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GC-MS COMPARATIVE CHEMICAL COMPOSITION OF ESSENTIAL OILS AND VOLATILE COMPOUNDS OF *ERYNGIUM PLANUM* L. USING CLASSICAL HYDRODISTILLATION, ULTRASOUND-ASSISTED HYDRODISTILLATION AND HEADSPACE SOLID-PHASE MICROEXTRACTION. ANTIMICROBIAL ACTIVITY[1]

Adriana-Maria ANDREICA[a,*], Ana BALEA[a], Maria POJAR-FENEȘAN[a], Rahela CARPA[b]

ABSTRACT. This study presents the extraction of essential oils and volatiles of *Eryngium planum* (*E. planum)* by classical hydrodistillation (HD), ultrasound-assisted hydrodistillation (UAHD) and headspace solid-phase microextraction (HS-SPME). The GC-MS analysis showed that the essential oil of *E. planum* obtained by UAHD contains the following majority compounds: β-copaene (11.97%), *cis*-chrysanthenyl acetate (10.14%), *(E)*-β-farnesene (6.79%), γ-gurjunene (6.53%), caryophyllene (5.73%), germacrene B (3.93%), (+)-*cis*-verbenol,2-methylpropionate (2.87%), β-selinene (2.73%). The GC-MS analysis showed that *E. planum* volatiles extracted by HS-SPME contains as majority compounds: *cis*-chrysanthenyl acetate (30.39%), *(E)*-β-farnesene (11.71%), γ-maaliene (7.69%), β-elemene (7.26%), caryophyllene (6.5%), β-selinene (4.72%), δ-cadinene (4.72%), β-copaene (4.61%), α-pinene (4.51%), γ-muurolene (2.98%). By using the UAHD method, the yield of *E. planum* oil was increased by 27.27% compared with the classical HD method. The essential oil of *E. planum* showed an excellent antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.*

Keywords: *Eryngium planum, essential oils, ultrasound-assisted hydrodistillation (UAHD), headspace solid-phase microextraction (HS-SPME), antimicrobial activity*

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[a] *Babeş-Bolyai University, "Raluca-Ripan" Institute for Research in Chemistry, 30 Fântânele str., RO-400294, Cluj-Napoca, Romania*

[b] *Babeş-Bolyai University, Faculty of Biology and Geology, Molecular Biology and Biotechnology Department, 1 Mihail Kogălniceanu str., RO-400084, Cluj-Napoca, Romania*

* *Corresponding author: adriana.andreica@ubbcluj.ro*

INTRODUCTION

The use of medicinal plants is becoming more popular in our society to prevent and treat many diseases [1–3].

Eryngium (E.) species that belong to the Apiaceae family are valuable for their use in traditional medicine due to their content in phenolic acids, saponins [4], flavonoids [5], coumarin derivatives [6, 7], essential oils [8] and acetylenes [9]. Some of these species such as *E. campestre* L., *E. caucasicum*, *E. creticum*, *E. foetidum* have been used in modern medicine for the treatment of several human diseases [10–13].

Eryngium planum L. (blue eryngo) is a perennial plant which in Romania was used in folk medicine in bronchitis, cough, wounds, urinary disorders, scars and burns as infusion [14]. It was also included in scientific studies for modern pharmaceuticals. The ethanolic extract of the aerial parts of *E. planum* was used for the treatment of active inflammatory periodontal disease in rats with potential therapeutic results [15]. The tincture from *E. planum* had dose-dependent anti-inflammatory properties in the rat paw-oedema test [16]. Ethanolic extracts from leaves and roots of three *Eryngium* species (*E. planum*, *E. campestre*, *E. maritimum*) showed a moderate antibacterial activity against *Staphylococcus aureus* and a significant antifungal activity [17]. The polyphenols and pectin from the aerial parts of the three mentioned *Eryngium* species were investigated, the results of their antimicrobial activity confirming that the tinctures obtained from these plants showed a high activity on *Pseudomonas aeruginosa,* a moderate activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, and no activity against *Escherichia coli* [18].

E. planum extracts are a source of biologically active compounds that have pharmaceutical and therapeutic potential applications. The bioactive compounds of *E. planum* were extracted with CO_2 under subcritical conditions using subterranean part of the plant [19], under supercritical conditions using aerial parts of the plant [20], by ultrasound-assisted alcoholic extraction using a sonication bath [21] or by methods such us percolation, maceration or microwave [22].

The composition of essential oils and the antimicrobial activity of some different *Eryngium* species such as *E. alpinum L., E. amethystinum L.* [23], *E. palmatum* [24], *E. campestre* [25], *E. maritimum L.* [26] were investigated by many researchers, but the essential oils from *Eryngium planum* were little investigated [27, 28]. These latter essential oils were obtained by classical hydrodistillation from different plant parts (inflorescences, stalk leaves, rosette leaves and root) and from *in vitro* shoot culture [28].

An improved hydrodistillation extraction was developed to isolate essential oil of fresh orange peel using a combination of hydrodistillation (HD) and ultrasound (UA) methods (Sono-Clevenger) [29]. Ultrasound-assisted hydrodistillation (UAHD) method was used to increase the extraction efficiency of essential oils from cinnamon barks [30]. Thus, the use of UAHD is a promising method for extracting essential oils from *E. planum*. Headspace solid-phase microextraction (HS-SPME) method was used for the isolation and determination of volatiles from plants [31, 32].

