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GC-MS AND HPLC CHROMATOGRAPHIC PROFILE OF MAJORITY VOLATILE AND PHENOLIC COMPOUNDS OF SOME MEDICINAL PLANTS FROM ROMANIA[1]

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ABSTRACT. This study reports the identification of volatile organic compounds (VOCs) and the phenolic composition for these medicinal plants: lemon balm (*Melissa officinalis*), lavender (*Lavandula angustifolia*), and elderflower (*Sambucus nigra*). The HS-SPME-GC-MS hyphenated technique was used to investigate the volatiles from the three plants in fresh and dried forms. The essential oils were obtained by hydrodistillation technique, followed by GC-MS analysis. Additionally, HPLC-UV/VIS detection was used to identify the phenolic compounds of these plants. The majority compounds identified in the fresh, dried and oil of lemon balm were *Z*-beta-ocimene, citronellal, citronellol, b-caryophyllene, (*E*)-citral, (*Z*)-citral and geraniol respectively. The aerial part of lavender contains mainly linalool, linalyl acetate, beta-myrcene, trans-beta-ocimene, lavandulyl acetate and caryophyllene. The most compounds identified in the fresh flowers of elderflower were linalool, cis-beta-ocimene, linalool oxide (II) pyran, cis-3-hexenyl isovalerate, while in the dry flowers the majority compounds were linalool oxide (II) pyran, cis-3-hexenyl isovalerate and hexenyl tiglate. The essential oil was rich in n-hexadecanoic acid, linoleic acid, and heneicosane. Majority phenolic compounds identified in the analysed species were vanillic, sinapic, ferulic, and p-coumaric acids, while the predominant flavonoids were rutin, quercetin and epicatechin. The profile of VOCs represents an indicator in the valorisation of medicinal plants.

Keywords: *medicinal plants, volatile organic compounds (VOCs), phenolic*

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compounds, HS-SPME-GC-MS, HPLC-UV/VIS

INTRODUCTION

Aromatic and medicinal plants have been an important concern of man during the development of civilization, currently preparations of vegetable origin occupy an increasingly large place in the sphere of therapeutic applications [1]. Medicinal plants are of particular importance, due to their active principles, being successfully used in traditional medicine (volatiles, phenolic compounds), in the cosmetic industry (essential oil extracts, natural antioxidants) and the food industry (spices, nutritional supplements, teas) [2].

Melissa officinalis *L*., commonly known as lemon balm, is a well-known medicinal plant of the *Lamiaceae* family. Lemon balm is an herbaceous, melliferous plant native to southern Europe [3], in our country being spread spontaneously, but mostly cultivated in the western and southern regions of the country [4]. The lemon balm herb acts in different ways, such as antioxidant, sedative, antidepressant, anxiolytic, antispasmodic, antiseptic, cholagogue, choleretic, carminative, digestive, hypoglycemic and antimicrobial [5–8].

Monoterpenes and sesquiterpenes, responsible for the flavour and medicinal use of this plant, were the main classes of volatile constituents. The majority volatile compounds were geranial, neral, citronellal, geraniol, and caryophyllene, the citrus flavour of lemon balm oil being given by these compounds. Additionally, phytochemical investigations have revealed the presence of triterpenes (ursolic acid, oleanolic acid), phenolic compounds (chlorogenic, caffeic, ferulic and rosmarinic acid) and flavonoids (luteolin, quercetin, rutin, hesperidin) [9, 10].

Lavandula angustifolia, commonly known as lavender, is one of the most useful plants of aromatic and medicinal properties from the *Lavandula* species which are perennial and robust plants from the *Lamiaceae* family, being cultivated specially in Europe, China and USA [11]. Lavender has a great commercial value for cosmetic, perfumery, pharmaceutical, and food industries, and also for aromatherapy [12–14]. This plant is also used in traditional herbal medicine for its sedative, anxiolytic, carminative, antifungal, bactericidal, antiseptic, and anti-inflammatory effects [15]. The essential oil is used in salves, balms, cosmetics, perfumes, and topical skin preparation. Tea prepared from dried lavender flowers is beneficial for relieving mood, insomnia, and abdominal disorders [16–18]. The chemical composition of lavender oil has been studied extensively [19, 20]. Literature data on the activity of lavender oil show differences in the chemical profile between the various species of lavender [21]. The main compounds of lavender oil are the linalool and linalyl acetate, followed by lavandulyl acetate, terpinen-4-ol, lavandulol, beta-caryophenyllene, α-pinene, limonene, α-terpineol, nerol, geraniol, etc. [22–24].

The phenolic compounds and flavonoids are known as secondary metabolites of plants with important biological action. Lavender flowers contain phenolic compounds such as p-hydroxybenzoic acid, vanillic acid, gallic acid, rosmarinic acid, caffeic acid, p-coumaric acid, ferulic acid, chlorogenic acid, sinapic acid, cinnamic acid and flavonoids such as apigenin and luteolin glycosides, catechin, vanillin, etc. [25, 26].

Sambucus nigra, known as elder, is a flowering plant in the *Adoxaceae* family. From these species, elderflowers and elderberries have been used in folk medicine to treat fever, cold, flu, cough, nasal congestion, herpes, ear infections, or as products with anti-inflammatory, analgesic, antimicrobial and diuretic effect or with gentle astringent effect for the skin [27, 28].

The oil extracted from the elderberries contain volatile organic compounds with important bioactive action, namely linalool, terpineol, limonene, beta-caryophyllene, carane, beta-damascenone, cis-rose-oxide and alkane hydrocarbons [29]. The lipophilic fraction of elderflower aqueous extract is represented mainly by the saturated and unsaturated fatty acids, like palmitic, stearic, behenic, oleic, and lignoceric acids [30]. These flowers mainly contain flavonoids such as rutin, quercetin, iso-quercetin, astragalin, hyperoside, nicotiflorin, isorhamnetin and kaempherol, followed by phenolic acids such as caffeoylquinic, dicaffeoylquinic, p-coumaroylquinic, respectively caffeic, ferulic acids, etc. [31, 32].

Research on finding new biologically active compounds from plants involves their isolation and purification through various extraction methods and identification/quantification by different chromatographic techniques, and then performing tests to determine their biological action [33]. The conventional extractive techniques used are maceration, infusion, percolation, and Soxhlet extraction. Among the modern extraction techniques, it is mentioned accelerated solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, called also sonication extraction, and supercritical fluid extraction [33].

Hydrodistillation (HD) is another conventional method that uses water or steam to extract bioactive compounds, mainly essential oils. This technique is regularly carried out by means of a setup recognized as a Clevenger apparatus or simple steam distillation [34, 35].

For the identification of compounds, extraction techniques are used in combination with different analytical techniques, such as the chromatographic (GC, HPLC), spectroscopic (IR, UV-VIS, NMR) or spectrometric (MS) techniques [36–38].

