The Essential Oil Composition of *Verbena officinalis* L. Herb from Different Origins

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Article

The Essential Oil Composition of *Verbena officinalis* L. Herb from Different Origins

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Abstract: The key objective of this study was to determine the yield and chemical composition of 9 samples of common vervain (*Verbena officinalis* L.) herb essential oil (EO), originating from 7 different countries and, consequently, to assess its potential for treating anxiety and depression. According to GC-MS analysis, 90 compounds were identified, 49 of which were discovered for the first time in *V. officinalis* EO. The plants with the highest oil content are from Greece (4.7 mL/kg) and South Carolina, USA (5.2 mL/kg). The chemical markers for the studied chemotypes of EO of *V. officinalis* are terpenoids *o*-cymene, *p*-cymene, L-carvone, thymol, carvacrol, *α*-curcumin, hexahydrofarnesyl acetone, phytol, *(E)*-β-ionone and phenylpropene anethole. The chemotype from UK demonstrated the greatest affinity to the continuum under study, it has the highest levels of similarity - 85.2% with the chemotype from Greece, 69.4% with the chemotype from the USA, 68.2% with the chemotype from Estonia (2), 58.7% with the chemotype from Germany and 58.6% with the chemotype from Hungary. Chemotypes identified that have the potential for use in the treatment of anxiety and depression.

Keywords: *Verbena officinalis*; essential oil; chemotypes

1. Introduction

Verbena officinalis L., common vervain or common verbena, is a perennial plant of the Verbenaceae J.St.-Hil. family that grows wild in Europe, where it particularly prefers the dry grasslands of Eastern, Central and Southern Europe. It is common to find the plant on roadsides and wastelands in these regions. The species has also spread from Europe to North Africa, Asia, and North America due to its use in rituals and traditional medicine, and it has become a weed following its cultivation [1]. The species is sold as an ornamental, but mainly for cultivation in herb gardens [2]. The Verbena flower is a beautiful plant with a healing effect that is also ideal for decorative purposes. Varieties of verbena differ primarily in the size and colour of the flowers. In the garden, verbena is often propagated by self-sowing.

Growing conditions and chemotype, as well as the time of collection, affect the amount of substances contained in the plant, therefore, common vervain has a high chemical variability depending on the origin. The extraction conditions are also important to obtain the necessary components from the plant. It has been found that aqueous extracts of common vervain are richer in phenolic compounds, flavonoids and phenolic acids than hydromethanolic extracts [3].

Common vervain mainly contains iridoids, phenylpropanoid glycosides, phenolic acids, flavonoids, terpenoids and essential oil [2]. The best-known and most characteristic iridoids in common vervain are the verbenaline and hastatoside glycosides. The plant also contains iridoid

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glycosides 3,4-dihydroverbenaline, 7-hydroxydehydrohastatoside, aucubin, and the secoiridoids verbeofflin I, verbenoside A, and verbenoside B [4–6].

The European Pharmacopoeia has had a monograph on common vervain since 2008. The raw material must be standardised according to the verbenaline content (minimum 1.5% of dry weight) [7] according to the pharmacopoeia requirements. It should be noted that in addition to the monograph *V. officinalis*, there is another monograph, "Leaf of lemon verbena, *Verbenae citriodorae folium*, *Verbena citriodora* (Palau) Cav." (*Aloysia citriodora* Palau, syn. *Aloysia triphylla* (L'Her.) Kuntze, *Verbena triphylla* L'Her., *Lippia citriodora* Kunth.]. The raw material is standardised according to the phenylethanoid, a derivative of tyrosol – acteoside (verbascoside) content: minimum 2.5% of dry weight, expressed as ferulic acid; – essential oil: minimum 3.0 mL/kg for the whole drug and minimum 2.0 mL/kg for the fragmented drug (dry weight) [7].

Of the phenylpropanoid glycosides, verbascoside, isoverbascoside and eukovoside are the most abundant in the plant, but isomers of leukoseptoside and cistanoside are also found [8]. Of the fatty acids, α-linolenic acid, palmitic acid, linoleic acid and oleic acid are the most abundant. Of the phenolic acids, the plant contains, among others, gallic acid, syringic acid, ferulic acid, cinnamic acid and protocatechuic acid, quinic, chlorogenic, rosmarinic acids, and dicaffeoylquinic acid derivatives have been identified [3,9].

