Four selected commercial seaweeds: biologically active compounds, antioxidant and cytotoxic properties

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ABSTRACT

The aim of this research work was to study the chemical characterisation, antioxidant and cytotoxic activity of ethanolic extracts of four commercial algae species Arame, Kombu, Hijiki and Wakame. The highest scavenging activity has been observed in Arame extract. Antioxidant potential of all extracts was in correlation with total phenol content (Arame extract: 319.15 ± 0.56 mg GAE/g d.w) and it was not in correlation with total carotenoids content (Wakame: 75.15 ± 0.20 mg/g). Polyphenols were quantified using LC-MS/MS technique. Baicalein and amentoflavone were identified in higher amount in relation to other phenols. Intracellular antioxidant activity and cytotoxicity of algae extracts were evaluated on the human prostate cancer cell line PC3. Although presented biomolecules in the extracts have demonstrated *in vitro* antioxidant activity, they did not show a significant effect on PC3 cells. However, this study opens up broad perspective for the further comprehensive investigation of these, commercial, seaweed's biopotential.

Introduction

Marine algae (seaweeds) represent a rich treasure of nutrients and bioactive compounds (Rodríguez-Bernaldo de Quirós et al. 2010; Stengel et al. 2011; Machu et al. 2015; Corsetto et al. 2020). Although they have been a part of the traditional diet of the Eastern people for centuries, their popularity is growing among the people of the Western world. They have a wide range of application in food, pharmaceutical and also cosmetic industry and contain several times more minerals, vitamins and numerous bioactive compounds that contribute to their pharmacological activity than terrestrial plants. According to their comprehensive healing properties (Wang et al. 2017), many researchers call them the food of the future. They have the ability to increase metabolism and purify blood from various toxins and heavy metals (Holdt and Kraan 2011). The seaweed world is diverse. The algal taxonomists try to discover new, interesting species every day. They are characterised by the type of pigments: red (Rhodophyta), brown (Phaeophyceae), green (Chlorophyta) and their appearance as well as chemical and nutritional compositiondepends on the place of growing, the depths of the sea, and others. Chlorophyll occurs only in green algae, carotenoid fucoxanthin is responsible for the colour of brown algae and phycoerythrin gives red algae their colour (Osório et al. 2020). The carotenoid fucoxanthin has beneficial functions and prevents and reduces the risk of various diseases (Kim and Pangestuti 2011). The brown seaweed originating in Japan such as Arame (*Ecklonia bicyclis*), Kombu (*Laminaria japonica*), Hijiki (*Sargassum fusiforme*), and Wakame (*Undaria pinnatifida*) are present in stores and markets around the world. The pharmacological activity of mentioned algae comes from the high content of diverse biologically active compounds such as phenolics, polysaccharides, carotenoids (Peng et al. 2011) and others (Stengel et al. 2011; Cardoso et al. 2015). They are classified into functional food given that they promote good health and reduce risk of different chronic diseases (Smit 2004; Holdt and Kraan 2011). In the last few years we have witnessed growing interest in use marine algae for anticancer propose, but these kinds of researches arestill limited. Thus, investigations related to the potential use of these species in the treatment of prostate cancer are particularly popular.

The incidence of prostate cancer within Asian population used to be much lower than in the Western population, due to genetic and life style differences. East and Southeast Asians consume more seafood, vegetables, with a lower proportion of animal protein and high-fat food in their daily intake (Chen et al. 2014).

Arame (A) is a species that inhabits the cold waters of the coasts of South Korea and Japan. It is mainly used in Asian cuisine but also represented in other country. They have slightly sweet taste and are increasingly used as salads, soups and as a supplement to various cooked vegetables combined with different seeds and tofu. It is known to the people as "sea oak" because their appearance resembles a list of leaves (Ermakova et al. 2013).

