

## Bioactive constituents of three algal species extracts and their anticancer activity against human cancer cell lines

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### Abstract:

This study was designed to identify the bioactive constituents of the red alga *Gracilaria dendroides* and one green alga *Chlorella vulgaris* and *Microcystis* sp. (cyanobacteria) by GC /Mass and HPLC analysis and assay the cytotoxic activity of the methanolic extract from the three species against three types of human cancer cell lines (liver HEPG-2, colon and breast MCV-7). The research was performed as an *in vitro* study. The effect of extract on proliferation of cell lines was measured by Methyl thiazolyl tetra zolium (MTT) colorimetric method. The results showed that the compounds identified by GC-MS were 19 compounds in *G. dendroides*, 11 compounds in *Chlorella* and 12 compounds in *Microcystis*. The most abundant compounds were fatty acids, methyl esters and terpenoids. HPLC analysis identified 4 compounds in *Gracilaria* and 6 compounds in *Chlorella* and *Microcystis*. Crude extract of *Gracilaria* had the strongest activity on HepG-2 cell lines with IC<sub>50</sub> value 15.46 µg/ml. Concerning MCF-7 cell lines, the most potent crude extract was the *Chlorella vulgaris* (IC<sub>50</sub> value of 15.53 µg/ml). On the other hand, evaluation the cytotoxic activity for the three algae species extracts against epithelial colorectal adenocarcinoma cells (CaCO-2) showed higher activity for *Chlorella vulgaris* with IC<sub>50</sub> = 14.63 µg/ml. Accordingly, we can say that *Chlorella* had the strongest activity on MCF-7 and (CaCO-2) cell lines while HepG-2 cell lines most affected by *Gracilaria* extract.

**Keywords:** Anticancer, Bioactive compounds, *Gracilaria*, *Chlorella*, *Microcystis*

### Introduction

Most of the anticancer drugs currently used in chemotherapy (the standard treatment method) are cytotoxic to normal cells and cause immune toxicity so scientists are interested in working on plants, marine organism and

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microorganisms natural compounds for the discovery and identification of new antitumor drug with low side effects (**Xu *et al.*, 2009**). Marine algae contain various biologically active compounds which have been used as source of food, feed and medicine. Until now, more than 2400 marine natural products have been isolated from seaweeds (**Manilal *et al.*, 2009**). The crude extract of red alga *Gracilaria corticata* had significant anticancer activity and it might be a good candidate for further investigations in order to develop a natural compound as an anticancer agent which can be used for the production of potential anticancer drug and novel pharmaceutical leads. The polysaccharides and peptides isolated from seaweeds have become a matter of great interest for cancer therapy. The mechanisms of their anticancer activity are related to their ability to suppress the growth of cancer cells (cytotoxic or cytostatic effects), to enhance the immune responses and to inhibit tumor angiogenesis (**Itoh *et al.*, 1993**; **Apryshko *et al.*, 2005**; **Matsubara *et al.*, 2005**). The anticancer potential of the polysaccharides extracted from 7 marine algae by using the cells of breast cancer (MCF7) and colon cancer (CoCa2) evaluated as mentioned by **Ghada *et al.* (2018)**. Microalgae synthesize compounds with known in vitro and in vivo biological activity against different tumor cell lines. The bioactive compounds which have the ability to inhibit cancer cell proliferation by activating apoptotic pathways can be employed as potential chemotherapeutic drugs. Cyanobacteria produce biologically active and chemically diverse compounds belonging to cyclic peptides, lipopeptides, fatty acid amides, alkaloids and saccharides. More than 50% of the marine cyanobacteria are potentially exploitable for extracting bioactive substances which are effective in killing cancer cells by inducing apoptotic death (**Ahmed *et al.*, 2017**). Microcystins are a group of chemically related cyclic peptides and most commonly studied group of cyanotoxins (**Kehr *et al.*, 2011**). The anticancer activity of *Chlorella vulgaris* against breast cancer cell lines was determined by means of MTT assay (a colorimetric assay) to determine cell viability by assessing the values of IC50 and the results showed that the MCF7 was the cancer cell lines that reach 50% of inhibition by algae (**Mohd *et al.*, 2012**). **Zhang *et al.* (2017)** showed that *Chlorella vulgaris* induces apoptosis of human non-small cell lung carcinoma. Recently the importance of strain selection taken into consideration as activity varied between strains of the same species as mentioned by **Ordog *et al.* (2004)**. **Michael *et al.* (2014)** showed that fatty acid derivatives often exhibit activity against cancer cells, but not

