26}	*n*-hexacosane	7.60	C20:0	arachidnic	1.08
C_{28}	*n*-octacosane	17.47	C24:0	lignoceric	8.07
C_{29}	*n*-nonacosane	2.71			
C_{30}	*n*-triacontane	1.36			
C_{29}	stigmasterol	18.84			
C_{29}	β-sitosterol	3.16			

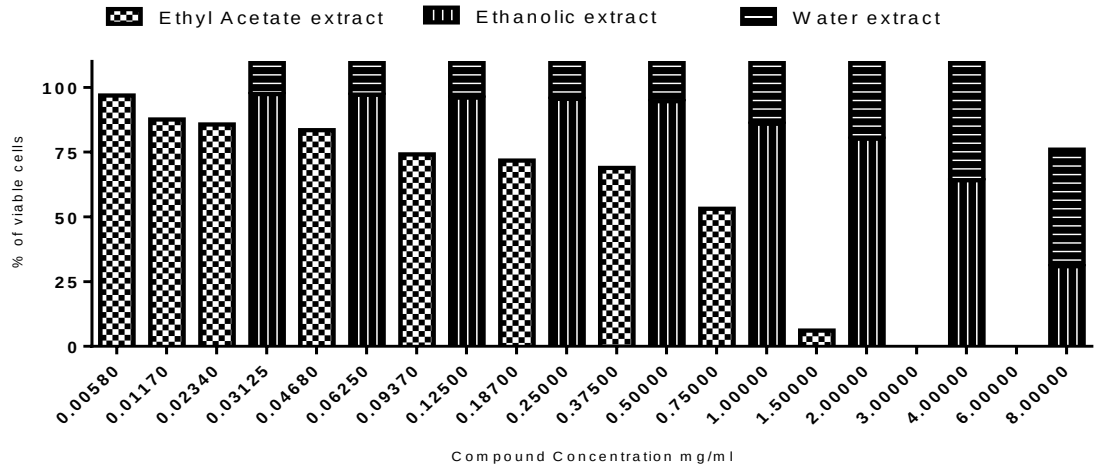

Fig. 1: Histogram representing cell viability of *HEp-2* cells at different concentrations of the Algae extract: Data were expressed as percentage of cell viability compared to the control (mean ± standard deviation SD).

Table 4
Antibacterial activities for extracts of *Enteromorpha prolifera* from various solvents.

Tested organism (Bacteria)	Inhibition zone diameter (mm)			
	Ethanol extract	Ethyl acetate extract	Water extract	Ampicillin
Vibrio parahaemolytic	9.33 ± 1.15	6 ± 0	-	17 ± 1
Staphylococcus aureus ATCC	18 ± 0	6.33 ± 0.57	-	26.66 ± 0.57
Aeromonas hydrophila ATCC	11.33 ± 0.58	7 ± 0	-	14 ± 0
Salmonella typhimurium	14.67 ± 0.58	6.67 ± 0.58	-	18 ± 1
Bacillus cereus ATCC 11778	8.67 ± 1.15	6 ± 0	-	26 ± 1
Escherichia coli ATCC 35218	22 ± 0	6 ± 0	-	12.33 ± 0.57
Listeria monocytogenes	6 ± 0	6 ± 0	-	12.33 ± 0.57
Pseudomonas aeruginosa	12 ± 0	6.67 ± 0.58	-	22.66 ± 0.57

- no inhibition zone observed.

Antimicrobial effect: The ethyl acetate, ethanol and water extracts of green algae (*Enteromorpha prolifera*) have been tested for their antimicrobial activity using disc-idiffusion method against eight bacteria (*Vibrio parahaemolytic, Salmonella typhimurium, Bacillus cereus ATCC 11778, Staphylococcus aureus ATCC, Escherichia coli ATCC 35218, Listeria monocytogenes, Aeromonas hydrophila ATCC* and *Pseudomonas aeruginosa*) and their potency was assessed by the presnece or absence of inhibition zones (Table 4).

Results showed that the ethanol extract exhibited an interesting antibacterial activity with inhibition zone values of 22 ± 0 against *Escherichia coli ATCC 35218*, 18 ± 0 against *Staphylococcus aureus ATCC*, 11.33 ± 0.38 against *Aeromonas hydrophila ATCC*, 14.67 ± 0.58 against *Salmonella typhimurium*, 12 ± 0 against *Pseudomonas aeruginosa*, 8.67 ± 1.15 against *Bacillus cereus ATCC 11778*, 6 ± 0 against *Listeria monocytogenes* and 9.33 ± 1.15 against *Vibrio parahaemolytic*. The ethyl acetate extract was found less active than the ethanol one against the eight bacteria with an inhibition zone diameter ranging from 6 to 7 mm. These obtained results are in a good agreement with previous works suggesting that the ethanol extract exhibits higher antimicrobial effect than the ethyl acetate one[9,10].

Conclusion

The work reported here provides an idea about the constituents of the Saudi Arabian green marine algae *Enteromorpha prolifera* when it is extracted with water, ethanol and ethyl acetate. This algae was found rich in *n*-hydrocarbons, fatty acids, sterols, favonoids and mineral constituents like sodium and calcium in diffrents proportions. The obtained marine algae extracts have been evaluated for their cytotoxic effects against the *HEp-2* cell (laryngeal epidermoid carcinoma) and their antimicrobial activity has also been examined against eight bacteria.

The ethanol extract has proven an interesting effect against all bacterial species while ethyl acetate extract was found less active with a diameter of growth inhibition ranging from 6 to 7 mm. However, no antimicrobial effect was observed with the water extract. This preliminary biological study suggests that the marine

algae *Enteromorpha prolifera* may be considered as an interesting vegetative species for therapeutic needs.

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