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RESEARCH ARTICLE

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ANTICANCER ACTIVITY OF SOME EGYPTIAN MARINE ALGAE EXTRACTS

ABSTRACT:

The chemical and biological diversity of the marine environment is immeasurable and therefore is an extraordinary resource for the discovery of new anticancer drugs. Marine algae are rich sources of new, biologically active compounds. Seaweeds have traditionally been used as food, but have also been used as folk medicine, particularly by coastal peoples. Recently, much attention has been paid to the antitumor activity of seaweeds. Thus, the extracts of some Egyptian marine algae were for their anticancer activity against Ehrlich Ascites Carcinoma (EAC) cell line. This paper highlight the anticancer activity of petroleum ether, chloroform and methanol extracts of the marine algae *Ulva rigida*, *Enteromorpha clathrata, Jania adherans*, *Corallina elongate in vitro* against Ehrlich Ascites Carcinoma (EAC) cell line. The results indicate that chloroform and methanol extracts of *Ulva rigida* has more activity than other extracts, of other algae *in vitro*. So methanol and chloroform extracts of *Ulva rigida* were tested *in vivo* and the results denote that maximal reductions of tumor volume (84.2 and 77.8) were observed when solid tumor-bearing mice were treated with methanol extracts 60 and 80 µg/ml, respectively. The maximal inhibitory action on the level of lipid peroxidation (64.8 and 69.6 at methanol 80,100 µg/ml, respectively). Treatment with Ulva rigida extracts leads to remarkable increases in of superoxide dismutase and catalase activities that associated with reduction in tumor volume revealing their antitumor activity, (65.0 and 69.0, respectively) and catalase levels (8.1 and 8.3, respectively) at the same concentrations.

KEY WORDS:

Anticancer, marine algae, *Ulva rigida, Enteromorpha clathrata, Jania adherans, Corallina elongate*

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INTRODUCTION:

Algae are simple photosynthetic eukaryotic which owing to their colonization of the oceans are responsible for up to 50% of the planet's atmospheric carbon fixation (Field *et al*., 1998, Croft *et al*., 2006). More than 60% of anticancer drugs have originated from natural materials, such as plants, microbes, and marine organisms (Newman *et al*., 1981). Compared with totally synthetic drugs, compounds derived from natural sources tend to have well-defined three-dimensional structures, fitting biological target structures that are conserved across species; thus, natural compounds may act more specifically and have fewer or less severe adverse side effects than synthetics (Paterson and Anderson, 2005). Marine organism–derived medicines have several features that make them particularly suitable for consideration as sources of antineoplastic agents. For example, the vast majority of marine invertebrates have only primitive immune systems, and thus, they produce toxic substances as a form of defense; these substances would be expected to have high potency and low solubility, given that they are immediately and tremendously diluted by water. Therefore, an increasing number of compounds derived from sponges, algae, mollusks, and other marine organisms are being tested for their therapeutic effects

against cancer and other diseases in clinical and preclinical trials (Paterson and Anderson, 2005; Newman and Cragg, 2004, Kingston, 2008; Newman and Cragg, 2009).

Apoptosis is a cell death mechanism by which many chemotherapeutic drugs kill cancer cells (Kaufmann and Earnshaw, 2000). This process is characterized by chromatin condensation and fragmentation, cell shrinkage, and membrane blebbing. The Bcl-2 family, which consists of antiapoptotic and proapoptotic proteins, is the central regulator of apoptosis (Cory and Adams, 2002). When apoptosis is triggered, proapoptotic proteins, such as Bax and Bak, translocate from the cytoplasm to the mitochondrial membrane, where they interact with antiapoptotic proteins, such as Bcl-2 and Bcl-XL, causing a loss of mitochondrial transmembrane potential and the release of cytochrome c. The release of cytochrome c, in turn, activates caspases, which then cause the apoptosis to occur (Iwamaru *et al.*, 2007).

