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Antimicrobial and antioxidant activities of solvent extracts and the essential oil composition of *Laurencia obtusa* and *Laurencia obtusa* var. *pyramidata*

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Abstract

The biological activities of Laurencia obtusa and Laurencia obtusa var. pyramidata from Cesme coasts (Turkey) were screened by using in vitro methods. The essential oils of L. obtusa and L. obtusa var. pyramidata were obtained by steam distillation and their chemical compositions were analyzed by GC/MS. The antimicrobial activities against bacteria (two specific pathogenic strains (methicillin-oxacillin resistant Staphylococcus aureus ATCC 43300, hemorrhagic Escherichia coli (O157: H7) RSSK 232)) and one yeast strain (Candida albicans ATCC 10239) were determined by using the disc-diffusion technique. The antimicrobial potential of the L. obtusa essential oil was found considerably strong. It showed inhibitory effect on two bacteria and one yeast strain. The hexane and chloroform extracts of L. obtusa and the methanol and chloroform extracts of L. obtusa var. pyramidata were generally found as moderate antioxidants in comparison with butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and α-tocopherol (Vitamin E). The chloroform extracts of both the red algae have high phenolic content compared to the other extracts and essential oils.

Key words: *Laurencia obtusa, Laurencia obtusa* var. *pyramidata,* antioxidant activity, antimicrobial activity, GC-MS.

Introduction

Red algae, especially many species of the genus *Laurencia* (order *Ceramiales*, family *Rhodomelaceae*) are proven to be rich sources of halogenated secondary metabolites. Many of these metabolites have been found to possess a variety of biological activities such as antifeedant (diterpenes) [1], antihelmintic *(β*-chamigrane-type sesquiterpenes) [2], antimalarial (brominated sesquiterpenes) [3], antifouling (sesquiterpenes) [4], antimicrobial (allolaurinterol) [5] and cytotoxic activities [6]. The cytotoxic properties of marine compounds belong to four structural types, such as polyketides, terpenes, nitrogen-containing compounds and polysaccharides [7]. A study by Kladi *et al*. [8] (sesquiterpenes) revealed that *L. obtusa* and *L. microcladia* exert an antitumor effect against lines CHO cells (ovaries biopsy, Chinese hamster) and the human tumor cell lines: K562 (a chronic myelogenous leukemia cell line), MCF7 (derived from a mammary adenocarcinoma), PC3 (derived from a prostate adenocarcinoma), HeLa (derived from cervix adenocarcinoma) and A431 (derived from epidermoid carcinoma). Brominated diterpenes isolated from *L. obtusa* were tested for cytotoxic activity against five human cell lines [9]. Some halogenated metabolites have been shown to exhibit antibacterial activities against terrestial bacteria[10]. Several studies have also examined the antioxidant activity of *Laurencia* [11, 12].

The present study covered an investigation on the antimicrobial and antioxidant activities of the methanol, chloroform, hexane extracts and the essential oils of the seaweeds *Laurencia obtusa* and *Laurencia obtusa* var. *pyramidata* from the Aegean Sea coast of Turkey.

Materials and methods

Algal material. The marine algae *Laurencia obtusa* (Hudson) J.V. Lamouroux (EGE No 40779) and *Laurencia obtusa* var. *pyramidata* Bory ex J. Agardh (EGE No 40778) were collected from the Cesme Coast of the Aegean Sea, Izmir at a depth of 0.5–1 m in September of 2007 and identified by Dr. Atakan Sukatar from Ege University, Faculty of Science, Department of Biology.

Preparation of algal extracts and essential oil. The collected algae specimens were initially freeze-dried (50 g dry weight) and then exhaustively extracted by soxhlet apparatus with methanol, chloroform and hexane [13]. For the essential oil, the dried samples of each alga (20 g) were exposed to steam distillation for 4 h utilizing a Clevenger-type apparatus according to the European Pharmacopoeia [14].

Gas chromatography-mass spectrometry (GC-MS) analysis. The steam-distilled components were analyzed by GC and GC-MS. A HP 6890 gas chromatograph equipped with a FID and a 5 m x □0.2 mm HP-1 capillary column (0.33 μm coating) was employed for the GC analysis. GC-MS analysis was performed on a HP 5973 mass selective detector coupled with a HP 6890 gas chromatograph, equipped with a HP-1 capillary column. Identification of the individual components was performed by comparison of mass spectra with literature data and by a comparison of their retention indices (RI) relative to a C8-C32 *n*-alkenes mixture [15]. A computerized search was carried out using the Wiley 275 L. GC-MS library and ARGEFAR GC-MS library was created with authentic samples.

