INTRODUCTION

Cancer is one of the most serious threats to human health in the world and chemotherapy is still the standard treatment method. 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 (Zandi et al., 2010). The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology (Xu et al., 2009). With this aim, many attentions have been paid to natural compounds in plants, marine organism and microorganisms.

Regarding the low side effects of plants and
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other natural compounds, scientists are interested in working on them to find new medications. Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. Various studies evidenced that algae act as antiviral, antibacterial, antifungal and antitumor (Harada et al., 1997) potentials, among numerous others. 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 trails (Newman and Cragg; 2004; Paterson and Anderson, 2005; Kingston, 2009).

It is now well recognized that apoptosis is a mode of cell death used by multi-cellular organisms to eradicate cells in diverse physiological and pathological settings. Recent evidence also shows that suppression of apoptosis by tumor promoting agents in pre-neoplastic cells is an important mechanism in tumor promotion (Shibata et al., 1996). In this context, it is noteworthy that apoptosis-inducing ability seems to have become a primary factor in considering the efficacy of chemo-preventive agents.

So the aim of the present, is to investigate the antitumor activity of two Egyptian marine algae (Colpomenia sinuosa and Cystoseira myrica) extract as natural agents against EAC cell line in vitro and in vivo.

**MATERIALS AND METHODS**

**Marine algae:**

Two marine algae Colpomenia sinuosa and Cystoseira myrica were examined for their antitumor activity in vitro and in vivo. Colpomenia sinuosa collected from the coastal area of Abou-Qir, Alexandria, Egypt, South East of the Mediterranean Sea Coast during May 2011; Cystoseira myrica from the coastal area of Ghardaqa, Egypt, Red Sea during October 2011. These algae were identified according to Taylor (1985) and Aleem (2001).

**Preparation of marine algae:**

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

**Extraction of algae**

Twenty gram. of each grinding alga were soaked in 100 ml methanol for 48 hours and then filtrated. The residues were repeatedly soaked in 100 ml methanol for 48 hours and filtrated (methanol extracts). The precipitate was soaked in distilled water with boiling for 30 min then filtrated (water extracts). The filtrate was 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 EAC. EAC was purchased from the National Cancer Institute, Cairo University, Cairo, Egypt. EAC cells were propagated in the laboratory by weekly intraperitoneal injection of 0.2 ml of 1:5 saline solution of freshly drawn ascitic fluid (2 *10^6 EAC cells) from a donor mouse bearing 6-8 days-old ascitic 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 exclusion assay (Bennett et al., 1967). Test extracts were titrated in duplicates with serial concentrations (2, 4, 6 and 8 mg/ml) for each *Colpomenia sinuosa* and *Cystoseira myrica* and then incubated at 37 °C for 2 hours. The cytotoxicity values were calculated according to Boyum (1968).

**In vivo assessment of antitumor activity:**

The antitumor efficacy of algae extracts was investigated in vivo (in mice bearing solid tumors). A total of 50 female Swiss mice were divided into 10 groups (five animals per group). Solid tumor was induced in all groups of study, except normal control group, by injection of $2 \times 10^6$ of EAC cells subcutaneously using female albino mice in their right thigh; the transplanted cells usually produce palpable tumors within a week. Group I served as the normal control. Group II served as the positive control (Mice-bearing solid tumor without any treatment). Group III was treated with 8 mg/ml water extract of *Colpomenia sinuosa* before inoculation in IP route. Group IV mice-bearing solid tumor treated with 6 mg/ml water extract of *Colpomenia sinuosa* after inoculation in IP route. Group V, mice-bearing solid tumor treated with 8 mg/ml water extract of *Colpomenia sinuosa* after inoculation in IP route. Group VI mice-bearing solid tumor treated with 10 mg/ml extract water of *Colpomenia sinuosa* after inoculation in IP route. Group VII mice treated with 6 mg/ml methanol extract of *Cystoseira myrica* before inoculation. Group VIII mice-bearing solid tumor treated with 4 mg/ml methanol extract of *Cystoseira myrica* post inoculation in IP route. Group IX mice-bearing solid tumor treated with 6 mg/ml methanol extract of *Cystoseira myrica* post inoculation in IP route. Group X mice-bearing solid tumor treated with 8 mg/ml extract of *Cystoseira myrica* post inoculation in IP route. In this study, extracts was given 0.1 ml daily for 9 days. At the end of the experimental period,
fasted mice were anesthetized by diethyl ether inhalation and sacrificed using a sharp razor blade. Blood was collected and the serum was obtained by centrifugation for 10 minutes at 4000 rpm. These samples were kept at -20°C until further investigation. Tumors were quickly excised, removed, washed with saline solution then kept in 10% formalin.

