Romanian Biotechnological Letters *Vol. , No. x,*

ORIGINAL PAPER

Evaluation of antioxidant, antimicrobial and anticancer effects of three selected marine macroalgae

Received for publication, August, 24, 2015
Accepted, May, 25, 2017

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Abstract

In the present investigation the acetone extracts of three marine macroalgae Laurentia obtusa, Codium elongatum and Cutleria multifida were tested for antioxidant, antimicrobial and cytotoxic potential. Antioxidant activity was evaluated by free radical scavenging, superoxide anion radical scavenging and reducing power. C. elongatum extract had most potent free radical scavenging activity (IC_{50}=382.45 µg ml^{-1}). Moreover, the tested extracts had effective reducing power and superoxide anion radical scavenging. Total content of phenol and flavonoid in extracts were examined using Folin-Ciocalteu reagent and by aluminium chloride method, respectively. Further, the antimicrobial potential was determined by a microdilution method. Among the tested species, extract of L. obtusa showed the best antimicrobial activity with minimum inhibitory concentration values ranging from 0.156 to 5 mg ml. Finally, the cytotoxic activity was tested using MTT method. Extract of L. obtusa expressed stronger cytotoxic activity toward tested cell lines with IC_{50} values ranging from 38.63 to 67.62µg ml^{-1}.

Keywords: Acetone extracts, Anticancer activity, Antimicrobial activity, Antioxidant activity, Algae.

1. Introduction

Seaweeds or marine macroalgae are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and grouped into different classes: green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae [1]. Interest in seaweeds has increased markedly through the world due to their value in nutrition and in medicine. Nutritionally valuable seaweeds are being used as fresh or dried vegetables or as ingredients in wide variety of prepared foods. From nutritional point of view they are low calorie food, with high concentration of minerals (Mg, Ca, P, K and I), vitamins, proteins, indigestible carbohydrates and low concentration of lipids [2]. Seaweeds also have attracted the attention of the pharmaceutical industry, due to the great diversity of species that are available and the ability to produce secondary metabolites with various pharmacological activities such as cytotoxic activity, antiproliferative, antimicrobial, antiviral, anti-allergic, anticoagulant and antioxidant activities [3-8]. As an aid to protect themselves against other organisms in their environment, macroalgae produce a wide variety of chemically active metabolites included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols that have a broad range of biological activities [9]. Some of these metabolites such as iodine, carotene, glycerol, alginates, carrageenans have been used in pharmaceutical industries [10,11]. In fact, the discovery of metabolites with biological and pharmaceutical activities, from seaweeds, has increased significantly in the past years [1].

Despite the diversity in quality and quantity of the Adriatic coast flora, with its large contains of marine organisms and seaweeds, most of them have not yet been investigated for pharmacological and biological activities [12]. Seaweeds *L. obtusa*, *C. elongatum* and *C. multifida* are poorly studied in relation to their biological activities and literature data on these species are mainly related to seas other than Adriatic Sea. Therefore, the objective of this research was to evaluate antioxidant, antimicrobial and anticancer activities of macroalgae *L. obtusa*, *C. elongatum* and *C. multifida* from the Adriatic coast of Montenegro.

2. Material and Methods

2.1. Algal samples

Algal samples of *L. obtusa* (Hudson) Lamouroux, *C. elongatum* (Turner) C. Agardh and *C. multifida* (Turner) Greville, were collected from the Adriatic Sea, in June of 2013. The voucher specimen of the algae (Voucher No. 22, 28 and 31) was deposited at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia. To determinate tested algae were used several standard keys [13,14].

2.2. Preparation of the algal extracts

Finely dry ground 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 ^{0}C until they were used in the tests. The extracts were dissolved in 5% dimethyl sulphoxide (DMSO) for the experiments. DMSO was dissolved in sterile distilled water to the desired concentration.