In this paper we have demonstrated, that one of natural products from some Egyptian marine algae, *Ulva rigdia*, Enteromorpha clathrata, Jania adherans, Corallina elongate can play principal role in the treatment of human cancer as concluded for the primary experiments data, which carried out using *in vitro* against Ehrlich Ascites Carcinoma (EAC) cell line.

MATERIAL AND METHODS:

Marine algae:

Four marine algae *Ulva rigida*, Enteromorpha clathrata, Jania adherans, Corallina elongata were examined for their antitumor activity *in vitro*. These algae were collected during outman 2005 from seashore of Alexandria Egypt at Abu-qir area. These algae were identified according to Taylor (1985), Aleem (1993) and Aleem (2001).

Preparation of marine algae:

Algal thallus were washed several times by tap water and dried for a week at room temperature, grind to get a powder.

Successive extraction:

Fifty gm of each alga were soaked in petroleum ether for 48h and filtrated, the residues were repeated soaked in petroleum ether for 48h and filtrated. The precipitate is soaked in chloroform for 48h and repeated after 48h soaked and filtrate and then by methanol. The filtrate is taken and concentrated in vacuum until drying; the residue is dissolved in hot saline solution and concentrated.

In vitro assessment of antitumor activity:

The antitumor activity of algae extracts was determined *in vitro* against Ehrlich Ascites Carcinoma (EAC) cell line, which was kindly provided by the National Cancer Institute (Cairo University). EAC cells were thereafter propagated in our laboratory by weekly I.P. injection of 0.2 ml (2x106/ml). Cells were withdrawn from a donor mouse bearing a 6-8 day old ascites tumor (Fahim *et al.*, 1997). Briefly, EAC cells were collected from the peritoneal of inoculated female Swiss mice and viability was checked using Trypan blue staining (El-khawaga *et al.*, 2003). Tested extracts were titrated in triplicates with serial volumes (0.01, 0.008, 0.004, and 0.002 g) and then incubated for 10 m in incubator at 37°C. Cells viability was checked by using Trypan blue staining and the cytotoxicity values were calculated (Boyum, 1968).

In vivo antitumor activity on solid tumor:

The antitumor efficacy of algae extracts was investigated *in vivo* on solid tumor-bearing mice. A total of 60 female Swiss mice were divided into 12 groups (5 animals per group). Solid tumor was induced in all groups of study, except normal control group; by injecting 2×105, EAC cells subcutaneously (S.C) between thighs of the lower limb (El-khawaga *et al.*, 2003). Twenty-four hours post EAC inoculation, 0.1 ml tested extracts was injected subcutaneously for three consecutive days. Mice were sacrificed, dissected and change in tumor volume (TV) compared to positive control group was assessed on day 12 after implantation of EAC cells. The following formula was used to calculate the volume of the developed tumor mass (Papadopoulos *et al.*, 1989):

$$\text{Tumor volume (mm3)} = 4\pi\,(A/2)2 \times (B/2)$$

Where: (A) is minor tumor axis and (B) is the major tumor axis

Preparation of liver homogenate and biochemical assays:

Liver from the sacrificed animals were quickly excised, rinsed in isotonic saline and blotted dry with a piece of filter paper. For each specimen 0.1g piece of liver tissue was homogenized in ice-cold saline using a glass homogenizer. The homogenate was then diluted with the homogenization medium to ultimately yield 10% (v/v) whole liver homogenate for determination of catalase activity (CAT) (Beutler, 1975), superoxide dismutase activity (SOD) (Rest and Spitznagel, 1977) and lipid peroxidation level (Ohkawa *et al.*, 1982)

Statistical analysis:

The statistical significance of the experimental biochemical results was determined by the Student's t test (Murray, 1982). For all analyses, $p<0.05$ was accepted as a significant probability level.

