Saudi Journal of Biological Sciences (2015) 22, 390–397

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Biological activities of two macroalgae from Adriatic coast of Montenegro

Marijana Kosanić [a,*], Branislav Ranković [a], Tatjana Stanojković [b]

[a] Department of Biology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, Kragujevac, Serbia
[b] Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia

Received 5 August 2014; revised 23 October 2014; accepted 3 November 2014
Available online 13 November 2014

KEYWORDS

Anticancer activity;
Antimicrobial activity;
Antioxidant activity;
Algae;
Ulva lactuca;
Enteromorpha intestinalis

Abstract In the present investigation the acetone extracts of macroalgae *Ulva lactuca* and *Enteromorpha intestinalis* were tested for antioxidant, antimicrobial and cytotoxic potential. Antioxidant activity was evaluated by measuring the scavenging capacity of tested samples on DPPH and superoxide anion radicals, reducing the power of samples and determination of total phenolic and flavonoid compounds in extracts. As a result of the study, *U. lactuca* extract was found to have a better free radical scavenging activity (IC_{50} = 623.58 μg/ml) than *E. intestinalis* extract (IC_{50} = 732.12 μg/ml). Moreover, the tested extracts had effective ferric reducing power and superoxide anion radical scavenging. The total content of phenol in extracts of *U. lactuca* and *E. intestinalis* was 58.15 and 40.68 μg PE/mg, while concentrations of flavonoids were 39.58 and 21.74 μg RE/mg, respectively. Furthermore, among the tested species, extracts of *U. lactuca* showed a better antimicrobial activity with minimum inhibitory concentration values ranging from 0.156 to 5 mg/ml, but it was relatively weak in comparison with standard antibiotics. *Bacillus mycoides and Bacillus subtilis* were the most susceptible to the tested extracts. Contrary to this *Aspergillus flavus*, *Aspergillus fumigatus* and *Penicillium purpurescens* were the most resistant. Finally, cytotoxic activity of tested extracts was evaluated on four human cancer cell lines. Extract of *E. intestinalis* expressed the stronger cytotoxic activity towards all tested cell lines with IC_{50} values ranging from 74.73 to 155.39 μg/ml.

1. Introduction

Marine organisms are source materials for structurally unique natural products with pharmacological and biological activities (Faulkner, 2001). Among the marine organisms, the macroalgae (seaweeds) occupy an important place as a source of biomedical compounds (Manilal et al., 2010). Marine macroalgae are the most interesting algae group because of their broad spectrum of biological activities such as antimicrobial (Zbakh

* Corresponding author. Tel.: +381 34336223; fax: +381 34335040.
E-mail address: marijanakosanic@yahoo.com (M. Kosanić).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2014.11.004
1319-562X

et al., 2012), antiviral (Bouhlal et al., 2011), anti-allergic (Na et al., 2005), anticoagulant (Dayong et al., 2008), anticancer (Kim et al., 2011), antifouling (Bhadury and Wright, 2004) and antioxidant activities (Devi et al., 2011). As an aid to protect themselves against other organisms in their environment, macroalgae produce a wide variety of chemically active metabolites including alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols that have a broad range of biological activities (Al-Saif et al., 2014). Some of these metabolites such as iodine, carotene, glycerol, alginates, and carrageenans have been used in pharmaceutical industries (Kharkwal et al., 2012; Kolanjinathan et al., 2014).

Ulva lactuca and *Enteromorpha intestinalis* are members of green macroalgae known as chlorophyceae. These macroalgae have already been studied for antioxidant and antimicrobial activities, but information on the anticancer activity is limited. Antioxidative potential was proved by measuring the 2,2-diphenyl-1-picrylhydrasyl (DPPH) radical scavenging activity, total content of phenolic compounds in extracts, reducing activity, superoxide anion scavenging activity and inhibition of lipid peroxidation (Zubia et al., 2007; Al-Amoudi et al., 2009). Their antioxidant activities have been attributed to various reactions and mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity, radical scavenging, etc. The previous studies of antimicrobial activity of *U. lactuca* and *E. intestinalis* (Soltani et al., 2012; Kolanjinathan and Stella, 2011) also showed promising antimicrobial activity against numerous bacterial, fungal, human, animal and plant pathogens, mycotoxin producers, and food spoilage agents.

In order to justify the long-term usage of algae as a potential food source as well as "medicinal plants", in this study we determined antioxidant, antimicrobial and anticancer activities in *U. lactuca* and *E. intestinalis* acetone extracts.

2. Material and methods

2.1. Algal samples

Algal samples of *U. lactuca* (Linnaeus) and *E. intestinalis* (Linnaeus) Nees were collected from the Adriatic sea, in June 2012. The voucher specimen of the algae (Voucher nos. 54 and 58) was deposited at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia. Identification of the tested algae was carried out with standard keys from Hardy and Guiry (2006) and Abbott and Hollenberg (1992).