**Collection of the blood and tumors from all groups:**

At the end of the experimental period, fasted mice were anesthetized by diethyl ether inhalation. Mice were sacrificed using a sharp razor blade. Blood was collected and the serum was obtained by centrifugation for 10 minutes at 4000 rpm. These samples were kept at -20°C for determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity (Reitman and Frankel 1957), serum albumin (Reinhold, 1953), serum creatinine (Henry, 1974), serum malondialdehyde level (Satoh, 1978) and serum nitric oxide level (Montgomery and Dymock 1961). A V-shaped incision was made from back to expose the internal organs. Tumors specimens were quickly excised, removed, washed with saline then kept in 10% buffered formalin for histopathological study using hematoxylin and eosin stain (H & E).

**Statistical analysis:**

The statistical significance of the experimental biochemical results was determined by the Student’s t test (Murray, 1982). For all analysis, P ≤ 0.05 was accepted as significant probability level.

**RESULTS**

**Antitumor activity of algal extracts (in vitro):**

Different concentrations (2, 4, 6 and 8 mg/ml) of water and methanol extracts of *Colpomenia sinuosa* and *Cystoseira myrica* were investigated on the viability of Ehrlich Ascites Carcinoma (EAC) cell line as shown in table(1). Results showed that 8 mg/ml of water extracts of Colpomenia sinuosa and 6 mg/ml of methanol extracts of *Cystoseira myrica* are more antiproliferative effects against EAC cell line.

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Algae conc.(mg/ml)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td><em>Colpomenia sinuosa</em></td>
<td>3.75%</td>
<td>25%</td>
<td>46.25%</td>
<td>93.75%</td>
</tr>
<tr>
<td></td>
<td><em>Cystoseira myrica</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Methanol</td>
<td><em>Colpomenia sinuosa</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Cystoseira myrica</em></td>
<td>55%</td>
<td>78.75%</td>
<td>97.5%</td>
<td>97.5%</td>
</tr>
</tbody>
</table>

**Effect of algal extracts on the volume of solid tumor:**

Further *in vivo* study for the highly toxic algal extracts after the end of experiment has been done on solid tumor bearing mice. As shown in table (2), all tested extracts caused significantly reduction in the tumor volume as compared to that of the positive control group. The maximal reduction of tumor volume (0.80 ± 0.09 mm³ and 0.87 ± 0.13 mm³)
was observed when mice-bearing solid tumor were treated with 8 and 6 mg/ml after inoculation of water extract of *Colpomenia sinuosa* and methanol extracts of *Cystoseira myrica* respectively.

**Table (2)**: Effect of tested algae extracts on the volume of solid tumor.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor Volume mm³ Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>1.52 ± 0.15</td>
</tr>
<tr>
<td>Pre-inoculation <em>Colpomenia sp</em> (8 mg/ml)</td>
<td>1.31 ± 0.13</td>
</tr>
<tr>
<td>Post-inoculation <em>Colpomenia sp</em> (6 mg/ml)</td>
<td>1.38 ± 0.13</td>
</tr>
<tr>
<td>Post-inoculation <em>Colpomenia sp</em> (8 mg/ml)</td>
<td>0.80± 0.09***</td>
</tr>
<tr>
<td>Post-inoculation <em>Colpomenia sp</em> (10 mg/ml)</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>Pre-inoculation <em>Cystoseira sp</em> (6 mg/ml)</td>
<td>1.21± 0.12**</td>
</tr>
<tr>
<td>Post-inoculation <em>Cystoseira sp</em> (4 mg/ml)</td>
<td>1.25± 0.10*</td>
</tr>
<tr>
<td>Post-inoculation <em>Cystoseira sp</em> (6 mg/ml)</td>
<td>0.87± 0.13***</td>
</tr>
<tr>
<td>Post-inoculation <em>Cystoseira sp</em> (8 mg/ml)</td>
<td>1.17± 0.07**</td>
</tr>
</tbody>
</table>

*: means significant value; P 0.05, ***: means highly significant value; P 0.01, ****: means very highly significant value; P **0.001.