RESULTS:

Table 1 demonstrate the in-vitro effect of the different concentrations of *Ulva rigdia*, Enteromorpha clathrata, Jania adherans,

Corallina elongata extracted by petroleum ether, chloroform and methanol respectively, on the viability of Ehrlich ascitis carcinoma cell line. Results showed varied inhibitory effects of algal extracts on the proliferation of EAC cell line. *Ulva rigdia* is more antiproliferative effects against EAC cell line were dose-dependent *in vitro*.

Further *in vivo* study for the highly toxic algal extracts has been done on solid tumor bearing mice. As shown in table 2, all tested extracts caused a significantly reduction in the tumor volume as compared to that of the control group. The maximal reductions of tumor volume (84.2 ± 7.1 and 77.8 ± 13.4) were observed when solid tumor-bearing mice were treated with 60 and 80 µg/ml of methanol extracts, respectively.

Effect of algal extracts on the level of hepatic lipid peroxidation:

Level of malondialdehyde as end product of lipid peroxidation was determined in the liver homogenate of tumor bearing mice treated with different algae extracts (Table 3).

Results indicate that development of solid tumor is associated with significant elevation of MDA in liver tissue as compared to that of normal control group. Treatment with different algal extracts resulted in significant reductions in lipid peroxidation. As shown in table 3, Met (80 and 100 µg/ml) showed the maximal inhibitory action on the level of lipid peroxidation (64.8 ± 4.5 and 69.6 ± 13.3, respectively).

Table 1. *In vitro* cytotoxic effect of different extracts of marine algae on Ehrlich Ascites Carcinoma (EAC) cells viability

	Cytotoxicity %											
extracts	Petroleum ether				Chloroform				Methanol			
Algae conc. (mg/ml)	0.01	0.008	0.004	0.002	0.01	0.008	0.004	0.002	0.01	0.008	0.004	0.002
Ulva rigdia	28.41 ±2.1	50.77 ±4.8	71.56 ±6.6	11.18 ±2.3	69.99 ±9.4	69.07 ±9.3	69.07 ±6.7	36.66 ±9.9	69.95 ±9.9	63.42 ±9.4	0 ±0	0 ±0
Enteromorpha clathrata	30.70 ±2.9	14.30 ±2.1	0 ±0	20.02 ±3.4	0 ±0	38.25 ±8.5	57.38 ±8.9	95.13 ±11.2	51.11 ±8.8	32.22 ±8.6	44.44 ±9.4	29.16 ±5.9
Corallina elongata	72.48 ±5.5	34.22 ±5.6	69.40 ±8.9	47.98 ±5.8	32.22 ±5.5	0 ±0	68.45 ±8.7	61.96 ±12.4	39.26 ±7.9	0±0	56.38 ±12.3	59.33 ±6.5
Jania adherans	60.09 ±7.9	48.09 ±5.7	61.95 ±8.3	98.28 ±11.5	63.33 ±7.7	86.66 ±10.1	78.88 ±8.3	46.66 ±9.6	42.2 ±10.5	47.77 ±5.9	62.22 ±12.4	38.88 ±4.8

Table 2. Effect of tested Algae extracts on the volume of solid tumor

Algae extracts	Methanol					Chloroform					Positive control
Concentration µg/ml	20	40	60	80	100	1	10	20	40	80	
Tumor volume (mm^3)	95.8 ± 11.6	164.8 ± 25.8	84.2 ± 7.1	77.8 ± 13.4	150.4 ± 5.7	186 ± 9.6	182.4 ± 22.0	154 ± 11.4	126.6 ± 64.5	117.8 ± 12.5	266 ± 19.7

Table 3. Effect of Algae extracts on hepatic lipid peroxidation.