Headspace solid-phase microextraction (HS-SPME) technique coupled to gas-chromatography with mass-spectrometry detection (GC-MS) is a hyphenated technique (HS-SPME-GC-MS) successfully used for sampling

and analysing the volatiles of a complex matrix from plants, animals or environmental samples, etc. [39]. Through this technique it is possible to easily establish the profile of volatiles and their variability in different stages of plant development, different anatomical parts of them (root, stem, leaves, flowers), different degrees of harvesting and processing (fresh, dried, oil) as well as the botanical and geographical origin of the plants [40,41].

In recent years, a re-evaluation of medicinal and aromatic plants is required, due to the modern concern developing new phytotherapeutic products based on nanotechnologies. Nano-formulations such as nano-emulsions, liposomes, micelles, hydrogels and nanoparticles are known as very good vehicles to obtain a high bioavailability of the active ingredients from plants [42–45].

The variation of the chemical profile of medicinal plants depending on the variety, the anatomical part of the plant, the stage of development, the types of products, the harvesting environment, requires a re-evaluation when it comes to their use in medicine, because the components can influence the pharmacological/therapeutic properties [41].

This study aimed to investigate by GC-MS the profile of the volatile organic compounds (VOCs) of three medicinal plants (lemon balm, lavender and elderflower) from Romania, Cluj County, each of them in three forms of presentation of the aerial part: fresh, dry, and essential oil. The VOC profile of the fresh and dried samples, respectively, was performed by HS-SPME-GC-MS while that of the essential oils, obtained by hydrodistillation of the dried aerial parts, by GC-MS. The content of some phenolic compounds and flavonoids in the alcoholic extracts of dried plants was performed by HPLC-UV/VIS analysis.

RESULTS AND DISCUSSION

In the present study, a comparative analysis on the abundance of volatile compounds in three forms of the plant, namely essential oil, fresh and dried plants, aims to trace their traceability. Phenolic and flavonoid compounds were also determined from the alcoholic extracts of the dried plants.

MELISSA OFFICINALIS – volatile profile

A total of 38 compounds were identified in all analysed samples. The GC-MS chemical composition of the HS-SPME extract of lemon balm from fresh and dried aerial part (leaves) includes mainly monoterpenes (55.19% and 72.95%) and sesquiterpenes (37.54% and 18.64%). The oxygenated

monoterpenes (50.85%) and the oxygenated sesquiterpenes (43.29%) are the main classes in lemon balm oil (Table 1). The fresh extract of lemon balm contains mainly: beta-caryophyllene (21.29%), *(E)*-citral (19.11%), (*Z)*-citral (13.07%), (*Z)*-beta-ocimene (11.12%), and citronellal (7.66%). The majority compounds identified in the dried extract were: (*E*)-citral (30.47%), (*Z*)-citral (19.03%), citronellal (15.61%) (*Z*)-beta-ocimene (3.59%), beta-caryophyllene (11.27%), while hydrodistilled essential oil was rich in (*E*)-citral (25.15%), beta-caryophyllene (22.47%), (*Z*)-citral (19.15%), germacrene-D (10.89%) and citronellal (3.26%).

Table 1. Volatile organic compounds identified in leaves (fresh, dried, and essential oil) of lemon balm

No.	**Compound**	**t_R (min)**	**LRIs**	**Lemon balm sample Normalised area (%)**		
				Fresh	Dried	Oil
1	***2***	***3***	***4***	***5***	***6***	***7***
1.	*E*-2-hexenal (OC)	9.776	835	nd	1.35	nd
2.	Benzaldehyde (OC)	13.107	955	nd	0.36	nd
3.	1-Octen-3-ol (OC)	13.533	971	1.03	nd	nd
4.	3-Octanone (OC)	13.694	976	nd	1.21	nd
5.	3-Heptanone, 5-methyl (OC)	13.715	977	3.36	nd	nd
6.	E,E-2,4-Heptadienal (OC)	14.534	1004	nd	0.17	nd
7.	E-beta-ocimene (MH)	15.209	1027	nd	0.14	nd
8.	Alpha-pinene (MH)	15.219	1028	0.75	nd	nd
9.	**Z-beta-ocimene (MH)**	**15.582**	**1040**	**11.12**	**3.59**	**1.60**
10.	(4E,6Z)-allo-ocimene (MH)	17.990	1123	0.50	0.15	nd
11.	1,5-Heptadiene-3,3-dimethyl (OC)	18.426	1139	0.26	0.56	nd
12.	**Citronellal (OM)**	**18.727**	**1149**	**7.66**	**15.61**	**3.26**
13.	Isoneral (OM)	18.945	1157	0.50	1.22	1.14
14.	Isogeranial (OM)	19.500	1177	0.70	1.23	1.50
15.	Methyl salicylate (OC)	20.003	1194	2.06	0.78	nd
16.	Nerol (OM)	20.849	1226	0.92	nd	nd
17.	**Z-citral (neral) (OM)**	**21.238**	**1240**	**13.07**	**19.03**	**19.15**
18.	Methyl citronellate (OM)	21.581	1253	0.35	4.92	0.65
19.	**E-citral (geranial) (OM)**	**22.058**	**1271**	**19.11**	**30.47**	**25.15**
20.	Methyl geranoate (OM)	23.303	1319	0.51	0.47	nd
21.	Alpha-cubenene (SH)	24.144	1352	0.62	0.33	nd
22.	Alpha-copaene (SH)	24.943	1384	1.34	0.79	0.77
23.	Beta-gurjunene (SH)	25.223	1395	1.08	0.46	1.28
24.	**Beta-caryophyllene (SH)**	**26.175**	**1434**	**21.29**	**11.27**	**22.47**

No.	Compound	t_R (min)	LRIs	Lemon balm sample Normalised area (%)		
				Fresh	Dried	Oil
1	*2*	*3*	*4*	*5*	*6*	*7*
25.	Trans-alpha-bergamotene (SH)	26.284	1439	1.51	0.65	nd
26.	Epi-bicyclosesquiphellandrene (SH)	26.338	1440	nd	nd	1.80
27.	Beta-farnasene (SH)	26.684	1456	nd	nd	0.23
28	Beta-copaene (SH)	26.865	1461	nd	nd	0.45
29.	Humulene (SH)	27.003	1467	1.35	0.64	1.31
30.	Gamma-muurolene (SH)	27.381	1485	1.83	0.83	nd
31.	Germacrene D (SH)	27.599	1494	3.30	1.68	10.89
32.	Alpha-farnesene (SH)	27.874	1505	1.81	0.80	1.44
33.	Gamma-cadinene (SH)	28.378	1522	2.35	0.95	1.67
34.	Trans-calamenene (SH)	28.487	1533	0.56	nd	nd
35.	Alpha-amorphene (SH)	28.829	1548	0.50	0.24	0.98
36.	Fenchone (OS)	29.829	1592	nd	nd	0.80
37.	Tau-cadinol (OS)	31.297	1660	nd	nd	1.31
38.	Alpha-cadinol (OS)	31.577	1673	nd	nd	2.08
	Monoterpene hydrocarbons (MH)			12.37	3.88	1.60
	Oxygenated monoterpenes (OM)			42.82	72.95	50.85
	Sesquiterpene hydrocarbons (SH)			37.54	18.64	43.29
	Oxygenated sesquiterpenes (OS)			0	0	4.19
	Other compounds (OC)			6.71	4.43	0
	Total %			**99.44**	**99.90**	**99.93**

t_R: retention time; LRIs: linear retention index (on HP-5ms column); nd: not detected;
The majority volatile organic compounds are written in bold.