**Effect of algae extracts on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity and albumin concentration:**

The chemopreventive effects of algae extracts on ALT, AST and albumin in different mice serum pre and post mice inoculation are shown in Table (3). The results revealed that the concentrations 8 mg/ml water extract of *Colpomenia sinuosa* pre inoculation and 6 and 8 mg/ml post inoculation had no effect on both enzymes activity and albumin level. However the applied of concentration 10 mg/ml water extract of *Colpomenia sinuosa* post inoculation had marked elevation on ALT and AST and reduction in albumin. In case of *Cystoseira myrica*, the result revealed that the concentrations 6 mg/ml methanol extract of *Cystoseira myrica* pre inoculation, 4 and 6mg/ml methanol extracts of *Cystoseira myrica* post inoculation had no effect on these liver enzymes and albumin. However concentration 8 mg/ml of *Cystoseira myrica* extract post inoculation had marked elevation on ALT and AST and reduction in albumin.
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**Table (2):** Effect of algal extracts on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity and albumin concentration.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (µ/l)</th>
<th>AST (µ/l)</th>
<th>Albumin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>159.75 ± 14.36</td>
<td>183.75 ± 6.75</td>
<td>2.40 ± 0.04</td>
</tr>
<tr>
<td>Positive Control</td>
<td>176.25 ± 5.37</td>
<td>189.25 ± 3.50</td>
<td>2.33 ± 0.05</td>
</tr>
<tr>
<td>Pre-inoculation <em>Colpomenia</em>  sp (8 mg/ml)</td>
<td>161.50 ± 15.24</td>
<td>192.25 ± 5.67</td>
<td>2.31 ± 0.04</td>
</tr>
<tr>
<td>Post-inoculation <em>Colpomenia</em> sp (6 mg/ml)</td>
<td>165.25 ± 9.43</td>
<td>176.50 ± 9.25</td>
<td>2.328 ± 0.07</td>
</tr>
<tr>
<td>Post-inoculation <em>Colpomenia</em> sp (8 mg/ml)</td>
<td>163.00 ± 32.09</td>
<td>180.00 ± 11.13</td>
<td>2.38 ± 0.04</td>
</tr>
<tr>
<td>Post-inoculation <em>Colpomenia</em> sp (10 mg/ml)</td>
<td>405.50 ± 22.60***</td>
<td>493.00 ± 93.41***</td>
<td>1.90 ± 0.12***</td>
</tr>
<tr>
<td>Pre-inoculation <em>Cystoseria</em> sp (6 mg/ml)</td>
<td>162.75 ± 19.13</td>
<td>184.25 ± 10.24</td>
<td>2.352 ± 0.07</td>
</tr>
<tr>
<td>Post-inoculation <em>Cystoseria</em> sp (4 mg/ml)</td>
<td>175.40 ± 12.56</td>
<td>178.40 ± 11.84</td>
<td>2.348 ± 0.04</td>
</tr>
<tr>
<td>Post-inoculation <em>Cystoseria</em> sp (6 mg/ml)</td>
<td>167.33 ± 4.22</td>
<td>182.00 ± 11.66</td>
<td>2.368 ± 0.04</td>
</tr>
<tr>
<td>Post-inoculation <em>Cystoseria</em> sp (8 mg/ml)</td>
<td>495.60 ± 30.72***</td>
<td>541.20 ± 50.85***</td>
<td>1.934 ± 0.09***</td>
</tr>
</tbody>
</table>

***: means very highly significant value; P □ 0.001.

**Effects of the tested algal extracts; *Colpomenia sinuosa* and *Cystoseria myrica*, on serum creatinine:**

Extracts and tumor had no significant effects on kidney function (creatinine) (Figure 1).
Effects of tested algae extracts on lipid peroxidation malondialdehyde (MDA) in control and different treated mice:

Level of MDA as end product of lipid peroxidation was determined in the serum of tumor-bearing mice treated with different algae extracts (Figure 2). Results indicate that development of solid tumor is associated with significant elevation of MDA as compared to that of negative control. Treatment with algal extracts resulted in significant reduction in lipid peroxidation. Both (8 and 10 mg/ml) water extract of Colpomenia sinuosa post inoculation and (6 mg/ml of methanol Cystoseira myrica pre inoculation and 8 mg/ml extracts of Cystoseira myrica post inoculation) showed maximal inhibitory effects.
Effects of tested algae extracts on serum nitric oxide (NO) of mice:

There was a reduction in NO concentration at all concentrations of *Colpomenia sinuosa* as shown in Figure(3); and this reduction was highly significant at concentration 8 mg/ml pre inoculation and 8 mg/ml post inoculation and extremely significant at 10 mg/ml post mice inoculation. In case of *Cystoseira myrica*, there was reduction in NO concentration at all concentrations were tested and this reduction was highly significant at concentration 6mg/ml pre inoculation and 6 mg/ml extracts post inoculation and extremely significant at concentration 8 mg/ml post inoculation.