The aim of the present study was to investigate the composition of essential oils and volatile organic compounds from *Eryngium planum* aerial parts (stalk leaves, rosette leaves and inflorescences) extracted by the UAHD and HS-SPME methods and to compare these results with the chemical composition of the essential oil extracted by the classical hydrodistillation (HD). The antimicrobial activity of *E. planum* essential oil was tested against gram-negative and gram-positive strains. To our knowledge, the comparative chemical composition of essential oils and volatiles from *E. planum* using UAHD and HS-SPME extraction methods and their antimicrobial activity have not been previously reported.

RESULTS AND DISCUSSION

To improve this yield, before classical HD, an ultrasound treatment was done by an ultrasonic device (Hielscher UP200S). To obtain the essential oil by this UAHD method, we studied the influence of the amplitude size set at 20%, 40% and 60%, respectively, for a duty cycle of 0.5 for 60 minutes. The UAHD yields for the three amplitude sizes were as follows: 0.20% for 20% amplitude, 0.28% for 40% amplitude and 0.27% for 60% amplitude. Therefore, the best yield of 0.28% of the essential oil was obtained with UAHD method at the amplitude set at 40%. After the ultrasound treatment, the essential oil was extracted as in the classical HD. Therefore, by the UAHD method, the extraction process was improved with 27.27% compared to the classical HD method, as it is mentioned in literature for the yield of other essential oils [30, 33].

The chemical composition of the essential oils extracted by HD and UAHD methods and the volatiles extracted by HS-SPME method were analysed by gas chromatography-mass spectrometry (GC-MS). Identification and percentage area (normalisation) of compounds were based on NIST 20.L mass spectral library searching using the internal library search algorithm for the Agilent's ChemStation (GC-MS) and Agilent's MassHunter software. The identified groups of chemical compounds of *E. planum* oil obtained by UAHD

method were sesquiterpene hydrocarbons (50.46%), followed by oxygenated sesquiterpenes (21.33%), oxygenated monoterpenes (14.28%), monoterpenes hydrocarbons (1.22%) and other compounds (8.5%) (Table 1). The majority compounds of the oil were β-copaene (11.97%), *cis*-chrysanthenyl acetate (10.14%), *(E)*-β-farnesene (6.79%), γ-gurjunene (6.53%), caryophyllene (5.73%), germacrene B (3.93%), (+)-*cis*-verbenol,2-methylpropionate (2.87%), and β-selinene (2.73%). The chemical profile of the two types of essential oils obtained by UAHD and HD methods was comparable with some minor differences.

By both extraction methods were identified in *E. planum* plant 66 compounds (Table 1). In the essential oil obtained by UAHD method were identified 60 compounds (95.79%; not identified compounds from the positions 19, 37, 48, 61, 66; see Table 1) while in the essential oil obtained by classical HD method were identified 61 compounds (95.39%; not identified compounds from the positions 5, 12, 13, 24, 64; see Table 1). It can be mentioned that the nonidentified compounds by the two methods are complementary.

Thiem et al. [28] extracted by hydrodistillation for the first time the essential oils from different parts of *E. planum* plant and the chemical composition was analysed by GC-MS. The classes of compounds identified in oils obtained from stalk leaves, rosette leaves and inflorescences, respectively, were monoterpene hydrocarbons (42.0%, 28.4% and 12.9%, respectively), oxygenated monoterpenes (10.3%, 36.6% and 51.2%, respectively), sesquiterpene hydrocarbons (20.0%, 24.4% and 18%, respectively) and oxygenated sesquiterpenes (10%, 2.4%, and 7.7%, respectively). The majority classes of compounds identified in oil obtained from root were monoterpene hydrocarbons (4.0%), sesquiterpene hydrocarbons (3.1%) and polyacetylenes (64.4%).

Table 1. The chemical composition of the essential oils of *Eryngium planum* obtained by the UAHD and HD methods and analysed by GC-MS

No.	Compounds	Retention time (min)		Identification probability (%)		Normalised area (%)	
		UAHD*	HD**	UAHD	HD	UAHD	HD
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***
1	Prenyl acetate (OC)	5.747	5.749	99.61	99.56	1.98	1.61
2	α-Pinene (MTH)	5.991	5.993	98.78	98.80	1.22	0.59
3	Oxalic acid, cyclohexyl pentyl ester (OC)	6.563	6.564	84.86	82.94	0.31	0.28
4	Octanal (OC)	7.229	7.231	98.87	99.32	0.38	0.34
5	*cis*-3-Hexenyl iso-butyrate (OC)	-	9.152	-	86.88	-	0.24
6	*cis*-Chrysanthenyl acetate (OMT)	12.070	12.075	97.73	97.63	**10.14**	**9.34*** **
7	Benzaldehyde, 2,4,6-trimethyl- (OC)	13.053	13.055	97.02	96.95	0.71	0.66
8	Benzaldehyde, 2,4,5-trimethyl- (OC)	13.742	13.744	98.73	98.67	1.37	1.22
9	Copaene (STH)	14.075	14.076	97.94	97.95	0.63	0.67