This species also belongs to the family of brown, marine macroalgae. It is characterised by an unusual structure in processing and consumption that is not characteristic of multiple plants. This property is conditioned by the high content of active components which possess a wide range of pharmacological activities such as antioxidant (Cornish and Garbary 2010; Vadlapudi et al. 2012), anti-inflammatory (Jung et al. 2013), anticancer (Ermakova et al. 2013), antiviral (Thomas and Kim 2011). Further, since it contains a high amount of calcium, Arame stimulates calcification of bones (Onofrejová et al. 2010; Mišurcová et al. 2012). Anti-allergic property of this species it is also described and is attributed to the existence of phlorotannins that have an impact on the immune response of the organism (Wijesekara et al. 2010).

Kombu (K) can be found in Japan, China, Russia, Australia, South Africa and Canada on rocky shores during the fall, at depths of 2–3 m attached to the rocks, but also at depths of 8–30 m (Mediterranean Sea). Since it is characterised by the content of all the necessary nutrients that are needed by modern populations in the world (Stengel et al. 2011), *L. japonica* is a highly regarded dietary supplement. Its composition includes a wide range of compounds and minerals for which it has a privileged status in nutrition. Regular consumption increases the intake of iodine and calcium absorption that acts on the secretion of angiotensin and other hormones involved in the regulation of blood pressure and kidney function, which prevents hypertension and renal dysfunction (Cardoso et al. 2015). It significantly reduces blood cholesterol level, indeed it is prescribed for patients with cardiovascular diseases (Yuan and Walsh 2006). This species showed potential antitumor activity on cervical carcinoma in mice and also inhibits proliferative activity on melanoma cell line (Lu et al. 2013; Peng et al. 2013; Zhai et al. 2014). Further, enzymatic hydrolysate exhibited antioxidant, neuro-protective and anti-inflammatory activities (Sevevirathne et al. 2012). Previous study indicated that this species inhibits proliferation of colon rectal cancer cells (Moussavou et al. 2014).

Hijiki (H) is a thin needle-like alga that is harvested in the spring when it is thought to be full of taste and has the most effective pharmacological effects (Machu et al. 2015). This species is primarily found in the waters along the coast of Japan, China and Hong Kong. Dry plant has a shape of black threads characterised by a bitter taste that are prepared in various ways like salads or cooked (Wells et al. 2017). It has traditionally been used in the fight against cancer (Okai et al. 1998). Some studies have shown that it could be used for therapeutic purposes due to the content of polysaccharides which possess antitumor (Chen et al. 2012; Zhu et al. 2016), antioxidant (Luo et al. 2010; Jin et al. 2014) and anti-hyperlipidemic activity, which improve immunity system (Chen et al. 2016). It is also characterised by activity in the prevention of osteoporosis because the algae extracts showed suppression of differentiation of osteoclasts and accelerated formation of osteoblasts in *in vitro* experiments (Machu et al. 2015).

Wakame (W) is very popular type of macroalgae cultivated in many countries of the world, especially in Japan, Korea and certain regions of China, France and Australia. This species is most commonly used in everyday diet, like salad, or it is an essential ingredient of the miso soup. Wakame belongs to the group of brown algae (Phaeophyceae) and is one of the best-studied representatives of marine macroalgae. *In vivo* studies carried out on mice have shown that this type of brown algae, when taken in the daily diet, sparking decreasing of the triacylglycerol concentration in the liver and serum by increasing the activity of the enzymes involved in the oxidation of fatty acids in the mitochondria (Liu et al. 2012). Also, antioxidant effect of some bioactive compounds in Wakame extract was noticed (Je et al. 2009; Fung et al. 2013). Several studies have reported on cytotoxic and anti-proliferative effects of polysaccharide fucoidan isolated from this species (Boo et al. 2013; Wang et al. 2014; Han et al. 2016).

Although carotenoids and polysaccharides are the most studied biocompounds present in marine brown algae that have significant pharmacological activity and beneficial effect on human health, these species also contain polyphenols that have very attractive

biological features. There are several studies that have addressed this topic (Jimenez-Escrig et al. 2001; Yuan and Walsh 2006; Rodríguez-Bernaldo de Quirós et al. 2010; Cox et al. 2014; Martelli et al. 2020). Therefore, the purposes of the present study were to determine phenolic compounds in these marine species and to investigate their antioxidant and potential anticancer activity, in order to establish the importance of brown algae utilisation in human diet and nutrition.