2.2. Preparation of the algal extracts

Finely ground dry thalli of the examined algae (100 g) were extracted using acetone (500 ml) in a Soxhlet extractor. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The Dry extracts were stored at −18 °C until they were used in the tests. Dimethyl sulphoxide (DMSO) was previously diluted in sterile distilled water to a concentration of 5%. The extracts were then dissolved in 5% DMSO for further testing.

2.3. Antioxidant activity

2.3.1. Scavenging DPPH radicals

The free radical scavenging activity of samples was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) as described by Dorman et al. (2004) with slight modifications. Two millilitres of methanol solution of DPPH radical in the concentration of 126.8 μM and 1 ml of test samples (1000, 500, 250, 125 and 62.5 μg/ml) were placed in cuvettes. The mixture was then shaken vigorously and was allowed to stand at room temperature for 30 min. DPPH solution is initially violet in colour which fades when antioxidants donate hydrogen. The change in colour is monitored by spectrophotometer ("Jenway" UK) at 517 nm against methanol as blank. Ascorbic acid was used as a positive control. Experiment was performed in triplicate. The DPPH radical concentration was calculated using the following equation:

$$\text{DDPH scavenging effect } (\%) = [(A0 - A1)/A0] \times 100$$

where A0 is the absorbance of the negative control (2 ml of methanol solution of DPPH radical + 1 ml of 5% DMSO) and A1 is the absorbance of reaction mixture or standard.

For both extracts and ascorbic acid, the inhibitory concentration (IC_{50}) at 50% was determined.

2.3.2. Ferric reducing power

The ferric reducing power of samples was determined according to the method of Oyaizu (1986). One millilitre of test samples (1000, 500, 250, 125 and 62.5 μg/ml) was mixed with 2.5 ml of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixtures were incubated at 50 °C for 20 min. Then, trichloroacetic acid (10%, 2.5 ml) was added to the mixture and the sample was centrifuged. Finally, the upper layer was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml; 0.1%). The absorbance of the solution was measured at 700 nm in a spectrophotometer ("Jenway" UK). Blank was prepared with all the reaction agents without extracts. Higher absorbance of the reaction mixture indicated that the reducing power was increased. Ascorbic acid was used as a positive control. Experiment was performed in triplicate.

2.3.3. Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of samples was detected according to the method of Nishimiki et al. (1972). Briefly, 0.1 ml of test samples (1000, 500, 250, 125 and 62.5 μg/ml) was mixed with 1 ml nitroblue tetrazolium (NBT) solution (156 μM in 0.1 M phosphate buffer, pH 7.4) and 1 ml nicotinamide adenine dinucleotide (NADH) solution (468 μM in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 100 μl of phenazine methosulphate (PMS) solution (60 μM in 0.1 M phosphate buffer, pH 7.4). The mixture was incubated at room temperature for 5 min, and the absorbance was measured at 560 nm in a spectrophotometer ("Jenway" UK) against the blank sample (phosphate buffer). Decreased absorbance indicated increased superoxide anion radical scavenging activity. Ascorbic acid was used as a positive control. Experiment was performed in triplicate. The inhibition percentage of superoxide anion generation was calculated using the following formula:

$$\text{Superoxide anion scavenging activity } (\%) = [(A0 - A1)/A0] \times 100$$

where A0 is the absorbance of the negative control (consisting of all of the reaction agents except the extract) and A1 is the absorbance of reaction mixture or standard.

For both extracts and ascorbic acid, the inhibitory concentration (IC_{50}) at 50% was determined.

2.4. Determination of total phenolic compounds

Total soluble phenolic compounds in the acetone extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1997) using pyrocatechol as a standard phenolic compound. Briefly, 1 ml of the extract (1 mg/ml) in a volumetric flask was diluted with distilled water (46 ml). One millilitre of 2 N Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, 3 ml of sodium carbonate (2%) was added and then allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (''Jenway'' UK). Experiment was performed in triplicate. The total concentration of phenolic compounds in the extract was determined as micrograms of pyrocatechol equivalents (PE) per milligram of dry extract by using the following equation that was obtained from a standard pyrocatechol graph as follows:

$$\text{Absorbance} = 0.0057 \times \text{total phenols } [\mu\text{g PE/mg of dry extracts}] - 0.1646$$

$$(R^2 = 0.9203)$$

2.5. Total flavonoid content

The total flavonoid content was determined using the Dowd method (Meda et al., 2005). Two millilitres of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (1 mg/ml). The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 415 nm in a spectrophotometer (''Jenway'' UK) against blank samples. Experiment was performed in triplicate. The total flavonoid content was determined as micrograms of rutin equivalents (RE) per milligram of dry extracts by using the following equation that was obtained from a standard rutin graph as follows:

$$\text{Absorbance} = 0.0296 \times \text{total flavonoid } [\mu\text{g RE/mg of dry extracts}] + 0.0204$$

$$(R^2 = 0.9595)$$

2.6. Antimicrobial activity

The bacteria used in this study were *Bacillus mycoides* (ATCC 6462), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 13883) were obtained from the American Type Culture Collection (ATCC). The fungi used as test organisms were: *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigatus* (ATCC 1022), *Candida albicans* (ATCC 10231), *Penicillium purpurescens* (DBFS 418) and *Penicillium verrucosum* (DBFS 262). They were from the American Type Culture Collection (ATCC) and the mycological collection maintained by the Mycological Laboratory within the Department of Biology of Kragujevac University's Faculty of Science (DBFS). Bacterial cultures were maintained on Müller-Hinton agar substrates (Torlak, Belgrade). Fungal cultures were maintained on potato dextrose (PD) agar and Sabourad dextrose (SD) agar (Torlak, Belgrade). All cultures were stored at 4 °C and subcultured every 15 days.