Among the flavonoids in the herb *V. officinalis* apigenin, luteolin, 5,7,4'-trihydroxy-8-methoxyflavone, scutellarein, scutellarein 7-glucoside, scutellarein-7-diglucuronide, scutellarein-7-glucuronide, pedalitin, pedalitin-6-galactoside, quercetin, kaempferol, isorhamnetin, diosmetin, and rutin [3,10–12].

The composition of the essential oil (EO) of common vervain varies greatly, depending on the plant's location and the part of the plant used for distillation. Mono-, di-, tri- and sesquiterpenoids have been identified in common vervain. Monoterpenoids include citral, limonene, eucalyptol, menthol, α-pinene, β-pinene, sabinene, β-phellandrene, diterpenoids include carnosol and rosmanol. The most abundant sesquiterpenoids in the plant are caryophyllene oxide, α-curcumin, β-caryophyllene, hexahydrofarnesylacetone, and spathulenol [2,13–15], while the most abundant triterpenoids are squalene, ursolic, barbinervic, and oleanoic acids [3,16]. The sterols such as β–sitosterol, γ-sitosterol, daucosterol, stigmasterol, campesterol, androst-5,15-dien-3-ol-acetate were isolated from *V. officinalis* aerial part [10,14–16].

Verbena has been used extensively throughout history and was considered sacred by the Egyptians, Romans, Persians and Druids. Roman soldiers wore the plant for protection and used it in religious ceremonies. Verbena has been used for nervous system disorders such as stress, anxiety, depression and insomnia. It has been used to relieve headaches and premenstrual tension. It has also been used to treat cramps, jaundice and asthma, among other conditions [17,18].

Verbena herb infusion is often used externally - for gargling with tonsillitis and stomatitis, and lotions for skin diseases. Verbena is a component of many preparations and part of the complex herbal preparation Sinupret® of the company "Bionorika, SE". Sinupret® is an effective treatment of acute viral rhinosinusitis in children, and it accelerates the relief of the main symptoms [19].

On the Ukrainian pharmaceutical market, packaged verbena herb and food supplements "Verbena Drops" (LLC "Botanika", Phytobiotechnologies, NVO, LLC), which are recommended for use to lower cholesterol levels and improve capillary blood circulation, are sold through pharmacy chains [20].

The tumour cell growth inhibitory effect of an aqueous extract of common vervain has been studied in vitro on rat and human colon adenocarcinoma cell lines. Thus, polysaccharides of *V. officinalis* significantly inhibited the invasion and metastasis of colorectal cancer cells [21]. The cytotoxic activity of two new phenylethanoid glycosides isolated from the plant was close to that of vinblastine sulfate used in chemotherapy [22]. Semi-purified fractions isolated from methanolic extracts of common vervain have shown tumour cell growth inhibitory activity on various melanoma cell lines [23]. The cytotoxic effect of the plant has also been studied in animal studies in the treatment

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of hepatocellular carcinoma. Administration of the aqueous extract to experimental animals inhibited tumour size by 38.78% compared to the control group [24].

The antioxidant, anti-inflammatory and hepatoprotective activity of aqueous and hydroalcoholic extracts of the plant was also demonstrated in another study [5,8,25,26]. In addition to the antioxidant activity, the antimicrobial and antifungal activity of the leaf extracts of common vervain was also investigated. The fraction containing caffeoyl derivatives showed the highest antifungal activity against *P. expansum* and *R. stolonifer* [27].

A study of EO's antioxidant and antibacterial effects on *S. aureus* and *E. coli* found that while gram-negative bacteria usually show lower sensitivity to EOs, *E. coli* was more sensitive to yarrow EO than gram-positive *S. aureus*. Increasing the concentration of the EO showed an increase in antioxidant activity [28]. Silver nanoparticles prepared from the extract of the leaves of the common vervain showed antibacterial activity against both gram-positive and gram-negative bacteria [29].

