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Antimicrobial activity of volatile component and various extracts of *Enteromorpha linza* (Linnaeus) J. Agardh from the coast of Izmir, Turkey

Atakan SUKATAR, N. Ulku KARABAY-YAVAŞOGLU*, Guven OZDEMIR, Zerrin HORZUM

Ege University, Faculty of Science, Department of Biology, 35100 Bornova, Izmir, Turkey

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Abstract - The methanol, dichloromethane, hexane, chloroform and volatile components of *Enteromorpha linza* were tested *in vitro* for their antimicrobial activity against five Gram-positive, four Gram-negative bacteria and *Candida albicans* ATCC 10239. GC-MS analysis of the volatile components of *E. linza* resulted in the identification of 35 compounds which constituted 84.76% of the total compounds. The volatile components of *E. linza* consisted of n-tetratriacontane (8.45%), 1-heptadecanamine (6.65%) and docosane (6.46%) as major components. The methanol and chloroform extracts showed more potent antimicrobial activity than hexane and dichloromethane extracts. The volatile oils of these algae did not remarkably inhibit the growth of tested microorganisms.

Key words: *Enteromorpha linza*; volatile compounds; extracts; antimicrobial activity.

INTRODUCTION

Antimicrobial agents are widely used for human medication and in veterinary field. Algae are important source of therapeutically useful substances. Marine algae have been used as food, as well as in industry and medicine for various purposes (Saleh *et al*., 1993). Algae have a diverse range of uses as sources of human food, especially in Japan, China and Korea (Nisizwa *et al*., 1987). Algal metabolites, derived from cyanobacteria or eukaryotic algae, are central to many source-water issues, and as such prokaryotic and eukaryotic algae are well known as sources of biologically active compounds (Cannell, 1993). In marine environment, the many ecological pressures prevailing on marine organisms, including competition for space, maintenance of unfouled surfaces, deterrence of predation and the ability to reproduce successfully, may have led to the prevalence of unique secondary metabolites (Konig *et al*., 1994). It is noticeable that seaweeds maintain the capacity to recover and regenerate rapidly after damage, suffering little attack from microbial pathogens. Antimicrobial activity of extracts from seaweeds originating in various parts of the world, for example, the coasts of France (Moreau *et al*., 1988), India (Rodrigues *et al*., 2004) and Bulgarian (Zamenarska, 2002) have been reported.

Recently, the production of secondary metabolites with antimicrobial activities by the macroalgae was studied under different conditions, although no trend for bioactivity was observed (Gonzalez del Val *et al*., 2001). Also, it is reported that, some substances extracted from marine green algae have been shown to have many pharmacological activities (Awad, 1998, 2000).

Ulva is a genus of common, green macroalgae found throughout the world in the upper intertidal zone of seashores and as a fouling organism on a variety of man-made structures including ships' hulls (Callow, 1996). The genus *Ulva* (syn. *Enteromorpha*; Hayden *et al*., 2003) are well known fouling organisms that colonise a variety of natural and artificial submerged substrata. The only chemical analysis of *Enteromorpha* concerns its metal content (Malea and Haritonidis, 1999). Antiviral compounds in extracts of *E. linza* (Linnaeus) J. Agardh have also been studied (Hudson *et al*., 1998).

Turkey has an extensive coastline along which marine algae are well represented. However, the distribution of such antimicrobial activity within algal thalli has not been studied. The present study investigated the antimicrobial activity within volatile components and various extracts from selected *E. linza* from Izmir bay, Turkey.

MATERIALS AND METHODS

The organisms. Field collections of seaweed were made from several reefs (depths of 1-2 m) along the Izmir coast during September and November 2004 and identified by Dr. Atakan Sukatar. Voucher specimens were deposited in the Hydrobiology Laboratory in Ege University, Faculty of Science, Department of Biology, Izmir, Turkey. Samples were frozen immediately after harvesting and stored at –30 °C until they were freeze-dried.