Bacterial inocula were obtained from bacterial cultures incubated for 24 h at 37 °C on Müller-Hinton agar substrate and brought up by dilution according to the 0.5 McFarland standard to approximately 10^8 CFU/ml (Andrews, 2005). Suspensions of fungal spores were prepared from fresh mature (3- to 7-day-old) cultures that grew at 30 °C on a PD agar substrate. Spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and then further diluted to approximately 10^6 CFU/ml according to the procedure recommended by NCCLS (1998).

The minimal inhibitory concentration (MIC) was determined by the broth microdilution method by using 96-well micro-titre plates (Sarker et al., 2007). A series of dilutions with concentrations ranging from 40 to 0.0047 mg/ml for extracts were used in the experiment against every microorganism tested. The starting solutions of test samples were obtained by measuring off a certain quantity of samples and dissolving this in DMSO. Twofold dilutions of test samples were prepared in Müller-Hinton broth for bacterial cultures and SD broth for fungal cultures. The MIC was determined with resazurin. Resazurin is an oxidation–reduction indicator used for the evaluation of microbial growth. It is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. The boundary dilution without any change in colour of resazurin was defined as the MIC for the tested microorganism at the given concentration. Streptomycin (for bacteria) and ketoconazole (for fungi) were used as the standards. The solvent control test has been performed to study an effect of 5% DMSO on the growth of a microorganism. All experiments were performed in triplicate.

2.7. Cytotoxic activity

2.7.1. Cell culture

Human colon carcinoma LS174 cells, human lung carcinoma A549 cells, malignant melanoma Fem-x cells and chronic myelogeneous leukaemia K562 cells (American Type Culture Collection, USA) were cultured as a monolayer in the RPMI 1640 nutrient medium, with 10% (inactivated at 56 °C) FBS, 3 mM of L-glutamine, and antibiotics, at 37 °C in humidified air atmosphere with 5% CO_2.

2.7.2. In vitro cytotoxic assay

In vitro assay for cytotoxic activity of investigated extracts was performed when the cells reached 70–80% confluence. Stock solution (50 mg/ml) of extracts was dissolved in the corresponding medium to the required working concentrations. Neoplastic LS174 cells (7000 cells per well), A549 cells (5000 cells per well), Fem-x cells (5000 cells per well) and K562 cells (5000 cells per well) were seeded into 96-well microtitre plates.

24 h later, after cell adherence, five different concentrations of investigated extracts were added to the wells, except for the control cells to which a nutrient medium was added only. Final concentrations achieved in treated wells were 200, 100, 50, 25, and 12.5 μg/ml. The cultures were incubated for the next 72 h. The effect on cancer cell survival was determined 72 h after the addition of extract using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Briefly, 20 μl of MTT solution (5 mg/ml PBS) was added to each well and incubated for a further 4 h at 37 °C in 5% CO_2 and humidified air. Subsequently, 100 μl of 10% SDS was added to solubilise the formazan crystals formed from MTT after the conversion by mitochondrial dehydrogenases of viable cells. Absorbencies proportional to the number of viable cells were measured using a microplate reader (Multiskan EX, Thermo Scientific, Finland) at 570 nm. The final concentration of DMSO solvent never exceeded 0.5%, which was non-toxic to the cells. Each experiment was performed in triplicate and independently repeated at least four times.

2.8. Flow cytometry analysis

Cellular DNA content and cell distribution were quantified by flow cytometry using propidium iodide (PI). Cells (3×10^5 cells/well) were seeded in 6-well plates and incubated with or without IC_{50} concentration of investigated extract for 24 h. After treatment, the cells were collected by trypsinisation, and fixed in ice-cold 70% ethanol at −20 °C overnight. After fixation, the cells were washed in PBS and pellets obtained by centrifugation was treated with RNase (100 μg/ml) at 37 °C temperature for 30 min and then incubated with propidium iodide (PI) (40 μg/ml) for at least 30 min. DNA content and cell cycle distribution were analysed using a Becton Dickinson FAC-Scan flow cytometer. Flow cytometry analysis was performed using a CellQuestR (Becton Dickinson, San Jose, CA, USA), on a minimum of 10,000 cells per sample (Clothier, 1995).

2.9. Data analyses

Data analyses were performed by using the EXCEL (Microsoft 2007) software and SPSS version 13.0 for Windows 2007. To determine the statistical significance of antioxidant activity, student's *t*-test was used. All values are expressed as mean ± SD of three parallel measurements.

