Macroalgae of Izmir Gulf: *Dictyotaceae* exhibit high in vitro anti-cancer activity independent from their antioxidant capabilities

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Abstract In this study, 24 marine macroalgae collected from the coastline of Izmir Gulf were examined for their antioxidant activities and their effects on cell proliferation. Crude extracts were obtained from samples with cold methanol extraction. Antioxidant activity was evaluated as 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity and total phenolic content (TPC); growth inhibitory effects of the extracts were determined by using WST-8. Amongst the species, *Polysiphonia denuata* (Rhodophyta) and *Cystoseira* species (*Phaeophyceae*) have been noticed by their high DPPH radical scavenging activities and TPCs. As expected, there was a strong correlation between these tests. *Dictyota dichotoma* (*Phaeophyceae*) showed the highest anti-cancer activity on MCF-7 cells with an IC_{50} of 17.2 ng mL^{-1} . Flow cytometry analyses demonstrated that *D. dichotoma* methanolic extract strongly induced apoptosis.

This extract exhibited moderate viability inhibition on MCF10A cells (IC_{50} : 49.3 ng mL^{-1}), suggesting a potential use of the extracts or its compounds for cancer therapy. There was no correlation between anti-cancer potential and antioxidant content of the extracts.

Keywords Macroalgae · Antioxidant activity · Anti-cancer activity · MCF-7 · MCF10A

Introduction

The medical potential of natural products is a focus of attention as they are inexpensive to produce and have relatively fewer side effects compared to drugs (Lee et al. 2010; Mohamed et al. 2012). Macroalgae are prolific sources for numerous natural compounds with promising potential for both prevention (Lee et al. 2013) and treatment of several diseases including cancer (Hiroishi et al. 2001; Rademaker-Lakhai et al. 2005) and diabetes (Nwosu et al. 2011). Many studies have attributed beneficial effects of macroalgae to their high antioxidant capacity (Zhang et al. 2007; Zubia et al. 2009; Murugan and Iyer 2013) thus naming their consumption “antioxidant phytomedicine” (Scalbert et al. 2005; Yuan and Walsh 2006; Gupta and Abu-Ghannam 2011; Nwosu et al. 2011). Dietary antioxidant intake and decreased oxidative stress are

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known to reduce the risk of type II diabetes (Valko et al. 2004) and cancer (Montonen et al. 2004) as both conditions are associated with oxidative stress. Since oxidative stress plays a role in many other diseases including neurodegenerative diseases and cardiovascular disease we can argue that antioxidant phytomedicine is an important treatment option that needs to be studied in detail.

Anti-cancer activity of the compounds derived from macroalgae is also studied extensively, demonstrating reduced cell viability and induction of apoptosis in cancer cells (Athukorala et al. 2006; Kong et al. 2009; Yang et al. 2010; Kang et al. 2012) and being used in clinical trials to treat different types of cancer (von Schwarzenberg and Vollmar 2013; Beesoo et al. 2014). Macroalgal crude extracts or their products have high cytotoxic activity on brain, renal and colon tumors (Fuller et al. 1992), inhibit the growth and invasion of several tumor cell lines, impedes lung metastasis and prolongs survival in mice (Hiroishi et al. 2001) or lead to apoptotic body formation in the CT-26 murine colon cancer cell line (Athukorala et al. 2006).

The role of antioxidants in cancer prevention and treatment is a double-edged sword. Anticancer activities of antioxidants are investigated thoroughly revealing the most common mechanism of action as the fenton reaction in which antioxidants at higher concentrations act as oxidants to induce apoptotic cell death in cancer cells (Matés and Sánchez-Jiménez 2000). On the other hand endogenous antioxidant capacity of cancer cells is known to be high, possibly to protect cells from oxidative stress induced cell death (Sun 1990). It is also clear that oxidative stress induced DNA damage, which can be prevented with antioxidants, is one of the most prominent drivers of the carcinogenesis (Valko et al. 2006). Taken together these findings suggest a complex relationship between antioxidants and cancer prevention/treatment, and that anti-cancer activities of natural antioxidants should be studied carefully.

Even though there are many studies conducted on macroalgae, there is no information with respect to the anti-cancer potential of the macroalgal members of the Gulf of Izmir. Therefore, in this study, we aimed to reveal their antioxidant and anti-cancer potential and the relationship between them.