Extraction of the volatile components of *Enteromorpha linza*. For the volatile compounds, the dried *E. linza* (50 g)

* Corresponding author. Phone: +90 232-3884000-1731; Fax: +90 232-3881036; E-mail: ulku.karabay@ege.edu.tr

were subjected to steam distillation for 4 h using a Clevenger-type apparatus. The obtained distillate was diluted with ethyl acetate and the volume was reduced 100 fold prior to analysis.

Preparation of various extracts of *Enteromorpha linza*. Freeze-dried samples were pulverised. Pulverised samples (15 g for each) were extracted as reported by Khan *et al*. (1988) and Vlachos *et al*. (1996) in 150 mL methanol, dichloromethane, hexane and chloroform for 24 h using a Soxhlet extraction apparatus (yield 12, 0.3, 0.9, 12%, respectively). The resulting extracts of *E. linza* from these different solvents were kept at 4 °C until use. All solvents used were of analytical reagent grade and obtained from Sigma Chemical Co. (St. Louis, USA)

Gas chromatography-mass-spectrometry (GC-MS) analysis. The steam-distilled components were analysed 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. The column temperature was programmed from an initial temperature of 70 °C to a final temperature of 280 °C at 10 °C/min. The injector temperature was 150 °C (1 µL injection size), whereas the detector temperature was 250 °C. The carrier gas was helium (2 mL/min). 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 (Adams, 1995). A computerised search was carried out using the Wiley 275 L. GC/MS library and ARGEFAR GC/MS library created with authentic samples.

Test microorganisms. *In vitro* antimicrobial studies were carried out against ten bacteria strains (*Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633*, Staphylococcus aureus* ATCC 6538 p*, Staphylococcus epidermidis* ATCC 12228*, Streptococcus faecalis* ATCC 8043*, Pseudomonas aeruginosa* ATCC 27853*, Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998*, Salmonella typhimurium* CCM 583) and one yeast strain (*Candida albicans* ATCC 10231) which were obtained from the Microbiology Department Culture Collection of Ege University, Faculty of Science.

Antimicrobial testing. The paper disc diffusion method was employed for determination of antimicrobial activities of volatile oils and extracts of samples (Collins and Lyne, 1989; Bradshaw, 1992). Briefly, sterile, 6 mm diameter filter paper discs (Schleicher and Schül, No. 2668, Dassel, Germany) were impregnated with 20-30 µL of three different concentrations (1, 2, 4 mg disc^{-1}) of the *E. linza.*

The bacteria strains were inoculated on nutrient broth (Oxoid) and incubated for 24 h at 37 °C, while the yeast strain was inoculated on malt extract broth (Oxoid) and incubated for 48 h at 28 °C. Adequate amounts of autoclaved Muller Hinton Agar (Oxoid) and Malt Extract Agar were dispensed into sterile plates, and allowed to solidify under aseptic conditions. The counts of bacteria strains and yeast strain were adjusted to yield approximately 1.0 x 10^7-1.0 x 10^8 mL^{-1} and 1.0 x 10^5-1.0 x 10^6 mL^{-1}, respectively, using the Standard McFarland counting method. The test organisms (0.1 mL) was inoculated with a sterile swab on the surface of appropriate solid medium in plates.

The agar plates inoculated with the test organisms were incubated for 1 h before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with the different extracts were placed on the agar plates. The bacterial plates were incubated at 37 °C for 24 h while the yeast plates were incubated at 28 °C for 48 h. After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times. Tobramycin discs (10 µg/disc) and nystatin discs (30 µg/disc) were used as positive controls.

RESULTS AND DISCUSSION

The composition of *E. linza* volatile components was analysed by employing GC-MS, leading to compare the relative retention times the mass spectra of oil components with those of authentic samples and mass spectra from data library. Thirty-five compounds were identified in the volatile oil of *E. linza*, constituting about 85% of the total component. The components are listed in Table 1. A lot of compounds in the

TABLE 1 – Volatile components of *Enteromorpha linza* (GC-MS analysis)