Materials and methods

Algae material and extracts preparation

Dry algae samples (*E. bicycles*, *S. fusiforme*, *L. japonica* and *U. Pinnatifida*), used in this study, were bought in Serbian Health food store. Every tested species was produced in Tokyo, Japan (MITOKU co. LTD). The ethanolic extracts (EtOH) were prepared as follows: 5 g of each algae species was mixed with 100 mL of 70% ethanol and macerated for 72 h at room temperature using incubator shaker (IKA®). After filtration, extracts were evaporated to dryness under reduced pressure at 40 °C (Buchi R-210, Switzerland). Finally, the residues were dissolved in DMSO to achieve the stock concentration of 100 mg/mL. All extracts were stored at +4 °C prior to the analysis.

LC-MS/MS analysis of phenolic compounds

The quantification of the selected phenolic compounds (Table 1) in ethanolic extracts of the four algae species was carried out using the LC–MS/MS method published by Orčić et al. (2014). To obtain the high selectivity and sensitivity, the selected reactions monitoring (SRM) acquisition mode was used,

Table 1. LC-MS/MS data for quantification of standard compounds.

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	Fragmentor voltage (V)
Hinic acid	0.52	191	85	20	150
Galic acid	0.61	169	125	10	90
Catechin	0.75	289	245	10	150
Protocatechuic acid	0.80	153	109	9	105
Epigallocatechingallate	0.80	457	169	16	165
Chlorogenic acid	0.84	353	191	10	100
Epicatechin	1.01	289	245	10	150
2,5-Dihydroxybenzoic acid	1.05	153	109	9	100
p-Hydroxybenzoic acid	1.11	137	93	10	80
Aesculetin	1.12	177	133	15	105
Caffeic acid	1.17	179	135	10	100
Vanillic acid.	1.26	167	108	15	100
Syringic acid	1.32	197	182	7	90
p-Coumaric acid	1.68	163	119	9	90
Scopoletin	1.73	191	176	8	80
Umbelliferon	1.76	161	133	19	120
Ferulic acid	1.89	193	134	11	90
Sinapic acid	1.89	223	193	17	100
Vitexin	1.91	431	311	22	200
Luteolin 7-O-glucoside	2.15	447	285	30	230
Hyperoside	2.18	463	300	30	200
Rutin	2.21	609	300	42	135
Quercetin 3-O-glucoside	2.25	463	300	30	210
Apiin	2.61	563	269	36	250
o-Coumaric acid	2.63	163	119	5	100
Myricetin	2.70	317	179	20	150
Apigenin 7-O-glucoside	2.71	447	284	30	190
Quercitrin	2.78	447	300	27	190
Kaempferol 3-O-glucoside	2.85	431	268	41	135
Secoisolariciresinol	2.87	361	165	26	130
3,4-Dimethoxycinnamic acid	3.00	207	103	7	110
Baicalin	3.36	445	269	22	140
Daidzein	3.43	253	208	31	145
Matairesinol	3.66	357	122	24	130
Quercetin	3.68	301	151	15	130
Naringenin	3.86	271	151	16	130
Cinnamic acid	3.91	147	103	5	100
Luteolin	3.96	285	133	25	135
Genistein	4.13	269	133	32	145
Kaempferol	4.52	285	285	0	130
Apigenin	4.64	269	117	25	130
Isorhamnetin	4.79	315	300	21	160
Chrysoeriol	4.81	299	284	20	125
Baicalein	5.15	269	269	0	165
Amentoflavone	5.81	537	375	35	220