Algae extracts	Methanol					Chloroform					Normal control	Positive control
Concentration µg/ml	20	40	60	80	100	1	10	20	40	80	82.4 ± 4.8	231.2 ± 20.4
MDA nmol /g tissue	197.2 ±11.2	163.8 ±25.4	92.2 ± 6.1	64.8 ± 4.5	69.6 ±13.3	164.8 ±26.2	145.4 ±23.9	119.2 ±18.7	81.0 ± 9.6	78.6 ± 6.5		

± Standard error of 3 replicates

Effect of marine algae extracts on the level of hepatic catalase and superoxide dismutase (SOD):

The activities of SOD and catalase as antioxidant enzymes were determined in the liver homogenate of solid tumor bearing mice treated with different extracts of algae (Table 4). Results demonstrate that antioxidant status was significantly diminished during tumor progression. Treatment with different algal extracts leads to remarkable increases in SOD and catalase activities that associated with reduction in tumor volume revealing their antitumor activity. From table 4, it can be concluded that Methanol (80 and 100 µg/ml) extracts showed the most potent stimulatory effect on SOD (65.0 ± 5.7 and 69.0 ± 4.7 respectively) and catalase levels (8.1 ± 0.3 and 8.3 ± 0.6, respectively).

Table 4. Effect of Algae extracts on the level of hepatic catalase and superoxide dismutase (SOD).

Algae extracts	Concentration µg/ml	SOD (U/g tissue)	Catalase (kU/mg tissue)
Methanol	20	37.2 ± 3.1	4.3 ± 0.6
	40	44.4 ± 3.9	5.0 ± 0.3
	60	47.4 ± 7.7	6.2 ± 0.6
	80	65.0 ± 5.7	8.1 ± 0.3
	100	69.0 ± 4.7	8.3 ± 0.6
Chloroform	1	27.8 ± 5.0	3.8 ± 0.1
	10	29.2 ± 7.2	4.1 ± 0.1
	20	37.0 ± 2.6	4.4 ± 0.5
	40	34.6 ± 7.0	4.6 ± 0.5
	80	57.4 ± 6.7	7.4 ± 0.7
Normal control		52.6 ± 2.9	6.7 ± 0.2
Positive control		22.8 ± 7.3	4.0 ± 0.5

DISCUSSION:

The decline in the output of the R&D programs of the pharmaceutical companies has been described as a “productivity crisis” by some; this has been attributed in part to disruption of laboratory activities by the spate of company mergers and acquisitions, the mounting costs of drug development, and FDA over caution in the drug approval process. Interestingly, no mention is made of the deemphasizing by many companies of the “tried and true” exploration of nature as the source of novel leads for drug development as a possible reason for this downturn. Though combinatorial chemistry continues to play a major role in the drug development process, it is noteworthy that there is a “growing trend toward the synthesis of complex natural product-like libraries”, and adoption of the diversity-oriented synthesis approach where natural product synthesis and combinatorial chemistry are combined. As has been eloquently stated by Danishefsky, “a small collection of smart compounds may be more valuable than a much larger hodgepodge collection mindlessly assembled”.

Results showed that extract of *Ulva rigdia* is more antiproliferative effects against EAC cell line were dose-dependent *in vitro*. These results are in agreement with (Serap Celikler *et al.*, 2008) who determine the *in vitro* possible clastogenic and cytotoxic activities of *Ulva rigida* crude extracts (URE), and identify their antigenotoxic and protective effects on chemotherapeutic agent mitomycine-C (MMC),They reported that, although URE itself is not a clastogenic or cytotoxic substance, it possesses strong antigenotoxic, anti-clastogenic, and protective effects on MMC *in vitro*. So *Ulva rigdia* extracts was tested *in vivo* on solid tumor-bearing mice. It was focused on the methanol extraction of alga, *Ulva rigdia* that inhibited malignant cell transformation by 77% for solid tumors in Swiss mice.

