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ORIGINAL ARTICLE

Chemical characterization, in vitro biological activity of essential oils and extracts of three *Eryngium* L. species and molecular docking of selected major compounds

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Abstract Many *Eryngium* species have been traditionally used as ornamental, edible or medicinal plants. The gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC–MS) analyses have shown that the major compounds in the aerial parts were spathulenol (in *E. campestre* and *E. palmatum* oils) and germacrene D (in *E. amethystinum* oil). The main compounds in the root oil were nonanoic acid, 2,3,4-trimethylbenzaldehyde and octanoic acid for *E. campestre*, *E. amethystinum* and *E. palmatum*, respectively. All the oils expressed the highest potential against Gram-positive bacteria *Staphylococcus aureus* as well as Gram-negative *Klebsiella pneumoniae* and *Proteus mirabilis*. Molecular docking analysis was used for determining a potential antibacterial activity mechanism of compounds present in the essential oils. Molecular docking confirmed that the binding affinity of spathulenol to the active site of tyrosyl-tRNA synthetase was the highest among the tested dominant compounds. Regarding the total phenolic content (determined by the Folin–Ciocalteu assay) and flavonoid content (evaluated using aluminum nitrate nonahydrate), the highest amount was found in the ethyl acetate extract of *E. palmatum*. The results of DPPH and ABTS assay indicated that the highest antioxidant activity was present in the water extract of *E. amethystinum*. Extracts of the aerial parts presented as minimum inhibitory concentration (MIC) expressed the activity in the range 0.004–20.00 mg/mL, with the highest activity exhibited by the acetone and ethyl acetate extracts against *Proteus mirabilis*. The obtained results suggest that *Eryngium* species may be considered a beneficial native source of the compounds with antioxidant and antimicrobial properties.

Keywords *Eryngium* species · Essential oil · Extracts · Chemical composition · Antioxidant and antimicrobial activity · Molecular docking

Introduction

The plants of genus *Eryngium* have been used in ethnopharmacology, as a nutrition source and for medical purposes. *Eryngium* is one of the most complex genera of the family Apiaceae with approximately 250 species, including annual, biennial, and perennial plants, widely found in Eurasia, America, North Africa and Australia (Thiem et al. 2011). This study was based on three taxa: *E. campestre* L., *E. amethystinum* L. and *E. palmatum* Pančić & Vis.

Eryngium campestre is a common species in Europe, extending to South England, whereas *E. amethystinum* grows in the Balkan Peninsula and the Aegean region, Italy and Sicily. *E. palmatum* is an endemic perennial plant, whose prevalence is restricted to the central part of the Balkan Peninsula (Chater 1968).

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In many countries *E. campestre* is extensively used in both traditional medicine and human diet. In Turkey the whole plant is used as an antitussive, diuretic, aperitif, stimulant and aphrodisiac (Güneş et al 2014), whereas in Italian folk medicine the root of *E. amethystinum* is used as a diuretic and laxative. Some recent studies have confirmed the beneficial results previously claimed by traditional medicinal uses. In experimental rats, ethanol extracts of *E. campestre* exhibited apparent anti-inflammatory and anti-nociceptive activity, as well as a positive anti-inflammatory effect on periodontitis, by reducing infiltration of leucocytes and nitro-oxidative stress (Küpeli et al. 2006; Conea et al. 2015). Previous results concerning methanolic extracts obtained from the fruit of *E. amethystinum* implied that this species had strong oxidation agents (Wojtanowski et al. 2013). Also, methanol and chloroform extacts from the aerial parts or the roots of *E. palmatum* expressed a significant antibacterial activity (Marčetić et al. 2014). A wide range of biological activities is conditioned by the presence of a large number of chemical compounds in *Eryngium* species: triterpenoid saponins, triterpenoids, sesquiterpenes, monoterpenes, flavonoids, coumarins, phenolics, steroids and acetylenes (Wang et al. 2012).

The molecular docking was chosen as the most appropriate method to determine the design of target metabolites, as well as the mechanism of action of the pharmacologically active molecules. Modeling and docking studies have been carried out to understand the interactions of the enzyme with the substrate which in turn gives information about the stability and activity of the psychrophilic enzyme in comparison with its counterparts (Ramya and Pulicherla 2015). Also, focus is determining a suitable geometry and binding affinity of the tested molecule (ligand) to the active site of the target macromolecules using "scoring" functions (Kroemer 2007).

The main objectives of this study were the comparison of the chemical compositions of EOs obtained from the aerial parts and roots, and evaluation of the antioxidant and antimicrobial activity of EOs and extracts, while an additional objective was to determine a potential mechanism of the dominant compounds' activity on *Staphylococcus aureus*, using molecular docking studies on *Eryngium campestre*, *E. amethystinum* and *E. palmatum*.

Materials and methods

Plant material

Aerial parts and roots of *E. campestre* and *E. palmatum* were collected in June 2012 in Serbia, at the localities of the City of Niš and Sićevo Gorge, respectively, while *E. amethystinum* plants were collected in June 2013 near Vitlište village (Macedonia). The voucher specimens for *E. campestre* (10802), *E. amethystinum* (10801), *E. palmatum* (10803) were deposited in the "Herbarium Moesiacum Niš", The University of Niš.

EO isolation

EOs were obtained separately by 3-hour hydro-distillation, using a Clevenger-type apparatus, from the previously dried aerial parts (490, 130, 297 g) and roots (47, 40, 76 g) of *E. campestre*, *E. amethystinum* and *E. palmatum*, respectively. Anhydrous sodium sulfate was used for the desiccation of oils which were stored at a temperature of 4 °C.

Gas chromatography-flame ionization detector (GC-FID) and gas chromatography–mass spectrometry (GC–MS)

The analysis of the studied oils included the use of GC-FID and GC–MS, where the GC analysis was performed using a GC HP-5890 II apparatus. The split-splitless injector was connected to HP-5 column (25 m × 0.32 mm, 0.52 μm film thicknesses) and suited to FID. The analytic conditions were as follows: flow rate of H_2-1 mL/min, split ratio-1:30, temperature of injector-250 °C, temperature of detector-300 °C, temperature of column-programed from 40° to 240 °C (at a rate of 4°/min). Solutions of EO were consecutively injected by ALS (1μL, splitless mode). The area percent reports, obtained as a result of standard processing of chromatograms, were used as the base for quantification purposes.

The same parameters were used for GC–MS analysis. HPG 1800C Series II GCD system (Hewlett-Packard, Palo Alto, CA, (USA) was also used with HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness). The transfer line was heated at 260 °C, whereas mass spectra were acquired in EI mode (70 eV), in m/z range 40–400, and scan time 1.50 s. Instead of hydrogen, helium was used as the carrier gas. Sample solutions were injected by ALS (1 μL, splitless mode).

The constituents were identified by comparison of their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1., National Institute of Standards and Technology-NIST, Standard Reference Data Program, Gaithersburg, MD, USA), compared to those from available literature and used as additional tool to approve MS findings (Adams 2007).

Extraction protocol and antioxidant activity

Air-dried, ground aerial parts of the plants (10 g) were used for extraction adding 100 mL of water (H_2O), methanol (MeOH), acetone (Acet) and ethyl acetate (EtOAc). All organic solvents (p.a.) were purchased from "Zorka pharma" company, Šabac, Serbia. After being left in an ultrasonic bath for 30 min, the mixture was kept in a dark place for 24 h and then filtered. Vacuum evaporator and freeze-dryer (for H_2O extract) were used to remove the solvents. All results were calculated per g of dry weight of plant extracts (DW) (Džamić et al. 2013).