Materials and methods

Collection of macroalgal material

Marine macroalgal species were collected from coastlines of Izmir Gulf, Republic of Turkey from March 2011 to March 2012. Specimens in the labelled plastic bags had an approximate wet weight of 0.5–1 kg and were immediately transported to the laboratory in cooled containers. To remove sand and epiphytes, algal thalli were gently rinsed in seawater. Taxonomic identification was done in terms of their morphology. Specimens had been stored at -20°C until experiments begun. The voucher specimens are preserved in the Herbarium of Ege University.

Preparation of macroalgal extracts

Wet tissues from each species were lyophilized. 1 g of powder was extracted with 10 mL of methanol by shaking in an orbital shaker at 300 rpm overnight at $+4^{\circ}\text{C}$. Extracts were then centrifuged at 4000 rpm for 10 min and supernatants were collected. Collected supernatants were placed in orbital shaker and extraction was performed again. This procedure had been repeated for 10 days. After 10 days of extraction/centrifugation cycle, solvent was removed by lyophilization and powders of the crude extracts were dissolved in DMSO (dimethyl sulphoxide; 10 mg crude extract per mL; Sigma-Aldrich, St. Louis, MO, USA) and stored at -20°C for further experiments.

Determination of DPPH radical scavenging activity

The free radical scavenging activity was measured by using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH; Sigma-Aldrich) method (Sharma and Bhat 2009). Briefly, 100 μL of 50 μM DPPH solution was added into 96-well plates containing 100 μL of sample (5 mg mL^{-1}) solutions or 100 μL of positive control ascorbate (10 $\mu\text{g mL}^{-1}$). After 30 min, the decline in absorbance was recorded at 520 nm against an individual blank. All measurements were repeated at least for two times and mean absorbance values were used to evaluate results. The capability to scavenge the

DPPH radical was calculated using the following equation:

$$\begin{aligned} \text{Scavenging activity}(\%) \\ = (\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}) \\ / \text{Absorbance}_{\text{blank}} \times 100 \end{aligned}$$

Determination of total phenol content

The total phenolic contents of macroalgae were determined by using Folin-Ciocalteu assay (Zhang et al. 2007) in 96-well plates. Methanol extracts (10 mg mL^{-1}) were loaded into 96-well plate containing $90 \mu\text{L}$ of deionized water and $10 \mu\text{L}$ 2N Folin–Ciocalteu's reagent (Sigma-Aldrich) in each well. Plates were incubated for 5 min at room temperature. After incubation $100 \mu\text{L}$ of sodium carbonate (7 %) was added to each well. Plates were shaken in the dark at room temperature for 90 min. The absorbance of the formed color was measured at 750 nm. All measurements were repeated three times and mean absorbance values were used to evaluate results. A calibration curve of gallic acid (ranging from 0.010 to 200 mg mL^{-1} ; Sigma-Aldrich) was drawn, and the results were expressed as mg Gallic Acid (GA) equivalents' ratio to dry weight (g).

Cell culture

All studied cell lines were kindly provided by Ege University, Medical School Department of Medical Oncology. Breast cancer cell line MCF-7 was cultured in RPMI medium (Lonza, Basel, CH) with 10 % fetal bovine serum (Lonza) and penicillin–streptomycin ($100 \text{ U mL}^{-1}/100 \mu\text{g mL}^{-1}$; Sigma-Aldrich) supplement. Immortalized mammary epithelial cell line MCF-10A was cultured in DMEM medium (Lonza) supplemented with 10 % fetal bovine serum (Lonza), penicillin–streptomycin ($100 \text{ U mL}^{-1}/100 \mu\text{g mL}^{-1}$; Sigma-Aldrich), EGF (20 ng mL^{-1} epidermal growth factor; New England Biolabs, Ipswich, MA, USA), hydrocortisone ($0.5 \mu\text{g mL}^{-1}$; Biochrom, Berlin, Germany) and insulin ($10 \mu\text{g mL}^{-1}$; Sigma-Aldrich). All cell lines were maintained in CO_2 incubator with standard incubation conditions. Cells were treated with different concentrations of macroalgal methanol extracts reconstituted in dimethyl sulphoxide (DMSO;

Sigma-Aldrich) and diluted with proper media for viability experiments. For each experimental procedure, appropriate concentration of vehicle (DMSO) was used as a carrier control.