3. Results

The scavenging DPPH radicals, superoxide anion radical scavenging and ferric reducing power of the studied extracts are represented in Tables 1 and 2. The extract from *U. lactuca* showed better antioxidant activities than *E. intestinalis*. In various antioxidant activities, there was a statistically significant difference between extracts and control ($P < 0.05$).

The total phenolic and flavonoid contents of the algal extracts are given in Table 3. The total phenolic content in extracts of *U. lactuca* and *E. intestinalis* was 58.15 and 40.68 μg PE/mg, while concentration of flavonoids was 39.58 and 21.74 μg RE/mg, respectively.

The antimicrobial activity of the algal extracts against the test microorganisms is shown in Table 4. The extract from *U. lactuca* inhibited all of the tested microorganisms, at concentrations ranging from 0.156 to 5 mg/ml. Extracts from *E. intestinalis* also inhibited all of the tested microorganisms, but at slightly higher concentrations.

The antimicrobial activity was compared with the standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi). The results showed that standard antibiotics had stronger activity than the tested samples as shown in Table 4. In the negative control, DMSO had no inhibitory effect on the tested organisms.

The data obtained for anticancer effect of *E. intestinalis* and *U. lactuca* extracts are shown in Table 5. *E. intestinalis* extract showed a much weaker cytotoxic activity against

Table 1 DPPH radical scavenging activity and superoxide anion scavenging activity of acetone extracts of *Ulva lactuca* and *Enteromorpha intestinalis*.

Algal species	DPPH radical scavenging IC_{50} (μg/ml)	Superoxide anion scavenging IC_{50} (μg/ml)
U. lactuca	623.58 ± 2.35**	785.48 ± 2.02**
E. intestinalis	732.12 ± 2.93**	913.52 ± 2.73**
Ascorbic acid	6.42 ± 0.18	115.61 ± 1.16

Values are expressed as mean ± SD of three parallel measurements. Significance was determined by using student's *t*-test.
** $P < 0.005$ compared to control.

Table 2 Ferric reducing power of acetone extracts of *Ulva lactuca* and *Enteromorpha intestinalis*.

Algal species	Absorbance (700 nm)				
	1000 μg/ml	500 μg/ml	250 μg/ml	125 μg/ml	62.5 μg/ml
U. lactuca	0.4686 ± .028**	0.3682 ± .024**	0.2862 ± .009**	0.1467 ± .006**	0.0894 ± .003**
E. intestinalis	0.3715 ± .015**	0.3123 ± .011**	0.2069 ± .008**	0.0969 ± .004**	0.0563 ± .003*
Ascorbic acid	2.113 ± .032	1.654 ± .021	0.0957 ± .008	0.0478 ± .008	0.0297 ± .004

Values are expressed as mean ± SD of three parallel measurements. Significance was determined by using student's *t*-test.
* $P < 0.05$.
** $P < 0.005$ compared to control.

Table 3 Total phenolics and flavonoid contents of acetone extracts of *Ulva lactuca* and *Enteromorpha intestinalis*.

Algal species	Phenolics content (μg PE/mg of extract)	Flavonoid content (μg RE/mg of extract)
U. lactuca	58.15 ± 1.065	39.58 ± 1.099
E. intestinalis	40.68 ± 1.013	21.74 ± 1.078

Values are expressed as mean ± SD of three parallel measurements. PE – pyrocatechol equivalents. RE – rutin equivalents. Significance was determined by using student's *t*-test. Means in each column are significantly different at $P < 0.05$.

Table 4 Minimum inhibitory concentration (MIC) of acetone extracts of *Ulva lactuca* and *Enteromorpha intestinalis*.

Algal species	*U. lactuca*	*E. intestinalis*	DMSO	S	K
Bacillus mycoides	0.156	0.312	na	7.81	–
Bacillus subtilis	0.312	0.156	na	7.81	–
Escherichia coli	2.5	5	na	31.25	–
Klebsiella pneumoniae	1.25	2.5	na	1.95	–
Staphylococcus aureus	0.625	1.25	na	31.25	–
Aspergillus flavus	5	10	na	–	3.9
Aspergillus fumigatus	2.5	10	na	–	3.9
Candida albicans	1.25	2.5	na	–	1.95
Penicillium purpurescens	5	10	na	–	3.9
Penicillium verrucosum	5	5	na	–	3.9

Values given as mg/ml for tested samples and as μg/ml for antibiotics. Values are the mean of three replicates. Antibiotics: K – ketoconazole, S – streptomycin, na: not active.

Table 5 Growth inhibitory effects of acetone extracts of *Ulva lactuca* and *Enteromorpha intestinalis* on Fem-x, A549, LS174 and K562 cell lines.

Cell lines Algal species	Fem-x IC_{50} (μg/ml)	A549	LS174	K562
U. lactuca	93.31 ± 0.74	> 200	> 200	169.54 ± 0.45
E. intestinalis	74.73 ± 0.58	155.39 ± 2.36	114.48 ± 1.37	82.24 ± 1.09
Cis-DDP	0.86 ± 0.33	4.91 ± 0.42	3.18 ± 0.29	2.22 ± 0.08

A459 and LS174 cells, whereas in Fem-x and K562 cells showed a significantly better to moderate cytotoxic effect (IC_{50} 74.73, and 82.24 μg/ml, respectively). *U. lactuca* extract shows very weak activity against Fem-x and K562 cells while cytotoxic activity against A569 and LS174 cells was not detected (IC_{50} > 200 mg/ml). Furthermore, both extracts showed significantly less activity compared to cis-DDP as a positive control.