normal cells. This appears to be a property that augers well for new anti-cancer drug development based on lipids. The available information does not provide a full understanding for this selectivity because the targeted pathways may be present in both cell types. The discovery and identification of new anti-tumor drugs with low side-effects on immune system has become an essential goal in immune pharmacology (Xu *et al.*, 2009). Regarding the reduced side-effects of plants and algae compounds, scientists are interested in working on them to find new formulations. In the present study therefore, *Gracilaria dendroides*, *Chlorella vulgaris* and *Microcystis sp* extracts were examined *in vitro* to assay the cytotoxic activity on proliferation of human cancer cell lines HEPG-2, colon and breast MCV-7.

## ***Materials and Methods***

### **Biological materials**

#### ***Gracilaria dendroides***

*Gracilaria* was manually collected from Mediterranean Coast at Baltim (31° 33' 14.3" N-31° 05' 42.0" E) during June 2015. Classification was done according to Aleem (1993). The alga (were collected in a considerable amount for assay in plastic bags and transferred to the laboratory. At the laboratory; the algal material was sorted to the species level, cleaned from epiphytes and were successively rinsed with sea water, fresh water and distilled water. After this operation it was air dried, weighed to the nearest gram and powdered using electric mill in preparation for the extractions.

#### **Microalgae and cyanobacteria growth conditions**

*Chlorella vulgaris* and *Microcystis sp.* were isolated from Nile River and purified. It was identified morphologically by light microscopy according to Prescott, 1982, and the growth conditions optimization were examined; the two organisms were cultivated in BG11 medium (Rippka *et al.*, 1979). For the production of biomass, exponentially growing algae culture was transferred into

fresh sterile medium [10% (v/v) of inoculums]. Cultures were illuminated by tubular fluorescent lamps with light intensity of  $25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Examination of the species growth optimization takes place as follow; pH intervals from 4,5,6,7,8,9 to identify the optimum initial pH, photoperiod (light/dark) intervals (continuous light, 12h:12h, 16:8), the different growth temperatures (15, 20, 25, 30, 35°C). The optimum incubation period was examined by measuring the organism growth every 3 days for 30 days. All experiments mentioned above were performed in triplicates using 1 L Erlenmeyer flasks. The growth of the organism was determined by dry weight and chlorophyll-a determination.

### **Preparation of Algal Extract**

One hundred grams of dried *Gracilaria dendroides* powder were suspended in 200 mL aqueous methanol 70% for 72 h then centrifuged at 5000 rpm for 10 minutes (Élica *et al*, 2013). To identify bioactive compounds of algal extracts GC-MS and HPLC analysis were takes place. The mass from these extracts was measured and stored under refrigeration for subsequent cytotoxicity assays

### **Microalgae and cyanobacteria**

One hundred milligram of dried algal cells harvested at stationary phase (after 21 days) were grinded by 70% methanol then centrifuged at 5000 rpm for 10 minute and the extract using for GC-MS and HPLC analysis to identify the bioactive compounds.

### **GC\MS Analysis and active compound identification:-**

GC/MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25  $\mu\text{m}$  film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 20:1, injection volume of 1  $\mu\text{l}$  and the following temperature program: 50 °C for 1 min; rising at 20 °C /min to 200 °C

and held for 5 min; rising at 3 °C/min to 230 °C and held for 15 min. The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization at 70 eV and using a spectral range of m/z 20-550 and solvent delay 1.8 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

#### **HPLC Analysis of algal extract:-**

The sample (100 µg/mL) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent of MeOH, filtered using a membrane disc filter (0.2 µm) then subjected to LC-ESI-MS analysis. Samples injection volumes (10 µL) were injected into the UPLC instrument equipped with m reverse phase C-18 column (ACQUITY UPLC - BEH C18 1.7 µm particle size - 2.1 × 50 mm Column). Sample mobile phase was prepared by filtering using 0.2 µm filter membrane disc and degassed by sonication before injection.

Mobile phase elution was made with the flow rate of 0.2 mL/min using gradient mobile phase comprising two eluents: A is H<sub>2</sub>O acidified with 0.1% formic acid and B is MeOH acidified with 0.1% formic acid. Elution was performed using the above gradient. The parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI between m/z 100–1000. The peaks and spectra were processed using the Maslynx 4.1 software and tentatively identified by comparing its retention time (Rt) and mass spectrum with reported data.