DPPH (2,2-dyphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assays were used to test the antioxidant activity of the extracts. All the measurements were set using Shimadzu, UV–visible PC 1650 spectrophotometer, while the extracts were soluted to concentrations of 2 mg/mL, except for EtOAc (5 mg/mL). The experiment chemicals such as anhydrous sodium carbonate, potassium acetate, potassium peroxidisulphate and L(+)-Ascorbic acid (Vitamin C) were purchased from AnalaR Normapur, VWR, Geldenaaksebaan, Leuven Belgium, while aluminum nitrate nonahydrate was obtained from Fluka Chemie AG, Buchs, Switzerland.

Total phenolic content (TPC)

TPC was determined applying FC-reagent method (1:10), given previously (Singh et al. 2016) which is a slightly modified form of the method originally reported by Singleton et al. (1999). The results were measured at 740 nm. The standards included BHA (3-tert-butyl-4-hydroxyanisole) and Vitamin C, while the blank was pure water. The calculated results were based on the gallic acid (Sigma Chemicals Co., St Louis, MO, USA) calibration curve (10–100 mg/L), expressed as gallic acid (GA)/g DW.

Flavonoid content (TFC)

The mixture used for determining TFC was prepared according to the procedure reported by Woisky and Salatino (1998) with some modifications (Matejic et al. 2016). The absorbance was measured at 415 nm on spectrophotometer. The quercetin hydrate (TCI Europe NV, Boerenveldsweg, Belgium) calibration curve was used for calculating the results (10–100 mg/L), expressed as quercetin equivalents (Qu)/g DW.

DPPH scavenging activity

The antioxidant activity of all the extracts and the two chosen standard compounds (Vitamin C and BHA) was evaluated according to so-called DPPH-test. The decreasing intensity of the purple of DPPH (Sigma Chemicals Co., St Louis, MO, USA) was measured at 517 nm (A_1) after 30 min (Blois 1958). The tested concentrations of the extract were: 0.50, 1, 2, 3, 4, 5, 6, 7, 8 mg/mL, where MeOH was used as blank (A_0). Scavenging activity (%) was calculated applying the following equation:

$$\text{Scavenging activity } (\%) = (A_0 - A_1) \times 100/A_0$$

The IC_{50} value was defined as the sample concentration causing 50% decrease of the initial DPPH-concentration, i.e. calculated *Scavenging activity*-50%.

ABTS radical scavenging activity

Experimental design was modelled after Miller and Rice-Evans (1997) as modified by Matejic et al. (2016). ABTS (TCI Europe NV, Boerenveldsweg, Belgium) solution was prepared by dissolving 19.2 mg ABTS in 5 mL potassium persulfate (2.46 mM), where the water was used as a blank. The measured absorbance was 734 nm and the results were calculated taking Vitamin C for the calibration curve (0.1–2 mg/L), expressed as Vitamin C (Vit C)/g DW.

Antimicrobial activity

Test microorganisms

Four Gram-positive and four Gram-negative bacterial strains were used to test the antibacterial activity of *Eryngium* EOs and its extracts: *Staphylococcus aureus* (ATCC 6538), *S. epidermidis* (ATCC 12228), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC 19433); *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus mirabilis* (ATCC 12453), *Klebsiella pneumoniae* (ATCC 10031). A human pathogenic yeast *Candida albicans* (ATCC 10231) was used to test antifungal activity. The bacterial strains were cultivated on Nutrient Agar (NA) at 37 °C, while the yeast was developed on Sabouraud Dextrose Agar (SDA) at 30 °C at The Microbiology Laboratory (Department of Biology, Faculty of Science and Mathematics, University of Niš).

Antimicrobial activity (microdilution method)

Antimicrobial activity was evaluated using the broth microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS 2003) with slight modifications (Sourmaghi et al. 2015). Overnight cultures (18 h) were used to make cell suspensions standardized to 0.50 McFarland turbidity, as measured on the McFarland Densitometer (DEN-1, Biosan). The 24 h

inoculation period was followed by incubation at 37 °C. Streptomycin and nystatin were used as the positive controls, while wells without inoculum and test substance represented the negative control, including test sterility of the medium. Visual reading of the bacterial growth was performed after adding triphenyltetrazolium chloride (TTC, 0.50%) aqueous solution. The lowest concentration of the test compound that inhibited growth was represented by a red-colored medium in the wells and considered the minimal inhibitory concentration (MIC). All experiments were performed in triplicate.

Molecular docking

Ligands data set

The compounds selected for docking studies had the highest percentage of EOs from the roots and herbal parts (spathulenol, germacrene D, nonanoic acid, octanoic acid and 2,3,4-trimethylbenzaldehyde). 3D structures of the studied analysis compounds in their neutral forms were constructed using Marvin 6.1.0, 2013, ChemAxon (http://www.chemaxon.com).

Docking studies

It is known that translation of genetic information into proteins is controlled by aminoacyl-tRNA synthetase enzymes. As tyrosyl-tRNA synthetase is fundamental in the biosynthesis of bacterial proteins, this enzyme invites a therapeutic target which is recommending as novel antibacterial agents (Lapointe 2013). Li et al. (2011) indicated that the most convincing explanation of the mechanism of action in the selected compounds can be achieved by molecular docking. The crystal structure of tyrosyl-tRNA synthetase was purchased from the Brookhaven Protein Data Bank http://www.rcsb.org/pdb (PDB entry: 1JIJ). All hydrogen bonds and hydrophobic interactions between the studied molecules and the amino acids from the enzyme's active site were identified by applying Molegro Virtual Docker (MVD v. 2013.6.0.1.). MVD software was used to calculate relevant binding energies and docking score functions (Thomsen and Christensen 2006), whereas the binding site was determined with a grid resolution of 0.30 Å. The number of runs was set to 100 in MolDock SE search algorithm. The docking procedure parameters were: population size − 50, the highest number of iterations − 1500, energy threshold − 100.00 and the maximum number of steps − 300. The largest number of docking runs was set to 10 and the estimation of ligand–receptor interactions was described by MVD-related scoring functions: E-Inter, Hbond, LE1, LE3, VdW, Steric, MolDock Score and Rerank Score. MolDock The optimizer algorithm was used for docking the ligand into the defined grid, while detailed energy estimates were used for monitoring its interactions. Each run included maximum population of 100, the highest iterations of 10,000 and five best positions.

Statistical analysis

All the values were measured three times and then presented as the average of these values ± standard deviation. OriginPro 8.0 software was used for analyzing the results which were also analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test ($P \leq 0.05$) carried out using the Minitab®17 software.

Results and discussion

Qualitative and quantitative analyses of the EOs (GC-FID and GC–MS)

A Clevenger-type apparatus was used for the isolation of EOs, with the following yields for aerial parts and roots: *E. campestre* (0.01%, 0.09%), *E. amethystinum* (0.06%, 0.08%), *E. palmatum* (0.05%, 0.08%), respectively.

The results of the chemical analysis of EOs in the three *Eryngium* species are presented in Table 1. Spathulenol was the main compound in *E. campestre* and *E. palmatum* oils obtained from the aerial parts, whereas germacrene D was a dominant constituent in the oil obtained from the aerial parts of *E. amethystinum*. The main compounds of the root oils were nonanoic acid, 2,3,4-trimethylbenzaldehyde and octanoic acid for *E. campestre*, *E. amethystinum* and *E. palmatum*, respectively. In a previous study, Çelik et al. (2011) analyzed the composition of the EOs from the aerial parts of three *Eryngium* species from Turkey. Among the 13 compounds identified in *E. campestre* oil, α-pinene (5.01%) had the highest values. Flamini et al. (2008) identified α-pinene, 2,3,6-trimethylbenzaldehyde and germacrene D as the main compounds of EOs obtained from the leaves, inflorescences and fruit of *E. amethystinum* from Italy. Furthermore, the EO from the aerial parts of *E. palmatum* from Serbia predominately contained sesquicineole (21.30%), caryophyllene oxide (16.00%), spathulenol (6.60%) and sabinene (4.40%) (Capetanos et al. 2007).