No.	Compounds	Retention time (min)		Identification probability (%)		Normalised area (%)	
		UAHD*	HD**	UAHD	HD	UAHD	HD
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***
10	β-Elemene (STH)	14.319	14.322	97.45	97.46	2.29	2.73
11	Caryophyllene (STH)	14.828	14.834	99.35	99.22	**5.73**	**6.05**
12	(+)-*epi*-Bicyclosesquiphellandrene (STH)	-	14.962	-	83.55	-	0.41
13	Sesquithujene (STH)	-	14.993	-	77.71	-	0.24
14	Himachalol (OST)	15.073	15.074	75.94	86.56	0.73	0.74
15	Aromadendrene (STH)	-	15.135	-	77.59	-	0.28
16	*(E)*-β-Farnesene (STH)	15.274	15.283	96.25	96.10	**6.79**	**7.11**
17	Humulene (STH)	15.366	15.367	97.22	97.09	1.47	1.51
18	β-Cubebene (STH)	15.445	15.447	84.80	88.10	0.64	0.62
19	β-Ylangene (STH)	15.634	-	86.98	-	1.24	-
20	γ-Selinene (STH)	15.686	15.690	95.06	96.34	1.45	1.51
21	β-Copaene (STH)	15.803	15.810	95.16	95.16	**11.97**	**11.73**
22	β-Selinene (STH)	15.890	15.896	97.60	97.25	**2.73**	**2.82**
23	γ-Gurjunene (STH)	16.030	16.035	96.15	96.16	**6.53**	**6.87**
24	*trans*-Verbenyl isovalerate (OMT)	-	16.123	-	73.34	-	0.37
25	Guaia-10(14),11-diene (STH)	16.188	16.195	96.09	96.10	2.58	2.5
26	γ-Cadinene (STH)	16.278	16.280	91.94	92.51	0.34	0.35
27	δ-Cadinene (STH)	16.387	16.389	94.63	95.03	1.13	1.22
28	Elemol (OST)	16.778	16.781	91.43	92.24	0.33	0.36
29	14-Hydroxycaryophyllene (OST)	16.867	16.869	89.53	90.19	0.81	0.77
30	Germacrene B (STH)	16.973	16.977	98.93	98.77	**3.93**	**4.14**
31	Mint oxide (OST)	17.111	17.114	86.80	86.84	0.41	0.39
32	(+)-Spathulenol (OST)	17.262	17.264	98.83	98.90	2.05	1.84
33	Isoaromadendrene epoxide (OST)	17.361	17.363	95.09	94.52	1.71	1.54
34	2,2,4-Trimethyl-1,3-pentadienol diisobutyrate (OC)	17.424	17.426	76.41	74.04	0.66	0.58
35	Salvial-4(14)-en-1-one (OST)	17.515	17.517	91.99	90.56	1.04	0.95
36	Longipinocarveol, trans- (OST)	17.730	17.732	89.92	89.19	1.24	1.14
37	2,4a,8,8-Tetramethyldecahydro cyclopropa[d]naphthalene (STH)	17.880	-	74.56	-	0.37	-
38	tau-Cadinol (OST)	17.939	17.941	91.21	91.52	1.13	1.11
39	(+)-*cis*-Verbenol, 2-methylpropionate (OMT)	18.072	18.076	80.17	80.20	**2.87**	**3.19**
40	(-)-Spathulenol (OST)	18.114	18.113	83.20	70.47	0.31	0.3
41	α-Cadinol (OST)	18.166	18.167	71.64	72.08	0.24	0.24
42	γ-Costol (OST)	18.220	18.221	86.68	86.81	0.82	0.82
43	Megastigma-4,6(Z),8(Z)-triene (OC)	18.284	18.288	74.10	75.37	0.39	0.4
44	Muurola-4,10(14)-dien-1.beta.-ol (OST)	18.351	18.355	77.06	71.17	1.01	0.98
45	Neointermedeol (OST)	18.384	18.386	70.45	73.56	1.02	1.15
46	1-Isopropyl12-oxatetracyclo [5.2.1.1(2,6).1(9,11)]	18.536	18.538	72.66	74.36	0.54	0.52

No.	Compounds	Retention time (min)		Identification probability (%)		Normalised area (%)	
		UAHD*	HD**	UAHD	HD	UAHD	HD
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***
	Dodecane (OC)						
47	Isospathulenol (OST)	18.585	18.587	87.04	86.36	1.17	1.21
48	α-Muurolene-14-hydroxy-(OST)	18.735	-	79.13	-	0.45	-
49	(1R,7S)-Germacra-4(15),5,10(14)-trien-1β-ol (OST)	18.806	18.809	94.81	94.56	1.65	1.65
50	Spiro[2.5]octane,3,3-dimethyl-2-(1-buten-3-on-1-yl)- (OC)	18.875	18.879	76.22	75.13	0.27	0.25
51	β-Costol (OST)	19.100	19.102	90.04	90.24	1.48	1.54
52	6-Isopropenyl-4,8a-dimethyl-1,2,3,4,5,6,7,8,8a-octahydronaphthalen-2-ol (OST)	19.212	19.213	89.28	90.53	0.43	0.43
53	α-Costol (OST)	19.258	19.262	86.82	86.48	0.69	0.77
54	Aristolene epoxide (OST)	19.303	19.306	89.55	91.28	1.22	1.32
55	Tricyclo[5.2.2.0(1,6)undecan-3-ol, 2-methylene-6,8,8-trimethyl- (OST)	19.347	19.350	89.59	90.52	0.28	0.3
56	α-Mintsulfide (STH)	19.583	19.586	77.73	77.63	0.36	0.35
57	β-Oplopenone (OST)	19.624	19.625	79.70	80.38	0.42	0.45
58	Ylangenol (OST)	19.728	19.729	69.69	73.74	0.31	0.34
59	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo [5.3.1]undec-1-ene (OST)	19.901	19.903	83.17	87.40	0.35	0.37
60	Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl- (OC)	19.935	19.937	75.20	82.40	0.38	0.44
61	Eremophilone (OST)	19.998	-	73.93	-	0.31	-
62	Neophytadiene (OC)	20.665	20.664	92.11	92.25	0.26	0.26
63	*cis*-Chrysanthenyl propionate (OMT)	20.803	20.804	82.55	82.40	1.27	1.54
64	n-Hexadecanoic acid (OC)	-	22.158	-	88.53	-	0.27
65	(+)-Falcarinol (OC)	23.060	23.063	96.93	97.75	0.97	1.47
66	1-Iodo-2-methylundecane (OC)	23.642	-	84.14	-	0.28	-
Total identified (TI)						**95.79**	**95.39**
Monoterpene hydrocarbons (MTH)						1.22	0.59
Oxygenated monoterpenes (OMT)						14.28	14.44
Sesquiterpene hydrocarbons (STH)						50.18	51.11
Oxygenated sesquiterpenes (OST)						21.61	20.71
Other compounds (OC)						8.50	8.54
Oil yield						**0.28**	**0.22**