#### **Cytotoxic activity of algal extract in vitro**

To determine the cytotoxicity of algal extract against studied cancer cell lines; 1mg of algal extract dissolved in 0.1m\l DMSO , and this extract tested against three types of human cancer cells (liver, colon and breast) by using Methyl thiazolyl tetrazolium (MTT assay) which used as a quantitative and approved method at VACSERA center. In this method, 10 µl of MTT stock solution (5 mg/ml in phosphate buffer solution) was added to 90 µl medium of wells

which were treated by different concentrations of algal extract for 72 hrs. The micro plate was incubated at 37°C for 4 hrs and then, the optical density of each well was read by microplate reader (ASYS – EXPERT 96) at 540 nm (**Van de Loosdrecht *et al.*, 1994**).

## ***Results and Discussion***

Growth optimization of the two species showed that the optimum initial pH of the BG11 medium was 7, the optimum (light/ dark) cycle was 16hrs: 8hrs at 27±2°C for the two species. The optimum incubation period was 21days for *Microcystis* sp. and 27 days for *Chlorella vulgaris*.

### **GC\MS analysis**

#### **GC\MS analysis of *Gracilaria dendroides*:-**

Methanol crude extract of *Gracilaria* resulted in the identification of different compounds which were predominated in Table 1. The spectrum analysis revealed the presence of 19 compounds; 9-octadeconic acid methylester with peak area of 38.97% followed by 9, 12-octadecadionic acid(z,z) methylester with 8.41% and Squalene 8.40% .

In our results fatty acids and methyl esters were represented the dominant compounds as mentioned by **Venkataraman *et al.* (2019)** showed that the analysis of *Gracilaria corticata* methanol extract revealed several bioactive compounds such as: fatty acids, free saturated fatty acids and methyl esters.

#### **GC\MS analysis of *Chlorella vulgaris*:-**

The spectrum analysis of *Chlorella* extract revealed the presence of 11 compounds, the most abundant compounds being 9-Octadeconic acid, Zmethyl ester (27.84%) represented the highest percentage in the extract followed by Exobornylacetate with 21.63% and pinocarvon 9.83% (Table 2).

**Table 1: GC\MS of methanol extract of *Gracilaria dendroides***

| No | Name of compound   | Common name                | Group                | Molecular formula | Molecular weight | Peak area%   | RT    |
|----|--|----------------------------|----------------------|-------------------|------------------|--------------|-------|
| 1  | Dodecane (CAS)   |                            | Alkane               | C12H26            | 170              | 0.93         | 10.41 |
| 2  | Eicosane   |                            | Alkane               | C20H42            | 282              | 1.83         | 16.77 |
| 3  | Isoborneol, trifluoroacetate ester   |                            | Ester                | C12H17F3O2        | 250              | 0.63         | 17.03 |
| 4  | 1,2,3-propentriol, triacetat   | Glyceryl triacetate        | Fatty acid           | C9H14O6           | 218              | 1.00         | 18.81 |
| 5  | 3-Trifluoroacetoxytetradecane  |                            |                      | C16H29F3O2        | 310              | 1.59         | 19.71 |
| 6  | Docosane   |                            | Alkane               | C22H46            | 310              | 1.84         | 22.27 |
| 7  | 5-tert-Butyl-4-isopropyl 2-methylphenol  |                            | Phenol               | C14H22O           | 206              | 0.4          | 22.84 |
| 8  | E-14-Hexadecenal   |                            |                      | C16H30O           | 238              | 2.11         | 24.53 |
| 9  | 1-octadecanol  | Stearyl alcohol            |                      | C18H38O           | 270              | 1.93         | 28.87 |
| 10 | Lucenin2   |                            |                      | C27H30O16         | 610              | 0.54         | 29.54 |
| 11 | Furoscrobiculin B  |                            |                      | C15H20O2          | 232              | 4.31         | 31.50 |
| 12 | 1,2-Benzendicarboxylic acid dibutyle ester   | Dibutyl phthalat           |                      | C16H22O4          | 278              | 0.73         | 32.38 |
| 13 | -6-Methyl-2-(3-phenyl-4-oxothiazolin-2-ylidene)aminopyridine   |                            |                      | C15H13N3OS        | 283              | 1.38         | 33.24 |
| 14 | 9,12-octadecadionic acid(z,z)methylester   | Linoleic acid methyl ester | Fatty acid           | C19H34O2          | 294              | 8.41         | 34.72 |
| 15 | 9-octadeconic acid methylester   | Methyl elaidate            | Fatty acid           | C19H36O2          | 296              | <b>38.97</b> | 34.83 |
| 16 | Phthalic acid, di(2-propylpentyl)ester   |                            | Plasticizer compound | C24H38O4          | 390              | 3.53         | 42.24 |
| 17 | Squalene   |                            |                      | C30H50            | 410              | 8.40         | 46.22 |
| 18 | 4H-Cyclopropa [5',6']benz[1',2':7,8]azuleno[5,6b]oxiren-4-one, 8-acetyloxy)-1,1a,1b,1c 2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl | Valtrate fatty acid ester  | Fatty acid           | C22H30O8          | 422              | 1.19         | 50.21 |
| 19 | Methyl-8,4,2-Diacetoxy-3-bromo-6-methoxy-9,10-dioxo-9,10-dihydro anthraquinon-2-(methyl)-4-(2-methyl[1,3]dioxolan-2-yl)-3-oxobutanoate   |                            |                      | C29H27BrO12       | 646              | 2.25         | 54.09 |