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). The method used was similar to that of DORMAN & *al.* [15] but was modified in its details. Two milliliters of methanol solution of DPPH radical in the concentration of 0.05 mg ml^{-1} and 1 ml of test samples (1000, 500, 250, 125 and 62.5 μg ml^{-1}) were placed in cuvettes. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. DPPH solution is initially violet in color which fades when antioxidants donate hydrogen. The change in color is monitored by spectrophotometer (“Jenway” UK) at 517 nm against methanol as blank. Ascorbic acid was used as positive control. The DPPH radical concentration was calculated using the following equation:

DPPH scavenging effect (%) = ((A0 - A1) / A0) x 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 extract and ascorbic acid, the inhibitory concentration (IC_{50}) at 50% was determined.

2.3.2. Reducing power

The reducing power of samples was determined according to the method of OYAIZU [16]. One milliliter of test samples (1000, 500, 250, 125 and 62.5 μg ml^{-1}) were mixed with 2.5 ml of phosphate buffer (2.5 ml, 0.2 mol l^{-1}, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixtures were incubated at 50^{o}C for 20 min. Then, trichloroacetic acid (10%, 2.5 ml) was added to the mixture and 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 spectrophotometer (“Jenway” UK). Blank was prepared with all the reaction agents without

extract. Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid was used as positive control.

2.3.3. Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of samples was detected according to the method of NISHIMIKI & *al.* [17]. Briefly, 0.1 ml of test samples (1000, 500, 250, 125 and 62.5 μg ml^{-1}) was mixed with 1 ml nitroblue tetrazolium (NBT) solution (156 μmol l^{-1} in 0.1 mol/L phosphate buffer, pH 7.4) and 1 ml nicotinamide adenine dinucleotide (NADH) solution (468 μmol/L in 0.1 mol l^{-1} phosphate buffer, pH 7.4). The reaction was started by adding 100 μL of phenazine methosulphate (PMS) solution (60 μmol l^{-1} in 0.1 mol/L phosphate buffer, pH 7.4). The mixture was incubated at room temperature for 5 min, and the absorbance was measured at 560 nm in spectrophotometer ("Jenway" UK) against blank sample (phosphate buffer). Decreased absorbance indicated increased superoxide anion radical scavenging activity. Ascorbic acid was used as positive control. The percentage inhibition of superoxide anion generation was calculated using the following formula:

Superoxide anion scavenging activity (%) = ((A0 - A1) / A0) x 100

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

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

2.3.4. 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 & SINGELTON [18] using pyrocatechol as a standard phenolic compound. Briefly, 1ml of the extract (1 mg ml^{-1}) in a volumetric flask diluted with distilled water (46 ml). One milliliter of 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 was allowed to stand for 2h with intermittent shaking. The absorbance was measured at 760 nm in spectrophotometer ("Jenway" UK). The total concentration of phenolic compounds in the extract determined as microgram of pyrocatechol equivalents (PE) per milligram of dry extract by using an equation that was obtained from a standard pyrocatechol graph as follows:

Absorbance = 0.0057 x total phenols (μg PE mg^{-1} of dry extracts) - 0.1646

(R^2 = 0.9203)

2.3.5. Total flavonoid content

The total flavonoid content was determined using the Dowd method [19]. Two milliliters of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (1 mg ml^{-1}). The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 415 nm in spectrophotometer ("Jenway" UK) against blank samples. The total flavonoid content determined as microgram of rutin equivalents (RE) per milligram of dry extract by using an equation that was obtained from a standard rutin graph as follows:

Absorbance = 0.0296 x total flavonoid (μg RE mg^{-1} of dry extracts) + 0.0204

(R^2 = 0.9595)

2.4. Antimicrobial activity

The bacteria used in this study: *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 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 inoculi 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^{-1}. 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^{-1} according to the procedure recommended by NCCLS [20].