Antimicrobial activity

Microorganisms.The following standard organisms were obtained from the Microbiology Department Culture Collection of Ege University, Faculty of Science, Turkey. The antibacterial activities of the extracts and essential oils of two red algae were tested against ten species of bacteria strains (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538p, *Enterococcus faecalis* ATCC 8043, *Staphylococcus epidermidis* ATCC 12228, *Enterobacter aerogenes* CIP 6069, *Enterobacter cloaceae* ATCC 13047, *Escherichia coli* ATCC 29998, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 6897, *Salmonella typhimurium* CCM 5445), two specific pathogenic strains (methicillin- oxacillin resistant *Staphylococcus aureus* ATCC 43300, hemorrhagic *Escherichia coli* (O157: H7) RSSK 232) and one yeast strain *Candida albicans* ATCC 10239.

Disc diffusion method.The paper disc diffusion method was performed[16, 17]. Briefly, sterile 6 mm diameter filter paper discs (Schleicher and Schül, Nr 2668, Dassel, Germany) were impregnated with 10, 20, 30 μL of methanol, chloroform and hexane extracts of the *L. obtusa* and *L.* var. *pyramidata* (0.5, 1 and 2 mg disc^{-1}) and three different concentrations of essential oils (0.1, 0.2 and 0.4 μL). The bacteria plates were incubated at 37°C for 24 h and the yeast plates were incubated at 28 ± 0.1°C for 48 h. Tobramycin discs (Bioanalyse, 10 μg disc^{-1}) and nystatin discs (Oxoid, 30 μg disc^{-1}) were used as positive controls.

Antioxidant activity

DPPH radical scavenging assay. DPPH (1,1-diphenyl-2-picryl hydrozyl) radical is considered to be a model of stable lipophilic radical [18]. The extracts and oils were tested by measuring of bleaching purple colored methanol solution of DPPH radical (0.04 %, w/v), spectrophotometrically at 515 nm. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and α-tocopherol (Vitamin E) were used as positive control.

β-carotene bleaching assay. This experiment was carried out by measuring the oxidation of β-carotene and linoleic acid system [19]. β-carotene solution in chloroform (2 mg/10 mL) was added to a mixture of linoleic acid and Tween 20. The chloroform in the preparation was gently removed under a rotary evaporator. Distilled water was added and mixed well with the residue to prepare an emulsion. β-carotene/linoleic acid emulsion were mixed with the methanol solutions of the extracts and commercial antioxidants, and incubated in a water bath at 50 °C for 120 min. Inhibition of the oxidation of the β-carotene/linoleic acid emulsion system by the extracts and the commercial antioxidants was monitored spectrophotometrically by measuring the absorbance at 470 nm.

ABTS radical cation decolorisation assay. The experiments were carried out using an improved ABTS (2,2'-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid) decolorisation assay [20]. The mixture of ABTS and potassium persulfate was left at room temperature in the dark for 16 h, then it was diluted with ethanol (70 %) and its absorbance was adjusted to 0.70 ± 0.02 at 734 nm. ABTS cation solutions were added to the extract solution and the decrease of absorption was measured against 70 % ethanol, 6 min later, at the end of the reaction at room temperature. Synthetic antioxidants were used as positive controls.

Total phenolic compounds. Concentrations of the total phenolic compound in the extracts were determined by using Folin-Ciocalteu reagent according to the method described by Zubia *et al*. [21]. The phenolic content was expressed as gallic acid equivalent.

Statistics. Antioxidant activity of the data are expressed as means ± SEM. Statistical analysis was performed by ANOVA with LSD test and Student's *t*-test; *p* value of 0.05 or less was taken to indicate statistical significance.

Results

GC-MS analysis. The chemical compositions of the essential oils are reported in Table 1. Twenty eight components were identified in the oil of *L. obtusa* which represented about 77.74 % of the total composition. Twenty seven components were identified in the oil of *Laurencia obtusa var. pyramidata* making up 83.42% of the total oil composition.