**Table 2: GC\MS of methanol extract of *Chlorella vulgaris***

| No | Name of compound                                      | Common name                | Group          | Molecular formula                              | Molecular weight | Peak area%   | RT    |
|----|---|----------------------------|----------------|--|------------------|--------------|-------|
| 1  | 1,8-cineole   | Eucalyptus oil             | Mono-terpenoid | C <sub>10</sub> H <sub>18</sub> O              | 154              | 1.00         | 9.68  |
| 2  | EXOBORNYL ACETATE                                     |                            | terpenoid      | C <sub>12</sub> H <sub>20</sub> O <sub>2</sub> | 196              | <b>21.63</b> | 17.02 |
| 3  | Phenol,2- methyl -5,(1-methylethyl)                   | Pinocarvon                 | phenol         | C <sub>10</sub> H <sub>14</sub> O              | 150              | 9.83         | 17.34 |
| 4  | trans-Caryophyllene                                   |                            | terpene        | C <sub>15</sub> H <sub>24</sub>                | 204              | 1.12         | 20.47 |
| 5  | 1-Heptadecene   |                            | Alkene         | C <sub>17</sub> H <sub>34</sub>                | 238              | 1.55         | 24.51 |
| 6  | Heptadecene   |                            | Alkene         | C <sub>17</sub> H <sub>36</sub>                | 240              | 5.19         | 26.89 |
| 7  | Hexadecanoicmethyl ester                              | Palmitic acid              | Ester          | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> | 270              | 1.87         | 31.57 |
| 8  | Octadecadienoic 9,12- acid (Z,Z)-, methylester        | Linoleic acid methyl ester | Ester          | C <sub>19</sub> H <sub>34</sub> O <sub>2</sub> | 294              | 6.83         | 34.73 |
| 9  | -Octadecenoic acid9- )Z)-, methyl ester               | Oleic acid methyl ester    | Fatty acid     | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub> | 296              | <b>27.84</b> | 34.83 |
| 10 | Benzenedicarboxyl1,2 ic acid,Bis(2- ethylhexyl) ester | phthalic acid ester        | Fatty acid     | C <sub>24</sub> H <sub>38</sub> O <sub>4</sub> | 390              | 0.57         | 42.22 |
| 11 | Suprene   |                            | lipid          | C <sub>30</sub> H <sub>50</sub>                | 410              | 1.04         | 46.21 |

**GC\MS analysis of *Microcystis* sp.**

From the results of *Microcystis* extract analysis twelve compounds were determined the highest peak area with high concentration, the highest is Trans-13-octadecenoic acid, methyl ester with 28.49%, followed by Exobornyl acetate with 14.54% and Heptadecane, 9-octyl with 12.40% (Table 3).

**Michael *et al.* (2014)** illustrated that from different cellular studies; fatty acid derivatives often exhibit activity against cancer cells, but not normal cells. According to this property the use of lipids for new anti-cancer drug development was preferred.