The data showed that the maximal reductions of tumor volume (84.2 ± 7.1 and 77.8 ± 13.4) were observed when solid tumor-bearing mice were treated with methanol extracts of at 60 µg/ml and 80 µg/ml and 100 µg/ml, respectively. Treatments with different algal methanol extracts at 80 µg/ml and 100 µg/ml showed the maximal inhibitory action on the level of lipid peroxidation (64.8± 4.5 and 69.6±13.3 respectively) and leads to remarkable increases in SOD and catalase activities that associated with reduction in tumor volume revealing their antitumir activity, (65.0± 5.7 and 69.0± 4.7) and catalase levels (8.1±0.3 and 8.3±0.6, respectively) at the same concentrations. These results are in agreements with Hideomi (2003) who reported that *Ulva pertusa*, *Enteromorpha prolifera*, *Codium fragile*, and *Scytosiphon lomentaria* are effective against Ehrlich Ascites Carcinoma (EAC) in mice by oral administration of 1600 mg seaweed powder/ Kg body wt/day for 28 days at an inhibition rate between 35 and 70%. Ren *et al.* (1995) concluded that algal polysaccharide from Gloiopeltis tenax significantly inhibited the growth of Ehrlich Ascites Carcinoma (EAC) and solid Ehrlich, Meth-A fibrosarcoma, and sarcoma-180 tumors. Cun *et al.*(1995) fractionated and purifed neutral and acidic polysaccharides and their protein complexes from the brown seaweed (Sargassum thunbergii). Thirty one polysaccharides fractions were obtained and tested for antitumor activity in mice with Ehrlich Carcinoma transplanted had such activity.

Finally, extracts of marine algae *Ulva rigdia*, *Enteromorpha clathrata*, *Jania adherans*, *Corallina elongate* possess significantly inhibited the growth of Ehrlich Ascites Carcinoma (EAC) *in vitro* and extracts of *Ulva rigdia* possess a remarkable inhibition of Ehrlich Ascites Carcinoma (EAC) *in vivo* and reduction in tumor volume.

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النشاط المضاد للأورام من بعض الطحالب المصرية

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من المعروف أن الطحالب من أغنى المصادر الطبيعية التي يتم الحصول منها على مركبات عديدة متنوعة وجديدة والتي لها تأثير بيولوجي على الكائنات الحية وخاصة المضادة للخلايا السرطانية. فقد تم اختيار لمجموعة من الطحالب البحرية لمعرفة نشاطات مستخلصاتها ضد الخلايا السرطانية؛ فبعد عملية تجميع الطحالب وتجفيفها تم اخذ اوزان محددة (50 جرام) من كل طحلب فى حجم معلوم من كل مذيب من المذيبات التالية (الميثانول - الكلوروفورم- الايثار البيترولى) لمدة 48 ساعة ، وتم تجفيف الراشح وبعد ذلك تم الذوبان فى كمية معلومة من محلول ملحى معقم وتم حفظها للخطوات التالية. أولا: تم اختبار هذه المستخلصات خارجيا in-vitro باستخدام Ehrlich Ascites Carcinoma (EAC) cell line (سرطان الثدي) وخلصت هذة الخطوة الى ان هناك اربع طحالب لها نشاط مضاد للاورام وهى *Enteromorpha clathrata, Jania adherans, Corallina elongata* للمذيبات التالية petroleum ether و chloroform و methanol. وكانت النتائج ان مستخلصات *Ulva rigida* افضل النتائج. وثانيا: تم اختبار*Ulva rigida,* الايجابى منها بحقنها في الفئران السويسرية In-vivo antitumor activity on solid tumor أوضحت النتائج أن مستخلصات تلك الطحلب تحد من انتشار الخلايا السرطانية بنسبة تتراوح من 7708 % إلى 8402 % لمستخلص الميثانول بتركيزات 60 و80 ميكروجرام على التوالى لطحلب أولفا ريجيديا *Ulva rigida* . وأوضحت النتائج أيضا إلى انخفاض كبير فى Hepatic liver peroxidation وزيادة فى مستوى كل من Hepatic catalase and superoxide dismutase (SOD) . ونخلص إلى أن هذه النتائج واعدة و تدعوا إلى الاستمرار ومحاولة لفحص هذه المستخلصات طبيا clinical evaluation مع الجهات المعنية.

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