Table 1. Chemical composition of Laurencia obtusa (Lo) and Laurencia obtusa var. pyramidata (Lp) essential oils (GC-MS Analysis)

Rt (min)	Compounds	Area (%)	
		Lo	Lp
10.70	Beta- Chamigrene	0.11	1.78
10.95	Longifolen	-	0.28
11.08	Beta-Patchoulene	-	1.04
11.68	Aromadendrene	-	0.65
12.06	Thujopsene	-	0.53
12.67	Alpha-guaiene	-	0.12
13.23	Isolongifolene	-	1.09
13.38	Benzene, 1,3,5-tris(1-methylethyl)	0.59	1.50
13.95	Epi-bicyclosesquiphellandrene	0.23	0.40
14.12	Dehydroaromadendrene	-	0.47
14.18	Beta-elemene	0.66	-
14.44	Trans-caryophyllene	-	0.29
16.39	3-phenylindole	0.24	0.87
16.55	Valencene	2.90	0.09
16.68	Alpha-selinene	1.63	-
16.99	2,4,6-trimethyl-3-(3′ methyl [1′-deuterio] buta-1′,2′-	1.77	-

	dienyl) aniline		
17.07	Trans-gamma-bisabolene	1.56	-
17.71	1-methoxy-6,6-dimethyl-3,4-benzotricyclo heptene	13.07	0.30
18.32	1,3,6-trimethyl-8-ethl-2,7-naphthyridine	1.28	-
18.48	Cuparene	2.21	-
18.60	Alpha-eudesmol	-	3.55
18.65	Laurene	2.23	-
18.95	7-methoxy-1,5,5-trimethyl-cyclohepta-1,3-diene	2.93	9.18
19.40	2,6-dimethyl-4-oxa-endo-tricyclo decane	15.10	48.22
19.62	Beta-eudesmol	1.23	2.42
20.23	1R,4s,7s,8R,11-R-2,2,4,8-tetramethyltricyclo undecan-7-ol	1.22	1.80
20.84	Dactylol	1.10	1.10
21.49	Nerolidol	4.11	0.70
21.95	Cyclohexyl-15-crown-5	-	0.31
22.33	5,10,13-trimethyl-2-oxatricyclo tridecane-9,11,13 triene	7.21	0.15
23.07	15-crown-5	0.81	0.88
23.45	10-demethylsqualene	-	5.28
24.32	18-crown-6-ether	4.16	0.17
25.03	3,6,9,12,15-pentaoxanonadecan-1-ol	-	0.25
25.07	Tetraethylene glycol	1.89	-
25.74	Pentaethylene glycol	0.84	-
27.46	Ethanol, 2-(2-ethoxyethoxy)	1,18	-
28.13	1-(3,4,5-trimethylphenyl) ethanone	3.61	-
28.85	12-crown-4	1.06	-
29.79	3,4-diethyl-6-methyl-pyrazolo pyridine quinone	2.81	-
	TOTAL	**77.74**	**83.42**

Antimicrobial activity. The antibacterial activities of the extracts and of the essential oils of *L. obtusa* and *L. obtusa* var. *pyramidata* were tested against Gram-positive and Gram-negative test bacteria, and against one yeast strain. The results of the paper disc diffusion assays are shown in Table 3 and 4. As evident from Table 3, the solvent extracts from *L. obtusa* exhibited weak activity against Gram-positive bacteria, only one Gram-negative bacterium, *E. cloaceae* and yeast strain *C. albicans*. However, the essential oil of *L. obtusa* was the most active showing a strong inhibitory effect against all tested Gram-positive bacteria including methicillin-oxacillin resistant *S. aureus* (MRSA), Gram-negative bacterium, *E. cloaceae* and yeast strain. The methanol extract and the essential oil of *L. obtusa* var. *pyramidata* showed neither antibacterial nor antifungal activity. On the other hand, the chloroform and hexane extracts showed low antimicrobial activity similar to that of *L. obtusa* extracts. Differently from the *L. obtusa* solvent extracts, the chloroform and hexane extracts of *L. obtusa* var. *pyramidata* exerted additionally low activity against *P. vulgaris*.