**Table 3: GC\MS of methanol extract of *Microcystis* sp**

| No | Name of compound  | Common name                | Group              | Molecular formula | Molecular weight | Peak area%   | RT    |
|----|---|----------------------------|--------------------|-------------------|------------------|--------------|-------|
| 1  | Benzene4-ethyl-1,2-dimethyl   |                            | Aromatic alkane    | C10H14            | 134              | 1.93         | 9.50  |
| 2  | 1,8-Cineole   |                            | Alcohol            | C10H18O           | 154              | 1.63         | 9.68  |
| 3  | Exobronyle acetate  |                            | terpenoid          | C12H20O2          | 196              | <b>14.54</b> | 17.03 |
| 4  | Docosane  |                            | Alkane             | C22H46            | 310              | 1.45         | 22.26 |
| 5  | Heptadecane,9-octyl   |                            | Alkane hydrocarbon | C25H52            | 352              | <b>12.40</b> | 26.86 |
| 6  | Flavone   |                            | flavonoid          | C27H30O15         | 594              | 0.35         | 29.81 |
| 7  | Octadecane,3-ethyl-5-(2-ethylbutyle)  |                            |                    | C26H54            | 366              | 1.59         | 31.48 |
| 8  | Hexadecanoic acid methyl ester  | Palmitic acid methyl ester | Fatty acid         | C17H34O2          | 270              | 1.62         | 31.59 |
| 9  | Trans-13-octadecanoic acid methyl ester   | Oleic acid methyl ester    | Fatty acid         | C19H36O2          | 296              | <b>28.49</b> | 34.84 |
| 10 | 1,2-Benzendicarboxylic acid, bis(ethylhexyl) ester                              | Phthalic acid ester        | Fatty acid         | C24H38O4          | 390              | 4.93         | 42.23 |
| 11 | 2,6,10,14,18,22-Tetraosahehexane,2,6,10,15,19,23-hexamethyl                     | Squalene                   |                    | C30H50            | 410              | 1.86         | 46.22 |
| 12 | Pregn-4-ene-3,20-dione,11 hydroxy-17,21-bis(trimethylsilyloxy)[bis(oxime)],11 á | Steroid                    | Sterol             | C29H52N2O5Si2     | 564              | 1.32         | 54.08 |

### HPLC analysis of algal extract

It was obvious from Table 4 that there are four bioactive compound in *Gracilaria dendroids*; the highest concentration of compounds was cholestyremyristate with 97% relative concentration at 8.01 RT, and then followed by phytol and Cholest-5-en-3-ol with percentage 50% and 19% at (21.85-0.72) RT, respectively. Finally, 2-hydroxy myristic acid appeared with 18% abundance at 5.72RT. Phytol act as antimicrobial potency and play role in insulin production for human. **Sheeja et al. (2016)** studied the methanolic extract of the *Gracilaria edulis* by Gas Chromatography mass spectral fragmentation

pattern and illustrated that the purified compound is Phytol which was analyzed against MCF-7 using MTT assay method denoting significant anti-proliferation activity and the IC<sub>50</sub> of phytol was 125 cell lines.

**Table 4: HPLC analysis of *Gracilaria dendroids*, *Chlorella vulgaris* and *Microcystis sp.***

|                               | Compound name          | Molecular weight | RT    | Relative concentration% |
|-------------------------------|------------------------|------------------|-------|-------------------------|
| <i>Gracilaria dendroids</i> , | Phytol                 | 296.5            | 21.85 | 50                      |
|                               | Cholesterylmyristate   | 597              | 8.01  | 97                      |
|                               | 2-hydroxymyristic acid | 244.37           | 5.72  | 18                      |
|                               | Cholest-5-en-3-ol      | 386.7            | 0.72  | 19                      |
| <i>Chlorella vulgaris</i>     | Lutein                 | 568              | 9.8 4 | 15                      |
|                               | $\alpha$ -Tocopherol   | 430.71           | 24.55 | 18                      |
|                               | Astaxanthine           | 596.84           | 23.77 | 45                      |
|                               | Ascorbic acid          | 176.12           | 30.41 | 44                      |
|                               | Zeaxanthine            | 568              | 22.38 | 8                       |
|                               | CuracinA               | 373.6            | 16.27 | 6                       |
| <i>Microcystis sp</i>         | Cyanopeptoline         | 957              | 15.94 | 29                      |
|                               | Microcystine           | 995              | 26.77 | 30                      |
|                               | Aeruginosin            | 635              | 0.77  | 6                       |
|                               | Micropeptine           | 987              | 12.05 | 75                      |
|                               | Nodularine             | 824.9            | 23.96 | 23                      |
|                               | Microgynon             | 608              | 27.13 | 25                      |