The minimal inhibitory concentration (MIC) was determined by the broth microdilution method using 96-well micro-titer plates [21]. A series of dilutions with concentrations ranging from 40 to 0.0047 mg ml^{-1} 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 it in DMSO. Two-fold 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 changing color 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 a positive control. A solvent control test was performed to study an effect of 5% DMSO on the growth of microorganisms. All experiments were performed in triplicate.

2.5. Cytotoxic activity

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

In vitro assay for cytotoxic activity of investigated extract was performed when the cells reached 70–80% of confluence. Stock solution (50 mg ml^{-1}) of extract was dissolved in 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 microtiter plates, and 24 h later, after cell adherence, five different, double diluted concentrations of investigated extract were added to the wells. Final concentrations of the extract were 200, 100, 50, 25, and 12.5 μg ml^{-1} except for the control wells, where only nutrient medium was added. The cultures were incubated for the next 72 h. The effect on cancer cell survival was determined 72 h after the addition of extract, by the MTT test [22]. Briefly, 20 μl of MTT solution (5 mg ml^{-1} 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. Each experiment was performed in triplicate and independently repeated at least four times.

2.6. Data analyses

Data analyses were performed with the EXCEL and SPSS softwares package. 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 and discussion

In the present study, *in vitro* antioxidant, antimicrobial, and anticancer activities of acetone extracts from the algae *L. obtusa*, *C. elongatum* and *C. multifida* were examined.

The scavenging DPPH radicals, superoxide anion radical scavenging and reducing power of the studied extracts are represented in Tab. 1 and Tab. 2. As a shown in tables, extract from *C. elongatum* showed larger antioxidant activities than *L. obtusa* and *C. multifida*. In various antioxidant activities, there was a statistically significant difference between extracts and control ($P<0.05$). Various antioxidant activities were compared to ascorbic acid. The results showed that standard antioxidant had stronger activity than tested samples.

The total phenolic and flavonoid contents of the algal extracts are given in Tab. 3. The total phenolics contents in extracts of *L. obtusa*, *C. elongatum* and *C. multifida* were 59.89, 86.72 and 76.72 μg PE mg^{-1}, while concentrations of flavonoids were 38.12, 72.31 and 59.48 μg RE mg^{-1}, respectively.

Table 1. DPPH radical scavenging activity and superoxide anion scavenging activity of acetone extracts of *Laurentia obtusa*, *Codium elongatum* and *Cutleria multifida*

Algal species	DPPH radical scavenging activity IC_{50} (μg ml^{-1})	Superoxide anion scavenging activity IC_{50} (μg ml^{-1})
Laurentia obtusa	593.81 ± 1.36	712.24 ± 2.12
Codium elongatum	382.45 ± 2.35	493.34 ± 2.02
Cutleria multifida	491.12 ± 2.93	589.31 ± 2.73
Ascorbic acid	6.42 ± 0.18	115.61 ± 1.16

Values are expressed as mean ± SD of three parallel measurements

Table 2. Reducing power of acetone extracts of *Laurentia obtusa*, *Codium elongatum* and *Cutleria multifida*

	Absorbance (700 nm)				
Algal species	1000 μg ml^{-1}	500 μg ml^{-1}	250 μg ml^{-1}	125 μg ml^{-1}	62.5 μg ml^{-1}
L. obtusa	0.5281 ± .031	0.3316 ± .025	0.2238 ± .008	0.1246 ± .004	0.0925 ± .002
C. elongatum	1.1137 ± .028	0.9982 ± .024	0.5579 ± .009	0.2263 ± .006	0.1192 ± .003
C. multifida	0.8733 ± .015	0.6621 ± .011	0.0970 ± .008	0.0542 ± .004	0.0365 ± .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

Table 3. Total phenolics and flavonoids content of acetone extracts of *Laurentia obtusa*, *Codium elongatum* and *Cutleria multifida*