Inhibition of cell viability

Growth inhibitory effects of extracts were investigated via mitochondrial dehydrogenase activity using the WST-8 colorimetric assay kit (Sigma-Aldrich). Briefly, MCF7 cells were seeded in a 96-well plate 10×10^3 cells per well and incubated overnight for both cell attachment and growth. Macroalgal extracts were added to wells in different final concentrations (0, 1, 5, 10, 25, 50 and $100 \mu\text{g mL}^{-1}$) with six repeats. Maximum DMSO concentration was 1 %. After 72 h of incubation, $10 \mu\text{L}$ of WST-8 reagent was added to wells and plates were incubated at 37°C for 15 min for the formation of color. Absorbance was measured at 450 nm on multi-well spectrophotometer. Untreated cell viability was considered as 100 % and extract- treated cell viabilities were calculated accordingly. IC_{50} (inhibitory concentration 50) values were calculated using CalcuSyn v2.0. A lower concentration range was used to calculate IC_{50} of *Dictyota dichotoma* extract which showed a high growth inhibitory potential.

Flow cytometric detection of apoptosis

To determine apoptosis, MCF-7 cells were treated with *D. dichotoma* methanol extract (17.25 ng mL^{-1}) for 72 h. Both treated and non-treated samples were washed with PBS, and stained with Annexin V- FITC and PI according to manufacturer's protocol of the Apoptosis Detection Kit (BD, Franklin Lakes, NJ, USA). Stained cells were analysed by flow cytometry (BD FACS Aria, USA). Annexin-V positive cells were considered apoptotic and cell percentages were calculated accordingly.

Statistical analyses

All statistical analyses were performed with GraphPad Prism 5.03 software. Spearman assay was used to evaluate correlations. Correlations with *P* values lower than 0.05 were considered significant. IC_{50} values were determined by CalcuSyn 2.0 software.

Results

Macroalgal material

Samples of 24 macroalgae belonging to *Chlorophyta*, *Rhodophyta* and *Phaeophyceae* were examined in the study. The scientific names, collection dates, collection places of specimens, their coordinates and IC₅₀ values are presented together with their voucher IDs in Table 1.

Phaeophyceae demonstrated high antioxidant capabilities

Our results suggest a high antioxidant capacity demonstrated via DPPH radical scavenging ability (Fig. 1a) and phenolic content for many of the extracts studied (Fig. 1b). Members of the *Phaeophyceae* especially demonstrated high antioxidant capabilities: while six of the ten highest DPPH scavengers were members of *Phaeophyceae*, seven of the ten highest gallic acid equivalence were also detected in *Phaeophyceae* members (Fig. 1a, b). *Polysiphonia variegata* (EGE41707) demonstrated highest antioxidant potential, scavenging 86.73 % of DPPH radical, followed by *Cystoseira crinita* (EGE41722), which scavenged 79.36 % of DPPH. Both of them had high phenolic contents: 245.94 304.38 mg GAE g⁻¹ dw, respectively. Highest phenolic content (570.31 mg GAE g⁻¹ dw) was detected in *Palisada perforata* (EGE41725), which scavenged 38.12 % of DPPH radical. It was followed by *Cystoseria barbata* (EGE41719) with a 465.31 mg GAE g⁻¹ dw and 58.22 % scavenging activity.

DPPH scavenging activity and total phenolic content of the samples correlated significantly (Fig. 2a) with a *P* value lower than 0.0001 (Spearman *r* = 0.8008), as expected.

Dictyotaceae members showed high anti-cancer activities in vitro

Inhibition of MCF-7 breast cancer cell line viability by 50 µg mL⁻¹ of the extracts is presented in Fig. 1c and IC₅₀ values calculated for each extract are shown in Table 1. Highest in vitro anti-cancer activity was determined for *D. dichotoma* (EGE41715), a member of *Phaeophyceae*. It inhibited almost all MCF-7 viability (91.32 %) at a concentration of 50 µg mL⁻¹. *D. dichotoma* was followed by *Laurencia obtusa* of

Rhodophyta (EGE41726) which inhibited 82.86 % of MFC-7 viability. Third most effective extract was that of *Dictyota spiralis* (EGE41708), another member of *Phaeophyceae*, with an inhibition rate of 82.14 % cell viability.