Comparison with the previous studies referenced in literature indicated similar EO compositions to the samples analyzed in our study, differing only in percentages of the main compounds. α-pinene was not recorded in our oil samples, which is completely different from the previously reported data. Numerous studies emphasized the influence of biotic and abiotic factors as potential causes of variation

Table 1 Composition of the EOs from the aerial parts and roots of *Eryngium* species

	Species			Ec	Ec	Ea	Ea	Ep	Ep
	Plant part			Herb	Root	Herb	Root	Herb	Root
	Constituents	KIE	KIL	%	%	%	%	%	%
1	*n*-Heptanal	917.7	901	0.08	0.17			0.11	0.09
2	Valeric acid	931.1	933		0.22				
3	Thuja-2,4(10)-diene	958.9	953	0.02					
4	Benzaldehyde	969.3	952	0.03					
5	*n*-Heptanol	979.0	959	0.15	0.55			0.10	0.23
6	1-Octen-3-ol	987.4	974	0.11	0.13				
7	Myrcene	994.8	987	0.20					
8	2-Pentyl furan	995.6	987		0.14				
9	Mesitylene	996.6	994				0.20		
10	*n*-Octanal	1006.4	998	2.05	3.74	0.07	0.04	**3.49**	0.66
11	Hexanoic acid	1006.5	1008		**6.82**				0.23
12	1,2,3-Trimethyl benzene	1023.9	1019				0.26		
13	*p*-Cymene	1024.0	1020	0.11	0.08	0.01		0.11	0.21
14	Limonene	1026.9	1024	0.07					
15	1,8-Cineole	1029.1	1026	0.01	0.10				
16	2-Ethylhexan-1-ol	1033.3	n/a	0.04					
17	3-Octen-2-one	1040.5	1030	0.04					
18	Benzene acetaldehyde	1045.7	1036	0.04					
19	β-Terpinene	1055.0	1056				0.02		
20	γ-Terpinene	1055.4	1054	0.05					
21	(2*E*)-Octen-1-ol	1058.0	1060	0.11					
22	*n*-Octanol	1074.8	1063	0.48	0.66	0.06		0.86	0.77
23	Fenchone	1083.8	1083		0.24				
24	2-Nonanone	1091.6	1087	0.72	0.48	0.02	0.03	0.19	0.02
25	*n*-Undecane	1096.7	1100	0.92	**8.09**				
26	*n*-Nonanal	1103.7	1100	1.53	1.00	0.11	0.04	1.22	0.10
27	Heptanoic acid	1108.8	1109		3.24				0.51
28	n.i.*	1121.3							
29	α-Campholenal	1122.6	1122	0.40				0.12	0.05
30	Nopinone	1132.8	1135	0.11					
31	*trans*-Pinocarveol	1135.8	1135	0.42					
32	*cis*-Verbenol	1139.6	1137	0.29	0.20				0.17
33	*trans*-Verbenol	1144.1	1140	0.55	0.22	0.02			0.65
34	Eucarvone	1155.4	1146				0.16		
35	(2*E*)-Nonen-1-al	1157.7	1157	0.55	1.13	0.03		0.57	0.47
36	(2*E*)-Nonenol	1159.5	1163						0.11
37	Borneol	1164.6	1165		0.22				
38	*p*-Mentha-1,5-dien-8-ol	1166.7	1166	0.19					
39	Terpinen-4-ol	1174.3	1174	1.12	0.37	0.01		0.18	0.16
40	Dec-1-en-3-ol	1181.3	1177	0.04	0.54			1.81	1.84
41	2,4-Dimethylbenzaldehyde	1183.8	1177				0.03		
42	Ethyl octanoate	1184.5	1190					0.09	0.12
43	*p*-Cymen-8-ol	1186.2	1179	0.10					
44	2-*n*-Heptylfuran	1188.1	1176		0.09				
45	Thuj-3-en-10-al	1190.8	1181	0.25		0.06			
46	Octanoic acid	1192.8	1191	0.44					**18.00**

Table 1 continued

	Species			Ec	Ec	Ea	Ea	Ep	Ep
	Plant part			Herb	Root	Herb	Root	Herb	Root
	Constituents	KIE	KIL	%	%	%	%	%	%
47	Myrtenol	1195.1	1194	0.36					
48	Safranal	1195.3	1197				0.24		
49	*n*-Decanal	1202.4	1201	0.15	0.26	0.03		0.62	
50	Verbenone	1205.0	1204	0.04					
51	*β*-Cyclocitral	1205.1	1208			0.04		0.28	
52	*trans*-Dihydrocarvone	1207.8	1210					0.32	
53	*trans*-Carveol	1218.4	1215	0.24					
54	*cis*-Carveol	1219.0	1226	0.62					
55	2,4,6-Trimethylphenol (mesitol)	1230.3	1226				0.09		
56	Pulegone	1233.7	1233					0.62	
57	2,4,5-Trimethylphenol	1247.8	n/a				**1.44**		
58	Vinyl octanoate*	1253.4	n/a						1.52
59	(2*Z*)-Decenal	1255.8	1252	0.25					
60	Dec-9-en-1-ol	1258.0	1263					0.13	0.22
61	(2*E*)-Decenal	1260.7	1260	1.89		0.15		0.62	1.29
62	Nonanoic acid	1262.3	1267		**28.42**				0.57
63	2-Butylcyclohexanone	1263.4	n/a	0.35				0.13	
64	Thymoquinone	1263.9	n/a				**2.83**		
65	5-Undecanone	1268.6	n/a		0.36				
66	*trans*-2-Decen-1-ol	1270.0	n/a					0.05	
67	1-Methyl-3-pentyl-cyclohexane	1272.7	n/a		0.55				
68	6-(5-Methyl-furan-2-yl)-hexan-2-one	1276.8	n/a		0.08				
69	Dihydroedulan I	1279.9	1273			0.13			
70	Isopulegyl acetate	1280.2	1275	0.09					
71	(*E*)-Anethole	1283.1	1282		**4.19**			0.08	
72	Tridecan	1283.8	1289					0.19	0.37
73	Thymol	1289.7	1289					0.93	1.50
74	2-Undecanone	1290.0	1293	0.06	1.56				
75	3-Undecanol	1297.2	1293		0.57				
76	Carvacrol	1298.3	1298	0.45				**3.46**	3.19
77	Undecanal	1302.6	1305		0.74				
78	2,3,4-Trimethylbenzaldehyde	1311.8	1313			0.90	**74.10**		
79	(2*E*,4*E*)-Decadienal	1313.8	1315	0.85	**4.67**				2.07
80	Piperitenone	1327.5	1340					0.12	0.42
81	*δ*-Elemene	1329.0	1335			0.31			
82	2,4,6-Trimethyl benzaldehyde	1334.2	1342						0.53
83	Undec-3-en-2-one	1339.2	1344	0.74	0.21				
84	*α*-Cubebene	1341.6	1345	0.34	0.95	0.21			
85	2,3,6-Trimethylbenzaldehyde	1355.9	1352				**1.83**	0.12	**3.98**
86	(2*E*)-Undecenal	1359.3	1357		0.54			0.10	0.31
87	*α*-Ylangene	1364.6	1373	0.58					
88	*α*-Copaene	1367.3	1374	0.75		0.89		0.52	
89	2,3,5-Trimethylbenzaldehyde	1369.0	1364				**15.16**		
90	Isoledene	1371.9	1374			0.28			
91	*β*-Bourbonene	1375.8	1387	0.69		0.27		0.18	
92	(*E*)-*β*-Damascenone	1379.8	1383	0.48					