In the case of this plant, fewer compounds were identified because its profile of volatiles is uniform, in the three samples (fresh, dried and essential oil) analysed. The main common compounds (6) of the three samples were: (*Z*)-beta-ocimene, citronellal, (*Z)*-citral, (*E)*-citral, beta-caryophylene, germacrene D in variable proportions (Figure 1). Other compounds common to the three samples were in smaller quantities: beta-gurjunene, humulene, alpha-farnasene, gamma cadinene, and S-citronellic methyl ester. A notable variability among the three samples would be the presence of (*Z*)-beta-ocimene in the fresh sample in a proportion of, 11.12%, compared to 3.59% in dried sample and 1.60% in the essential oil. (*Z*)-beta ocimene is a common monoterpenoid found in almost all fresh green plants with the role of attracting pollinators [46]. The oil sample contains 43.29% sesquiterpene compounds compared to the other samples, where the proportion of sesquiterpenes was 37.54% for fresh sample and 18.64% for dried sample. Another important thing to mention would be that

the lemon balm oil sample contains Gemacrene-D in a percentage of 10.89%, compared to the other samples where the amount found was lower.

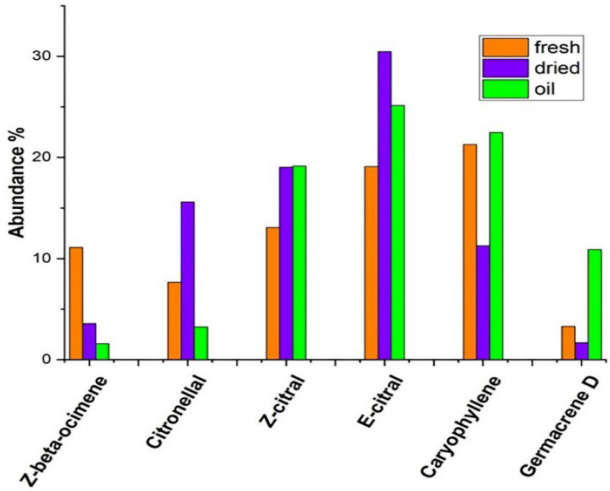

Figure 1. The six majority compounds found in lemon balm samples: fresh, dried and essential oil

In the literature, there are few data regarding the identification of volatiles from the leaves and oil of *Melissa officinalis* by the HS-SPME-GC-MS respecively HD-GC-MS techniques. A comparative study presents the collection of volatiles from fresh lemon balm leaves by the HS-SPME-GC-MS technique and from lemon balm oil obtained by hydrodistillation. The main compounds identified in both fresh leaves and essential oil were: citronellal (31.1% and 10.2%), (*E*)-citral (11.9% and 11.2%), (*Z*)-citral (9.6% and 19.6%), beta- caryophyllene (12.0% and 13.2 %) [47].

Ieri et al. [48] presents a study on HS-SPME-GC-MS analysis of the dried powdered foliar sample of *Melissa officinalis*. Terpenes were the most representative class of compounds monoterpenes (71.91%) beside the sesquiterpenes (19.01%). The most abundant compounds were citronellal (27.54%), α-citral, (25.00%), beta-caryophyllene (9.24%) and beta-citral (7.61%).

The chemical composition of lemon balm oil is mainly represented by compounds such as E/Z-citral, citronellal, caryophylene, in variable proportions [49, 50]. These compounds that give the smell and aroma of the plant [51]. Besides their use in perfumery, these compounds have antimicrobial, anxiolytic and antidiabetic action [52]. The other compounds found in the oil such as thymol [53], sesquiterpene alcohol (nerolidol) [54], are not specific to lemon balm oil. The volatile lemon balm oil from Romania was characterized [55] by a

higher content of monoterpenes (mainly *E* and *Z*-Citral, citronellal, ~32%) and by the presence in *Mellisa* of trans-anethole and estragole (26.44%) [55]. In this study trans-anethole and estragole were not found in the *Mellisa* samples.

LAVANDULA ANGUSTIFOLIA – volatile profile

A total of 41 compounds were identified in all the studied samples (fresh, dried and essential oil) of lavender aerial part (flowers). (**Table 2**) The bouquet of fresh and dried flowers of lavender contains mainly: linalyl acetate (19.70% and 16.48%), linalool (15.57% and 21.03%), caryophyllene (12.78% and 8.28%), trans-beta-ocimene (10.44%, and 9.28%), beta-myrcene (8.42% and 9.96%), lavandulyl acetate (5.81% and 6.15%) and allo-ocimene (6.05% and 4.88%). Extraction of essential oil from the dried lavender flowers was carried out by hydrodistillation, using a Clevenger-type apparatus. The majority components found were: linalool (21.91%), linalyl acetate (14.54%), E-beta-ocimene (8.11%), beta-caryophyllene (7.31%), and beta-myrcene (6.19%).

Table 2. Volatile compounds identified in the aerial part of *lavender*

No.	Compound	t_R (min)	LRIs	Lavender sample Normalised Area %		
				Fresh	Dried	Oil
1	***2***	***3***	***4***	***5***	***6***	***7***
1	Alpha-pinene (MH)	12.223	926	nd	nd	0.15
2	Camphene (MH)	12.770	944	nd	0.41	0.30
3	**Beta-myrcene (MH)**	**13.959**	**985**	**8.42**	**9.96**	**6.19**
4	Alpha-phellandrene (MH)	14.545	1005	0.56	0.60	0.65
5	**E-beta-ocimene (MH)**	**15.308**	**1031**	**10.44**	**9.28**	**8.11**
6	Z-beta-ocimene (MH)	15.697	1044	5.59	4.28	3.94
7	Gamma terpinene (MH)	16.038	1056	nd	0.20	nd
8	3-Carene (MH)	16.916	1078	0.41	nd	0.55
9	**Linalool (MH)**	**17.388**	**1100**	**15.57**	**21.03**	**21.91**
10	**Allo-ocimene (MH)**	**18.151**	**1129**	**6.05**	**4.88**	**5.00**
11	Camphor (OM)	18.442	1154	0.75	0.60	0.25
12	Borneol (OM)	19.147	1164	0.74	0.29	0.20
13	Lavandulol (OM)	19.292	1164	nd	0.98	nd
14	Terpinen-4-ol (OM)	19.884	1184	0.27	1.82	2.08
15	Cryptone (OM)	20.091	1196	nd	0.62	0.61
16	Alpha-terpineol (OM)	20.359	1207	nd	nd	6.12
17	**Linalyl acetate (OM)**	**21.513**	**1251**	**19.70**	**16.48**	**14.54**