Common vervain has also been studied in the treatment of hyperlipidemia. The crude extract of the plant reduces total cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins in vivo. A similar result was also shown by the administration of atorvastatin, which was used as a comparator [30]. Evidence suggests that *V. officinalis* regulates lipid metabolism. Biologically active components of *V. officinalis* herb, such as quercetin, luteolin, and kaempferol, have been considered key ingredients for the treatment of atherosclerosis [10].

An animal study has also investigated the effect of the plant's aqueous extract on physical stress. The study showed, among other things, antioxidant changes in red blood cell membranes and a significant effect against physical stress. Common vervain extract is seen as a potential ingredient in sports supplements to accelerate post-exercise recovery [31].

In addition to what has already been mentioned, several studies have been conducted on the effects of vervain on anxiety, depression, and insomnia [32–34].

The research aimed to analyse the chemical composition of verbena essential oil (EO) and, consequently, to assess its potential for treating anxiety and depression. To the best of our knowledge, this is the first systematic study of *V. officinalis* EO chemotypes.

2. Materials and Methods

2.1. Plant Material

Nine different dried herbs of common vervain originating from seven different countries (Table 1) were used in the study. Eight of them are commercial herbs ordered online. The herb *V. officinalis* (sample Ukraine 1) was collected in the wild in the village of Isakiv, Ivano-Frankivsk region, Ukraine (coordinates 40°44′382″ N, 33°06′099″ E), accounted for and stored at the Department of Pharmaceutical Management, Drug Technology and Pharmacognosy, Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine (vouchers no. 487-489). The studied herb samples mostly had a rich stem composition and brownishgreen colour. The exception was the samples of German origin, consisting mainly of green leaves, and sample Ukraine 1, containing, in addition to leaves, rather large fragments of stems.

Table 1. Plant material of *Verbena officinalis* herb and content of EO.

Country of origin	Company	Webpage	Yield of EO, mL/kg
Estonia 1	Kubja Herbal Farm (2023)	https://kubja.ee/	1.51
Estonia 2	Kubja Herbal Farm (2024)	https://kubja.ee/	1.85
UK	Clinic Naturae	https://clinicnaturae.com/	1.23
Greece	You Herb It	https://www.youherbit.com/	4.68

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USA, South Carolina	Trifecta Botanicals	https://www.trifectabotanicals.com/	5.15
Germany	Greek Herbay	https://greekherbay.com/	3.69
Hungary	Herba Peru - Luci Vita	https://herbaperu.eu/	0.32
Ukraine 1	Collected from nature	Collected from nature	1.21
Ukraine 2	PhytoBioTechnologies	https://www.goldenfarm.com.ua/en/fitobiotehnologii-ukraina/	0.31

Note. “nd” – not detected.

2.2. Hydrodistillation of Essential Oil

The EO hydrodistilled from the dried herbs of *V. officinalis* using the method described in the European Pharmacopoeia [7]. The plant materials (35 g) with 300 mL of purified water were hydrodistilled in a 1000 mL round-bottom flask for 3 hours (2–3 ml/min). Hexane (0.5 mL) was added to a graduated tube to remove the distilled oil.

2.3. Gas Chromatography/Mass Spectrometry

The samples of EO were analysed on Agilent 6890/5973 GCMS system run by MSD Chemstation. 1 µL of the sample was introduced into Agilent HP-5MSUI column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) using split mode (20:1). The injector temperature was 280 °C and carrier gas (He) flow was kept constant 1 mL/min throughout whole analysis. The oven was held at 50 °C for 2 min, followed by a ramp of 4 °C/min to a final temperature of 280 °C and was kept there for 5 minutes.

The MSD was operated in EI mode at 70 eV, scanning across the mass range of 29 – 400 m/z with a delay time of 4 min and a scan speed of 3.8 scans per second. The data were analyzed by Agilent Masshunter Software package applying deconvolution algorithm at different window size factors. Resulted compounds were identified by using NIST23 library with Match Factor ≥ 90 and by retention indexes (relative to n-alkanes C8 – C30) or obtained by the analysis of the reference compounds. The area percentages of each peak were calculated from the total areas in the chromatograms without using correction factors [35,36].

3. Results

The component composition of EOs of *V. officinalis* varies depending on the place of plant growth and soil and climatic conditions. Plants from different growing areas all belonged to different chemotypes, i.e., their highest content of components was different (Table 2). The same can be said about the overall composition, which varied greatly.