Since *D. dichotoma* extract was the only one effective at the nanogram scale, we have run another experiment using lower concentrations (10–100 ng mL⁻¹) of the extract to determine its effect on MCF-7 cancer cells and the MFC-10A normal breast epithelial cell line (Fig. 3). Extract was more effective against MCF-7 cells compared to MCF-10A cells (Fig. 3). We have then calculated the IC₅₀ of the extract as 17.2 ng mL⁻¹ for MCF-7, and used that concentration for flow cytometric apoptosis analysis to determine the mechanism of growth inhibition. Flow cytometric Annexin-V/PI analysis of the extract revealed induction of apoptosis approximately in 90 % of the cells (Fig. 4).

Antioxidant potentials of *D. dichotoma* (EGE41715), *L. obtusa* (EGE41726) and *D. spiralis* (EGE41708) were either low or moderate compared to others (Table 1.). There was no correlation between inhibition of viability and antioxidant capabilities (Fig. 2b, c).

Discussion

Antioxidant and anti-cancer properties of macroalgal extracts is a popular research topic due to their potential use as pharmaceuticals, phytomedical drugs, cosmetic products as well as food supplements (Murugan and Iyer 2013). In this study we have evaluated the antioxidant and anti-cancer effects of marine macroalgae collected from Izmir Gulf which is located in the western part of the Anatolian Peninsula and is partially separated from Aegean Sea by Karaburun Peninsula (Sayin 2003).

Our results suggest that extracts of macroalgae especially *Phaeophyceae* members demonstrated high antioxidant capabilities which was shown both as DPPH scavenging capacity and total phenol content. Antioxidant capabilities correlated significantly with phenolic content in accordance with current knowledge (Yuan and Walsh 2006; Farasat et al. 2014). However antioxidant capabilities and anti-cancer abilities did not correlate well. Antioxidants are known to act as pro-oxidants and inhibit cell viability via Fenton reaction (Dai and Mumper 2010) exhibiting anti-cancer properties. In our study extracts with