Table 1 continued

	Species			Ec	Ec	Ea	Ea	Ep	Ep
	Plant part			Herb	Root	Herb	Root	Herb	Root
	Constituents	KIE	KIL	%	%	%	%	%	%
93	n.i.*	1373.5							
94	(*2E*)-Undecenol	1373.7	1365					0.31	
95	*β*-Elemene	1385.3	1389			1.55			
96	4-(4-Methylphenyl)pentanal**	1386.0	n/a					0.11	0.60
97	Methyl decyl ketone**	1388.6	1388					0.16	
98	Decanoic acid	1392.0	1387		0.15				0.23
99	9-Decenyl acetate**	1392.3	1399		0.19			0.15	
100	*β*-Longipinene	1398.6	1400		0.13			0.40	0.11
101	Dodecanal	1403.2	1401		1.19				
102	(*Z*)-Caryophyllene	1406.5	1408	0.86					
103	Italicene	1408.8	1408					0.24	0.13
104	(*E*)-Caryophyllene	1411.5	1417	**3.50**					
105	n.i.*	1413.1							
106	(*2E*,*4E*)-Undecadienal	1416.1	1415		0.18				
107	α-Gurjunene	1416.2	1409			**10.87**			
108	α-Barbatene	1419.9	1407				0.78	0.28	0.24
109	*β*-Copaene	1420.4	1430	0.58		2.23			
110	*cis*-Thujopsene	1428.0	1431					1.45	1.20
111	α-*trans*-Bergamotene	1433.0	1432	0.09				0.58	0.24
112	6,9-Guaiadiene	1436.8	1442			0.92		0.96	0.21
113	(*E*,*Z*)-Iridolactone	1441.0	1443		0.72				
114	α-Humulene	1446.7	1452			2.08			
115	Geranyl acetone	1447.7	1453		0.21				
116	n.i.*	1450.5							
117	(*E*)-*β*-Famesene	1456.4	1454	**6.74**		**4.61**			
118	(*2E*)-Dodecenal	1456.6	1464					2.62	**5.14**
119	Ethyl-(*2E*,*4Z*)-decadienoate	1458.9	1467					1.31	1.42
120	*n*-Dodecanol	1467.8	1469		2.29				
121	trans-*β*-ionone	1468.7	1468					0.53	0.39
122	n.i.*	1472.4							
123	γ-Muurolene	1472.6	1478	1.30		**8.06**			
124	*ar*-Curcumene	1479.9	1479	1.88	0.30				1.54
125	Germacrene D	1476.5	1484	2.09		**23.44**			
126	2,4,6-Trimethylbenzoic acid	1477.0	n/a				0.23		
127	*δ*-Selinene	1489.7	1492			2.46			
128	γ-Amorphene	1490.5	1495	0.18		3.40	0.12		
129	α-Muurolene	1493.8	1500	0.25	0.51				
130	Epizonarene	1494.3	1501	0.41	0.24		0.36		
131	α-Chamigrene	1495.4	1503					0.55	
132	Cuparene	1495.5	1504					2.62	3.57
133	Sesquicineole	1494.8	1507					5.45	
134	*β*-Bisabolene	1507.3	1505	**5.37**					0.98
135	Cubebol	1513.8	1514	0.16		0.45			
136	2,4-Ditert-butylphenol	1518.0	1519					0.15	
137	*δ*-Cadinene	1520.0	1522	2.27		1.56		0.22	
138	*trans*-Cadina-1,4-diene	1526.7	1533	0.08					

Table 1 continued

	Species			Ec	Ec	Ea	Ea	Ep	Ep
	Plant part			Herb	Root	Herb	Root	Herb	Root
	Constituents	KIE	KIL	%	%	%	%	%	%
139	α-Cadinene	1531.4	1537	0.20		0.61			
140	n.i.*	1532.0							
141	α-Calacorene	1537.0	1544	0.30				0.11	
142	n.i.*	1541.4							
143	Italicene epoxide	1546.1	1547	0.53					
144	Selina-3,7(11)-diene	1548.8	1545			0.76			
145	Salviadienol	1552.3	1549		1.92	0.75	0.91	0.84	
146	Silphiperfol-5-en-3-ol A	1555.5	1557	1.22					
147	Germacrene B	1559.3	1559		0.17	0.44			
148	*trans*-Nerolidol	1567.2	1561	0.83	0.20	0.45		0.82	0.69
149	(*3Z*)-Hexenyl benzoate	1569.7	1565			1.01			
150	γ-Undecalactone	1570.0	1569		0.42				
151	*cis*-3-Hexenyl benzoate	1571.9	1565	1.11					
152	Caryophyllene oxide	1575.2	1582				0.16		
153	*ar*-Tumerol	1575.5	1582		0.35				
154	Spathulenol	1581.9	1577	**12.33**	0.10	**4.67**		**38.61**	**12.35**
155	Salvial-4(14)-en-1-one	1592.0	1594	2.32	0.54	2.49	0.09	0.27	0.10
156	α-Alasken-8-ol	1594.6	1600		0.75	0.55		0.31	0.36
157	Torilenol	1599.6	1599			0.50			0.39
158	Dodecyl acetate	1603.0	1607		0.05			0.66	
159	Humulene epoxide II	1605.4	1608	1.16	0.27	0.97	0.06	**3.11**	1.56
160	Guaia-6,10(14)-diene-4-β-ol	1611.2	1610			1.94			
161	β-Atlantol	1611.5	1608	2.07					
162	*cis*-Isolongifolanone	1613.7	1612	0.64					
163	1,10-di-epi-Cubenol	1607.8	1618					0.13	
164	n.i.*	1616.9							
165	n.i.*	1619.1							
166	α-Colocalene	1618.5	1622	0.39		0.49	0.04	0.49	
167	1-epi-Cubenol	1624.3	1627	0.67		1.08		0.22	
168	γ-Eudesmol	1624.6	1630		0.20				
169	Muurola-4,10(14)-dien-1β-ol	1629.1	1630					0.23	0.54
170	Selina-3,11-dien-6α-ol	1635.4	1642					0.35	0.12
171	Caryophylla-4(12),8(13)-dien-5-α-ol	1636.0	1639	1.63	0.06				
172	epi-α-Cadinol (t-cadinol)	1638.2	1638			0.94			
173	4-Phenyl undecane	1638.9	1643	0.60	0.14				
174	β-Eudesmol	1642.7	1649		0.31				
175	α-Eudesmol	1645.9	1652		0.34				
176	Cedr-8(15)-en-10-ol	1647.8	1650	1.39		1.74	0.05	0.42	0.55
177	Cedr-8(15)-en-9α-ol	1651.3	1650					0.57	
178	(Z)-Methyl dihydrojasmonate	1651.3	1654	0.78					
179	α-Cadinol	1654.9	1654	0.74	0.42	2.82			
180	1-(2,4-Dimethylphenyl)-3-(tetrahydrofuryl-2)propane	1662.2	n/a	1.04					
181	14-hydroxy-(*Z*)-Caryophyllene	1668.9	1666	0.71	0.48	0.93		1.83	1.13
182	Hexyl salicilate	1671.3	1674			0.91			0.32
183	14-hydroxy-9-epi-(*E*)-Caryophyllene	1675.6	1668	1.13			0.06		
184	Eudesma-4(15),7-dien-1β-0l	1679.2	1685	0.43	0.08	2.43		0.50	0.42