Results of cell cycle analysis of cancer cell lines treated with extracts for 24 h are given in Fig. 1. We checked whether the cytotoxic effect was associated with any cell cycle phase-specific arrest. After treatment with *E. intestinalis* and *U. lactuca*,

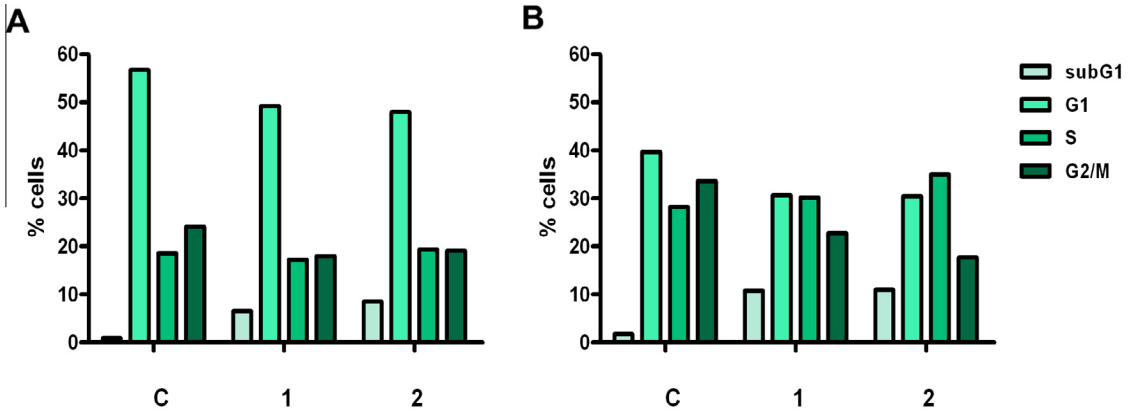

Figure 1 Effect of extracts on cell cycle phase distribution. Fem-x (A) and K562 (B) cell lines were exposed to extracts *Ulva lactuca* (1) and *Enteromorpha intestinalis* (2) (IC_{50} μg/ml) for 24 h and then collected for analysis of cell cycle phase distribution using flow cytometry. (C) as the control, untreated cells.

the proportion of sub-G1 phase in the treated cells was insignificantly higher compared to the untreated cells. This was accompanied with a concomitant decrease of the cells in the G1 phase and a slight accumulation of cells in S phase. Our results show that the lower *in vitro* antiproliferative activity is in accordance with the effects on cell cycle progression after treatment with algae extracts.

4. Discussion

The tested algal extracts have moderate antioxidant activity using various antioxidant bioassays. The DPPH fast test (H-donor method) was used as a principal test in antioxidant activity studies, while the other two methods are used to know and understand the mechanism of antioxidant activity (reducing power, superoxide anion radical scavenging activity).

The recorded activity was shown to correlate with the total phenolic content in the algal extracts with high correlation coefficient (r) of −0.995. In most algae, phenols are important anti oxidants because of their ability to scavenge free radicals such as singlet oxygen, superoxide and hydroxyl radicals (Shanab et al., 2011). Numerous researches found high correlations between antioxidative activities of algae and phenolic content (Sivakumar and Rajagopal, 2011; Demirel et al., 2011). On the other hand, few studies as Heo and Cha (2005) reported that the antioxidant activity (determined by different methods), using large number of algal species (10 green and 25 brown seaweed species) may not necessarily be correlated with the total phenolic content in each algal extract. These results may indicate the possible participation of other active substances which exhibit antioxidant activity such as pigments (chlorophyll, carotenoids), essential oils, and low molecular weight polysaccharides (Murthy et al., 2005).

Previous researches (Al-Amoudi et al., 2009; Abd El-Baky et al., 2009) have found antioxidant activity for the algae *U. lactuca*. Heo and Cha (2005) and Zubia et al. (2007) have also found strong antioxidant capacity for an extract of *E. intestinalis*. From their research they determined antioxidant activity for the above mentioned species but by using other extraction solvents such as aqueous, methanol, hexane, dichloromethane/methanol. Different extraction solvents, according to their polarity, may have extracted various compounds including pigments (chlorophyll a, b, carotenoids), alkaloids, and phenolic compounds, as well as essential oil which can participate in the great antioxidant activity (Shanab et al., 2011). This means that synergistic effects may occur between these constituents leading to the pronounced antioxidant activity of algal extract (containing the antioxidant active components). In contrast, extracts that not only have lower pigment contents but also a lower content of phenolic compounds have reduced antioxidant activity. Also, in experiments with other algae carried out by numerous researchers (Shanab et al., 2011; Sivakumar and Rajagopal, 2011; Uma et al., 2011), it was found that the tested species exhibit different activities depending on the extraction solvents used. Compared with their results, the results of this study suggest that the acetone extracts of *U. lactuca* and *E. intestinalis* demonstrated a relatively powerful antioxidant activity, probably due to the higher content of antioxidant active compounds in extracts.