Table 3. Antimicrobial activity of *Laurencia obtusa* extracts and essential oil

Microorganisms	**Gram**	**µL/disc**			**mg/disc**									**µg/disc**	
		Lo-EO			**Lo-ME**			**Lo-CE**			**Lo-HE**			**Tob**	**Ny**
		0.1	**0.2**	**0.4**	**0.5**	**1**	**2**	**0.5**	**1**	**2**	**0.5**	**1**	**2**	**10**	**30**
B. subtilis	+	13	14	15	-	6.5	7	6.5	7	7.5	-	6.5	7	24	nt
S. aureus	+	11	12	13	6.5	7	8	-	6.5	7.5	-	6.5	7	16	nt
S. aureus *[a]	+	13	14	15	-	-	-	-	-	6.5	-	-	6.5	7	nt
E. faecalis	+	9	10	11	-	-	-	-	6.5	7	-	6.5	7.5	9	nt

S. epidermidis	+	9	10	11	-	-	-	-	-	6.5	-	-	6.5	7	nt
E. aerogenes	-	-	-	-	-	-	-	-	-	-	-	-	-	19	nt
E. cloaceae	-	10	11	12	-	-	-	-	-	-	-	-	6.5	13	nt
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	10	nt
*E. coli**[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	25	nt
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	12	nt
P. vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	13	nt
S. typhimurium	-	-	-	-	-	-	-	-	-	-	-	-	-	10	nt
C. albicans		11	12	13	-	-	-	-	6.5	7	-	6.5	7	nt	30

Zone of inhibition (mm), including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; Tob, Tobramycin (10 µg/disc); Ny, Nystatin (30 µg/disc); nt, Not tested; –, No activity; Lo, *Laurencia obtusa* ; EO, Essential Oil; ME, Methanol Extract; CE, Chloroform Extract, HE, Hexane Extract; *[a]Methicillin-Oxacillin resistant and *[b] Hemorrhagic, O157:H7.

Table 4. Antimicrobial activity of *Laurencia obtusa* var. *pyramidata* extracts and essential oil

Microorganisms	**Gram**	**µL/disc**			**mg/disc**									**µg/disc**	
		Lp-EO			**Lp-ME**			**Lp-CE**			**Lp-HE**			**Tob**	**Ny**
		0.1	**0.2**	**0.4**	**0.5**	**1**	**2**	**0.5**	**1**	**2**	**0.5**	**1**	**2**	**10**	**30**
B. subtilis	+	-	-	-	-	-	-	-	-	6.5	-	-	6.5	24	nt
S. aureus	+	-	-	-	-	-	-	-	6.5	7	-	6.5	7	16	nt
S. aureus *[a]	+	-	-	-	-	-	-	-	6.5	7	-	-	6.5	7	nt
E. faecalis	+	-	-	-	-	-	-	-	6.5	7.5	-	-	6.5	9	nt
S. epidermidis	+	-	-	-	-	-	-	-	-	6.5	-	-	6.5	7	nt
E. aerogenes	-	-	-	-	-	-	-	-	-	-	-	-	-	19	nt
E. cloaceae	-	-	-	-	-	-	-	-	-	6.5	-	-	6.5	13	nt
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	10	nt
*E. coli**[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	25	nt
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	12	nt
P. vulgaris	-	-	-	-	-	-	-	-	6.5	8.5	-	-	6.5	13	nt
S. typhimurium	-	-	-	-	-	-	-	-	-	-	-	-	-	10	nt
C. albicans		-	-	-	-	-	-	-	-	6.5	-	-	6.5	nt	30

Zone of inhibition (mm), including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; Tob, Tobramycin (10 µg/disc); Ny, Nystatin (30 µg/disc); nt, Not tested; –, No activity; Lp, *Laurencia obtusa* var. *pyramidata*; EO, Essential Oil; ME, Methanol Extract; CE, Chloroform Extract, HE, Hexane Extract *[a] Methicillin-Oxacillin resistant and *[b] Hemorrhagic, O157:H7.

Antioxidant activity. The *L. obtusa* and *L. obtusa* var. *pyramidata* extracts and the essential oils exhibited free radical scavenging activity against the stable free radical DPPH, decolorising activity of ABTS radical cation and also inhibition activity of the β-carotene bleaching at different proportions (Table 5 and 6). As for the antioxidant test results, the essential oils of *L. obtusa* and *L. obtusa* var. *pyramidata* showed rather similar activity, whereas, their total phenolic compounds were differently determined as 48.15±0.9 GAE mg/ 1g, 12.31±0.9 GAE mg/ 1g, respectively. As for the ABTS and β-carotene bleaching assay, solvent extracts of both algae showed consistent activity. In the present study, the algae extracts and oils exhibited approximately 100-fold weaker antioxidant activity than BHT, BHA and Vitamin E, because the extracts probably include various biologically active components as well as antioxidant compounds. DPPH radical scavenging activity of the chloroform extracts was found to be high for both algae, 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 extracts. When concerning the ABTS assay, *L. obtusa* showed a bit stronger antioxidant activity than *L. obtusa* var. *pyramidata*. The chloroform and hexane extract of *L. obtusa* and the methanol extract of *L. obtusa* var. *pyramidata* inhibited the bleaching percent of β-carotene-linoleat system, in excess of 50 %. Lastly, it was clarified that, both chloroform extracts of the algae include high amounts of phenolic compounds.