Table 1 continued

	Species			Ec	Ec	Ea	Ea	Ep	Ep
	Plant part			Herb	Root	Herb	Root	Herb	Root
	Constituents	KIE	KIL	%	%	%	%	%	%
185	2α-Hydroxyamorpha-4,7(11)-diene	1683.2	1678						0.57
186	Massoiadodecalactone*	1685.3	1685				0.09		
187	Germacra-4(15),5,10(14)-trien-1-α-ol	1688.1	1685			3.43			
188	(*E*)-γ-Atlantone	1688.8	1681						0.39
189	(*Z*)-α-*trans*-Bergamotol	1690.1	1690	**4.59**	0.08				0.19
190	n.i.*	1699.9							
191	n.i.*	1702.5							
192	n.i.*	1707.9							
193	n.i.*	1716.0							
194	(1-Pentylheptyl)-benzene	1720.1	n/a	0.24	0.12				0.07
195	(1-Butyloctyl)-benzene	1726.7	n/a	0.16	0.11				0.14
196	(1-Propylnonyl)-benzene	1738.0	n/a	0.15	0.13	0.63	0.26		0.33
197	(*E*)-2-Hexyl-cinnamaldehyde	1742.9	1748	0.15	0.17				
198	2-Ethylhexyl benzoate	1755.4	n/a	0.48	0.36	0.46	0.03		
199	Octyloctanoate	1757.5	1753						0.21
200	Benzyl benzoate	1762.3	1759	0.69	0.48	0.55			
201	14-Oxy-α-Muurolene	1767.0	1767	0.16		0.35	0.02		
202	Octyl benzoate	1767.6	1792			0.07		0.16	0.36
203	14-Hydroxy-α-Muurolene	1775.8	1779	0.15		0.16			0.42
204	n.i.*	1783.9							
205	Octadecane	1789.7	1800				0.03		0.05
206	n.i.*	1796.6							
207	14-Hydroxy-δ-cadinene	1799.0	1803			0.20		0.26	
208	2-Ethylhexyl salicylate	1799.8	1807	0.45	0.24				
209	n.i.*	1805.6							
210	n.i.*	1814.3							
211	(1-pentyloctyl) benzene (6-phenyl-tridecane)	1818.5	n/a	0.57	0.62				
212	n.i.*	1828.5							
213	Neophytadiene (isomer II)	1832.6	1830			1.14			
214	Hexahydrofarnesyl acetone	1843.6	1845	**3.51**	0.53	0.20		2.00	0.66
215	n.i.*	1853.9							
216	n.i.*	1881.8							
217	Nonadecane	1888.5	1900				0.03		
218	Eudesma-5,11(13)-dien-8,12-olide	1889.1	n/a		0.05				
219	(*5E*,*9E*)-Farnesyl acetone	1895.1	1913					0.35	0.18
220	Methyl hexadecanoate	1918.3	1921	0.11	0.05				0.04
221	Isoalantolactone	1931.1	n/a		0.06				
222	Isophytol	1939.5	1946	0.10					
223	Ethyl hexadecanoate	1986.7	1992	0.31				0.21	0.11
224	Eicosane	1988.0	2000				0.02		
225	(*Z*)-Falcarinol	2037.7	2035	0.90	1.25			**8.74**	**15.42**
226	(*6E*,*10E*)-Pseudo phytol	2061.8	2058				0.03		
227	Sclareolide	2063.7	2065	0.10	3.15				
228	*n*-Octadecanol	2070.6	2077		1.01				
229	Methyl linoleate	2085.2	2095	0.09	0.04				
230	Phytol	2110.5	2116	1.21		0.49		0.39	0.15

Table 1 continued

	Species			Ec	Ec	Ea	Ea	Ep	Ep
	Plant part			Herb	Root	Herb	Root	Herb	Root
	Constituents	KIE	KIL	%	%	%	%	%	%
231	Ethyl linoleate	2155.3	2159	0.11					
232	Falcarinol (isomer)**	2171.3	n/a	1.17					
233	Tricosane	2287.9	2300	0.04					
234	Tetracosane	2387.5	2400	0.02					
235	Pentacosane	2489.0	2500	0.20					
236	Hexacosane	2586.3	2600	0.02					
237	Heptacosane	2687.5	2700	0.10					
238	Nonacosane	2884.6	2900	0.05					
	Monoterpene hydrocarbons			0.45	0.08	0.01	0.02	0.11	0.21
	Oxygenated monoterpenes			8.57	11.01	0.31	0.40	9.47	11.56
	Sesquiterpene hydrocarbons			28.85	2.30	65.44	1.30	8.60	8.22
	Oxygenated sesquiterpenes			33.90	6.24	26.85	1.35	53.92	19.78
	Sesquiterpene lactones			0.10	3.26	0.00	0.00	0.00	0.00
	Oxygenated diterpenes			1.31	0.00	0.49	0.00	0.39	0.15
	Aldehyde			4.43	8.86	1.11	91.20	8.34	11.88
	Ketone			5.66	3.43	0.22	0.03	3.23	1.25
	Esters			4.13	1.41	3.00	0.03	2.58	4.10
	Alcohol			2.96	6.46	0.06	0.03	10.01	16.53
	Fatty acids			0.44	38.48	0.00	0.00	0.00	19.54
	Others			3.16	11.36	1.9	5.48	0.34	0.96
	Total			93.96	92.89	99.39	99.84	96.99	94.18
	Number of constituents			113	81	62	34	71	72

Bold values represent high percentage of main compound in essential oil

Ec = *Eryngium campestre*; Ea = *Eryngium amethystinum*; Ep = *Eryngium palmatum*; KIE = Kovats (retention) index experimentally determined (AMDIS); KIL = Kovats (retention) index—literature data (Adams 2007), n.i. = not identified, n/a = not available

*Tentative identification

in the chemical composition of EOs (Sivropoulou et al. 1997).

TPC and TFC

The aerial parts of *E. campestre*, *E. amethystinum* and *E. palmatum* were treated with different solvents, and the yields of the obtained extracts are presented in the following order: MeOH > H_2O > EtOAc ≥ Acet. Solvent polarity is a major factor that leads to the variation in extract yields (Ouerghemmi et al. 2016).

The amounts of TPC and TFC are in a positive correlation with the extracts' ability for free radical scavenging. The results are presented in Table 2.

TPC was determined by the Folin–Ciocalteu method. The amount of phenolic compounds varied from 47.3 to 146.8 mg GA/g DW and the highest content of phenols was detected in the EtOAc extracts of *E. campestre* (111.9 mg GA/g DW) and *E. palmatum* (146.8 mg GA/g DW), except for *E. amethystinum* where the highest content of these compounds was detected in the H_2O extract (74.5 mg GA/g DW). The standard antioxidant values were 63.0 mg GA/g (BHA) and 40.9 mg GA/g (Vitamin C). The recent study by Marčetić et al. (2014) pointed that the TPC was higher in the MeOH extract of *E. palmatum* aerial parts (29.0 mg GA/g DW) than in the equivalent extracts of the roots (13.9 mg GA/g DW).