Rt (min)	Compound	Area (%)
6.26	n-Octane	2.58
6.51	n-Heptane	2.75
6.71	2,4-Dimethyl-1-heptene	3.87
10.12	n-Undecane	0.96
10.98	n-Tridecane	0.84
14.65	n-Pentadecane	1.19
14.95	n-Tetradecanol	0.50
15.18	n-Pentadecanol	1.34
15.35	1-Octene	1.65
15.55	n-Hexadecane	3.41
15.75	n-Heptadecane	1.13
15.95	n-Octadecane	0.58
18.60	n-Nonadecane	0.79
18.88	n-Eicosane	4.13
19.05	n-Heneicosane	0.83
19.32	Phenol, 2,4 bis(1,1-dimethyl ethyl)	3.30
19.40	Methyloctadecanoate	1.83
19.57	Cyclohexylheptadecane	2.22
19.69	n-Docosane	6.46
19.89	n-Tricosane	3.25
20.03	n-Tetracosane	1.91
20.11	n-Pentacosane	1.45
20.26	n-Hexacosane	1.52
20.36	Thiophene-D3	0.29
20.46	n-Octacosane	0.67
22.55	n-Nonacosane	1.07
22.73	n-Ethanol, 2-(dodecyloxy)	1.06
22.88	n-Triacontane	4.05
23.05	n-Tritriacontane	1.47
23.47	2-Fluoro-5-phenylpyrimidine	2.39
23.73	n-Tetratriacontane	8.45
24.05	1-Heptadecanamin	6.65
24.24	n-Pentatriacontane	4.86
24.52	3-Ethyl-3-methylheptane	3.42
24.77	Decanedioic acid didecylester	1.89
TOTAL		84.76

TABLE 2 – Antimicrobial activity of *Enteromorpha linza* volatile oil compared with standard tobramycin and nystatin

Microorganism	Gram	Inhibition zone (mm)[a]		
		E. linza (0.015 µg/disc)	Tobramycin (10 µg/disc)	Nystatin (30 µg/disc)
Staphylococcus epidermidis	+	–	7	nt
Staphylococcus aureus	+	7	16	nt
Streptococcus faecalis	+	–	9	nt
Bacillus subtilis	+	–	24	nt
Salmonella typhimurium	–	8	10	nt
Pseudomonas aeruginosa	–	–	12	nt
Enterobacter cloacae	–	7	13	nt
Escherichia coli	–	7	10	nt
Candida albicans	nt	–	nt	18

[a] Zone of inhibition, including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; –, no activity; nt, not tested.

volatile oil of *E. linza* were identified as hydrocarbon compounds. The major components were n-tetratriacontane (8.45%), 1-heptadecanamine (6.65%) and n-docosane (6.46%). The other most abundant components were identified as n-pentatriacontane (4.86%), n-eicosane (4.13%), and n-triacontane (4.05%). Heptadecane and hexadecane have been reported to be common major volatile components in many other macro and micro algae (Yamamoto *et al*., 2001; Tellez *et al*., 2001; Ozdemir *et al*., 2004). Chemical composition studies of *E. linza* are very limited. However, aldehydes such as pentadecadienal, (8Z)-8-heptadecanal, (8Z,11Z)-8,11-heptadecadienal, (8Z,11Z,14Z)-8,11,14-heptadecatrienal, and (7Z,10Z,13Z)-7,10,13-hexadecatrienal are the most important flavour components among a variety of volatile oil found in marine green alga *Ulva pertusa* (Akakabe *et al*., 2005). Also, in essential oil of *Ulva conglobata*, short-chain aldehydes such as hexanal, (2E)-2-hexenal, (3Z)-3-nonenal, (2E)-2-nonenal and (2E,6Z)-2,6-nonadienal were found and identified by GC-MS (Akakabe *et al*., 2003). Here we report for the first time on the essential oil composition of *E. linza*.