Table 5. Antioxidant activity of *Laurencia obtusa* extracts and essential oil

Concentrations (mg/mL)		% $ABTS^+$ decolorisation	% β-carotene bleaching	% DPPH radical scavenging	Total phenolics (GAE mg / 1g)
Lo-ME	0.5	9.83±2.3	11.58±2.6	1.65±0.8	1.80±0.4
	1	18.38±0.3	24.02±8.0	2.35±0.08	11.42±1.0
	2	29.62± 1.2	45.64±0.9	3.62±0.2	32.22±1.9
Lo-CE	0.5	22.55±0.9	28.42±4.1	-	22.82±1.7
	1	29.89±1.7	35.43±11.5	4.53±0.6	45.72±0.9
	2	44.42±0.4	64.84±1.8	9.08±1.1	77.76±0.8
Lo-HE	0.5	18.89±3.6	32.62±1.6	-	21.53±1.3
	1	32.77±0.9	47.05±1.6	-	40.77±0.1
	2	43.94±2.3	66.51±3.2	1.99±0.3	65.11±1.4
Lo-EO	0.5	9.72±3.5	33.03±1.9	-	15.17±2.8
	1	26.08±0.2	37.37±0.6	0.19±0.8	25.83±0.6
	2	31.80±4.0	38.17±2.6	1.4±1.8	48.15±0.9
BHA	0.1	97.90±0.4	92.85±8.6	87.93±0.2	nt
BHT	0.1	94.28±3.1	96.63±4.2	87.38±0.4	nt
Vit E	0.1	84.74±4.1	93.45±1.5	81.39±2.6	nt

Lo; *Laurencia obtusa*; EO, Essential Oil; ME, Methanol Extract; CE, Chloroform Extract, HE, Hexane Extract; BHA, Butylated Hydroxyanisole; BHT, Butylated Hydroxytoluene; Vit E, α-tocopherol; ±SEM, nt; Not tested.

Table 6. Antioxidant activity of *Laurencia obtusa var. pyramidata* extracts and essential oil

Concentrations (mg/mL)		% $ABTS^+$ decolorisation	% β-carotene bleaching	% DPPH radical scavenging	Total phenolics (GAE mg / 1g)
Lp-ME	0.5	3.8±0.9	26.5±3.5	0.7±1.7	6.56±1.3
	1	13.0±2.6	37.3±4.0	5.6±0.6	19.04±5.2
	2	20.2±3.2	59.4±6.9	11.9±0.8	40.96±1.3
Lp-CE	0.5	15.9±0.9	27.9±8.6	3.4±0.5	25.67±2.2
	1	16.2±1.9	27.2±9.8	6.1±0.7	50.64±0.8
	2	27.5±1	46.7±2.3	9.6±0.6	96.17±9.1
Lp-HE	0.5	10.8±0.8	20.8±10.5	-	1.37±1.2
	1	17.5±0.8	38.5±4.7	1.1±0.3	13.04±1.8
	2	25.2±0.4	43.4±4.1	4.2±0.9	45.47±4.8
Lp-EO	0.5	12.3±0.8	5.7±8.0	0.05±1.2	1.06±3.2
	1	23.1±0.2	10.8±2.2	1.2±0.4	11.48±1.0
	2	30.8±1.7	40.8±4.3	2.4±0.2	12.31±0.6
BHA	0.1	97.90±0.4	92.85±8.6	87.93±0.2	nt
BHT	0.1	94.28±3.1	96.63±4.2	87.38±0.4	nt
Vit E	0.1	84.74±4.1	93.45±1.5	81.39±2.6	nt

Lp; *Laurencia obtusa* var. *pyramidata*; EO, Essential Oil; ME, Methanol Extract; CE, Chloroform Extract, HE, Hexane Extract; BHA, Butylated Hydroxyanisole; BHT, Butylated Hydroxytoluene; Vit E, □□α-tocopherol; ±SEM, nt; Not tested.