Table 1 Macroalgal data

Familia	Voucher ID	Species	Date	Station	Coordinates	TPC (mg GA g ⁻¹ dw) ±SD	DPPH (%) ±SD	IC ₅₀ (μg mL ⁻¹)
<i>Ulveaceae</i>	EGE41706	<i>Ulva linza</i> Linnaeus	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	76.56 ± 8.40	23.12 ± 3.01	199.95
	EGE41705	<i>Ulva rigida</i> C.Agardh	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	132.50 ± 9.72	43.66 ± 1.82	41.05
	EGE41711	<i>Codium fragile</i> (Suringar) Hariot	September 2011	Urla	38°08'57,52"N26°46'49,10"E	18.75 ± 0.88	0.79 ± 1.12	28.49
<i>Cladophoraceae</i>	EGE41703	<i>Chaetomorpha aerea</i> (Dillwyn) Kützinger	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	90.63 ± 1.77	4.01 ± 2.87	ND
	EGE41700	<i>Cladophora</i> sp.	March 2012	Urla	38°08'57,52"N26°46'49,10"E	63.44 ± 3.09	0.45 ± 0.35	ND
	EGE41704	<i>Osmundea pinnatifida</i> (Hudson) Stackhouse	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	128.13 ± 4.42	32.92 ± 0.91	ND
<i>Rhodomeleaceae</i>	EGE41726	<i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux	March 2012	Urla	38°22'04,21"N26°49'45,90"E	190.31 ± 1.33	9.60 ± 4.48	99.1
	EGE41725	<i>Palisada perforata</i> (Bory de Saint-Vincent) K.W.Nam	March 2012	Urla	38°28'20,32"N26°36'45,97"E	571.31 ± 21.26	38.12 ± 0.28	70.4
	EGE41707	<i>Polysiphonia denudata</i> (Dillwyn) Greville ex Harvey	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	245.94 ± 1.33	86.73 ± 0.14	ND
<i>Gracilariaceae</i>	EGE41712	<i>Acanthophora nayadiformis</i> (Delile) Papenfuss	September 2011	Urla	38°08'57,52"N26°46'49,10"E	30.94 ± 2.21	1.83 ± 0.63	21.04
	EGE41701	<i>Gracilaria gracilis</i> (Stackhouse) M.Steenhof, L.M.Irvine & W.F.Farnham	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	152.19 ± 9.28	10.05 ± 2.73	ND
<i>Ceramiales</i>	EGE41716	<i>Ceramium virgatum</i> Roth	March 2012	Urla	38°22'04,21"N26°49'45,90"E	109.69 ± 1.33	7.48 ± 3.99	89.9
	EGE41717	<i>Gelidium spinosum</i> (S.G. Gmelin) P.C. Silva	March 2012	Urla	38°22'04,21"N26°49'45,90"E	65.31 ± 2.21	0.99 ± 1.40	ND
	EGE41702	<i>Gelidium pulchellum</i> (Turner) Kützinger	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	71.56 ± 3.09	16.63 ± 1.82	ND
<i>Scytosiphonaceae</i>	EGE41709	<i>Petatonia fasciata</i> (O.F.Müller) Kuntze	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	173.75 ± 3.54	16.14 ± 5.04	720.1
	EGE41710	<i>Scytosiphon lomentaria</i> (Lyngbye) Link	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	169.38 ± 2.65	41.09 ± 0.70	ND
	EGE41833	<i>Colpomenia sinuosa</i> (Mertens ex Roth) Derbès & Solier	September 2011	Urla	38°08'57,52"N26°46'49,10"E	15.94 ± 1.33	0.00 ± 0.00	ND
<i>Sypocaulaceae</i>	EGE41834	<i>Padina pavonica</i> (Linnaeus) Thivy	September 2011	Urla	38°08'57,52"N26°46'49,10"E	251.56 ± 1.33	70.50 ± 1.40	320.3
	EGE41708	<i>Dictyota spiralis</i> Montagne	March 2011	Inciraltı	38°24'39,16"N27°02'13,67"E	102.81 ± 10.16	9.36 ± 6.93	35.9
	EGE41715	<i>Dictyota dichotoma</i> (Hudson) J.V. Lamouroux	March 2012	Urla	38°22'04,21"N26°49'45,90"E	177.50 ± 0.00	4.01 ± 4.13	0.0172
<i>Sargassaceae</i>	EGE41718	<i>Halopteris flicina</i> (Grateloup) Kützinger	March 2012	Urla	38°22'04,21"N26°49'45,90"E	284.69 ± 11.93	47.33 ± 46.91	ND
	EGE41719	<i>Cystoseira barbata</i> (Stackhouse) C. Agardh	March 2012	Urla	38°18'57,52"N26°42'08,00"E	465.31 ± 8.40	58.22 ± 2.66	ND
	EGE41721	<i>Cystoseira compressa</i> (Esper) Gerloff & Nizamuddin	March 2012	Urla	38°18'57,52"N26°42'08,00"E	277.81 ± 9.28	65.00 ± 0.77	ND
<i>Sargassaceae</i>	EGE41722	<i>Cystoseira crinita</i> Duby	March 2012	Urla	38°18'57,52"N26°42'08,00"E	304.38 ± 0.00	79.36 ± 0.35	ND

TPC, Total phenolic content of the extract

DPPH, Inhibition percentage of extract for 2,2-diphenyl-1-picryl-hydrazyl radical

IC₅₀, the half maximal inhibitory concentration of extract

Fig. 1 *Phaeophyceae* has higher antioxidant capacities. DPPH radical scavenging activity was calculated as percentage inhibition against ascorbate (a) and TPC was expressed as mg GAE per dry weight (b). Macroalgal methanolic extracts have variable effects on MCF-7 cell viability at 50 $\mu\text{g mL}^{-1}$ (c). Cells were seeded onto 96-well plates, incubated overnight for attachment and treated with increasing concentrations of the extract. Viability was determined with WST-8 reagent according to the manufacturer's instructions. Viability of untreated cells was considered as 100 % and viability of treated cells were calculated accordingly. Error bars represent standard deviation. Each of macroalgal species was presented with their voucher ID number

the highest inhibitory potential on MCF-7 breast cancer cell viability had low to moderate DPPH scavenging activity as well as low to moderate phenolic content. Similarly extracts with highest antioxidant capacities demonstrated relatively low viability inhibitory potential. Taken together these results suggest that the pro-oxidant behavior of antioxidants is not sufficient to explain anti-cancer activities of the extracts. The role of antioxidant in cancer therapy is a subject of debate. While there are numerous studies focusing growth inhibitory potential

of antioxidant compounds (Murugan and Iyer 2013; Guner et al. 2015), there are others claiming a protective role for those substances as cancer cells are known to require antioxidants for their survival (Jeon et al. 2012; Lee et al. 2013; Mezghani et al. 2013). Surely oxidative stress is one of the most common inducers of carcinogenesis (Valko et al. 2006), however it is also known that once transformation is completed, cancer cells utilize antioxidant machinery to prevent further damage caused by reactive oxygen species (Saeidnia and Abdollahi