due to the fact that only ions specific to the targeted analytes were monitored. Extracts and standards were analysed using Agilent Technologies 1200 Series high-performance liquid chromatograph coupled with Agilent Technologies 6410 A Triple Quad tandem mass spectrometer with electrospray ion source, and controlled by Agilent Technologies MassHunter Workstation software-Data Acquisition (ver. B.03.01). Compounds were separated on Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 µm) rapid resolution column held at 50 °C. Mobile phase was delivered at flow rate of 1 mL/min in gradientmode (0 min 30% B, 6 min 70% B, 9 min 100% B, 12 min 100% B, re-equilibration time 3 min). Eluted compounds were detected by MS, using the ion source parameters as follows: nebulisation gas (N_2) pressure 40 psi, drying gas (N_2) flow 9 L/min and temperature 350 °C, capillary voltage 4 kV, negative polarity. Data were acquired in dynamic MRM mode, using the optimised compound-specific parameters (retention time, precursor ion, product ion, fragmentor voltage, collision voltage) given in previously published research by Orčić et al. (2014). For all the compounds, peak areas were determined using Agilent MassHunter Workstation software Qualitative Analysis (ver. B.03.01). Calibration curves were plotted and concentrations of samples calculated using the OriginLabs Origin Pro (ver. 8.0) software.

Determination of total phenol content (TPC)

The total phenolic content (TPC) in algae extracts of four algae species was determined with Folin-Ciolcateu reagent according to the method of Singleton et al. (1999). Standard curve was prepared for gallic acid (GA), and TPC was expressed as milligrams of gallic acid equivalents per g dry weight (mg GAE/g d.w.).

Determination of total carotenoid content (TCC)

To determinate the amount of total carotenoid content (TCC), approximately 0.1 g of each grinded algae sample was weighted in glass tubes and added 10 mL of cold 80% (v/w) acetone (de Carvalho et al. 2012). The mixture was shakenfor 10 minutes at 25 °C, using Vortex shaker (IKA®Vortex 3). The supernatant was filtered using Buchner flask under vacuum, made up to the 10 mL (the initial volume) and the procedure was repeated four times (until the samples became colorless). The experiment was performed in dark. The absorbance was read at 440 nm using MultiscanMicroplate Spectrophotometer (Thermo Scientific®). The total carotenoid content was calculated using modified equation: TCC (mg/g) = $A \times V$ (mL) $\times 10^4 / A_{1cm}^{1\%} \times P$(g), where: A-absorbance (440 nm); V-total extract volume; $A_{1cm}^{1\%}$ – specific extinction coefficient in acetone; P-sample weight.

Antioxidant activity assays

Spectrophotometric determination of free radical scavenging capacity (RSC) was measured according to the methods described by Espin et al. 2000 (DPPH assay) and Arnao et al. 2001 (ABTS assay). Antioxidant potential of the extracts in both assays was expressed as milligrams of Trolox equivalents per gram of dry weight (mg TE/g d.w.), calculated according to the Trolox standard calibration curve.

The reducing power was determined according to method of Benzie and Strain (1999) and expressed as mg of ascorbic acid equivalents per g of dry weight (mg AAE/g d.w.).

All the assays which were used for evaluation of scavenging effect, reducing power and determination of total phenolics in the extracts were carried out in triplicate and the mean values were calculated.

Cell culture

Human androgen-independent prostate cancer cell line PC3 was established from grade 4 prostatic adeno-carcinoma from 62 years old Caucasian male and derived from the metastatic site in bones. Cells were cultured in 25 cm^2 flasks at 37° C in a 5% CO_2 humidified atmosphere. The culture medium consisted of RPMI-1640 supplemented with 10-% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 100 IU/mL penicillin and 100 mg/mL streptomycin.

Cell growth inhibition assay

Living cells were measured by a colorimetric 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-s ulfophenyl)-2H-tetrazolium (MTS) assay (CellTiter 96 Aqueous One Solution Assay, Promega, USA). PC3 cells were seeded at 5×10^3 in a 96-well plate in complete culture medium. After 24 h, the medium was removed from the wells by aspiration and the cells were treated with designed concentrations of algae extracts (0.01–30 ug/mL) for 72 h at 37 °C in a humidified chamber. The total medium volume in each well was 200 µL. Each condition was repeated in quadruplicate. Equal amount of DMSO

(0.1% v/V) was used in control wells. MTS reagent (20 µL, 10 mg/mL in PBS) was added to each well and incubated for 1 additional hour. Absorbance was recorded on a microplate reader at 490 nm. Data were analysed using GraphPad Prism 7.