No.	Compound	t_R (min)	LRIs	Lavender sample Normalised Area %		
				Fresh	Dried	Oil
1	*2*	*3*	*4*	*5*	*6*	*7*
18	Piperitone (OM)	22.157	1270	nd	0.21	nd
19	**Lavandulyl acetate (OM)**	**22.520**	**1289**	**5.81**	**6.15**	**3.64**
20	Bornyl acetate (OM)	22.707	1295	0.39	0.66	0.71
21	p-cymen-7-ol/cuminol (OM)	22.800	1299	nd	0.14	nd
22	Neryl acetate (OM)	24.357	1360	2.06	1.60	2.26
23	Geranyl acetate (OM)	24.912	1382	3.13	3.05	3.25
24	Alpha-cubenene (SH)	25.213	1388	0.20	nd	nd
25	**Beta-caryophyllene (SH)**	**26.245**	**1438**	**12.78**	**8.28**	**7.31**
26	Beta-farnasene (SH)	26.774	1458	4.14	3.83	2.12
27	Humulene (SH)	27.086	1471	0.58	0.46	0.33
28	Biciclosesquiphellandrene (SH)	27.193	1476	0.21	0.30	nd
29	Gamma-muurolene (SH)	27.470	1488	nd	nd	0.37
30	Germacrene D (SH)	27.646	1496	0.44	0.20	nd
31	Beta-bisabolene (SH)	28.094	1515	0.25	nd	nd
32	Gamma-muurolene (SH)	28.367	1527	1.21	2.11	0.98
33	Gamma-cadinene (SH)	28.476	1529	0.18	nd	nd
34	Caryophyllene oxide (OS)	30.007	1600	nd	0.12	0.79
35	Epicubenol (OS)	30.666	1630	nd	0.11	0.50
36	Tau-cadinol (OS)	31.288	1657	nd	1.17	4.14
37	Muurol-5-en-4-one (OS)	32.319	1708	nd	nd	0.22
38	Epicubenol (OS)	33.367	1723	nd	nd	0.15
39	2-Pentadecanone, 6,10,14-trimethyl (OC)	34.659	1802	nd	nd	0.29
40	Nonadecane (OC)	36.039	1900	nd	nd	1.27
41	Eicosane (OS)	36.325	2000	nd	nd	0.12
	Monoterpene hydrocarbons (MH)			31.47	29.61	24.89
	Oxygenated monoterpenes (OM)			48.42	53.63	55.57
	Sesquiterpene hydrocarbons (SH)			19.99	15.18	11.11
	Oxygenated sesquiterpenes (OS)			0	1.4	5.80
	Other compounds (OC)			0	0	1.68
	Total			**99.88**	**99.82**	**99.05**

t_R-retention time; LRIs: linear retention index (on HP-5ms column); nd: not detected;
The majority volatile organic compounds are written in bold.

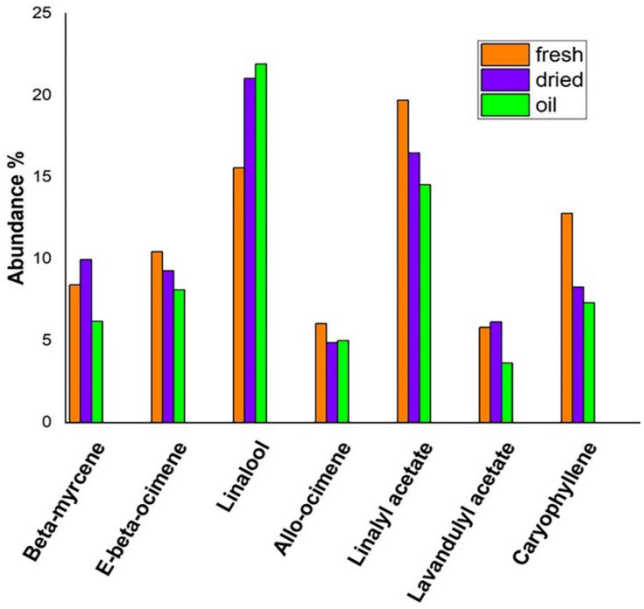

Figure 2. Majority compounds in lavender samples: fresh, dried and oil

There are numerous studies that have analysed the chemical composition of Lavender in all its forms (inflorescences, leaves, fresh, dried, oil) obtained through different extraction techniques and analysis methods [56–58].

The most characteristic and valuable constituents for lavender (*Lavandula angustifolia*) presented by Zagorcheva [59] and J. Fu [60] were linalool (20–35%), linalyl acetate (30–35%), and lavandulyl acetate (5–6%). Surprisingly, different studies also reported other main components such as eucalyptol (8.50% and 31.9%), borneol (15.21% and 24%) and camphor (2.00% and 16.1%), in the lavender aerial parts [61]. In our samples camphor and borneol were identified in traces, and eucalyptol is missing. Thus, a continuous evaluation of this plant with applications in medicine, cosmetic industry and aromatherapy is necessary, because it presents a great variability of biologically active compounds. These compositional variabilities can be dictated by environmental conditions, soil characteristics, harvesting time and drying/processing methods [62].

SAMBUCUS NIGRA – volatile profile

Through the GC-MS analysis of the aerial part (flowers) and the oil of elderflower, a total of 55 compounds were identified, grouped as follows: monoterpenes, sesquiterpenes, esters, acids, hydrocarbons and other compounds. In the case of this plant, in addition to the great variability of the main volatile compounds in the three samples, we also observe a great variability in the classes of compounds. In the flower samples monoterpenes predominate, they exist in a proportion of 83.05% in the fresh elderberry plant, 71.31% in the dry plant, while in the oil sample, monoterpenes represented only 9 .5%.

The esters were found in a proportion of 11.59% in the fresh sample, 22.85% in the dried sample and only 5.02% in the essential oil. Fatty acids were identified in a proportion of 59.84% and hydrocarbons 16.3% only in the essential oil sample (**Table 3**).