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Table 2. Composition (>0.01%) of essential oils in *Verbena officinalis* herbs from different countries.

Compound	RI	Content in essential oil, %										Mentioned in previous studies
		Library RI	Estonia 1	Estonia 2	UK	Greece	USA	Germany	Hungary	Ukraine 1	Ukraine 2	
Hexanal	800	801	0.10	0.09	0.53	0.02	0.22	0.02	0.16	0.03	0.09	
1-Hexanol	865	868	0.11	0.08	0.01	0.04	0.01	0.00	0.21	0.00	0.01	
p-Xylene	866	865	1.81	0.01	0.15	0.05	0.03	0.00	0.03	0.20	0.17	
α-Pinene	932	932	0.18	0.53	0.05	0.02	0.16	0.57	0.45	0.20	0.71	[13,37,39]
(E)-2-Heptenal	955	958	0.02	0.01	0.16	0.09	0.02	0.01	0.04	0.04	0.01	
Benzaldehyde	958	962	0.33	0.27	0.26	0.13	0.13	0.03	0.29	0.05	0.14	[15]
α-Sabinene	973	974	0.06	0.05	0.01	0.02	0.03	1.01	0.11	0.10	0.10	[13,39]
1-Octen-3-ol	978	980	1.17	1.15	1.02	2.49	0.56	1.25	7.76	2.29	6.04	[38]
6-Methyl-5-hepten-2-one	987	986	0.19	0.14	0.11	0.04	0.06	0.84	0.16	0.00	0.00	[28]
β-Myrcene	991	991	0.08	0.13	0.02	0.00	0.07	0.13	0.13	0.32	0.95	
2-Pentyl-furan	991	993	0.13	0.31	1.16	0.12	0.24	0.10	0.26	0.00	0.18	
(Z)-2-(2-Pentenyl)furan	1002	1002	0.47	0.00	0.00	0.04	0.02	0.00	0.11	0.28	0.84	
(E,E)-2,4-Heptadienal	1010	1012	0.15	0.09	0.34	0.16	0.17	2.54	0.36	0.00	0.16	
o-Cymene	1024	1022	0.37	0.55	0.21	0.09	5.75	3.03	0.38	0.04	0.14	[13,39]
p-Cymene	1024	1025	0.37	0.55	0.21	0.09	5.75	3.03	0.38	0.04	0.14	[37]
D-Limonene	1028	1031	1.66	1.41	0.32	0.15	0.32	5.43	0.42	1.05	3.22	[28,37–39]
Eucalyptol	1030	1032	0.24	0.22	0.03	0.06	0.45	1.62	0.49	0.04	0.05	[28,37–39]
Benzeneacetaldehyde	1043	1045	0.26	0.17	0.36	0.55	0.24	0.00	0.83	0.18	0.55	[38]
(E)-2-Octenal	1057	1060	0.03	0.04	0.16	0.03	0.05	0.01	0.08	0.01	0.03	
γ-Terpinene	1059	1060	0.05	0.05	0.06	0.03	0.39	0.41	0.07	0.00	0.02	[13,37,39]
Artemisia ketone	1058	1062	0.03	0.02	0.02	0.00	0.04	0.04	0.06	0.00	0.00	
1-Octanol	1070	1070	0.07	0.07	0.10	0.10	0.16	0.05	0.14	0.02	0.02	
Linalool	1100	1099	2.28	1.76	1.04	2.02	0.77	0.89	2.09	0.10	0.23	[28,38,39]
Nonanal	1104	1104	0.08	0.16	0.24	0.10	0.23	0.08	0.48	0.09	0.20	
α-Thujone	1105	1103	0.27	0.16	0.26	0.21	0.11	0.78	0.38	0.00	0.03	[38]
β-Thujone	1105	1114	0.07	0.05	0.04	0.11	0.02	0.06	0.07	0.00	0.00	[38]
Camphor	1145	1145	1.76	1.08	0.23	1.10	0.11	0.13	0.91	0.09	0.02	[38]
L-Menthone	1154	1164	4.80	3.88	0.64	0.60	0.08	0.11	0.66	0.00	0.02	[38]
DL-Menthol	1172	1173	3.29	3.42	0.45	1.00	0.54	0.05	0.73	0.01	0.05	[15,38]
Terpinen-4-ol	1178	1177	0.75	0.55	0.34	0.51	0.15	0.67	0.30	0.01	0.04	[13,37–39]
Acetophenone	1184	1183	0.11	0.08	0.08	0.12	0.07	0.41	0.11	0.01	0.03	
α-Terpineol	1191	1189	0.66	0.53	0.30	0.64	0.26	1.03	0.70	0.01	0.06	[13,38,39]
Methyl salicylate	1194	1192	0.12	0.10	0.26	0.09	1.15	0.03	0.08	0.01	0.06	
(E)-Dihydrocarvone	1197	1201	0.44	0.29	0.04	0.17	0.02	0.13	0.20	0.00	0.01	
Estragole	**1199**	**1196**	**8.17**	**6.53**	**0.52**	**0.87**	**0.25**	**0.23**	**0.53**	**0.00**	**0.01**	**[38]**
Decanal	1206	1206	0.10	0.08	0.08	0.07	0.09	0.05	0.13	0.05	0.06	
β-Citronellol	1228	1220	0.18	0.23	0.15	0.44	0.07	0.49	0.37	0.01	0.04	
Anisole	1236	1235	0.09	0.09	0.03	0.04	0.78	0.02	0.08	0.00	0.01	
Pulegone	1240	1237	2.31	1.51	0.43	0.53	0.25	0.24	0.74	0.00	0.06	
L-Carvone	**1245**	**1245**	**20.36**	**16.27**	**3.77**	**3.04**	**0.36**	**2.87**	**5.82**	**0.05**	**0.15**	**[38]**
Piperitone	1255	1253	1.31	0.89	0.46	0.36	0.10	0.92	4.98	0.00	0.00	[37]
(E)-2-Decenal	1262	1263	0.15	0.03	0.44	0.03	0.04	0.06	0.16	0.15	0.02	
(E)-Cinnamaldehyde	1270	1270	0.55	0.43	1.19	0.11	0.09	0.09	0.32	0.00	0.00	
(E)-Citral	1272	1270	0.25	0.31	0.00	0.33	0.20	3.33	0.21	0.00	0.00	[13,37,39]

