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

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Biological activities of two macroalgae from Adriatic coast of Montenegro

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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.
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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

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2014.11.004
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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:

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