*UAHD – ultrasound-assisted hydrodistillation; **HD – hydrodistillation;
***Majority compounds are written in bold.

By the HS-SPME method, 21 volatile organic compounds of *E. planum* were extracted.

The major groups of the identified compounds were sesquiterpene hydrocarbons and oxygenated monoterpenes (Table 2). The following majority compounds identified were: *cis*-chrysanthenyl acetate (30.39%), *(E)*-β-farnesene (11.71%), γ-maaliene (7.69%), β-elemene (7.26%), caryophyllene (6.5%), β-selinene (4.72%), δ-cadinene (4.72%), β-copaene (4.61%), α-pinene (4.51%), and γ-muurolene (2.98%). γ-Maaliene and γ-muurolene were not found in the essential oils extracted by UAHD and HD methods.

Table 2. Chemical composition of volatiles of *Eryngium planum* obtained by HS-SPME and analysed by GC-MS

No.	Compounds	Retention time (min)	Identification probability (%)	Normalised area (%)
1	***2***	***3***	***4***	***5***
1	α-Pinene (MTH)	5.445	98.54	**4.51***
2	*cis*-Chrysanthenyl acetate (OMT)	12.053	98.35	**30.39**
3	α-Cubebene (STH)	13.603	95.61	0.64
4	Copaene (STH)	14.069	96.85	1.34
5	β-Elemene (STH)	14.314	98.31	**7.26**
6	Caryophyllene (STH)	14.819	99.47	**6.5**
7	Isogermacrene D (STH)	14.968	94.33	1.29
8	Aromadendrene (STH)	15.128	94.76	0.99
9	*(E)*-β-Farnesene (STH)	15.264	97.15	**11.71**
10	Humulene (STH)	15.360	96.35	1.05
11	γ-Muurolene (STH)	15.680	97.09	**2.98**
12	β-Copaene (STH)	15.782	86.56	**4.61**
13	4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene (STH)	15.821	84.99	1.11
14	β-Selinene (STH)	15.880	97.97	**4.72**
15	γ-Maaliene (STH)	16.009	97.06	**7.69**
16	(+)-Valencene (STH)	16.157	94.56	0.72
17	γ-Cadinene (STH)	16.272	98.74	2.03
18	δ-Cadinene (STH)	16.388	97.31	**4.72**
19	α-Cadinene (STH)	16.618	95.78	1.06
20	Germacrene B (STH)	16.962	98.17	0.99
21	1-Hexadecanol (OC)	23.502	92.88	0.81
Total identified (TI)				**97.12**
Monoterpene hydrocarbons (MTH)				4.51
Oxygenated monoterpenes (OMT)				30.39
Sesquiterpene hydrocarbons (STH)				61.41
Oxygenated sesquiterpenes (OST)				-
Other compounds (OC)				0.81

*Majority compounds are written in bold.

The main classes of compounds identified by GC-MS in the essential oils and volatiles obtained by the UAHD, HD and HS-SPME methods from the aerial parts of *E. planum* plant, are presented in Figure 1.

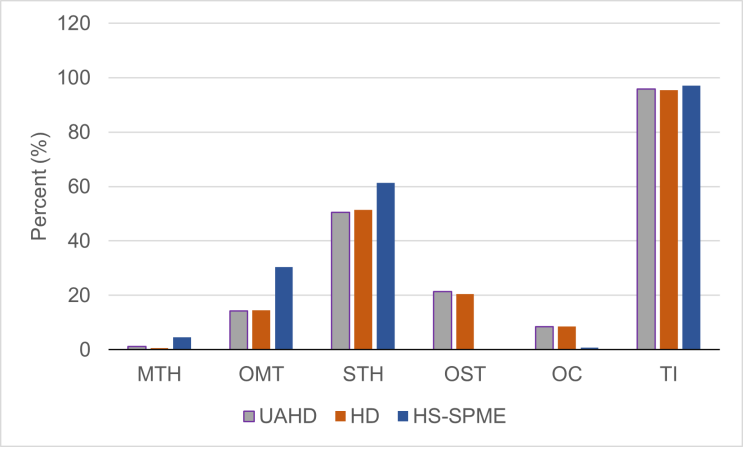

Figure 1. Main classes of compounds found by GC-MS in the essential oils and volatiles obtained by the UAHD, HD and HS-SPME extraction methods: MHT – Monoterpene hydrocarbons; OMT – Oxygenated monoterpenes; STH – Sesquiterpene hydrocarbons; OST – Oxygenated sesquiterpenes; OC – Other compounds; TI – Total identified.