The fatty acid 2-hydroxy myristic acid is long chain and act as emulsifier and surfactant for industrial also used as energy source .Cholesterol myristate has been used in the composition of lipid nanoparticles as drug carrier systems for drugs with low water solubility. **Yosie et al. (2016)** isolated different compounds from *G. changii*, *G. manilaensis* and *Gracilaria* sp. and revealed the presence of hexadecanoic acid (palmitic acid) as a major compound and cholest-5-en-3-ol from the diethyl ether extracts. Moreover, 2-hydroxymyristic acid and cholesterylmyristate. In *Chlorella vulgaris* astaxanthine represent 45% abundance, followed by ascorbic acid with 44% relative abundance and also found that  $\alpha$ -tocopherol with 18%.Lutein with 15% abundance and the minor abundance components were zeaxanthine and curacin A with (8-6)%, respectively. The obtained compounds have biological activity; Lutein acts as an antioxidant, protecting cells against the damaging effects of free radicals. Tocopherols have been suggested to reduce the risk of cancer due to their strong antioxidant properties. Curacin A has been characterized as potent antiproliferative cytotoxic compound with notable anticancer activity for several cancer lines including renal, colon, and breast cancer (**Verdier et al., 1998 and Gu et al., 2007**).Ascorbic acid is water soluble vitamin act as antioxidant and free radical scavenger. Astaxanthine is a lipid-soluble pigment has anticancer properties as mentioned by **Palozza et al. (2009)** which showed that astaxanthine inhibited the growth of fibrosarcoma, breast, and prostate cancer cells and embryonic fibroblasts. The cytotoxic effects of zeaxanthin(lipid soluble antioxidants) on two human uveal melanoma cell lines (SP6.5 and C918) measured by MTT assay which revealed that zeaxanthin reduced the cell viability of melanoma cells (**Ming-Chao et al, 2013**). Finally as illustrated in Table 4 the highest concentration in *Microcystis* sp. extract was micropeptine with 75%abundance, then microcystine with 30% abundance, followed by cyanopeptoline and microgynon with 29 and 25% relative abundance respectively. The minor components were nodularine 23% abundance and aeruginosin 6%. Microcystins are stable hydrophilic cyclic heptapeptides with a potential to cause cellular damage following uptake *via* organic anion-transporting polypeptides (OATP),developing analogues of microcystin cyanotoxins for efficiently targeting the OATP-expressing metastatic cancers, which are resistant to conventional chemotherapy treatment represent a potential novel targets for anticancer drugs (**Sainis et al., 2010**).

### Anticancer effect of algal extracts

The *in vitro* cytotoxic activity of the methanolic crude extracts of *Chlorella vulgaris*, *Microcystis* sp. and *Gracilaria dendroides* was evaluated against liver cancer cell line (HepG-2), human breast adenocarcinoma cell line (MCF-7) and epithelial colorectal adenocarcinoma cells (CaCO-2). Different concentrations of the tested extracts were used to calculate the values of IC<sub>50</sub> (the half-maximal inhibitory concentration). Doxorubicin (DOX) has been used as a positive control. The MTT assay results (Table 5 and Fig. 1) showed that crude extract of *Gracilaria dendroides* had the strongest activity on HepG-2 cell lines with IC<sub>50</sub> value 15.46 µg/ml. Concerning MCF-7 cell line, the most potent crude extract was the *Chlorella vulgaris* (IC<sub>50</sub> value of 15.53 µg/ml) followed by the *Microcystis* sp. (IC<sub>50</sub> value of 23.70 µg/ml) and *Chlorella vulgaris* (IC<sub>50</sub> value of 76.29 µg/ml). On the other hand, evaluation the cytotoxic activity for the three algae species extracts against epithelial colorectal adenocarcinoma cells (CaCO-2) showed higher activity for *Chlorella vulgaris* with IC<sub>50</sub> = 14.63 µg/ml followed by extracts of *Microcystis* sp. (IC<sub>50</sub> value of 15.86 µg/ml) and *Gracilaria dendroides* (IC<sub>50</sub> value of 16.50 µg/ml). Our results on *Chlorella vulgaris* consistent with **Balaji *et al.* (2017)** which showed that the extracts of *Chlorella vulgaris* induced concentration-dependent cytotoxic effects (84.11%) of cell viability in MCF7 with 100 µg/ml after an exposure of 48 hours. **Prakash *et al.* (2017)** reported that *Chlorella vulgaris* possessed cytotoxic activity against human breast adenocarcinoma cell line (MCF-7). Certain bioactive compounds such as curacin-A detected in *Chlorella vulgaris* suppress colon and breast cell lines. Several studies have been demonstrated that *Chlorella* extracts exhibit cytotoxic effects in various human cancer cell lines and has ability to modulate apoptosis signaling pathways. In the hepatoma cell line HepG2, the crude extract of *Chlorella vulgaris* inhibited cell proliferation and induced apoptosis cascades (**Yusof *et al.*, 2010**).