Table 3. Volatile constituents identified in aerial part of *elderflower:* fresh, dried, and essential oil

No.	Compound	t_R (min)	LRIs	Elderflower sample Normalised area %		
				Fresh	Dried	Oil
1	***2***	***3***	***4***	***5***	***6***	***7***
1.	3-Hexen-1-ol (OC)	9.818	838	1.29	nd	nd
2.	Hexenyl tiglate (E)	9.839	837	nd	**6.95**	nd
3.	1,5-Heptadiene-2,6-dimethyl (H)	10.574	865	nd	nd	1.03
4.	Pentanoic acid, 3 methyl-2-oxo, methyl ester (OC)	13.118	953	nd	1.16	nd
5.	Beta-myrcene (MT)	13.839	981	1.59	nd	nd
6.	*E*- β –ocimene (MT)	15.199	1027	1.24	nd	nd
7.	L-isoleucine, methyl ester (E)	15.341	1031	nd	2.34	nd
8.	***Z*- β-ocimene (MT)**	**15.551**	**1039**	**20.08**	**nd**	**nd**
9.	3-Carene (MT)	15.858	1051	0.37	nd	nd
10.	**Cis-Linalool oxide (MT)**	**16.366**	**1067**	**7.57**	**0.96**	**nd**
11.	Trans-Linalool oxide (MT)	16.833	1083	0.55	nd	nd
12.	**Linalool (MT)**	**17.165**	**1094**	**24.68**	**0.73**	**0.56**
13.	Hotrienol (MT)	17.258	1097	nd	2.73	nd
14.	Nonanal (OC)	17.378	1101	nd	nd	2.51
15.	Trans-rose oxide (MT)	17.489	1102	0.41	nd	**4.19**
16.	Phenylethyl alcohol (OC)	17.624	1110	nd	0.76	nd
17.	Trans-alloocimene (MT)	17.974	1123	0.90	nd	nd

No.	Compound	t_R (min)	LRIs	Elderflower sample Normalised area %		
				Fresh	Dried	Oil
1	*2*	*3*	*4*	*5*	*6*	*7*
18.	Cis-rose oxide (MT)	18.032	1134	nd	nd	2.01
19.	Cis-alloocimene (MT)	18.344	1136	1.00	nd	nd
20.	Citronellal (MT)	18.898	1148	0.52	nd	nd
21.	Nerol oxide (MT)	18.772	1151	nd	nd	0.44
22.	2,4-Dimethylfuran (OC)	18.903	1156	nd	3.57	nd
23.	**Linalool oxide (II) (pyran) (MT)**	**19.303**	**1170**	**14.18**	**63.56**	**0.65**
24.	Terpenediol (I) (MT)	19.682	1183	nd	0.53	nd
25.	**Citronellol (MT)**	**20.745**	**1222**	**8.89**	**1.60**	**0.65**
26.	**Cis-3-hexenyl isovalerate (E)**	**20.849**	**1226**	**9.88**	**9.06**	**nd**
27.	Butyl 2-methylbutenoate (E)	20.963	1230	nd	0.40	nd
28.	Geraniol (MT)	21.394	1246	1.10	nd	0.29
29.	Citral (MT)	21.913	1282	1.21	1.2	nd
30.	Dihydroedulan (OC)	22.855	1295	nd	nd	0.78
31.	(Z)-hexenyl angelate (E)	23.319	1319	1.71	2.94	1.73
32.	Beta-damascenone (OC)	24.938	1356	nd	nd	0.26
33.	Cis-jasmone (ST)	25.259	1396	0.72	nd	nd
34.	1,3,8-Menthatriene (MT)	25.285	1397	nd	nd	1.36
35.	Benzene-4-ethyl-1,2-dimethyl (H)	25.819	1421	nd	nd	0.43
36.	Beta - caryophyllene (MT)	26.095	1431	0.86	1.31	0.27
37.	Germacrene D (ST)	27.387	1485	0.20	nd	nd
38.	Trans-2-hexenyl isovalerate (E)	27.952	1490	nd	nd	0.86
39.	1,3-Benzenediol, 5-pentyl (OC)	28.324	1520	nd	nd	0.82
40.	3-Hexen-1-ol benzoate (E)	29.564	1580	nd	nd	1.21
41.	Benzoic acid, hexyl ester (E)	29.708	1581	nd	nd	0.14
42.	Tetradecanal (H)	30.370	1590	nd	nd	0.50
43.	Pentadecanal (H)	32.596	1725	nd	nd	0.26
44.	Tetradecanoic acid (OA)	33.572	1761	nd	nd	1.45
45.	Octadecanal (OC)	34.370	1800	nd	nd	0.74
46.	2-Pentadecanone-6,10,14-trimethyl (OC)	34.687	1852	nd	nd	1.72
47.	Nonadecane (H)	35.394	1900	nd	nd	**4.04**
48.	Citronellyl tiglate (E)	35.752	1934	nd	nd	1.08
49.	Geranyl vinyl ether (OC)	35.932	1942	nd	nd	1.46
50.	**n-Hexadecanoic acid (OA)**	**36.024**	**1964**	**nd**	**nd**	**45.13**
51.	9-Nonadecene (H)	37.593	1968	nd	nd	1.05

No.	Compound	t_R (min)	LRIs	Elderflower sample Normalised area %		
				Fresh	Dried	Oil
1	***2***	***3***	***4***	***5***	***6***	***7***
52.	Heneicosane (H)	37.974	2100	nd	nd	**6.62**
53.	**Linoleic acid (OA)**	**38.937**	**2140**	**nd**	**nd**	**9.33**
54.	Oleic acid (OA)	39.056	2147	nd	nd	**3.93**
55.	Docosane (H)	39.424	2200	nd	nd	2.37
	Monoterpenes (MT)			83.05	71.31	9.5
	Sesquiterpenes (ST)			1.78	1.31	0.53
	Esters (E)			11.59	22.85	5.02
	Organic Acids (OA)			0	0	59.84
	Hydrocarbons (H)			0	0	16.30
	Other compounds (OC)			2.53	4.33	8.68
	Total			**98.95**	**99.80**	**99.87**

t_R-retention time; LRIs: linear retention index (on HP-5ms column); nd: not detected; The majority volatile organic compounds are written in bold.

The main compounds from elderflower*,* fresh aerial part were found: cis- beta-ocimene (20,08%), linalool (24,68%), linalool oxide II (14,18%), cis-hexenyl-isovalerate (9,88%) and citronellol (8,89%). The main compounds from elderflower dried were identified: linalool oxide II (63,56%), cis-hexenyl-isovalerate (9,06%) and hexenyl tiglate (6,95%). The specific compounds in the elderflower oil were found: n-hexadecanoic acid (45,13%), heneicosane (6,62%), linoleic acid (9,33%), oleic acid (3,93%) and docosane (2,37%) (Figure 3).

Common compounds were identified in the three forms of *elderflower* thus: linalool, citronellol, linalool oxide pyran, citronellol, (*Z*)-hexenyl angelate and caryophyllene in different percentages.

Similar studies, which use the sampling of elder flowers, through the HS-SPME-GC-MS technique, have highlighted important compounds that give the characteristic aroma such as: linalool, hotrienol, linalool oxide, 2-hexanone, eugenol, 3-hexen-1-ol [63, 64]. Among the dominant compounds of the essential oil of elderflowers, hydrocarbons (nonadecane, tricosane, eicosane, pentacosane, heneicosane), followed by fatty acids such as n-hexadecanoic and linoleic acid are listed. Cis-linalool oxide, linalool, epoxy-linalool, rose oxide, carvacrol, and citronellol were the most prominent oxygenated monoterpenes found [65-67].