Numerous algae have been screened for antimicrobial activity in search of new antimicrobial agents (Omar et al., 2012; Ibtissam et al., 2009). In our experiments, the tested algal species show relatively strong antimicrobial activity. The intensity of the antimicrobial effect depended on the species of algae, its concentration and the tested organism. The extract of *U. lactuca* had the stronger antimicrobial activity, inhibiting the tested bacteria and fungi at low concentrations. Differences in antimicrobial activity of different species of algae are probably a consequence of the presence of different components with antimicrobial activity (Jeyanthi Rebecca et al., 2012). However, it is necessary to understand that extracts are mixtures of natural compounds, and their antimicrobial activity is not only a result of different activities of individual components but may be the result of their interactions, which can have different effects on the overall activities of extracts. Similar to our results, numerous researchers (Zbakh et al., 2012; Soltani et al., 2012; Kolanjinathan and Stella, 2011; Vallinayagam et al., 2009) found strong antimicrobial activity for the algae *U. lactuca* and *E. intestinalis*, but with other extraction solvents, with other methods and against other species of microorganisms. In correlation with the results obtained by the other researchers, we also noticed a strong antimicrobial activity for acetone extracts of the tested *U. lactuca* and *E. intestinalis*, which suggests that these species contain components toxic to microorganisms and, therefore, responsible for their antimicrobial activity.

The extracts used in this study, had stronger antibacterial than antifungal activities. The probable reason is the difference in the composition and permeability of their cell walls. The cell walls of gram-positive bacteria are made of peptidoglycans and teichoic acids, while the cell walls of gram-negative bacteria are made of peptidoglycans, lipopolysaccharides, and lipoproteins (Kosanić et al., 2012; Heijenoort, 2001). The lipid portion of the outer membrane of gram-negative bacteria is poorly permeable to antimicrobials, hence the reason for their greater resistance. The cell wall of fungi is poorly permeable and consists of polysaccharides such as hitchin and glucan (Farkaš, 2003). This observation is in accordance with many other studies (Albouchi et al., 2013; Kosanić et al., 2012; Yang and Anderson, 1999), focused on antimicrobial activity which has demonstrated that structure and permeability of the cell wall are reasons for different sensitivities in gram-positive bacteria, gram-negative bacteria and fungi.

Our results are the first published reports on the cytotoxic activity of extracts of green algae *E. intestinalis* and *U. lactuca* on LS174, A549, Fem-x and K562 cell lines. Exceptionally, it has been shown that the polysaccharide from *E. intestinalis*, although not directly demonstrates cytotoxic activity *in vitro*, may be associated with its potent immunostimulating effect and increased anti-tumour response in mice (Jiao et al., 2009). Furthermore, other studies and findings reported that algal components are responsible for anticancer activities of algae (Nakajima et al., 2009; Go et al., 2010; Ermakova et al., 2011). However, it is difficult to determine the contribution of individual components for the overall anticancer effects. Often, the activities of the extracts may be the result of synergistic or antagonistic effects of several compounds.

In the literature there are no data on anticancer activity of extracts from *U. lactuca* and *E. intestinalis,* but anticancer activity of some other algal extracts were studied by other researchers. Salem and Ibrahim (2011) found anticancer activity for different extracts of the *Ulva rigida* on the Ehrlich ascites carcinoma (EAC) cell line. Zandi et al. (2010) reported

significant anticancer effects of extracts of brown alga *Sargassum oligocystum* against K562 and Daudi cell lines. Sundaram et al. (2012) explored anticancer properties of ethanol extract of *Gracilaria edulis*. Compared with their results, the findings of this research suggest that the tested algal species showed moderate anticancer activity.

The importance of algae as anticancer agents is confirmed in recent years, which suggests that algae can be used as biological agents in the treatment of cancer. The mechanism of action of the tested extracts is yet to be tested. Thus, further research will be necessary to fractionate in order to identify compounds responsible for the observed anti-tumour effects, and to establish the opportunities reinforcement activities as well as to improve the selectivity.

In conclusion, it can be stated that tested algal extracts have a certain level of antioxidant, antimicrobial and anticancer activities *in vitro*. Based on the results, tested macroalgae appear to be good natural antioxidant, antimicrobial and anticancer agents. Identification of the active compounds of these algal species will lead to their evaluation of considerable commercial potential in medicine, food production and cosmetic industry.

Acknowledgements

This work was financed in part by the Ministry of Science, Technology, and Development of the Republic of Serbia and was carried out within the framework of projects nos. 173032 and 175011.

References

Abbott, I.A., Hollenberg, G.J., 1992. Marine Algae of California. Stanford University Press, Stanford.

Abd El-Baky, H.H., El-Baz, F.K., El-Baroty, G.S., 2009. Natural preservative ingredient from marine alga *Ulva lactuca* L.. Int. J. Food Sci. Technol. 44, 1688–1695.