Fig. 2 A significant correlation is observed between total phenolic content and DPPH radical scavenging activity. DPPH and TPC values of the samples were evaluated for Spearman correlation. Results suggest a strong correlation between parameters with a P value (two-tailed) less than 0.0001 and r : 0.8006 (a). There is no correlation between inhibition of cell viability and DPPH or TFC (b), (c)

2013). Protection of the cells by antioxidant components of the extracts may explain the lack of correlation between antioxidant and anti-cancer capacities of the extracts in our experiments.

Dictyota dichotoma, a member of *Phaeophyceae*, exhibited highest anti-cancer capability with an IC_{50} of 17.3 ng mL^{-1} which is very low (suggesting a very high anti-cancer potential) compared to all other extracts. Further studies revealed the mechanism of viability inhibition as apoptosis and that almost all of

Fig. 3 *D. dichotoma* strongly inhibits viability of MCF7 cells and is less effective against MFC-10A, normal breast epithelial cells. Cells were seeded onto 96-well plates, incubated overnight for attachment and treated with increasing concentrations of the extract. Viability was determined with WST-8 reagent according to the manufacturer's instructions. Viability of untreated cells was considered as 100 % and viability of treated cells were calculated accordingly. Error bars represent standard deviation

the cells undergo apoptosis following treatment of cells with the indicated concentrations of extracts. It is also important to note that the extract was less effective on MCF-10A normal breast epithelial cells, proposing a potential chemotherapeutic use for the extract or for the active compounds it contains. In a previous study, *D. dichotoma* was suggested to have an anti-cancer effect on lung and cervix cancer cell lines and leukemia cell line (Guedes et al. 2013). The investigators have tested the effects of different extracts including chloroform, dichloromethane, methanol and water extracts on the mentioned cell lines and determined high IC_{50} values (microgram per mL scale) compared to our results in all experiments. The lack of effectiveness of the extracts would either be due to the cell lines or the extraction method used. It is known that different cancer cells may react differently to the same stimuli and it is also known that cold extraction may produce a different composition protecting heat sensitive compounds compared to room temperature extraction (Heffernan et al. 2014). In another study, dichloromethane extract of *D. dichotoma* was found to have an IC_{50} of 600 ng mL^{-1} in MCF-7 cell line, a concentration 35 fold higher than what we have determined. Since the same cell line is used in both studies, the difference should be attributed to the extraction method used which is Soxhlet extraction for the mentioned study (Abou-El-Wafa et al. 2013). Another member of *Dictyotaceae* family, *Dictyota*

Fig. 4 *D. dichotoma* methanolic extract induces apoptosis in MCF-7 cells. Cells were treated with *D. dichotoma* extract for 72 h, collected, stained with Annexin-V-FITC and propidium iodide, and 20,000 cells were counted in flow cytometer. **(a)** Non-treated cells, **(b)** DMSO control, **(c)** 17.2 ng mL⁻¹ *D. dichotoma* methanolic extract-treated. *Left panels* shows flow cytometer graphs: each *dot* represents a cell, Q3 represents viable (low FITC, low PI signal), Q1 represents necrotic (low FITC, high PI) and Q2 + Q3 represents apoptotic (high FITC) cell populations. *Right panels* demonstrate percentages of relevant cell populations

cilliolata, was also tested for its anticancer potential in different cell lines including MCF-7 (Caamal-Fuentes et al. 2014) and in this study, the extract was effective only at microgram per mL concentrations. Furthermore in several studies focusing on the anti-cancer potential of pure compounds isolated from *Dictyota* species in different cell lines including MCF-7, IC₅₀ values calculated for all of the compounds were at microgram per mL scale (Jongaramruong and Kongkam 2007; Ayyad et al. 2011; Abou-El-Wafa et al. 2013; Pardo-Vargas et al. 2014). Since our extract was much more effective compared to all counterparts and to all pure compounds mentioned, we believe either the extract itself or the active compounds of the extract have a strong potential to be used as anti-cancer agent.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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