Other studies followed changes in the profile of volatiles before and after harvesting the flowers [68]. Drying for further use is an important preservation method for plant material, as it inhibits enzymatic degradation and limits microbial growth [69].

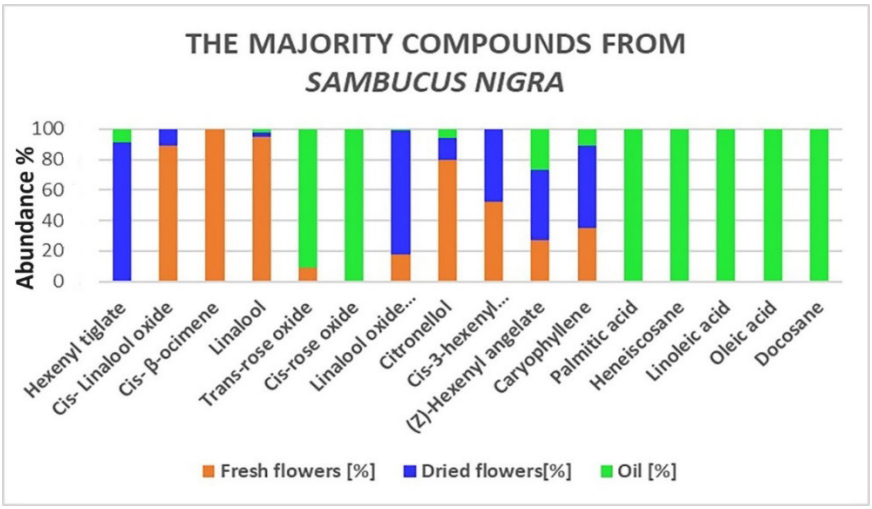

Figure 3. Majority compounds from aerial part (flowers) of elderflower: fresh, dried and essential oil

The studies regarding the use of this species in traditional medicine are focused more on the fruits and less on the effectiveness of the elder flowers.

The predominant compounds in the three samples, such as linalool, linalool oxide (II), pyran, citronellol and fatty acids show biological activity.

Linalool is a racemic mixture of both enantiomers, being present not only in the essential oil of plants but also in some fruits. The linalool has anti-inflammatory, anticancer, anti-hyperlipidemic, antimicrobial, antinociceptive, analgesic, anxiolytic, antidepressant and neuroprotective properties [70]. Using *in vitro* and *in vivo* models, linalool demonstrated to hold a broad spectrum of bioactive properties, that can be exploited by the pharmaceutical industry. Inhaled linalool showed anxiolytic properties in the light/dark test, increased social interaction and decreased aggressive behavior [71]. The antitumor activity of linalool has been studied *in vitro* and *in vivo* and its role as a modulator that increases the antitumor activity of some drugs and reduces the effect of cytotoxicity has been highlighted [72]. The linalool oxide can be used as natural flavoring and was tested by inhalation in case of animal model (mouse), without causing any motor deficit. These results suggest that inhaling of linalool oxide can be used against anxiety [73].

A systemaic review have highlighted the biological activities of citronellol, including antibiotic and antifungal effects *in vitro*, and pointed out various properties, including analgesic and anticonvulsant effects *in vivo*, in addition to showing low toxicity [74].

Fatty acids such as n-hexadecanoic acid, and linoleic acid have proven antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumoniae*, *Mycobacteria*, *Helicobacter pylori*, etc. [75, 76] and also anti-inflammatory activity [77, 78].

PHENOLIC COMPOUNDS ANALYSIS

Phenolic compounds from plants of bioactive compounds with antioxidant activities. This study reports the content of phenolic acid and flavonoid contents of these three medicinal plants from Cluj County, Romania, including lemon balm (*Melissa officinalis*), lavender (*Lavandula angustifolia*) and elderflower (*Sambucus nigra*).

The majority compounds present in *lemon balm* extract were: rutin (364.05 µg/g), catechin (33.4 µg/g), sinapic acid (108.75 µg/g), vanillic acid (42.95 µg/g) and p-coumaric acid (36.95 µg/g).

Vanillic (2023.8 µg/g), sinapic (247.2 µg/g) and ferulic (171.55 µg/g) acids, epicatechin (341.45 µg/g) in *lavender* extract. Other compounds identified in low concentration were: p-coumaric (27.25 µg/g) and luteolin (142.95 µg/mg) and quercetin (82.7 µg/g).

For elderflower extract, the HPLC-UV/VIS technique revealed that the majority compounds were vanillic acid (444.1 µg/g), catechin (304 µg/g) and quercetin (77.3 µg/g), while the least abundant was gallic acid (2.1 µg/g) (Table 4).

Table 4. HPLC-UV/VIS determination of polyphenols from herbal materials: lemon balm, lavender, and elderflower

Polyphenols	Herbal materials		
	Lemon balm	Lavender	Elderflower
	Polyphenols (µg/g)		
Gallic acid*	0.00	0.00	2.1
Catechin**	33.4	0.00	304
Epicatechin**	16.8	341.45	0.00
Vanillic acid*	42.95	2023.8	444.1
Caffeic acid*	18.05	0.00	51.9
p-Coumaric acid*	36.95	27.25	5.5
Sinapic acid*	108.75	247.2	30.25
Ferulic acid*	9.2	171.55	0.00
Rutin**	364.05	20.1	24.35
Quercetin**	9.45	82.7	77.3
Luteolin**	0.00	142.95	0.00
SUM	**639.6**	**3057**	**939.5**

*Phenolic acid; **Flavonoid

The phenolic acids and flavonoids were identified by comparison with the standards followed by their quantification based on the HPLC-UV/VIS analysis (Figure 4).