Effect of algal extracts on histopathological examination (haematoxylin and eosin stain) of solid tumors:

There was a regression in tumor grade by using *Colpomenia sinuosa* and *Cystoseira myrica* extracts (figures 4-15).

![Fig. (3) : Effect of algal extracts on serum nitric oxide (NO).](image)
Fig. (4): Section of normal mouse skin showing hair follicles (HF) extended to the dermis. Epidermis (Epi), sebaceous glands (Seb), dermal papillae (DP). Connective tissue in the dermis is stained with hematoxylin and eosin (H&E).

Fig. (5): Section in tumor of positive control showing grade III tumor (H&E, X 100).

Fig. (6): Section in tumor of group IV showing grade II tumor (H&E X 100).

Fig. (7): Section in tumor of group III showing grade II tumor (H&E X 100).

Fig. (8): Section in tumor of group VI showing grade I tumor (H&E X 100).

Fig. (9): Section in tumor of group V showing grade I tumor (H&E X 100).
**Fig. (10):** Section of normal mouse skin showing hair follicles (HF) extended to the dermis. Epidermis (Epi), sebaceous glands (Seb), dermal papillae (DP). Connective tissue in the dermis is stained with H&E.

**Fig. (11):** Section in tumor of positive control showing grade III tumor (H&E, X 100).

**Fig. (12):** Section in tumor of group VIII showing grade II tumor (H&E X 100).

**Fig. (13):** Section in tumor of group VII showing grade II tumor (H&E X 100).

**Fig. (14):** Section in tumor of group X showing grade I tumor (H&E X 100).

**Fig. (15):** Section in tumor of group IX showing grade I tumor (H&E X 100).
DISCUSSION

The mounting coasts of anticancer drug development, and FDA over caution in the drug approval processes, it is noteworthy that there is a growing trend towards the synthesis of complex natural products. Marine algae produce a wide range of new secondary metabolites with various biological activities. In an attempt to find new anticancer drugs, most seaweeds, including the brown algae; have exhibited a cytotoxic activity against cancer cell lines.

In vitro assay, the brown algae extract of Colpomenia sinuosa killed 93.75 % at a concentration of 8 mg/ml/10^6 EAC cells. This is in agreement with Abourriche et al. (1999); Bennamara et al. (1999); Ayyad et al. (2003), Who found that Cystoseira (Cystoseiraceae) is a widely distributed genus of brown algae have antibacterial, antifungal, and cytotoxic activity. On the other hand, this is in disagreement with Khanavi et al. (2010); who did not note any significant cytotoxic activity for total extract and fractions of Colpomenia sinuosa.

The data obtained from the present investigation revealed that the administration of 8 mg/ml extract of Colpomenia sinuosa post inoculation (0.8096 ± 0.09542) and 6 mg/ml extract of Cystoseira myrica post inoculation (0.8742 ± 0.1367) at the tenth day of tumor inoculation induced maximal reduction of tumor size. This reduction is in agreement with that obtained by El - khawaga et al. (2001). They found that the treatment of mice with injection of extracts of brown algae resulted in both a significant inhibition of tumor size compared with mice that did not receive extracts of brown algae.

Colpomenia sinuosa and Cystoseira myrica extracts had no significant effects on liver enzymes (ALT & AST) and albumin except at a concentration of 10 mg/ml of Colpomenia sinuosa extract and a concentration of 8 mg/ml of Cystoseira myrica extract post inoculation which show increased values of liver enzymes (ALT & AST) and decreased values of liver albumin. Colpomenia sinuosa and Cystoseira myrica extracts had no significant effects on kidney function (Creatinine). This means that algal extracts had no harmful effect on general health of mice.