Algal species	Phenolics content (μg PE $mg^{-1}$of extract)	Flavonoid content (μg RE $mg^{-1}$of extract)
Laurentia obtusa	59.89 ± 1.065	38.12 ± 1.099
Codium elongatum	86.72 ± 1.013	72.31 ± 1.078
Cutleria multifida	76.72 ± 1.002	59.48 ± 0.991

Values are expressed as mean ± SD of three parallel measurements
PE - pyrocatechol equivalents
RE - rutin equivalents

The tested algal extracts have moderate antioxidant activity using various antioxidant bioassays. Antioxidant efficiency of all three extracts was carried out by DPPH method. This method was used as a principle antioxidant as fast test (H-donor method) and 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 phenolics in the algal extracts. In most algae, phenols are important antioxidants because of their ability to scavenge free radicals such as singlet oxygen, superoxide and hydroxyl radicals [23]. Numerous researches found a high correlations between antioxidative activities of algae and phenolics content [24,25]. On the other hand, few studies as HEO & CHA [26] reported that the antioxidant activity (determined by different methods), using large number of algal species (10 green and 25 brown seaweed species), not always correlate with the total phenolics content in each algal extract. These results may indicate the possible participation of other active substances which exhibit antioxidant activity as pigments (chlorophyll, carotenoids), essential oils, and low molecular weight polysaccharides [27].

In previous researches it was found antioxidant activity for the algae *L. obtusa*. For example, the methanol, diethylether and hexane extracts of *L. obtusa* were evaluated for their antioxidant activity by ANGGADIREDJA & *al.* [28]. They found that the hexane extract of L. obtusa was more active than the diethylether and methanol extracts, may be due to phenolic compounds present in significant amounts in this extract. DEMIREL & *al.* [25] found that the *L. obtusa* methanol, chloroform and hexane extracts exhibited free radical scavenging activity against the stable free radical DPPH, decolorising activity of 2,2'-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid (ABTS) radical action and also inhibition activity of the β-carotene bleaching at different proportions. As for the antioxidant test results DPPH radical scavenging activity of the chloroform extracts was found to be high, while methanol and hexane extracts showed very little activity. On the other hand, to the ABTS radical cation decolorisation assay, % activity of the chloroform and the hexane extracts were higher than the activity of the methanol extract. The above-mentioned authors determined antioxidant activity for these species, but for other extraction solvents used. In this study, the antioxidant activity of selected algae was confirmed by acetone algal extracts. Different extraction solvents, according to the their polarity, may 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 [23]. This means that synergistic effects may occur between these constituents leading to the pronounced antioxidant activity of algal extract (containing the antioxidant active components). On the contrary, extracts that have a lower pigment contents but also lower content of phenolic compounds have reduced antioxidant activity. Also, in experiments with other algae

[23,24,29], it was found that the tested species exhibit different activity depending on the extraction solvents used. Compared with their results, the results of this research suggest that the acetone extracts of *L. obtusa*, *C. elongatum* and *C. multifida* showed a relatively powerful antioxidant activity, probably due to the higher content of antioxidant active compounds in extracts. No data exist in the literature about antioxidant activity of *C. elongatum* and *C. multifida* species.

The antimicrobial activity of the algal extracts against the test microorganisms is shown in Tab. 4. Extract from *L. obtusa* showed the best antimicrobial activity. They inhibited all the tested microorganisms, at concentrations from 0.156 to 5 mg ml^{-1}. Extracts from *C. elongatum* and *C. multifida* also inhibited all 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 tested samples as shown in Tab. 4. In a negative control, DMSO had no inhibitory effect on the tested organisms.