Cellular antioxidant activity (CAA) assay

Intracellular antioxidant activity exerted by algae extract was detected modifying the method of Wolf and Liu 2007. Namely, PC3 cells were seeded at a density of 2.0×10^4/well on a 96-well microplate in 100 µL of growth medium/well. The peripheral wells of the plate were not used. 48 h after seeding, the growth medium was removed and the cells were washed with 100 µL PBS. After this step, the cells were treated with 100 µL of uncompleted DMEM supplemented with 2 mM glutamine and 25 µM DCFH2-DA without (blank and control) or with algae extracts (0.01–10µg/mL) for 1 h in incubator. The solutions were removed by aspiration, and the cells were washed with PBS. Then 5 mM ABAP in HBSS was added. Samples treated with (HBSS) alone were used as blanks. Samples were maintained in incubator for further 60 min before endpoint fluorescence measurement (λ_{Em} =538 nm; λ_{Ex} =485 nm).

In order to quench the extracellular fluorescence, 50 µL of 0.033% Trypan blue solution in HBSS were added to each well. For all sample condition, 6 replicates were used. Quantification of CAA: after blank subtraction, the CAA value at each experimental condition was calculated as follows: CAA unit (%) = 100-(AFU Sample/AFU Control) x 100, where AFU is arbitrary fluorescence unit.

Statistical analysis

All the results are expressed as means values ± standard deviation (SD) of three different trials. The data were subjected to analysis of analysis of variance (one-way ANOVA) and a probability value of $p < 0.01$ was considered significant. The differences between experimental and control samples were determined via Tukey's test.

Results and discussion

In order to quantify amounts of the selected phenol compounds, all tested edible algae extracts were analysed by LC-MS/MS technique. The obtained results presented in Table 2. The detected polyphenols possess plenty biological activity such as antioxidant and anticancer potential, which was observed on different human cancer cell lines (Chan et al. 2000; Zhang et al. 2012). The results presented in the Table 2 showed that amentoflavone is the most abundant and dominant phenol compound occurring in Kombu (1365.96 ng/mL), followed by baicalein that was determined in Arame (1179.15 ng/mL), Kombu (1272.81 ng/mL) and Wakame (1227.80 ng/mL) in similar amount. Amentoflavone has been reported as an antioxidant, anti-inflammatory and anti-diabetes agents, while there were evidences that baicalin has appreciable ability to inhibit the growth of various type of cancers (Liu et al. 2016; Yu et al. 2017). Further, several phenolic acids were noticed in the algae samples. Gallic acid is a strong antioxidant which was found in all algae samples in notable amount (109.08–236.95 ng/mL). This acid has also earlier reported as abundant bioactive compound in brown algae extracts (Rodríguez-Bernaldo de Quirós

Table 2. Phenolic quantification using LC-MS/MS technique.