In Figure 1, differences observed between the HS-SPME *versus* the UAHD and HD extraction methods consist in the absence of the oxygenated sesquiterpenes (OST) in the volatiles extracted by HS-SPME method, but also in the presence of the sesquiterpene hydrocarbons (STH) and oxygenated monoterpenes (OMT) after the extraction by all three methods, more increased in the first case. Regarding the total identified compounds by GC-MS after extraction by these three methods, it can state that the percentage values are very close.

The majority compounds identified by GC-MS in the essential oils and volatiles obtained by the UAHD, HD and HS-SPME methods from the aerial parts of *E. planum*, are presented in Figure 2. Differences observed between the UAHD and HD extraction methods were small but should be mentioned an increase in *cis*-chrysanthenyl acetate. Differences observed between the HS-SPME *versus* UAHD and HD extraction methods consist in the presence of γ-maaliene and γ-muurolene in the volatiles extracted by HS-SPME method. *Cis*-chrysanthenyl acetate followed by *(E)*-β-farnesene were the main compounds extracted by the HS-SPME method, while β-copaene

followed by *cis*-chrysanthenyl acetate were the main compounds extracted by both UAHD and HD methods. In the literature [28], the essential oils of *E. planum* were extracted by HD method from stalk leaves, rosette leaves, inflorescences and root. The majority compounds identified in stalk leaves and rosette leaves were α-pinene, β-pinene, β-phellandrene, limonene, camphene. The majority compounds identified in inflorescences and root were *cis*-chrysanthenyl acetate and *(Z)*-falcarinol. The chemical composition of the essential oil from aerial parts was quite different from that previously reported for essential oils from different parts of the plant. This variation in the chemical composition of essential oils can be attributed to the plant organ, soil composition, climate, etc. [34].

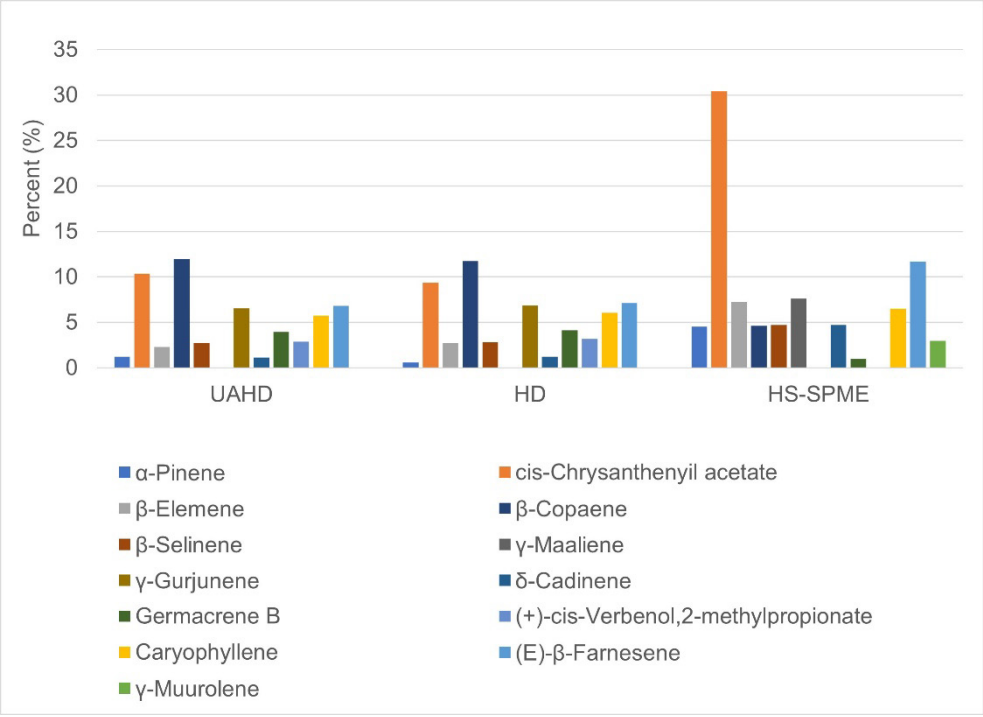

Figure 2. Majority compounds (%) found by GC-MS in the essential oils and volatiles obtained by the UAHD, HD and HS-SPME extraction methods

The essential oil of *E. planum* obtained by the UAHD method was chosen for antimicrobial evaluation because the UAHD extraction yield was

improved by 27.27%, the total identified compounds increased by 0.4%, and the highest increase for one of the main compounds was 0.8%, namely for *cis*-chrysanthenyl acetate.

Antimicrobial activity evaluation of UAHD E. planum essential oil

The antimicrobial activity of extracts was described for some *Eryngium* species in literature against gram-negative and gram-positive bacteria, some species of fungi, yeast and viruses [19, 35].

In this work, the antimicrobial activity of *E. planum* essential oil was tested against gram-negative and gram-positive bacteria using the agar-well diffusion method. After the end of the incubation period at 37ºC, the inhibition zones (mm) of the tested bacterial strains were determined. It was observed that in both bacterial strains, *E. planum* essential oil inhibited growth and the magnitude of the diameter of inhibition varied with the test strain. In the case of *Escherichia coli ATCC 25922*, the gram-negative bacterium strain indicated a very high inhibition reaching the inhibition diameter of 40 mm (Figure 3).