Discussion

Fresh and dry seaweeds are extensively consumed by people because they contain carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. Seaweeds have some pharmaceutically active components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations [22]. In addition, marine algae are researched for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan [22, 23].

Laurencia is known as a rich source of valuable secondary metabolites which possess a variety of biological activities, such as antimicrobial, antifeedant, anthelmintic, and cytotoxic activities [6]. More than 300 compounds, mainly sesquiterpenes, C_{15}-acetogenins, and a few di- and triterpenes have been identified from some 40 species of the red algae genus *Laurencia,* collected in various parts of the world since the 1960s [24]. Elatol and iso-obtusol isolated from methanol extract of *L. majuscula* were shown to exert antibacterial activity. It was reported that elatol exerted significant antibacterial activities against *S. epidermis*, *K. pneumonia* and *Salmonella* sp, iso-obtusol inhibited to *K. pneumonia* and *Salmonella* sp., significantly [25]. Suzuki *et al*. [26] studied the antibacterial activities of the crude extract from *L. pannosa* against marine bacteria isolated from algal habitats in the Malaysian waters. Also, antibacterial activity of pannosanol, which is the major halometabolites of this species was noticed [26]. Mao and Guo [27], reported that cuparene-derived sesquiterpenes, laureperoxide, 10-bromoisoaplysin, isodebromolaurinterol and 10-hydroxyisolaurene were isolated from *L. okamurai*. Sun *et al*. [28], isolated from *L. tristicha* three naturally new sesquiterpenes named 10-hydroxyepiaplysin, 10-hydroxyaplysin and 10-hydroxybromoepiaplysin.

In the present study, solvent extracts and essential oils of *Laurencia obtusa* and *L. obtusa* var. *pyramidata* have been analyzed for their antimicrobial and antioxidant properties. The chemical composition of their essential oils and the total phenolic contents of all the extracts were also determined. In recent years, many articles have been concerning the volatile compounds of different marine algae [13, 29, 30]. The essential oil, specifically and the extracts of *Laurencia obtusa* were found to possess strong antimicrobial activity against the test microorganisms. The essential oil and the methanol extracts of *L. obtusa* var. *pyramidata* were found to be antimicrobially ineffective against tested bacteria and yeast strain, in spite of little antimicrobial activity of the chloroform and hexane extracts. To the results of GC-MS analysis of volatile oils, various sesquiterpens such as nerolidol, dactylol, valencene, β-chamigrene and β-eudesmol were identified in the volatile compounds of *L. obtusa* and *L. obtusa* var. *pyramidata* by GC-MS. Additionally, β-elemene was identified in *L. obtusa* in this assay, and it was known as an antitumor drug for different tumors, including glioblastoma [31]. Some species of *Laurencia* biosynthesize a characteristic set of secondary metabolite that usually is not widely used within the genus. Hence, secondary metabolite chemistry can supply as a standard for taxonomy in the genus *Laurencia* [8]. The concentration of terpenoids is lower in *L. obtusa* var. *pyramidata* compared to *L. obtusa*.

Bansemir *et al*. [32], found that the chloroform extract of *Laurencia chondrioides* was better than methanol and water extract against fish pathogenic and human pathogenic bacteria, because of chamigrene-type sesquiterpenes. In addition, it was reported in many studies that marine algae exerted higher antimicrobial activity on gram positive bacteria when compared with gram negative ones, because the cell wall structure of Gram-negative bacteria is more complex than that of Gram-positive ones [23, 33]. Also, in our study, it was seen that Gram positive bacteria were more susceptible to the algal solvent extracts and volatile oils of *L. obtusa* and hexane and chloroform extracts of *L. obtusa* var. *pyramidata*. However

antimicrobial activity spectrum of methanol, hexane and chloroform extracts of *L. obtusa* and hexane and chloroform extracts of *L. obtusa* var. *pyramidata* increased according to the concentration values, due to the effect on both Gram-negative and Gram-positive bacteria.