		Amount of compound detected (ng/mL of algae sample)			
Class	Compound	Arame	Kombu	Hijiki	Wakame
Hydroxybenzoic acids	Gallic acid	149.59 ± 0.06	111.17 ± 0.1	236.95 ± 0.33	109.08 ± 0.31
	Protocatechuic acid	10.05 ± 0.02	12.28 ± 1.19	42.55 ± 0.01	7.06 ± 0.08
	p-Hydroxybenzoic acid	n.d.	2.34 ± 1.27	52.43 ± 0.21	17.89 ± 1.19
	Caffeic acid	n.d.	n.d.	35.04 ± 0.02	36.41 ± 0.02
Cyclohex.Acid	Quinic acid	82.35 ± 0.03	n.d.	113.34 ± 0.02	n.d.
Hydroxycinnamic acids	p-Coumaric acid	n.d.	2.02 ± 0.03	n.d.	n.d.
Flavones	Apiin	n.d.	4.53 ± 2.09	n.d.	n.d.
	Baicalein	1179.15 ± 0.06	1272.81 ± 0.	n.d.	1227.80 ± 0.9
	Chysoeriol	n.d.	>6	n.d.	>6
Flavanon	Liquiritigenin	3.66 ± 0.12	10.86 ± 0.50	n.d.	7.02 ± 2.10
	Isoliquiritigenin	5.30 ± 0.03	25.87 ± 0.11	n.d.	14.56 ± 0.37
Biflavonoid	Amentoflavone	321.46 ± 0.03	1365.96 ± 0.12	321.46 ± 1.13	437.47 ± 1.88
Isoflavonoids	Formononetin	1.33 ± 0.11	3.90 ± 0.04	n.d.	2.79 ± 0.01
	Diosmetin	2.74 ± 0.27	15.02 ± 0.08	1.45 ± 2.09	7.05 ± 0.03
Flavonols	Quercetin-hexosides	7.94 ± 0.97	10.67 ± 1.07	n.d.	9.25 ± 2.22
	Quercitrin	8.51 ± 0.66	9.44 ± 0.12	n.d.	8.59 ± 0.14
	Kaempferol	>98	>98	>98	>98

Concentration is lower than the LoQ (limit of qui), but higher than the LoD (limit of detection); n.d.-not detected

Table 3. Antioxidant activity, TP and TC contentantioxidant activities of Arame, Kombu, HijikiandWakame extracts.

	Arame	Kombu	Hijiki	Wakame
DPPH(mg TE/g d.w.)	24.68 ± 0.93^{a}	1.34 ± 0.14^{c}	11.15 ± 1.33^{b}	1.95 ± 0.05^{c}
ABTS (mg TE/g d.w.)	55.53 ± 0.22^{a}	0.66 ± 0.15^{b}	53.80 ± 2.90^{a}	0.73 ± 0.08^{b}
FRAP (mgAAE/g d.w.)	90.78 ± 0.55^{a}	1.90 ± 0.05^{c}	32.59 ± 1.48^{b}	2.62 ± 0.04^{c}
TPC (mg GAE g/d.w.)	319.15 ± 0.56^{a}	2.77 ± 0.05^{d}	23.53 ± 1.15^{b}	7.90 ± 0.14^{c}
TCC (mg/g)	38.46 ± 0.38^{b}	26.96 ± 0.31^{d}	33.75 ± 0.13^{c}	75.15 ± 0.20^{a}

TPC- total phenol content; TCC-total carotenoid content. Data are reported as mean ± SD of triplicates
[a–d] Different letters in the same row indicate significant difference between extracts ($p < 0.01$)

et al. 2010). This acid possesses a strong cytotoxic activity on prostate and fibrosarcoma cell lines (Russel et al. 2011; Filipiak et al. 2014). In some previous study (Machu et al. 2015), the content of phenolic acids investigated in brown algae such as protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, caffeic acid (73–1800 ng/mL) was generally higher than that in the present study (2.34–236.95 ng/mL) (Table 2).

The lowest amount of all selected phenolics was detected in the brown algae Hijiki, although the content of the hydroxybenzoic acids were higher than in other extracts.

Total content of antioxidants and antioxidant potential of selected algae extracts

Given their increasingly commercial applications, interest in studying biological activity of brown sea macroalgae is growing. A wide range of various bioactive compounds is responsible for their high antioxidant activity. The results of examined antioxidant activity, total phenol and carotenoid content were presented in Table 3. Based on the obtained results, it can be observed that there were significant differences in the content of total phenols as well as carotenoid content among the extracts. TPC is the highest in extract of Arame (319.15 mg GAE/g d.w.) and the lowest in the extract of Kombu (2.77 mg GAE/g d.w). It has earlier reported that the highest total phenol content was noticed in Arame species (Machu et al. 2015; Sivagnanam et al. 2015). Martelli et al. showed in their study that quantities of phenolics found in brown algae extracts were significant lower in respect to the other seaweed extracts (red and green algae)(Martelli et al. 2020). The TPC in their study ranged from 0.15 to 1.5 mg GAE in brown algae extracts. The total phenol content in brown algae species was lower or similar in respect to the results in this study (Chandini et al. 2008; Cox et al. 2014) in other reports. On the basis of the obtained results, it was noticed that the best "scavenging" activity to DPPH and ABTS radical forms and reduction potential was noticed in Arame, following by extract of Hijiki which also showed good antioxidant potential (Eom et al. 2012; Machu et al. 2015). Some previous studies show that extracts of brown algae were effective in scavenging DPPH and hydroxyl radical (Chandini et al. 2008). The results of antioxidant potential indicated that antioxidant capacity and total phenolic content is not correlated to the phenolic composition of algae extracts performed by LC-MS/MS technique. The highest sum amount of all quantified phenol compounds was increasing in following order Hijiki < Arame < Wakame < Kombu, respectively.