In this study there was a reduction in MDA at all concentrations and maximal inhibitory effect at (8 and 10 mg/ml extracts of Colpomenia sinuosa post inoculation and 6 mg/ml pre inoculation, 8 mg/ml extracts of Cystoseira myrica post inoculation). These results are in agreement with Hideomi (2003) who reported that brown algae extracts showed a reduction in MDA. Hamza et al. (2011) reported that the treatment with different algal extracts results in significant reduction in MDA. The treatment with different algal extracts showed a reduction in NO level and the maximal inhibitory effect occurs at 8 mg/ml pre inoculation, 8 and 10 mg/ml extracts of Colpomenia sinuosa post inoculation. It was recorded that 6 mg/ml of Cystoseira myrica applied before inoculation, 6 and 8 mg/ml post inoculation caused a significant reduction in NO level. In accordance Shalaby et al. (2011) who reported that the potent antioxidant activity of polar algal extracts and the aqueous and ethanol extracts of brown algae showed highly antioxidant activity and could act as electron or hy-
drogen donors for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

The present histopathological studies revealed that there was a regression in tumor grade from grade III in positive control to grade II in 8 mg/ml extracts of *Colpomenia sinuosa* pre inoculation and 6 mg/ml extracts of *Colpomenia sinuosa* post inoculation and to grade I in 8 mg/ml extracts of *Colpomenia sinuosa* post inoculation and 10 mg/ml extracts of *Colpomenia sinuosa* post inoculation. In *Cystoseira myrica* there was a regression of tumor grade from grade III in positive control to grade II in 6 mg/ml extracts of Cystoseira myrica pre inoculation and 4 mg/ml extracts of *Cystoseira myrica* post inoculation and to grade I in 6 mg/ml extracts of *Cystoseira myrica* post inoculation and 8 mg/ml extracts of *Cystoseira myrica* post inoculation. This associated with a reduction in tumor volume revealing their antitumor activity.

Finally, these aqueous extracts of *Colpomenia sinuosa* and *Cystoseira myrica* exhibited antioxidant and anticancer activities which possess a significantly inhibition of the growth of EAC in vitro and in vivo with causing a reduction in tumor volume. So its effect is amplified by these biological efficiencies which are very important for human health; for the manufacture of pharmaceutical drugs (anticancer and antioxidant).

**REFERENCES**


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الملخص العربي

"نشاط اثنين من الطحالب البنية ضد الخلايا السرطانية (الإرلش) في الفئران البيضاء السويسرية"

أحمد صالح 1, أسامة محمد بدر 2, رجاء حمودة 3

ميرفت حسين 4, أميمة خميس 5

1 معهد الهندسة الوراثية والتكنولوجيا الحيوية - جامعة مدينة السادات
2 أقسام تكنولوجيا الحيوية (و قسم البيوتكنولوجيا الميكروبية)
3 أقسام النبات - كلية العلوم - جامعة المصورة

تعتبر الطحالب من المصادر الطبيعية الغنية بمركبات متنوعة والتي لها تأثير بيولوجي على الكائنات الحية وخاصة المضادة للخلايا السرطانية. وقد تم اختبار نوعين من الطحالب البحرية لمعرفة كفاءة مستخلصاتها ضد الخلايا السرطانية، تم جمع الطحالب وتف cresification ثم أخذ أوران محددة 0.07 جرام من كل طحلب، ثم وضعها في 100 مللي ميتران لفترة 48 ساعة ثم تجفيف الرياح وبعد ذلك تم الذوبان في Ehrlich كمixture من الماء المفطر وحفظها للاختبارات التالية. أولاً: تم اختبار هذه المستخلصات خارجية (في الخلايا الإرلش) وخلصت هذه النتائج إلى أن لها تأثير مضاد للأورام. وأدت النتائج أن تركيز 8 ملی M. Ascites Carcinoma EAC) وأفضل النتائج، ثانياً: تم اختيار هذه Cystoseira myrica و1 مللي غرام / ملي من Colpomenia sinuosa غرام/ ملي غرام / ملي من المستخلصات بتقريب نتائج تلك الطحالب بعد انتشار الخلايا السرطانية وتؤدي إلى إخفاق كبير في نشاط إنزيمات الكبد ومحتوى أكسيد النيتروجين كما يقلل من الدهون المكتسبة. و أظهر الفحص الهرمونولوجي للورم الانتحار في درجة ورم الذي بسرعة مع انخفاض حجم الورم. ويوصي البحث بإجراء مزيد من الدراسات المستقبلية لفهم وكشف الفوائد المحتملة لهذه الطحالب واستخدامها كعقار طبيعي في المجال.
ANTITUMOR ACTIVITY OF TWO BROWN ALGAE AGAINST EHRlich ASCITES CARCINOMA IN SWISS ALBINO MICE

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