Our results showed that *Gracilaria dendroides* has cholesterylmyristate (97%) and cholest-5-en-3-ol (19%). **Kim *et al.* (2013)** illustrated that the cells treated with cholesterol derivatives showed chromatin condensation and DNA fragmentation, which are typical morphologic changes associated with apoptosis. Indeed, cholesterol induced apoptosis in A2058 cell via the mitochondria-

mediated pathway. **Yosie *et al.* (2016)** showed that the selected edible seaweeds (*G. changii*, *G. manilaensis* and *Gracilaria* sp.) have cytotoxic activity against HL-60 and MCF-7 cell lines and showed good activity as antibacterial and antioxidant agents. So that; the high cholesterol content of *Gracilaria dendroides* in our results may be the most effective anticancer agents.

**Table 5:** Cytotoxic activity (IC<sub>50</sub>, µg/ml) of the methanolic extracts of three algae species against HepG-2, MCF-7 and CaCo-2 cell line.

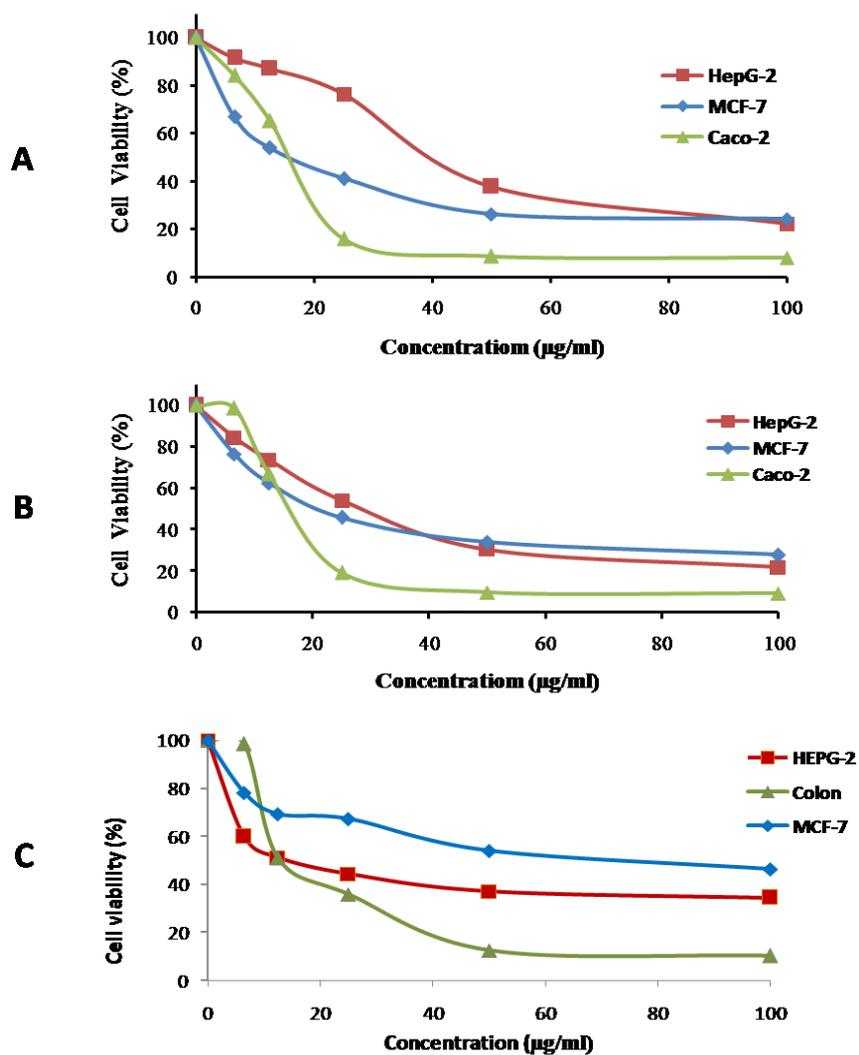
| Extract                      | MTT assay IC <sub>50</sub> (µg/ml) |       |        |
|------------------------------|------------------------------------|-------|--------|
|                              | HepG-2                             | MCF-7 | CaCo-2 |
| <i>Chlorella vulgaris</i>    | 42.45                              | 15.53 | 14.63  |
| <i>Microcystis</i> sp.       | 28.40                              | 23.70 | 15.86  |
| <i>Gracilaria dendroides</i> | 15.46                              | 76.29 | 16.50  |
| Doxorubicin                  | 1.28                               | 0.32  | 0.33   |