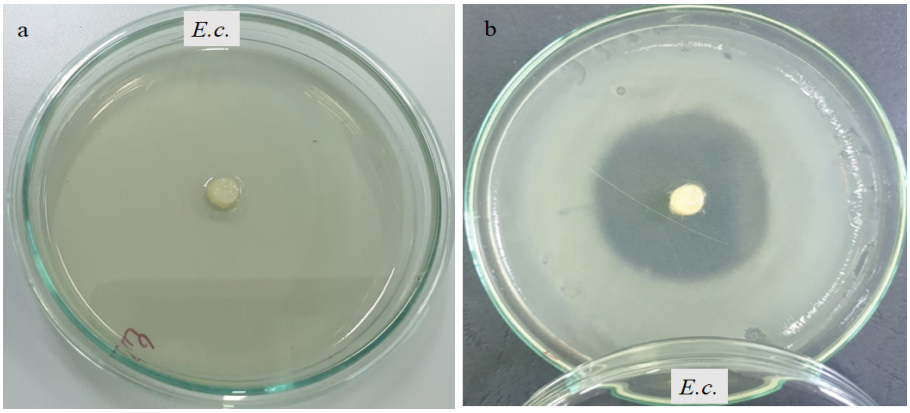

Figure 3. Effect of *Eryngium planum* essential oil on *Escherichia coli* (*E.c.*) ATCC 25922 (gram-negative bacterium strain): a) initial moment; b) after inhibition period

In the case of *Staphylococcus aureus ATCC 25923*, the gram-positive bacterium strain indicated a very high inhibition of the tested oil with an average diameter of 35 mm (Figure 4).

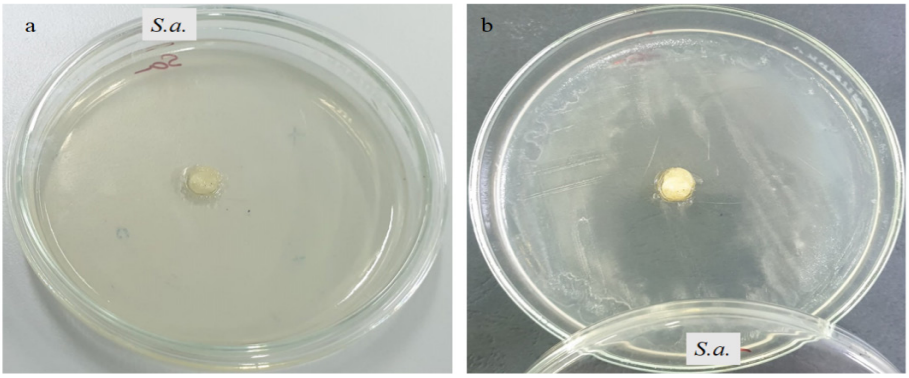

Figure 4. Effect of *Eryngium planum* essential oil on *Staphylococcus aureus* (*S.a.*) ATCC 25923 (gram-positive bacterium strain): a) initial moment; b) after inhibition period

Following these experiments, we can conclude that *E. planum* essential oil exhibits excellent antimicrobial activity against gram-negative and gram-positive bacterial strains.

CONCLUSIONS

The present study reports a comparative chemical composition of essential oils and volatile organic compounds extracted from *Eryngium planum* by different methods, as well as the antimicrobial activity capacity of the oil.

Methods such as classical hydrodistillation, ultrasound-assisted hydrodistillation, and headspace solid-phase microextraction were used to extract essential oils and volatiles from the aerial parts of *Eryngium planum*. The basic chemical composition of these essential oils extracted by the UAHD and HD methods was similar. Using the UAHD method, the oil yield from *E. planum* increased by 27.27% compared to the classical HD method, fact due to the optimisation of the extraction method by adding an ultrasound pretreatment.

The GC-MS analysis showed that β-copaene, a sesquiterpene hydrocarbon, was the majority compound of the *E. planum* oil obtained by both UAHD (11.97%) and HD (11.73%) methods. Within the *E. planum* volatiles extracted by HS-SPME method, *cis*-chrysanthenyl acetate (30.39%), an oxygenated monoterpene, was the majority compound.

The antimicrobial activity of *E. planum* essential oil extracted by UAHD was tested against gram-negative *(Escherichia coli)* and gram-positive *(Staphylococcus aureus)* bacterial strains using the agar-well diffusion method. The results obtained showed the capability of this essential oil to have excellent antimicrobial activity against both gram-negative and gram-positive bacterial strains.

The essential oil of *E. planum* due to its antimicrobial activity is promising for use and benefits in natural medicine, aromatherapy, cosmetics.

EXPERIMENTAL SECTION

Materials

The samples of *E. planum* plant were collected in July 2023 during the flowering period from Florești village, Cluj County, Romania. These plant materials were also identified at the "Alexandru Borza" Botanical Garden from Cluj-Napoca, and the voucher specimen (no. 673003) was deposited in the CL Herbarium, Babeș-Bolyai University, Cluj-Napoca. Aerial parts (stalk leaves, rosette leaves and inflorescences) of *E. planum* were shade-dried at room temperature and ground to powder before the extraction procedures.

Extraction of essential oil by classical HD

Essential oil of *E. planum* was extracted by hydrodistillation using a Clevenger-type apparatus. 100 g of dried aerial parts of *E. planum* were boiled in 1 L distilled water for 3 hours until no essential oil was released. The collected essential oil was dried over anhydrous $MgSO_4$ and stored in dark vials at 4ºC until use.