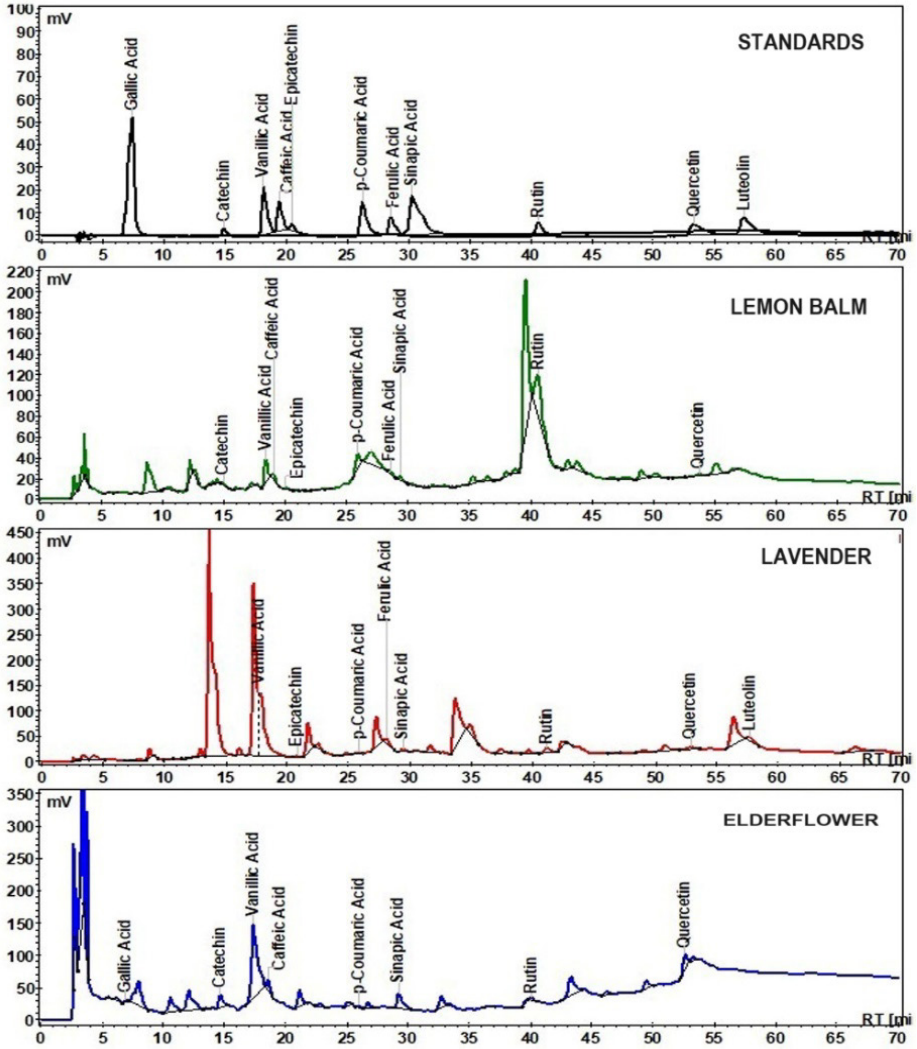

Figure 4. HPLC-UV-VIS chromatograms of polyphenols: standards and compounds found in the analysed samples (lemon balm, lavender, elderflower

HPLC parameters of the determination of polyphenols from the plant extracts are presented in Table 5.

Table 5. Validation parameters of HPLC method for determination of polyphenols from plant extracts

Compound	[a]t_R (min)	[b]Calibration curves	[c]Regression Coefficient R^2	[d]LOD (3 × S/N, µg/mL)	[e]LOQ (10 × S/N, µg/mL)
Gallic acid	7.40	A=0.023C+0.012	0.998	0.037	0.123
Catechin	14.88	A=0.122C+0.091	0.994	0.152	0.506
Vanillic acid	18.14	A=0.732C+0.887	0.997	0.081	0.270
Caffeic acid	19.41	A=0.354C-0.066	0.998	0.730	2.433
Epicatechin	20.47	A=0.064C-0.026	0.996	0.840	2.800
p-Coumaric Acid	26.19	A=0.174C-0.017	0.998	0.329	1.096
Ferulic acid	28.52	A=0.422C+0.357	0.997	0.103	0.343
Sinapic acid	30.27	A=0.521C+0.134	0.998	0.198	0.660
Rutin	40.56	A=0.309C+0.355	0.996	0.241	0.803
Quercetin	53.27	A=0.575C-0.032	0.998	0.321	1.070
Luteolin	57.38	A=0.586C+0.531	0.998	0.387	1.290

[a]t_R (min), Retention time; [b]Calibration curves; A, peak area; C, concentration of analyte (µg/mL); [c]Regression coefficient R^2; [d]LOD (3 × S/N, µg/mL), limit of detection; [e]LOQ (10 × S/N, µg/mL), limit of quantification; S/N, signal to noise ratio.

Our research was consistent with literature studies done on lemon balm. The polyphenols from lemon balm reported by Virchea et al. [79] were luteolin, quercetin, rhoocitrin, those reported by Miraj et al. [80] were rosmarinic, caffeic and protocatechuic acids, while those reported by Ordaz et al. [81] were astragalin and apigenin and vanillic, ferulic and caffeic acids.

The literature data [82–85] presents a multitude of HPLC methods for the analysis of ethanolic and aqueous extracts from fresh or dried flowers of *Lavandula angustifolia* using different detectors such as: UV-VIS, MS, DAD, UHPLC-DAD, UPLC-ESI-MS/MS. Thus, through these techniques, the polyphenolic markers of lavender flowers were identified the following phenolic acids such as rosmarinic, ferulic, caffeic, vanillic, chlorogenic, sinapic and p-coumaric acids and flavonoids such as apigenin, luteolin, catechin, naringenin, epicatechin and rutin.

Some of the phenolic compounds identified by us were also identified in different elderflower herb extract, but in different concentrations [86]. The difference in concentrations is due primarily to the way the samples were processed and secondly to the extraction method used.

Vanillic acid is a phenolic acid that has previously been attributed with antioxidant, anti-inflammatory, and neuroprotective features. It displays a variety of bioactivities that may be utilized to treat neurological, cardiovascular, and other chronic diseases [87].

Quercetin is considered beneficial against different types of cancers, including pancreatic cancer, osteosarcoma, breast cancer, cervical cancer, leukemia, colon cancer, gastrointestinal cancer, ovarian cancer, and oral cancer [88].

Using catechin and quercetin can reduce the amount of malondialdehyde which is the end product of lipid peroxidation during physical exercise and may create a protective effect against free radicals and increase the levels of antioxidant enzymes and strengthen the antioxidant defense systems of the cells and have a positive effect on exercise performance [89].

CONCLUSIONS

This study aimed to investigate the volatile chemical profile (VOC) from the aerial parts of three Romanian medicinal plants collected from Cluj county lemon balm (*Melissa officinalis*) (*Lavandula angustifolia*), and elderflower (*Sambucus nigra*). The studied plants were in fresh and dried form. The essential oils obtained by hydrodistillation from these plants were also studied.

HS-SPME is a simple and low-cost extraction technique which allows the obtaining of good results for the analysis of volatiles by GC-MS. In each plant we identified common compounds in varying proportions from the three samples (fresh, dry and essential oil) analysed.

For lemon balm and lavender, experimental data revealed the typical volatile constituent pattern for the *Lamiaceae* family: alpha-pinene, *Z*-beta-ocimene, *E*-beta-ocimene, beta-caryophyllene, beta-farnasene, humulene, etc.

In the elderflower plant, in the three samples, we observed a great variability for the classes of compounds obtained, such as monoterpenes, sesquiterpenes, esters, acids and hydrocarbons.

Specific markers for each type of plant indicate the originality/authenticity of floral. The profile of volatiles can be used as an indicator in the valorization of medicinal plants.

Majority phenolic compounds identified in the analysed samples were vanillic, sinapic, ferulic, and p-coumaric acids, while predominant flavonoids were rutin, quercetin and epicatechin.