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Anethole	**1287**	**1287**	**15.38**	**20.48**	**25.64**	**6.41**	**12.64**	**6.48**	**6.80**	**0.02**	**0.05**	**[13,38,39]**
L-Bornyl acetate	**1288**	**1285**	**0.08**	**0.14**	**0.11**	**0.14**	**0.04**	**0.22**	**0.17**	**7.43**	**15.86**	**[39]**
Thymol	**1292**	**1291**	**2.69**	**3.39**	**2.41**	**2.13**	**6.44**	**1.38**	**1.20**	**0.00**	**0.10**	**[28,37]**
Menthyl acetate	1295	1295	0.22	0.28	0.08	0.15	0.03	0.00	0.07	0.00	0.00	
Carvacrol	**1302**	**1299**	**5.16**	**4.64**	**22.98**	**18.49**	**7.39**	**11.51**	**3.16**	**0.07**	**0.10**	**[37]**
(E,E)-2,4-Decadienal	1317	1317	0.10	0.16	1.22	0.29	0.52	0.04	0.60	0.06	0.12	
α-Terpinyl acetate	1351	1350	0.21	0.31	0.47	0.52	0.02	0.41	0.36	0.00	0.00	
Eugenol	1359	1357	0.97	0.78	0.11	0.62	2.52	1.81	0.12	0.00	0.01	
n-Capric acid	1369	1373	0.09	0.11	0.32	0.28	0.39	0.00	0.05	0.01	0.03	
Copaene	1378	1376	0.09	0.26	0.05	0.05	1.02	1.34	0.12	0.20	0.00	[13,37,39]
L-β-Bourbonene	1387	1384	0.03	0.12	0.01	0.01	0.08	2.43	0.16	0.63	2.15	
Methyleugenol	1406	1402	0.18	0.17	0.12	1.45	0.22	0.77	0.26	0.00	0.02	
Caryophyllene	1423	1419	0.26	0.82	0.06	0.12	2.62	0.85	0.46	1.61	2.74	[28,39]
(Z)-β-Copaene	1438	1432	0.07	0.19	0.01	0.01	0.09	0.50	0.00	3.01	4.03	
(E)-Geranylacetone	1454	1453	0.43	0.66	1.10	0.78	1.12	0.41	2.34	0.41	0.76	
Humulene	**1457**	**1454**	**0.28**	**1.00**	**0.00**	**0.01**	**5.59**	**0.55**	**0.41**	**2.31**	**5.00**	**[39]**
γ-Muurolene	1479	1477	0.09	0.28	0.03	0.02	0.05	0.50	0.09	48.82	0.00	[39]
***α*-Curcumene**	**1485**	**1483**	**0.72**	**2.13**	**0.18**	**0.27**	**0.28**	**8.04**	**1.52**	**14.78**	**16.76**	**[28,37]**
***(E)*-β-Ionone**	**1488**	**1486**	**0.73**	**0.82**	**2.16**	**2.51**	**1.48**	**7.54**	**2.35**	**1.81**	**5.41**	
Bicyclogermacren	1500	1496	0.04	0.09	0.01	0.00	0.23	0.45	0.11	0.54	2.23	[13,37,39]
β-Bisabolene	1511	1509	0.16	0.56	0.11	0.06	0.10	0.11	0.62	0.00	0.00	[28]
γ-Cadinene	1517	1513	0.09	0.31	0.04	0.09	0.14	1.90	0.16	0.23	3.52	[2]