TFC was evaluated using aluminum nitrate nonahydrate, whereas the amount of flavonoid compounds ranged from 14.1 to 222.5 mg Qu/g DW. TFC from the extracts isolated in the aerial parts is presented in the following order for all three *Eryngium* species: EtOAc > Acet > MeOH > H_2O.

The highest amounts of TPC and TFC were observed in EtOAc extracts. This extract concentration (5 mg/mL) was

Table 2 TPCs, TFCs and antioxidant activities for aerial part extracts isolated from *Eryngium* species (mean ± SD)

Eryngium species	Extracts	TPC (mg GA/g)	TFC (mg Qu/g)	DPPH IC_{50} (mg/mL)	ABTS (mg VitC/g)
E. campestre	H_2O	56.3 ± 0.02^{fgh}	14.1 ± 0.01^{h}	1.9 ± 0.01^{j}	2.4 ± 0.01^{ef}
	MeOH	85.9 ± 0.07^{cde}	35.9 ± 0.03^{g}	1.9 ± 0.10^{d}	2.6 ± 0.03^{f}
	EtOAc	111.9 ± 0.11^{b}	164.5 ± 0.05^{c}	5.2 ± 0.03^{i}	2.1 ± 0.02^{de}
	Acet	70.8 ± 0.07^{efg}	73.1 ± 0.07^{f}	4.4 ± 0.01^{g}	1.7 ± 0.02^{c}
E. amethystinum	H_2O	98.7 ± 0.02^{bc}	16.8 ± 0.00^{h}	1.7 ± 0.01^{c}	3.6 ± 0.01^{h}
	MeOH	94.8 ± 0.06^{bcd}	43.9 ± 0.00^{g}	2.2 ± 0.07^{f}	2.5 ± 0.01^{f}
	EtOAc	74.5 ± 0.19^{def}	195.4 ± 0.00^{b}	8.3 ± 0.01^{e}	1.0 ± 0.01^{a}
	Acet	81.2 ± 0.11^{cde}	123.2 ± 0.05^{d}	5.0 ± 0.01^{a}	1.4 ± 0.01^{bc}
E. palmatum	H_2O	53.1 ± 0.01^{gh}	17.2 ± 0.00^{h}	4.2 ± 0.02^{b}	3.0 ± 0.02^{g}
	MeOH	47.3 ± 0.05^{h}	98.5 ± 0.09^{e}	5.9 ± 0.01^{cd}	1.2 ± 0.02^{ab}
	EtOAc	146.8 ± 0.12^{a}	222.5 ± 0.02^{a}	1.0 ± 0.01^{h}	1.6 ± 0.02^{c}
	Acet	80.6 ± 0.05^{cde}	161.4 ± 0.16^{c}	6.0 ± 0.04^{k}	1.8 ± 0.00^{cd}
BHA		63.3 ± 0.00	–	0.1 ± 0.00	2.7 ± 0.00
Vitamin C		40.9 ± 0.00	–	0.1 ± 0.00	–

Different letters above the bars indicate statistically significant differences only among the treatments performed for each assay according to the Tukey test ($P \leq 0.05$)

2.5 times higher than the concentrations of other extracts (2 mg/mL), so this solvent had the lowest amount of phenolics.

Antioxidant capacity by DPPH and ABTS assays

Free radical scavenging capacities of the tested extracts were measured by DPPH assay. This method was chosen since radical scavenging is the main mechanism of antioxidant activity in food. The highest activity with IC_{50} of 1.7 mg/mL was recorded in the H_2O extract of *E. amethystinum* and the lowest in the EtOAc extract obtained from *E. palmatum* with IC_{50} of 10.0 mg/mL (Table 2). IC_{50} value of the synthetic antioxidants BHA and Vitamin C was 0.1 mg/mL, which was determined in parallel experiments.

The results of the ABTS assay are presented in Table 2. The amounts ranged from 0.9 to 3.6 mg VitC/g DW. The highest activity was recorded in the H_2O extract and the lowest in the EtOAc extract from *E. amethystinum*, whereas the standard antioxidant BHA value was 2.7 mg VitC/g DW.

Generally, the highest antioxidant activities in both assays (DPPH and ABTS) were recorded for the H_2O and MeOH extracts obtained from all three *Eryngium* species, which is in accordance with the previous results.

The evaluation of the radical scavenging and antioxidant activity of *E. campestre* ethanol: H_2O extract (7:3, V/V) from Kosovo expressed a higher radical-scavenging activity against DPPH-radical in the ethanol extract of the root (IC_{50} = 0.7 mg/mL) than in the aerial parts of the plant (IC_{50} = 1.1 mg/mL) (Nebija et al. 2009). The result of the DPPH assay for *E. palmatum* MeOH extracts obtained from the aerial parts was 0.6 and 0.7 mg/mL for the roots (Marčetić et al. 2014).

Antimicrobial activity

This paper includes the results of a study of the antimicrobial potential of EOs isolated from the plant material (roots and/or aerial parts), as well as the MeOH, EtOAc, Acet and H_2O extracts, of three *Eryngium* species. The results are presented in Table 3.

The tested EOs from all three *Eryngium* species proved significantly efficient, with pronounced inhibitory action against two Gram-negative strains (*K. pneumoniae* and *P. mirabilis*) and one Gram-positive bacteria strain (*S. aureus*) in all tested concentrations. All tested EOs isolated from *Eryngium* were inactive against *S. pyogenes*. Among the oils of the three species, those isolated from *E. palmatum* had the highest inhibitory effect. In addition, the oils isolated from the aerial parts exhibited a higher activity than those obtained from the underground (root) parts, where the main compounds from the aerial parts were spathulenol and germacrene D. Individual components of the EOs such as spathulenol demonstrated a potent antibacterial activity as presented in previous studies (Bougatsos et al. 2004, Pichette et al. 2006). It was proven that germacrene D also had high antibacterial and antifungal activities (Sahin et al. 2004).

Eryngium campestre extracts have shown activity in the range 0.004–20.00 mg/mL, where the highest activity was

Table 3 Antimicrobial activity of the three *Eryngium* species extracts and EOs, expressed as minimal inhibitory concentration (MIC)

		S. aureus	*S. pyogenes*	*E. faecalis*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*C. albicans*
E. campestre (mg/mL)	1	> 20.00	20.00	2.50	20.00	0.07	> 20.00	10.00	20.00	0.03
	2	5.00	20.00	5.00	2.50	0.07	10.00	5.00	20.00	0.03
	3	0.03	> 5.00	> 5.00	5.00	0.01	2.50	0.007	> 5.00	0.007
	4	0.01	> 2.50	> 2.50	2.50	0.01	1.25	0.004	> 2.50	0.02
	5	0.004	> 1.25	0.007	0.01	0.31	< 0.0005	< 0.0005	1.25	0.15
	6	0.007	> 1.25	0.15	0.07	0.31	< 0.0005	< 0.0005	1.25	0.31
E. palmatum (mg/mL)	1	> 20.00	10.00	10.00	20.00	0.07	> 20.00	5.00	> 20.00	20.00
	2	10.00	2.50	5.00	1.25	0.07	2.50	0.07	20.00	20.00
	3	0.02	1.25	0.01	1.25	0.01	0.62	0.004	0.31	1.25
	4	0.03	2.50	0.03	0.62	0.07	0.31	0.01	0.62	1.25
	5	0.01	> 2.50	0.007	0.31	0.01	< 0.001	< 0.001	0.62	0.15
	6	0.007	> 1.25	0.15	0.62	2.50	< 0.0005	< 0.0005	> 1.25	0.15
E. amethystinum (mg/mL)	1	20.00	20.00	10.00	10.00	> 20.00	20.00	> 20.00	10.00	10.00
	2	2.50	> 20.00	2.50	2.50	20.00	2.5	5.00	5.00	2.50
	3	0.62	> 2.50	0.62	0.31	0.62	0.31	0.62	0.62	0.62
	4	1.25	> 10.00	1.25	2.50	2.50	0.62	2.50	2.50	2.50
	5	0.002	> 1.25	0.15	0.62	0.62	< 0.0005	< 0.0005	1.25	0.31
	6	0.007	> 1.25	0.007	1.25	0.62	< 0.0005	< 0.0005	1.25	0.62
AB (μg/mL)		0.04	0.04	0.09	0.09	0.09	0.04	0.09	0.09	0.06