In the case of the total carotenoid content, the highest amount was observed in Wakame extract and it was significant higher compared to other three extracts. One of the most abundant carotenoid in brown algae is fucoxanthin and many biological properties, such as antioxidant, can be attributed to this bioactive compound (Kim and Pangestuti 2011). There can easily be concluded that the content phenolics and total carotenoids was not significantly responsible for antioxidant potential, but amount of these biological active compounds could contribute to some other biological or pharmacological activities of these algae species.

Cytotoxicity and cellular antioxidant activity

PC3 cells are androgen-independent cell line, they are a model for prostatic small cell neuroendocrine carcinoma (SCNC), a variant form of prostate cancer. PC3 xenografttumors proliferate rapidly and are more invasive compared to other prostate cancer (Tai et al. 2011).

Anti-proliferative effect of algae extracts on PC3 prostate cancer cell line was evaluated. As shown in Figure 1, the algae extracts did not show significant effect on cell viability at any concentration applied. No data are available in the literature concerning the cytotoxicity of these extracts, neither in PC3 nor in other types of cells, so it is not possible to compare them with other results. Furthermore, it was not possible to increase the concentrations of the extracts due to the toxic effect of DMSO.

It has been described that PC3, when compared with other prostate cancer cells, generically produce higher levels of reactive oxygen species, such as H_2O_2 and superoxide anion radical, and that these are responsible for cell proliferation and vitality (Kumar et al. 2008). The ethanolic extracts of four edible macroalgae were used in order to evaluate the antioxidant potential in PC3 cancer cells. In our experimental conditions, low concentration of extract was used in order to mimic the serum physiological amount.

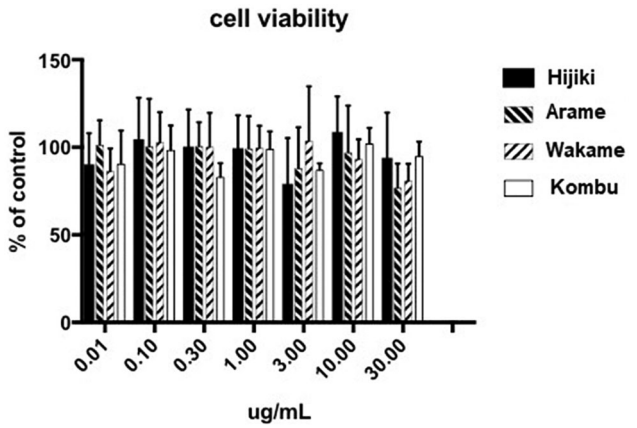

Figure 1. Cytotoxic effect of algae extractson PC3 cell line. Data are means of 2 indipendent experiment with 4 replicates for each condition. Data are expressedas % of control (100% viability).

Figure 2 shows the data concerning the antioxidant effect of ethanolic extracts in PC3 cells. Again, we have not seen any statistically significant antioxidant effect. Due to the higher production of ROS, it has been shown that in such cells there is a greater concentration of reduced glutathione and a higher activity of glutathione reductase (GR). Together, they can help to balance endogenous and exogenous oxidative stress (Freitas et al. 2012).

Indeed, Koren and colleagues have highlighted how the administration of small molecules with antioxidant activity, though absorbed by the cells (also the PC3), do not lead to an effective change/improvement in the cell's capacity to respond to an oxidative insult (Koren et al. 2008).