Free radicals which cause oxidative stress in the body and lipid peroxidation resulted in damaging of cellular membrane; proteins, nucleic acids and eventually cell death [34] are constantly generated in the body during the normal metabolic processes and interaction with environmental excitation [34]. Lipid peroxidation is also a handicap for food sector, thus, many products with antioxidant properties, mainly of synthetic origins, phenol derivatives such as, butylhydroxy toluene (BHT) and butyl hydroxyanisol (BHA), are widely used to increase the shelf life by preventing of foodstuffs from spoilage, loss of vitamins and rancidity.

Macroalgae synthesize antioxidant molecules such as ascorbate and glutathione (GSH), as well as more stable molecules including carotenoids, mycosporine-like amino acids, catechins, phlorotannins [31, 33-35]. Phenolic compounds are supposed to protect algal thalli from negative effects of UV radiation and to exhibit free-radical scavenging properties. Moreover high phenolic levels are coupled with antioxidant and antibacterial activities [33]. Our results showed that generally the antioxidant activity of hexane and chloroform extracts of *L. obtusa* and *L. obtusa* var. *pyramidata* were higher than that of methanol and essential oil. There was not a direct positive correlation between the amount of phenolics and the antioxidant activity.

DPPH radical scavenging assay is used to determine the test materials antioxidant capacity as proton radical scavengers or hydrogen donors, and not specific to any particular antioxidants [36]. Chew *et al*. [36] found a positive correlation between the total phenolic content and the concentration of seaweed extracts required to reduce DPPH radicals by 50. Rhodophyta have been reported to exhibit weak $DPPH^{\bullet}$ quenching activity when obtained by using water, ethanol or methanol as solvents for the extraction. On the other hand, chloroform, ethyl acetate and acetone extracts from several Rhodomelaceae genera have been reported to exhibit strong $DPPH^{\cdot}$ quenching activity *in vitro* [35]. To the present study, DPPH radical scavenging activities of the solvent extracts and the essential oils of *L. obtusa* and *L. obtusa* var. *pyramidata* were too low when compared to the other methods used and to synthetic antioxidants, and varied nearly between 1.5 and 12 %.

In the β-carotene bleaching assay, depending on the presence of antioxidant constituents which could neutralize the linoleate free radicals, the rate of decomposition of highly unsaturated β-carotene by peroxyl free radicals generated via oxidation of linoleic acid or prevention could be measured by monitoring the bleaching amount of orange color of β-carotene spectrophotometrically [36]. Chew *et al*. [36] found in their research that three different seaweed species have similar β-carotene bleaching antioxidant activity and probably the presence of approximately similar amounts of lipophilic antioxidants in all three species, in spite of very large differences in total phenolic content. As for our research, β-carotene prevent capabilities of *L. obtusa* and *L. obtusa* var. *pyramidata* were found to be very close to each other with little differences, and approximately activity range was found as 40-65 %, besides, unrelated with their phenolic content. Most studies showed there was no correlation between total phenolic content and β-carotene bleaching although a positive correlation has been reported. This is due to the different types of antioxidants that are assayed by the two methods, it means that total phenolic content gives an indication of the levels of both lipophilic and hydrophilic compounds, whereas β-carotene bleaching only gives an indication of the levels of lipohilic compounds [36].

As usual, the ABTS assay is used to determine the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxyl radicals) [37] for the plant samples. In the present study, the macroalgae samples of *L. obtusa* and *L. obtusa* var. *pyramidata* showed a low antioxidant activity ranging from about 20 to 45 %. Indeed, all the extracts and essential oils showed rather similar amount of ABTS radical decolorization activity. Some differences between the antimicrobial test results obtained in previous studies were reported by Rajasulochana *et al*. [22], underlining that several factors like intraspecific variability in the production of secondary metabolites, rarely seasonal variations, active metabolites achieving capability of extraction protocols. As in all biochemical research, the test materials may have trace impurities and the assay methods may have different susceptibilities for the target strains.

Consequently, *L. obtusa* essential oil showed moderate antimicrobial activity against Gram-positive and Gram-negative bacteria, and against the yeast strain, when compared to the *L. obtusa* var *pyramidata*. The extracts of the *Laurencia* samples showed little antimicrobial activity, and varied antioxidant activity according to the features of the test methods and the chemical content of the solvent extracts and the essential oils. Actually, the active components responsible for the antioxidant and antimicrobial activities need to be evaluated for achieving novel valuable compounds. Therefore, it is suggested that further studies should be performed on the isolation and identification of the antioxidant and antimicrobial components in *Laurencia* species for their industrial and pharmaceutical applications.

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