Extraction of essential oil by UAHD

Essential oil of *E. planum* was extracted by ultrasound-assisted hydrodistillation in two stages: (i) ultrasound treatment using a Hielscher UP200S (24 kHz, 200 W) ultrasonic device equipped with a titanium sonotrode of 14 mm (Hielscher Ultrasonics GmbH, Germany); (ii) hydrodistillation using a Clevenger-type apparatus. *E. planum* powder (50 g) was mixed with 0.5 L distilled water then the sonotrode was immersed in the sample at room temperature. The amplitude (power level of ultrasound) was set at 40%. The duty cycle was set at 0.5. After 60 minutes of ultrasound treatment, the sample was subjected to hydrodistillation for 3 hours. The collected essential oil followed the same procedure as in the case of classical HD extraction.

Extraction of volatiles by HS-SPME

A SPME fibre for manual use and a DVB/CAR/PDMS (50 µm DVB layer; 30 µm CAR/PDM layer) fibre (Supelco, USA) used. The collection of volatiles from the *E. planum* sample was done from the head-space on the SPME fibre: 1 g sample, 8 mL distilled water and 0.5 g NaCl were placed in a 20 mL vial at a temperature of 55ºC for 10 min, followed by adsorption of volatile organic compounds on the SPME fibre for 30 min. These compounds were immediately thermally desorbed in the injector port of GC-MS equipment for their separation on column and MS identification.

Gas chromatography-mass spectrometry (GC-MS) analysis

For GC-MS analysis of essential oils was used an Agilent 8890/ 5977B/2019 (Agilent Technologies, Santa Clara, California, USA) equipment. The analysis was performed in scan mode using a capillary column HP5-MS UI:2556856 (30 m × 250 µm × 0.25 µm) (Agilent Technologies, Santa Clara, California, USA) with Helium (grade 6.0) as carrier gas (Linde Gaz, Cluj-Napoca, Romania) at a flow rate of 1 mL/min. Temperature programme was: 40ºC hold 1 min, ramp to 220ºC with 8ºC/min rate, and then ramped up to 240ºC with 20ºC/min rate and hold at 240ºC for 5 min. The injection volume was 1µL. The GC/MS operated under the following temperature conditions: injector, 200ºC; MS transfer line, 280ºC; MS source, 230ºC and MS Quad 150ºC. Data acquisition and processing were performed using Agilent MassHunter Workstation Software (Agilent Technologies, Santa Clara, California, USA) which allows the qualitative and percentage area evaluation of the analysed compounds. For the structure identification/confirmation of components, the NIST library 20.L (Agilent Technologies, Santa Clara, California, USA) was used.

Antimicrobial activity evaluation

The antimicrobial activity of the *E. planum* essential oil was tested regarding *Gram-negative bacteria, Escherichia coli (ATCC 25922)* and *Gram-positive bacteria, Staphylococcus aureus (ATCC 25923)* at the Microbiology Laboratory, Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania using the agar-well diffusion method [36]. Each bacterial strain was grown for 24 hours, at 37ºC, on Nutrient Agar medium [37]. Then a dilution of 0.5 McFarland turbidity standard according to EUCAST 2013 was made from each bacterial strain in sterile physiological serum [38]. From these dilutions, each Petri dish is inoculated with the help of a sterile swab spreading over the entire surface of the solid culture medium (Mueller Hinton-Oxoid).

Agar-well diffusion method. Petri dishes with Mueller Hinton Agar (M-H Agar) medium (Thermo Fisher Scientific, UK) were inoculated with each bacterial strain and left at 37ºC, for 15 min to infiltrate. Subsequently, 6 mm diameter wells were carved into the agar using a cut sterile pipette tip. The wells were then filled with sterile cotton beads, each bead being loaded with 100 µL of the *E. planum* essential oil. The plates were incubated at 37ºC, for 24 hours. The zones of inhibition were then measured, using a zone of inhibition scale [36]. All assays were performed in triplicate, under aseptic conditions and the mean value was calculated.