The hypothesis proposed states that the total antioxidant capacity of cells is maintained under tight regulation and is not easily modulated (Koren et al. 2008).

Conclusion

Although the significant amount of phenolics and carotenoids have been detected in Kombu and Wakame

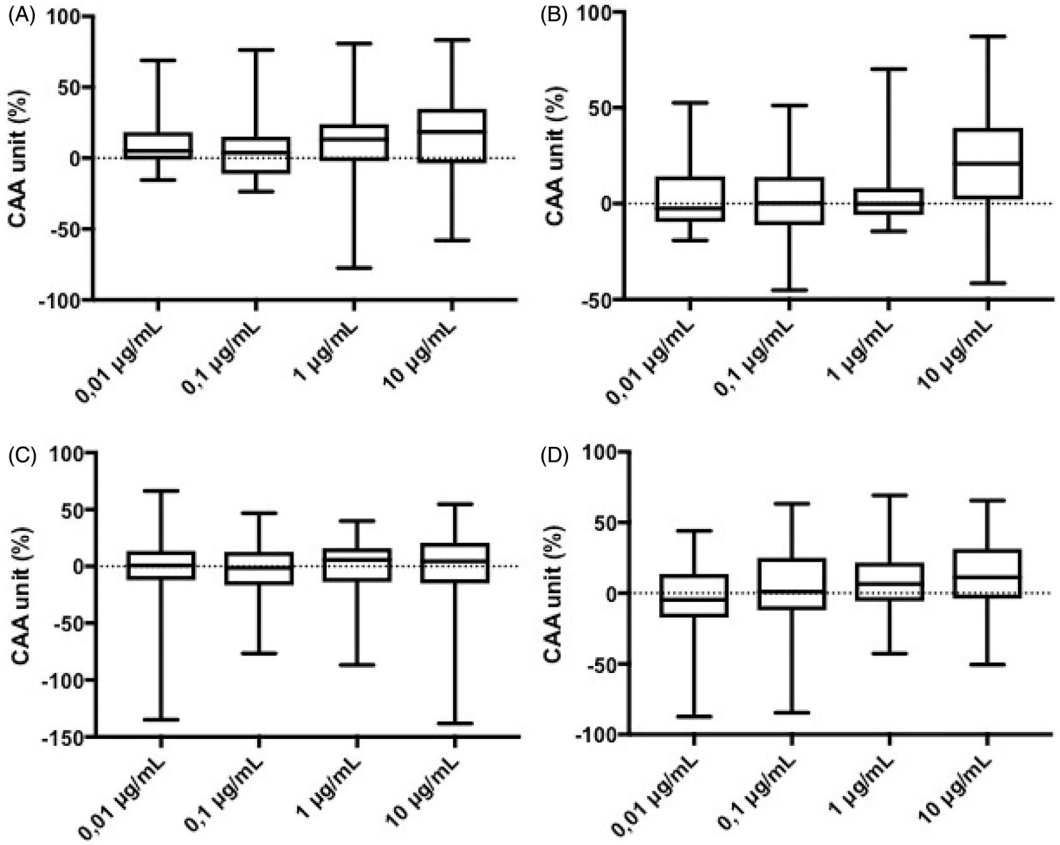

Figure 2. Intracellular antioxidant activity of of Arame (A), Kombu (B), Hijiki (H) and Wakame (D) extracts.

extracts, Arame extract was recognised to possess the greatest antioxidant activity. Thus, the new opportunities for expanding investigations are opened in terms of more detailed chemical characterisation of tested marine seaweed extracts. Although the extracts of commercial marine algae investigated in this study have not shown a significant antioxidant and cytotoxic effects on prostate cancer cell lines (PC3), these results show that these extracts are not harmful, thus they could be safely included in human diet as functional food. For their content of bioactive compounds, these species should be further investigated for their safety and potential beneficial effects on other cell types. Thus, they could contribute to the development of some new bioactive compounds and their application in some disease prevention.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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