Al-Amoudi, O.A., Mutawie, H.H., Patel, A.V., Blunden, G., 2009. Chemical composition and antioxidant activities of Jeddah corniche algae, Saudi Arabia. Saudi J. Biol. Sci. 16, 23–29.

Albouchi, F., Hassen, I., Casabianca, H., Hosni, K., 2013. Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) swingle leaves. S. Afr. J. Bot. 87, 164–174.

Al-Saif, S.S., Abdel-Raouf, N., El-Wazanani, H.A., Aref, I.A., 2014. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. Saudi J. Biol. Sci. 21, 57–64.

Andrews, J.M.BSAC Working Party on Susceptibility Testing, 2005. BSAC standardized disc susceptibility testing method. J. Antimicrob. Chemother. 56, 60–76.

Bhadury, P., Wright, C.P., 2004. Exploitation of marine algae: biogenic compounds for potential antifouling application. Planta 219, 561–578.

Bouhlal, R., Haslin, C., Chermann, J.C., Colliec-Jouault, S., Sinquin, C., Simon, G., Cerantola, S., Riadi, H., Bourgougnon, N., 2011. Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (*Rhodophytha*, *Gigartinales*) and *Boergeseniella thuyoides* (*Rhodophyta*, *Ceramiales*). Mar. Drugs 9, 1187–1209.

Clothier, R.H., 1995. The FRAME cytotoxicity test. Methods Mol. Biol. 43, 109–118.

Dayong, S., Jing, L., Shuju, G., Lijun, H., 2008. Antithrombotic effect of bromophenol, the alga-derived thrombin inhibitor. J. Biotechnol. 136, 577–588.

Demirel, Z., Yilmaz-Koz, F.F., Karabay-Yavasoglu, N.U., Ozdemir, G., Sukatar, A., 2011. Antimicrobial and antioxidant activities of solvent extracts and the essential oil composition of *Laurencia obtusa* and *Laurencia obtusa* var. *pyramidata*. Rom. Biotechnol. Lett. 16, 5927–5936.

Devi, G.K., Manivannan, K., Thirumaran, G., Rajathi, F.A.A., Anantharaman, P., 2011. *In vitro* antioxidant activities of selected seaweeds from southeast coast of India. Asian Pacific J. Trop. Med. 4, 205–211.

Dorman, H.J., Bachmayer, O., Kosar, M., Hiltunen, R., 2004. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J. Agric. Food Chem. 52, 762–770.

Ermakova, S., Sokolova, R., Kim, S.M., Um, B.H., Isakov, V., Zvyagintseva, T., 2011. Fucoidans from brown seaweeds *Sargassum hornery, Eclonia cava, Costaria costata*: structural characteristics and anticancer activity. Appl. Biochem. Biotechnol. 164, 841–850.

Farkaš, V., 2003. Structure and biosynthesis of fungal cell walls: methodological approaches. Folia Microbiol. 48, 469–478.

Faulkner, D.J., 2001. Marine natural products. Nat. Prod. Rep. 18, 1–49.

Go, H., Hwang, H.J., Nam, T.J., 2010. A glycoprotein from *Laminaria japonica* induces apoptosis in HT-29 colon cancer cells. Toxicol. In Vitro 24, 1546–1553.

Hardy, F.G., Guiry, M.D., 2006. A Check-List and Atlas of the Seaweeds of Britain and Ireland. British Phycological Society, London.

Heijenoort, J., 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. Glycobiology 11, 25–36.

Heo, S.J., Cha, S.H., 2005. Antioxidant activities of chlorophyta and phaeophyta from Jeju Island. Algae 20, 251–260.

Ibtissam, C., Hassane, R., José, M.L., Josè Francisco, D.S., Josè Antonio, G.V., Hassan, B., Mohamed, K., 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr. J. Biotechnol. 8, 1258–1262.

Jeyanthi Rebecca, L., Dhanalakshmi, V., Thomas, T., 2012. A comparison between the effects of three algal extracts against pathogenic bacteria. J. Chem. Pharm. Res. 4, 4859–4863.

Jiao, L., Li, X., Li, T., Jiang, P., Zhang, L., Wu, M., Zhang, L., 2009. Characterization and anti-tumor activity of alkali-extracted polysaccharide from *Enteromorpha intestinalis*. Int. Immunopharmacol. 9, 324–329.

Kharkwal, H., Joshi, D.D., Panthari, P., Pant, M.K., Kharkwal, A.C., 2012. Algae as future drugs. Asian J. Pharm. Clin. Res. 5, 1–4.

Kim, S.K., Thomas, N.V., Li, X., 2011. Anticancer compounds from marine macroalgae and their application as medicinal foods. Adv. Food Nutr. Res. 64, 213–224.

Kolanjinathan, K., Stella, D., 2011. Comparative studies on antimicrobial activity of *Ulva reticulate* and *Ulva lactuca* against human pathogens. Int. J. Pharm. Biol. Arch. 2, 1738–1744.