Myristicin	1524	1519	0.64	0.84	0.37	0.30	0.52	0.16	1.59	0.00	0.00	
δ-Cadinene	1526	1524	0.26	0.69	0.17	0.26	0.36	0.96	0.64	0.08	0.13	[37]
D-Spathulenol	1581	1576	0.10	0.15	0.10	0.17	0.36	3.32	0.46	0.09	0.35	[37]
Caryophyllene oxide	1587	1581	0.16	0.25	0.14	0.36	1.04	3.92	0.59	0.20	0.26	[28,37]
Cedrol	1605	1599	0.02	0.05	0.06	0.02	0.04	0.20	0.00	0.00	0.00	
α-Humulene epoxide II	1613	1606	0.06	0.12	0.06	0.12	0.54	0.68	0.11	0.00	0.20	
β-Asarone	1624	1626	0.03	0.03	0.00	0.11	2.24	3.21	0.04	0.00	0.00	
Benzophenone	1629	1635	0.01	0.01	0.04	0.05	0.49	0.14	0.05	0.00	0.03	[15]
Selin-11-en-4-*α*-ol	1658	1653	0.09	0.16	0.07	0.09	0.07	0.11	0.17	0.40	0.79	
ar-Turmerone	1668	1664	0.02	0.04	0.15	1.08	1.34	0.12	0.26	0.00	0.00	
Asarone	1683	1678	0.04	0.03	0.03	0.02	3.60	0.04	0.79	0.00	0.00	
Apiol	1685	1682	0.27	0.30	0.51	0.00	0.20	1.72	0.00	0.00	0.08	
ent-Germacra-4(15),5,10(14)-trien-1β-ol	1690	1690	0.05	0.08	0.04	0.09	0.08	0.09	0.10	0.16	0.61	
Acorenone B	1693	1701	0.13	0.30	0.27	0.05	0.36	0.27	0.01	0.10	0.40	
Myristic acid	1765	1768	0.04	0.17	2.12	1.05	0.61	0.00	0.01	0.00	0.00	
Phenanthrene	1776	1776	0.04	0.05	0.28	0.62	0.11	0.06	0.39	1.48	2.55	
Hexahydrofarnesyl acetone	**1846**	**1844**	**1.18**	**2.60**	**8.99**	**3.89**	**1.56**	**0.47**	**4.79**	**4.35**	**5.96**	**[15]**
Phthalic acid	1870	1869	0.10	0.19	6.03	0.66	0.70	0.15	0.62	0.63	1.05	
Farnesyl acetone	1920	1918	0.15	0.31	0.65	0.43	0.36	0.55	0.81	0.15	0.27	
Methyl palmitate	1927	1926	0.23	0.16	1.95	0.13	0.23	0.04	1.21	0.37	0.64	
Dibutyl phthalate	1964	1965	0.10	0.00	0.22	0.42	0.67	0.19	1.37	1.58	3.45	
Palmitic acid	**1977**	**1968**	**8.78**	**7.17**	**0.11**	**35.02**	**13.49**	**0.00**	**20.00**	**0.00**	**2.95**	**[38]**
Methyl linolenate	2097	2099	0.30	0.14	0.72	0.16	0.17	0.02	1.79	0.54	0.93	
Phytol	**2109**	**2114**	**0.22**	**0.42**	**0.46**	**0.52**	**0.55**	**2.01**	**7.01**	**1.60**	**4.23**	**[38]**
Hexacosane	2594	2600	0.13	0.22	0.22	0.97	0.24	0.14	1.46	0.78	1.28	