1—H_2O extract; 2—MeOH extract; 3—EtOAc extract; 4—Acet extract; 5—EO from aerial parts; 6—EO from root; AB—antibiotic, Streptomycin for bacterial and Nystatin for yeast species, given in μg/mL

Table 4 Score values (kcal/mol) and indentified hydrogen bonds (amino acids and bond length) for all studied compounds

	E-Inter total	HBond	LE1	LE3	VdW	Steric	MolDock score	Rerank score	Indentified hydrogen bonds (amino acids and bond length)
Spathulenol	− 105.72	− 624.43	− 718.80	− 569.84	− 338.96	− 994.82	− 115.01	− 911.74	Tyr36 (2.81 Å)
Germacrene D	− 97.19	0.00	− 728.00	− 532.99	− 239.20	− 971.95	− 109.20	− 799.49	–
Nonanoic acid	− 98.93	− 118.36	− 907.17	− 753.49	− 276.63	− 870.98	− 99.78	− 828.84	Thr75 (2.84 and 2.89 Å)
									Asn124 (2.83 Å)
Octanoic acid	− 92.87	− 117.90	− 929.50	− 780.06	− 263.58	− 810.84	− 92.95	− 780.06	Thr75 (2.77 and 2.86 Å)
									Asn124 (2.95 Å)
2,3,4-trimethylbenzaldehyde	− 87.20	− 5.00	− 724.83	− 634.71	− 274.64	− 822.05	− 79.73	− 698.18	Gln174 (3.10 Å) Tyr170 (3.10 Å)

E-Inter—Inter Energy of Pose

LE1—Ligand Efficiency calculated as MolDock Score divided by Heavy Atoms count

LE3—Ligand Efficiency calculated as Rerank Score divided by Heavy Atoms count

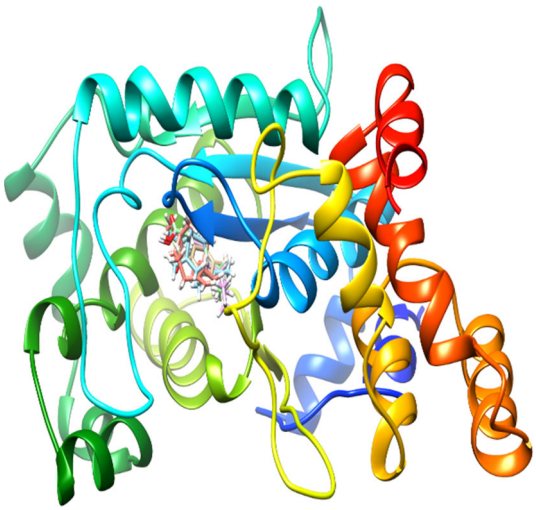

Fig. 1 The most optimal calculated poses for all the studied compounds inside the active site of *S. aureus* tyrosyl-tRNA synthetase

expressed by the Acet extract. The highest activities of all four extracts were against the yeast *Candida albicans*. Although two Gram-positive strains, *S. pyogenes* and *E. faecalis,* demonstrated a higher resistance to the action of the extracts, other MIC values did not have significant differences related to the cell wall structure. *E. palmatum* extracts were efficient in the same range of concentrations as *E. campestre* extracts (0.004–20.00 mg/mL). However, the activity of these extracts was higher than that of *E. campestre* extracts, since they mostly inhibited the growth of the same strains even in concentrations only half as high. The EtOAc extract had the strongest antimicrobial effect, followed by the Acet extract. Among the tested strains the most sensitive ones were *E. coli* and *P. mirabilis* and the highest tolerance to the action of these extracts was found in *K. pneumoniae*, *P. aeruginosa* and the yeast *C. albicans*. The H_2O extract expressed the highest resistance in the tested concentrations. Contrary to the previous results, the extracts of *E. palmatum* demonstrated the lowest activity toward the tested fungal organism. The extracts of *E. amethystinum* expressed an activity in the range between 0.31–20.00 mg/mL, while the extract obtained from EtOAc had the highest antimicrobial effect. Again, *S. pyogenes* was reported as the most resistant species, which was not inhibited even by the most potent, EtOAc extract. The H_2O extract demonstrated a relatively weak activity, acting as an inhibitory agent only at the highest tested concentrations. MeOH and Acet extracts expressed similar activities, with the Acet extract's being slightly higher. The strains most sensitive to the extracts of *E. amethystinem* was *K. pneumonia.* The results of the extract activity indicated a high potential in all three species, while the EtOAc and Acet extracts demonstrated the highest effect. This may be explained by the content of flavonoids and phenolic compounds in general, the second highest for both phenolic compound types, right after the EtOAc extracts of the same species.

Previous studies on the antimicrobial activity of the *Eryngium* species observed in this paper were relatively scarce and provided data only for *E. palmatum* and *E. campestre*. To the best of our knowledge, these results represent the first study of the antimicrobial activity of *E. amethystinum*. Usta et al. (2014) studied the antimicrobial and antitumor activity of the MeOH, ethanol and H_2O extracts of *E. campestre*, where it was determined that the