Kolanjinathan, K., Ganesh, P., Saranraj, P., 2014. Pharmacological importance of seaweeds. World J. Fish Mar. Sci. 6, 01–15.

Kosanić, M., Ranković, B., Stanojković, T., 2012. Antioxidant, antimicrobial and anticancer activity of 3 *Umbilicaria* species. J. Food Sci. 77, T20–T25.

Manilal, A., Sujith, S., Sabarathnam, B., Kiran, G.S., Selvin, J., Shakir, C., Lipton, A.P., 2010. Bioactivity of the red alga *Asparagopsis taxiformis* collected from the south-western coast of India. Braz. J. Oceonogr. 58, 93–100.

Meda, A., Lamien, C.E., Romito, M., Millogo, J., Nacoulma, O.G., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91, 571–577.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Murthy, K.N.C., Vanilha, A., Rajesha, J., Swamy, M.M., Swmya, P.R., Ravishankar, G.A., 2005. *In vitro* antioxidant activity of

carotenoids from *Dunaliella salina* – a green microalga. Life Sci. 76, 1381–1390.

Na, H.J., Moon, P.D., Lee, H.J., Kim, H.R., Chae, H.J., Shin, T., Seo, Y., Hong, S.H., Kim, H.M., 2005. Regulatory effect of atopic allergic reaction by *Carpopeltis affinis*. J. Ethnopharmacol. 101, 43–48.

Nakajima, K., Yokoyama, A., Nakajima, Y., 2009. Anticancer effects of a tertiary sulfonium compound, dimethylsulfoniopropionate, in green sea algae on Ehrlich ascites carcinoma-bearing mice. J. Nutr. Sci. Vitaminol. 55, 434–438.

NCCLS (National Committee for Clinical Laboratory Standards), 1998. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-forming Filamentous Fungi: Proposed Standard M38-P. NCCLS, Wayne, PA, USA.

Nishimiki, M., Rao, N.A., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849–853.

Omar, H.H., Shiekh, H.M., Gumgumjee, N.M., El-Kazanm, M.M., El-Gendy, A.M., 2012. Antibacterial activity of extracts of marine algae from the Red Sea of Jeddah, Saudi Arabia. Afr. J. Biotechnol. 11, 13576–13585.

Oyaizu, M., 1986. Studies on products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–314.

Salem, T.A., Ibrahim, A.M., 2011. Anticancer activity of Egyptian marine alga *Ulva rigida*. Int. J. Health Sci. 5, 6–8.

Sarker, S.D., Nahar, L., Kumarasamy, Y., 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods 42, 321–324.

Shanab, S.M.M., Shalaby, E.A., El-Fayoumy, E.A., 2011. *Enteromorpha compressa* exhibits potent antioxidant activity. J. Biomed. Biotechnol.. http://dx.doi.org/10.1155/2011/726405.

Sivakumar, K., Rajagopal, S.V., 2011. Radical scavenging activity of green algal species. J. Pharm. Res. 4, 723–725.

Slinkard, K., Slingleton, V.L., 1997. Total phenolic analyses: automation and comparison with manual method. Am. J. Enol. Vitic. 28, 49–55.

Soltani, S., Ali Ebrahimzadeh, M., Khoshrooei, R., Rahmani, Z., 2012. Antibacterial and antihemolytic activities of *Enteromorpha intestinalis* in Caspian Sea Coast Iran. J. Med. Plants Res. 6, 530–533.

Sundaram, M., Patra, S., Maniarasu, G., 2012. Antitumor activity of ethanol extract *of Gracilaria edulis* (Gmelin) Silva on Ehrlich ascites carcinoma-bearing mice. Zhong Xi Yi Jie He Xue Bao 10, 430–435.

Uma, R., Sivasubramanian, V., Niranjali Devaraj, S., 2011. Evaluation of *in vitro* antioxidant activities and antiproliferative activity of green microalgae, *Desmococcus olivaceous* and *Chlorococcum humicola*. J. Algal Biomass Utln. 2, 82–93.

Vallinayagam, K., Arumugam, R., Ragupathi, K.R., Thirumaran, G., Anantharaman, P., 2009. Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. Global J. Pharmacol. 3, 50–52.

Yang, Y., Anderson, E.J., 1999. Antimicrobial activity of a porcine myeloperoxidase against plant pathogenic bacteria and fungi. J. Appl. Microbiol. 86, 211–220.

Zandi, K., Tajbakhsh, S., Nabipour, I., Rastian, Z., Yousefi, F., Sharafian, S., Sartavi, K., 2010. *In vitro* antitumor activity of *Gracilaria corticata* (a red alga) against jurkat and molt-4 human cancer cell lines. Afr. J. Biotechnol. 9, 6787–6790.

Zbakh, H., Chiheb, H., Bouziane, H., Motilva Sánchez, V., Riadi, H., 2012. Antibacterial activity of benthic marine algae extracts from the Mediterranean coast of Morocco. JMBFS 1, 219–228.

Zubia, M., Robledo, D., Freile-Pelegrin, Y., 2007. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J. Appl. Phycol. 19, 449–458.