Bold – not less than 5%.

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4. Discussion

The EO content of plants from different countries varies significantly (Table 1). The plants with the highest EO content are from Greece (4.68 ml/kg) and South Carolina, USA (5.15 ml/kg). The reason for the abundance of EO probably lies in the place where the plants grow, as both places (Mediterranean and humid subtropical climates) are quite warm and sunny. A sunny place promotes increased EO production [40]. A fairly high content of EO in the plant (3.9 mL/kg) was also identified in a study conducted in Italy [13], which again confirms the assumption that plants grown in sunny climates produce more EO. For comparison, a plant grown in Estonia contained 1.51 mL/kg of EO, or more than half as much as foreign analogues. The raw material sample from Germany is the third in terms of EO content. The plant collected from the wild in Ukraine had a lot of leaves, but also many coarse stem fragments. The EO content also reflected the general abundance of stems and the low odour of the drug. The lower EO content in the remaining plants may result from climatic and general growing conditions and the low proportion of leaves in the studied material [41].

The diversity of the component composition of EOs of the same plant species *V. officinali,s* is influenced by a large number of environmental parameters - geophysical, geochemical, biological, anthropogenic, climatic and other factors, therefore, multiple correlations of the chemical composition of EOs of objects made it possible to establish the degree of their relationship, as well as to determine the main chemotypes of *V. officinalis* inherent in European and American samples. The content of different groups of compounds in the studied samples of EO differs significantly. Comparable concentrations of terpenoids (52.46-59.0%), aromatic (26.65-36.2%) and aliphatic (10.38-17.52%) compounds were found in samples from Estonia 1, Estonia 2, UK and the USA (Figure 1). The highest content of terpenoids is found in wild raw materials (Ukraine 1 - 91.12%), industrial samples of raw materials from Ukraine 2 (78.0%) and Germany (79.19%). The highest content of aromatic compounds was found in the EO of the verbena herb from UK, Estonia 1 and Estonia 2. Samples of raw materials from Greece and Hungary are distinguished by the highest content of aliphatic compounds (41.23% and 35.17%).

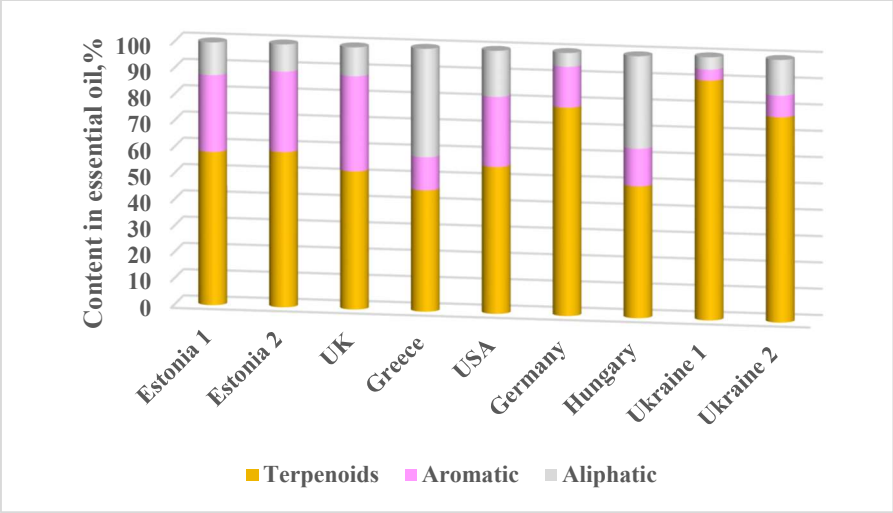

Figure 1. Content of BAS groups in essential oils of *V. officinalis* herbs from different countries.