REFERENCES

1. R. K. Gautan; K. Roy; G. Thapa; D. Arora; S. Parashar; B. Gurung; L. C. Deb; *Indian J. Pharm. Sci.*, **2020,** *82*, 741–765.
2. M. Altamish; M. Khan; M. S. Baig; B. Pathak; V. Rani; J. Akhtar; A. A. Khan; S. Ahmad; A. Krishnan; *ACS Omega*, **2022**, *7*, 24048–24065.
3. E. P. Pérez-Muñoz; M. Antunes-Ricardo; M. Martinez-Ávila; D. Guajardo-Flores; *Front. Nutr.*, **2022**, *9*, 878306.
4. Z. Zhang; S. Li; S. Ownby; P. Wang; W. Yuan; W. Zhang; R. Scott Beasley; *Phytochemistry*, **2008**, *69*, 2070–2080.
5. T. Kartnig; J. Wolf; *Planta Med.*, **1993**, *59*, 285–285.
6. C.A. J. Erdelmeier; O. Sticher; *Planta Med.*, **1985**, *51*, 407–409.
7. M. Pinar; M. P. Galan; *J. Nat. Prod.*, **1985**, *48*, 853–854.
8. J. J. Brophy; R. J. Goldsack; L. M. Copeland; J. Palá-Paúl; *J. Essent. Oil Res.*, **2003**, *15*, 392–397.
9. J. Lam; L. P. Christensen; T. Thomasen; *Phytochemistry*, **1992**, *31*, 2881–2882.
10. S. Bouzidi; N. Benkiki; M. Hachemi; H. Haba; *Current Bioactive Compounds*, **2017**, *13*, 340–346.
11. S. H. Eslami; M. A. Ebrahimzadeh; A. H. Moghaddam; S. F. Nabavi; N. Jafari; S. M. Nabavi; *Arch. Biol. Sci.*, **2011**, *63*, 157–160.
12. S. Nusair; M. Ahmad; T. EL-Elimat; K. Al-Essa; L. Abu-Qatouseh; R. Khasawneh; *J. Res. Pharm.*, **2022**, *26*, 770–780.
13. X. Zhang; J. Chen; S. Zhou & H. Zhao; *Nutrition and Cancer*, **2022**, *74*, 2996–3006.
14. I. Tiță; G. D. Mogoșanu; M. G. Tiță; **2009**, *Farmacia*, *57*, 141–156.
15. S. Conea; A. E. Pârvu; M. A. Taulescu; L. Vlase; *Dig. J. Nanomater. Bios.,* **2015**, *10*, 693–704.
16. S. Conea; O. Vostinaru, L. Vlase; *Dig. J. Nanomater. Bios.,* **2014***, 9,* 1039–1045.
17. B. Thiem; O. Goślińska; M. Kikowska; J. Budzianowski; *Herba Polonica*, **2010**, *56*, 52–58.
18. S. Conea; L. Vlase; I. Chirilă; *Cellulose Chem. Technol.*, **2016**, *50*, 473–481.

19. A. B. Arykbayeva; G. O. Ustenova; K. O. Sharipov; U. T. Beissebayeva; I. E. Kaukhova; A. Myrzabayeva; N. G. Gemejiyeva; *Int. J. Biomater.*, **2023**, *2023*, 4702607.
20. E. M. Suleimenov; S. Machmudah; M. Sasaki; M. Goto; *Chem. Nat. Compd.*, **2010**, *46*, 826–827.
21. G. Paun; E. Neagu; V. Moroeanu, C. Albu; S. Savin; G. L. Radu; *Biomed Res. Int.*, **2019**, *2019*, 3692605.
22. E. Shabani; M. Mahmoudisourestani; M. Y. Mahen; *Iran. J. Plant Physiol.*, **2022**, *12*, 4223–4230.
23. D. Kremer; M. Z. Končić; I. Kosalec; I. J. Košir; T. Potočnik; A. Čerenak; S. Srečec; V. Dunkić; E. Vuko; *Horticulturae*, **2021**, *7*, 364.
24. M. Marcetic; S. Petrovic; M. Milenkovic; Lj. Vujisic; V. Tesevic; M. Niketic; *Chem. Nat. Comp.*, **2014**, *49*, 1140–1142.
25. A. Medbouhi; F. Benbelaïd; N. Djabou; C. Beaufay; M. Bendahou; J. Quetin-Leclercq; A. Tintaru; J. Costa; A. Muselli; *Molecules*, **2019**, *24*, 2575.
26. O. Elkiran; C. Avsar; A. Veyisoglu; E. Bagci; *J. Essent. Oil-Bear. Plants*, **2023**, *26*, 566–575.
27. E. Korbel; A. Bighelli; A. Kurowska; D. Kalemba; J. Casanova; *Nat. Prod. Commun.*, **2008**, *3*, 113–116.
28. B. Thiem; M. Kikowska; A. Kurowska; D. Kalemba; *Molecules*, **2011**, *16*, 7115–7124.
29. D. Pingret; A. S. Fabiano-Tixier; F. Chemat; *Food Anal. Methods*, **2014**, *7*, 9–12.
30. G. Chen; F. Sun; S. Wang; W. Wang; J. Dong, F. Gao; *Chin. J. Chem. Eng.*, **2021**, *36*, 38–46.
31. C. Xing; C. Qin; X. Li; F. Zhang; R. J. Linhardt; P. Sun; A. Zhang; *LWT-Food Sci. Technol.*, **2019**, *104*, 38–44.
32. A. A. Taherpour; S. Khaef; A. Yari; S. Nikeafshar; M. Fathi; S. Ghambari; *J. Anal. Sci. Technol.*, **2017**, *8*, 11.
33. M. Vinatoru; *Ultrason. Sonochem.*, **2001**, *8*, 303–313.
34. M. G. Miguel; J. Duarte; A. C. Figueiredo, J. G. Barroso, L. G. Pedro; *J. Essent. Oil Res.*, **2005**, *17*, 422–426.
35. S. A. Erdem; S. F. Nabavi; I. E. Orhan; M. Daglia; M. Izadi; S. M. Nabavi; *DARU J. Pharm. Sci.*, **2015**, *23*, 53.
36. R. Carpa; M. Drăgan-Bularda; V. Muntean; Microbiologie Generală Lucrări Practice (General Microbiology, Practical Works), Cluj University Press Publishing House, **2014**.
37. R. M. Atlas; *Handbook of Microbiological Media*, 4th ed., CRC Press, New York, **2010**.
38. R. Leclercq; R. Canton; D. F. J. Brown; C. G. Giske; P. Heisig; A. P. MacGowan; J. W. Mounton; P. Nordmann; A. C. Rodloff; G. M. Rossolini; C. J. Soussy; M. Steinbakk; T. G. Winstanley; G. Kahlmeter; *Clin. Microbiol. Infect.*, **2013**, *19*, 141–160.