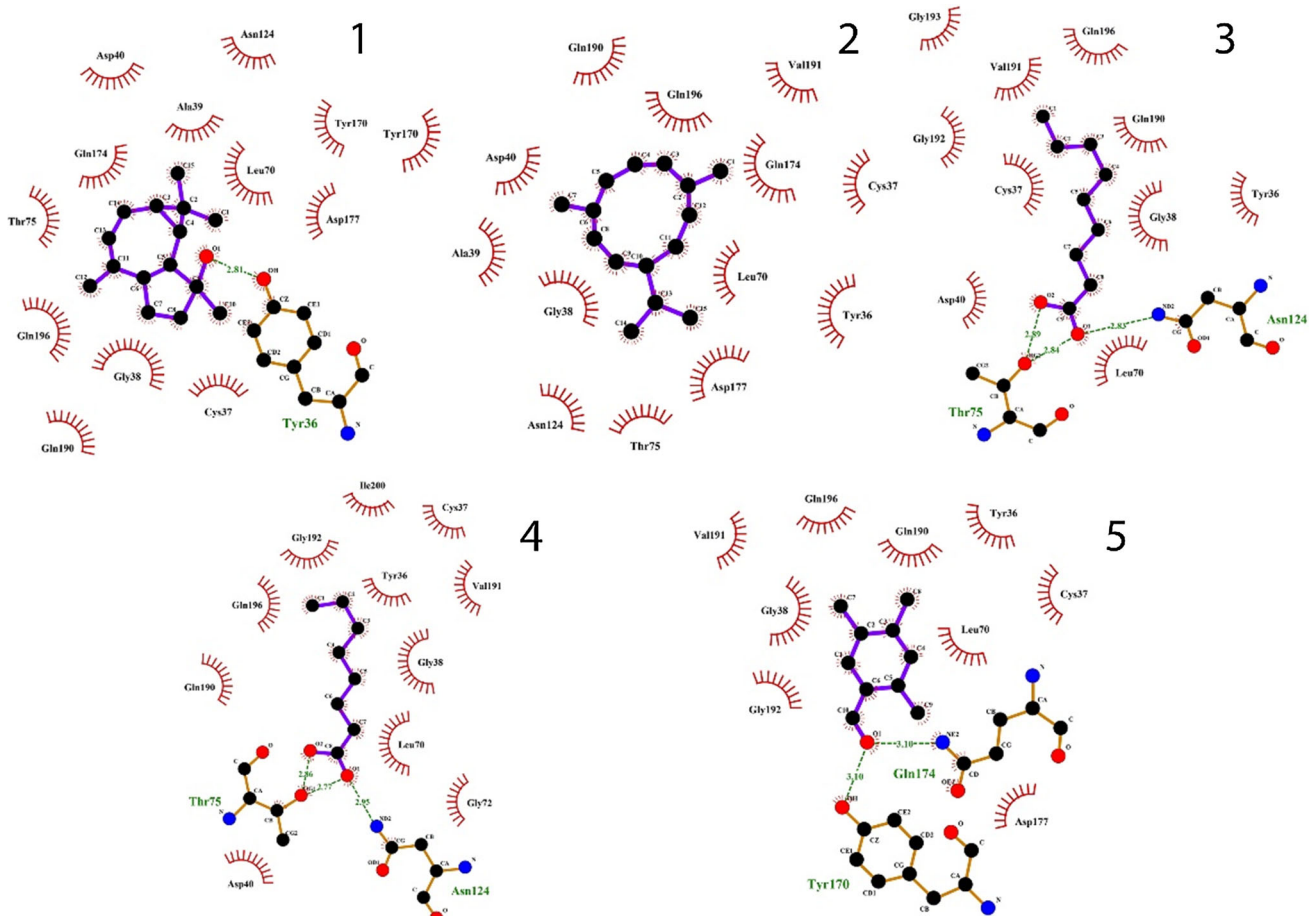

Fig. 2 Two dimensional representations of the best docking pose for (1) spathulenol, (2) germacrene D, (3) nonanoic acid, (4) octanoic acid and (5) 2,3,4-trimethylbenzaldehyde inside the active site of *S. aureus* tyrosyl-tRNA synthetase

ethanol extract had the highest activity, followed by the MeOH extract, whereas the H_2O extracts were the least effective, which matches our results. Also, the species most sensitive to the activity of the MeOH and ethanol extracts was *E. coli* which also demonstrated a high sensitivity to all *E. campestre* extracts in our study. Conea et al. (2016) reported the antimicrobial efficacy results of *E. campestre* ethanol extracts isolated from the aerial parts, whereas confirmed a moderate effect on *Staphyloccocus aureus* and *S. epidermidis*, as well as a high bacteriostatic effect on *Pseudomonas aeruginosa*. The only previous study concerning an antimicrobial activity of *E. palmatum*, performed by Marčetić et al. (2014), involved testing the MeOH and chloroform extracts of this species against eight bacterial strains and one yeast species. The extracts inhibited the growth of both Gram-positive and Gram-negative bacteria, with MICs in the 0.0035–0.0156 mg/mL range. The highest activity have shown by MeOH extracts (against *Micrococcus luteus* at 0.0035 mg/mL), which, according to the authors of the study, was a consequence of its high and specific flavonoid content comprised of kaempferol, apigenin and its glycosides. The MeOH root extract, expressing the activity at 0.0078–0.0156 mg/mL, contained catechin which has already been confirmed as an antimicrobial compound. Although catechins are known for higher activity against Gram-positive strains (Cushnie and Lamb 2005), in the study by Marčetić et al. the MeOH extract obtained from the roots initiated the same level of inhibition in both bacterial groups. This activity is caused by the synergistic activity of the phenolic compounds. On the other hand, extracts obtained from the same plant, using a non-polar solvent (chloroform), also expressed a very high activity, which is related to the presence of linoleic and palmitic acids (in the aerial parts of the plants) and saturated alcohols (in the corresponding root extract).

It is highly important to note that the EOs and extracts have shown different modes of activity, whereas the oil failed to express selective action toward the yeast strain, which is contrary to the action demonstrated by all four extracts.

Molecular docking

It was necessary to determine the binding energy between the tested compounds and the active site of *S. aureus* tyrosyl-tRNA synthetase. The results obtained from the applied docking score functions and identified hydrogen bonds between ligands and the active site of the enzyme are presented in Table 4. The best calculated poses for all the studied compounds inside the active site of the enzyme are presented in Fig. 1. The two-dimensional representation of the interactions between the studied compounds and amino acids inside the binding pocket of the enzyme is presented in Fig. 2.

Five dominant components were analyzed: spathulenol (oxygenated sesquiterpene) and germacrene D (sesquiterpene hydrocarbon) as the main compounds in the aerial parts, as well as nonanoic acid, octanoic acid (fatty acids) and 2,3,4-trimethylbenzaldehyde (aldehyde) as the main compounds in the roots. The results indicate that the highest intra-binding energy with the enzyme was that of spathulenol, while the lowest was that of 2,3,4-trimethylbenzaldehyde. The binding energies were determined by Van der Waals interactions and steric energy. Using the both parameters, it was determined that spathulenol had the highest value, while octanoic acid had the lowest.

The activity of the oils isolated from the aerial parts was higher than that of the oils isolated from the underground (root) parts. These results were confirmed by molecular docking, indicating that octanoic acid had the lowest steric arrangement inside the binding pocket of the enzyme and that the best "fit" inside the binding pocket was obtained for spathulenol. According to the both ligand efficiency parameters (LE1 and LE2) the lowest results were obtained for octanoic acid, while the results from LE1 and LE2 identified spathulenol and germacrene D, respectively, as the ligands with the highest efficiency. It is possible to determine the binding affinity of a ligand for the active site of the enzyme by using the score values obtained by applying the scoring functions from the molecular docking method. Both *MolDock* and *Rerank* score values indicated that the highest binding affinity to the active site of tyrosyl-tRNA synthetase was that of spathulenol, while the lowest binding affinity was determined for 2,3,4-trimethylbenzaldehyde. The ligand effect on the studied activity is strongly influenced by the number, bond length and bond energy of the hydrogen bonds formed between the ligand and the enzyme. *Hbond* value is determined as the total energy of hydrogen bonds between the ligand and the amino acids in the active site of the enzyme. *Hbond* values for the tyrosyl-tRNA synthetase demonstrate that the interaction was the strongest for spathulenol which formed one hydrogen bond with Tyr36 (2.81 Å). Among the oils studied in this work, the ones isolated from *E. palmatum* had the highest inhibitory effect on microbial strains, with spathulenol as the main compound (38.61%). The great antimicrobial effect of this oil was also recorded by molecular docking.

Conclusion

Eryngium species analyzed in this paper have demonstrated significant antioxidant and antimicrobial activity. The high antioxidant activity is the result of high concentrations of flavonoids and other phenolic compounds in the extracts. As spathulenol is the main compound, it may be regarded as an important molecule for good antimicrobial activity against *S. aureus*, as demonstrated through molecular docking simulation for tyrosyl-tRNA synthetase enzyme. The results of this study indicate that *Eryngium* species may produce powerful bioactive compounds with therapeutic potential, while they also retain a high potential in being used as natural food or cosmetic preservatives.

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