The antimicrobial activities of *E. linza* volatile oils and extracts against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameters. In this study, it was reported that the volatile oils of *E. linza* did not remarkably inhibit the growth of tested microorganisms. The antibacterial activities are reported in Tables 2 and 3. Dichloromethane and hexane extracts (except for *E. coli*) did not exhibit antimicrobial activity, but methanol and chloroform extracts were found to have moderate and strong activity against the most of microorganisms tested (Table 3). These remarkable differences between our results may be due to several factors. There could be differences in the capability of the extraction protocols to recover the active metabolites and differences in the assay methods. Some studies showed that methanol extraction yielded higher antimicrobial activity than hexane

TABLE 3 – Antimicrobial activity of *Enteromorpha linza* extracts

Microorganism	Diameter of zone of inhibition (mm)[a]											
	Methanol (mg/disc)			Dichloromethane (mg/disc)			Hexane (mg/disc)			Chloroform (mg/disc)		
	1	2	4	1	2	4	1	2	4	1	2	4
Staphylococcus epidermidis	–	–	–	–	–	–	–	–	–	–	–	–
Staphylococcus aureus	8	15	25	–	–	–	–	–	–	–	–	8
Streptococcus faecalis	–	–	–	–	–	–	–	–	–	–	–	–
Bacillus cereus	–	–	9.5	–	–	–	–	–	–	–	8	9.5
Bacillus subtilis	–	–	9	–	–	–	–	–	–	–	7	9.5
Salmonella typhimurium	–	–	7.5	–	–	–	–	–	–	–	7	10
Pseudomonas aeruginosa	–	–	–	–	–	–	–	–	–	7	10	11
Enterobacter cloacae	–	–	–	–	–	–	–	–	–	–	10	13
Escherichia coli	–	–	9	–	–	10	–	–	–	–	–	9
Candida albicans	–	–	–	–	–	–	–	–	–	–	–	–

[a] Zone of inhibition, including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; –, no activity.

and ethyl acetate (Febles *et al*., 1995). In others chloroform was better than methanol and benzene (Sastry and Rao, 1994). The results of our study were mostly similar the results of Febles *et al*. (1995) and Sastry and Rao (1994) studies. It is noted that most of the chloroform extracts showed significant antimicrobial activities than methanol extract. It may arised from the antagonistical effect. It is known that antagonistical and synergistical effects can change the activities of the extracts. In traditional medicine, liquid extracts are most often made by boiling or infusion or alcohol maceration (Phongpaichit *et al*., 2005). It is shown that the use of organic solvents always provides a higher efficiency in extracting antimicrobial activities.

The methanol extract of *E. linza* showed more potent antimicrobial activity than hexane, chloroform and dichloromethane extracts. Generally, when compared with the standard antibiotic, tobramycin, especially methanol extracts (4 mg/disc) exhibited more antimicrobial activity. For example, 2 mg/ kg of methanol extract of *E. linza* showed same antibacterial activity against *S. aureus* (inhibition zone is 15 mm) with tobramycin, while 4 mg/ kg of methanol extract of *E. linza* showed the more potent antibacterial activity against *S. aureus* (inhibition zone is 25 mm) than tobramycin (inhibition zone is 16 mm).

It has previously been shown that antimicrobial component-producing marine macroalgae inhibited to growth of some bacteria (Reichelt and Borowitzka, 1984; Ballantine *et al*., 1987; Gonzalez del Val *et al*., 2001; Lustigman *et al*., 1992; Sastry and Rao, 1994). Green macroalgae (Chlorophyta) showed a high percentage of species (73%) with antimicrobial activity. It was previously reported that, the methanol extract of *Enteromorpha compressa* had no antimicrobial activity against tested organisms while the methanol extract of *Enteromorph muscoides* had strong antimicrobial activity against only *Staphylococcus aureus* and weak antifungal activity against the filamentous fungus *Aspergillus fumigatus* (Gonzalez del Val *et al*., 2001). Extracts and volatile oil tested of *E. linza* did not showed activity on the yeast *Candida albicans.* Similarly, the methanol extracts of *E. compressa, E. muscoides* and *Ulva rigida* were not presented activity the fungus *Candida albicans* (Gonzalez del Val *et al*., 2001).

In summary, our results indicate that methanol and chloroform extracts of *E. linza* collected from the coast of Izmir (Turkey), present a significant capacity to show a variety of antimicrobial activities. Antimicrobial activity depends on algal species and efficiency on extraction of their active(s) principle(s). The chemical nature of active principles in lipid-soluble extracts of marine algae is not so far totally identified.

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