The determination of volatile profile and polyphenol content from plant is important because it provides an assessment of their bioavailability according to their biological activity given by its majority compounds.

Regarding our three studied plants, under the three forms of presentation (fresh, dried and oil), the obtained results show that lemon balm is especially valued in dry form, lavender in all three forms, and elderberry in both fresh and dry form.

These researches will be useful for further studies in the formulation of new phytotherapeutic products, with applications in natural herb medicine.

EXPERIMENTAL SECTION

1. Plant material and chemicals

The fresh aerial part of *Melissa officinalis* (*Lamiaceae* family), *Lavandula angustifolia* (*Lamiaceae* family) and *Sambucus nigra* (*Adoxaceae* family) were collected during full flowering stage from Romanian flora (Cluj County), in June 2021. The aerial parts of these plants were submitted to research as follows: leaves for lemon balm, flowers and stems for lavender and flowers grouped in inflorescences for elderflower.

The analysis on fresh flowers was performed on the same day. The vegetal herbal material was air dried at room temperature in shade, in thin layers, in a well-ventilated place until they reached a constant weight (after 7 days). From 3.5 kg fresh plant 1 kg of dry matter was obtained.

GC-MS chemicals were purchased as follows: hexane 99% pure p.a from Chempur (Piekary Slaskie, Poland), methanol p.a from Penta (Prague, Czech Republic), alkane mixture containing C8-C20 alkanes (40 mg/mL in hexane) from Sigma Aldrich, St. Louis, MO, USA) and Helium 6.0 purity as carrier gas from Linde Gas (Romania).

HPLC chemicals: the standards of flavonoids (catechin, epicatechin, rutin, quercetin and luteolin), phenolic acids (gallic, vanillic, caffeic, p-coumaric, ferulic and sinapic) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2. Profile analysis of volatile organic compounds

2.1 Headspace solid-phase microextraction (HS-SPME)

A 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/ CAR/PDMS) fibre purchased from Supelco (Bellefonte, PA, USA) was chosen to extract the volatile compounds from all samples. For each extraction, the

SPME fibre was preconditioned in the injection port of the Agilent 7890 gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA, USA) at 220ºC for one hour.

Extraction of volatile organic compounds. In headspace vial, with a volume of 20 mL, 1 g of of aerial part of each plant (leaves for lemon balm, flowers and stems for lavender and flowers grouped in inflorescences for elderflower), fresh and dried and 8 mL distillated water together with 0.5 g of NaCl were placed. The sample bottle was preheated at 50ºC for 20 min. The fibre was then exposed to the sample headspace for 30 min prior to thermal desorption of the constituents at 240ºC into the splitless injection port of the GC-MS for 5 min.

2.2 Extraction of essential oil by hydrodistillation method (HD)

The aerial parts of the studied plants were dried in shadow at room temperature for one week, cut into pieces of size over the range 1–4 cm and grounded to a homogeneous powder. Extraction of es sential oils were carried out by hydrodistillation, using a Clevenger-type apparatus. Two distillations were carried out by boiling 100 g of dried material of each plant in 1 liter of distilled water during 3 hours. The yield of essential oils was determined in relation to the dry matter (1.1% w/w). The obtained essential oils were collected and dried over anhydrous $MgSO_4$, and stored in dark glass bottles at 4ºC prior to use.

2.3 GC-MS analysis

The quantification of volatile organic compounds was done using the GC-MS method presented in [90]. The volatile compounds were analysed using a GC-MS instrument, Model Agilent 7890 & 5975 Series MSD (Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with a HP-5MS (5% phenyl)-methyl polysiloxane fused silica column Agilent (30 m $\times$ 0.25 mm $\times$ 0.25 µM) (Agilent Technologies, Inc., Palo Alto, CA, USA) Volatile compounds adsorbed on the SPME fibre were immediately thermally desorbed in the injector port of the GC and then separated on the GC column. Each essential oil sample (0.1 g) was diluted in n-hexane (1 mL) and a volume of 1 µl was injected into the GC instrument. GC-MS data were obtained in splitless mode under the following conditions: helium (He 6.0) as a carrier gas, flow rate of 1 mL/min, and injector temperature of 260ºC. The temperature programme was: oven temperature was set as 40ºC for 1 min with an increase of 5ºC/min up to 200ºC and from 200ºC to 240ºC the increase was with 20ºC /min and then it was maintained at 240ºC for 5 min. Mass spectra conditions: electron impact (EI+) mode, 70 eV, and ion source temperature of 230ºC. Mass spectra were

recorded over 50–500 a.m.u. range in scan mode. All analyses were carried out in triplicate. Data acquisition and processing were performed using MSD ChemStation software (Agilent Technologies,Inc., Palo Alto, CA, USA). NIST library (Agilent Technologies, Inc., Palo Alto, CA, USA) was used for identification/confirmation of the structure of compounds. In addition, an alkane standard solution C8–C20 (Sigma Aldrich, St. Louis, MO, USA) was used as analytical standard in the measurement of retention indices for the identification of alkanes co-existing in essential oils for GC-MS analysis. Based on this, the calculation of the linear retention indices (LRIs) was made, as well as the comparison of the experimental values with those reported in literature for similar chromatographic columns, in the same conditions. For the compounds with retention time $t_R < 5.690$ and $t_R > 29.978$, LRI_s was reported from Nist Library Spectra. The quantitative analysis was based on the percent area of each peak of the sample compounds.

3. Determination of polyphenolic compounds

HPLC analysis

The analysis of phenolic compounds (flavonoids and phenolic acids) was carried out by high-performance liquid chromatography (HPLC) on a Jasco Chromatograph (Jasco Corporation, Tokyo, Japan) equipped with UV/VIS detector and an injection valve equipped with a 20 µL sample loop (Rheodyne). The ChromPass software (Jasco Corporation, Tokyo, Japan) was used to control the HPLC system and to collect and process the chromatographic data.

Determination of the individual flavonoids and phenolic acids respectively was carried out using the HPLC gradient analysis method described by Filip et al. in [91]. The polyphenolic compounds were extracted in 80% methanolic solution. At 1 g of plant sample grinded was added 5 mL of extraction solution and was well stirred. Then, the mixture was sonicated for 60 minutes, centrifuged at 4500 rpm for 20 min and the supernatant was filtered through a 0.45 µm syringe filter and injected into HPLC. Separation of these compounds was carried out on the Lichrosorb RP-C18 column (25 × 0.46 cm) (Merck, Germany) at 22ºC column temperature and UV detection at 270 nm. The mobile phase was a mixture of methanol (A, HPLC grade) and 0.1% formic acid solution (Millipore ultrapure water). For the elution of compounds, the following gradient was applied: 0–10 min, linear gradient 10–25% A; 10–25 min, linear gradient 25–30% A; 25–50 min, linear gradient 35–50% A; 50–70 min, isocratic 50% A. The flow rate was 1 mL/min.

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