### Conclusion

The evaluation of cytotoxic activity for the three algae species methanolic extracts against epithelial colorectal adenocarcinoma cells (CaCO<sub>2</sub>) showed higher activity for *Chlorella vulgaris* with IC<sub>50</sub> = 14.63 µg/ml. Accordingly, we can say that *Chlorella* had the strongest activity on MCF-7 and (CaCO-2) cell lines while HepG-2 cell lines most affected by *Gracilaria*.

### Acknowledgment

The authors would like to express their thanks for the support of production of biofuels from algae and plants project (Implemented and funded by Helwan Univ. A18252).



**Fig.1:** Cytotoxic activity of the methanolic extracts of A: *Chlorella vulgaris*, B: *Microcystis* sp. and C: *Gracilaria dendroides* against HepG-2, MCF-7 and CaCo-2 cell lines

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## المكونات النشطة بيولوجيا من مستخلصات ثلاثة أنواع من الطحالب ونشاطها المضاد للسرطان ضد خطوط الخلايا السرطانية البشرية

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تم إجراء هذه الدراسة للتعرف على المكونات النشطة بيولوجيا للطحلب الأحمر *Gracilaria dendroides* ولأثنين من الطحالب الدقيقة *Chlorella vulgaris* (طحالب خضراء) و *Microcystis sp.* (طحالب خضراء مزرقة) من خلال تحليل GC / Mass و HPLC و عمل دراسة في المختبر لمعابنة النشاط السام لخلايا الأنواع الثلاثة بعد الاستخلاص بمذيب الميثانول ضد ثلاثة أنواع من خطوط الخلايا السرطانية البشرية (الكبد HEPG-2 ، القولون ، والثدي MCV-7). تم قياس تأثير المستخلصات على تكاثر خطوط الخلايا عن طريق طريقة MTT اللونية. وقد أظهرت النتائج أن عدد المركبات المحددة بواسطة GC-MS كانت 19،11 و 12 في الجلاسيلاريا، الكلوريليا ثم الميكروسيستس على التوالي. وكانت المركبات الأكثر وفرة هي الأحماض الدهنية ، واسترات الميثيل و terpenoids. أما بالنسبة لتحليل HPLC حدد 4 مركبات في جراسيلاريا و 6 مركبات في كلا من الكلوريليا و الميكروسيستس. أظهر المستخلص الخام للجلاسيلاريا أقوى نشاط على خطوط الخلايا HepG-2 بقيمة IC50 تساوى 15.46 ميكروجرام / مل. فيما يتعلق بخطوط الخلايا MCF-7 ، كان مستخلص الخام الأكثر قوة هو مستخلص الكلوريليا (IC50 تساوى 15.53 ميكروجرام / مل). من ناحية أخرى ، أظهر تقييم نشاط السمية الخلوية لمستخلصات أنواع الطحالب الثلاثة على خلايا الورم الحميد للقولون والمستقيم الظهاري (CaCO-2) نشاطاً أعلى للكلوريليا حيث كانت قيمة IC50 تساوى 14.63 ميكروجرام / مل. وفقاً لذلك ، يمكننا القول أن الكلوريليا كان له أقوى نشاط على خطوط الخلايا MCF-7 و (CaCO-2) بينما خطوط الخلايا HepG-2 هي الأكثر تأثراً بمستخلص الجراسيلاريا.