Table 4. Minimum inhibitory concentration (MIC) of acetone extracts of *Laurentia obtusa*, *Codium elongatum* and *Cutleria multifida*

Algal species	*L. obtusa*	*C. elongatum*	*C. multifida*	S - K
Bacillus mycoides	0.625	0.625	0.625	7.81 -
Bacillus subtilis	0.156	0.312	0.312	7.81 -
Eschrichia coli	0.156	2.5	0.156	31.25 -
Klebsiella pneumoniae	2.5	1.25	2.5	1.95 -
Staphylococcus aureus	0.625	0.625	0.625	31.25 -
Aspergillus flavus	5	2.5	5	- 3.9
Aspergillus fumigatus	2.5	2.5	2.5	- 3.9
Candida albicans	1.25	1.25	1.25	- 1.95
Penicillium purpurescens	5	2.5	5	- 3.9
Penicillium verrucosum	2.5	2.5	2.5	- 3.9

Values given as mg ml^{-1} for tested samples and as μg ml^{-1} for antibiotics. Values are the mean of three replicate.
Antibiotics: K – ketoconazole, S – streptomycin

Numerous algae have been screened for antimicrobial activity in search of new antimicrobial agents [30,31]. 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 organisms. Differences in antimicrobial activity of different species of algae are probably a consequence of the presence of different components with antimicrobial activity [32]. However, it is necessary understand that extracts are mixtures of natural compounds, and their antimicrobial activity is not only a result of the different activities of individual components but may be the result of their interactions, which can have different effects on the overall activity of extracts. Similar to our results, numerous researchers [25,33,34] found strong antimicrobial activity for the algae *L. obtusa*, *C. elongatum* and *C. multifida*, but with other extraction solvents, with other methods and against other species of microorganisms. In correlation with results obtained with the other researchers, we also noticed strong antimicrobial activity for acetone extracts of tested *L. obtusa*, *C. elongatum* and *C. multifida*, 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 activity. The probable reason for this is 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. The lipid portion of the outer membrane of gram-negative bacteria is poorly permeable to antimicrobials, and it is the reason for their greater resistance. The cell walls of fungi are the least permeable and consist of polysaccharides such as hitchin and glucan. This observation is in accordance to many other studies [35] focused on the antimicrobial activity which has demonstrated that structure and permeability of the cell wall are the reason for different sensitivity gram-positive bacteria, gram-negative bacteria and fungi.

The data obtained for anticancer effect of *L. obtusa*, *C. elongatum* and *C. multifida* extracts are shown in Tab. 5. The best cytotoxic effect showed the extract from *L. obtusa* (IC_{50}=62.21, 67.62, 43.32 and 38.63 μg ml^{-1} respectively), while *C. elongatum* and *C. multifida* extracts showed a slightly weakest cytotoxic activity. Furthermore, all extracts showed less activity compared to cis-DDP as a positive control.

Table 5. Growth inhibitory effects of acetone extracts of *Laurentia obtusa*, *Codium elongatum* and *Cutleria multifida* on LS174, A549, FemX and K562 cell lines

Cell lines	LS174	A549	Fem-x	K562
Algal species	IC_{50} (μg ml^{-1})			
L. obtusa	62.21 ± 1.97	67.62 ± 3.84	43.32 ± 2.66	38.63 ± 1.91
C. elongatum	124.55 ± 2.53	129.81 ± 2.11	69.56 ± 1.79	50.91 ± 2.12
C. multifida	100.11 ± 2.45	122.65 ± 0.78	105.37 ± 2.11	103.69 ± 1.93
cis-DDP	3.18 ± 0.29	4.91 ± 0.42	0.86 ± 0.33	2.22 ± 0.08

Values are expressed as mean ± SD of three parallel measurements

In case of the anticancer activity, in literature no data about tested species. Only, ALARIF & *al.* [33] examined anticancer activity of three laurene-type sesquiterpenes isolated from the organic extract of the alga *L. obtusa.* They revealed that isolated compounds to have very promising activity in an in vitro model of Ehrlich ascites Carcinoma.

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. The further research will be necessary to fractionation in order to identify compounds responsible for the observed anti-tumor effects, and to establish the opportunities reinforcement activities as well as to improve the selectivity.

4. Conclusion

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 marine 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 in considerable commercial potential in medicine, food production and the cosmetic industry.

5. 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 no. 173032 and 175011.

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