The terpenoid composition of the EO of the studied samples of raw materials also has significant differences (Figure 2). Despite the comparable values of the total content of terpenoids in the samples of Ukraine 1 and 2 and Germany, sesquiterpenoids prevail in the Ukrainian samples. In contrast, in the sample from Germany, the dominant group is monoterpenoids. In all studied samples, except for Ukraine 1 and Ukraine 2, the dominant group of terpenoids is monoterpenoids with a content of 29.15% (Hungary) to 53.25% (Estonia 1). In the oil from wild raw materials (Ukraine 1), the maximum content of sesquiterpenoids (77.66%) is noted; in the sample of industrial raw materials Ukraine 2 -

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45.40%, while in the samples from Estonia 1, Estonia 2 and Greece contain 4.05%, 10.51% and 7.20% respectively.

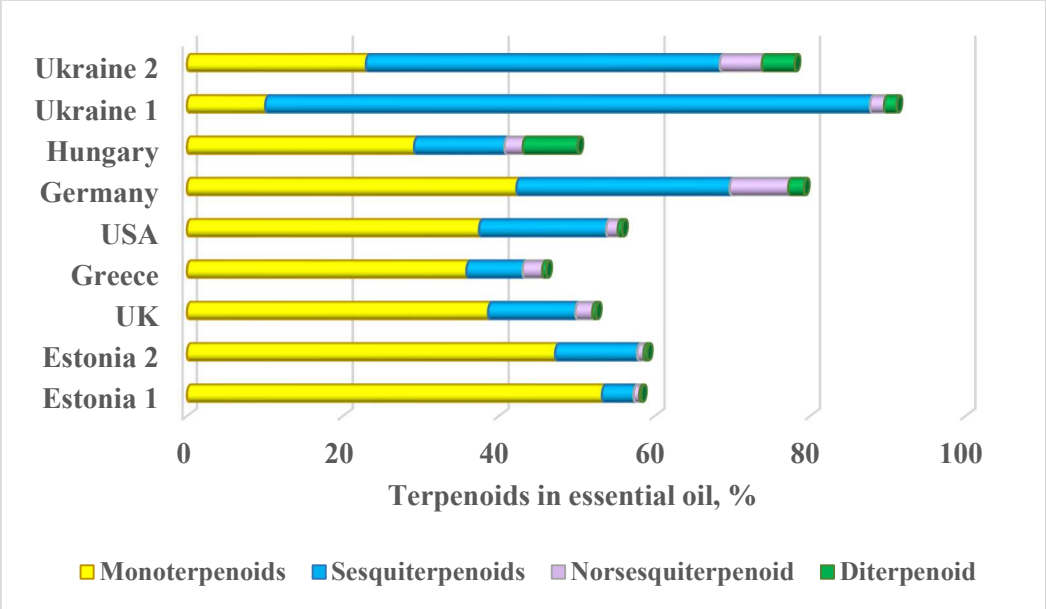

Figure 2. Content groups of terpenoids in essential oils of *V. officinalis* herbs from different countries.

The highest content of components in plants from Estonia was L-carvone (20.36, 16.27%), anethole (15.38, 20.48%) and palmitic acid (8.78, 7.17%). Carvone and anethole have shown some antibacterial activity [41]. Carvone is effective in the treatment of skin and breast cancer [42,43]. Anethole is a potential compound for the treatment of several chronic diseases, such as inflammation, type 2 diabetes, and neurodegenerative diseases [44]. Antidepressant effects have been observed in animals with both anethole [45,46] and carvone [47]. Both compounds have also shown anti-anxiety effects [48,49].

The characteristic components of the plant from the USA were palmitic acid (13.49%), anethole (12.64%), carvacrol (7.39%), isothymol (6.44%), thymol (6.44%), humulene (5.59%) and asarone (3.60%). Carvacrol possesses a wide range of bioactivities that are useful for clinical applications such as antimicrobial, antioxidant, and anticancer [50,51]. Therapeutic properties of carvacrol have been demonstrated as diabetes prevention, cardioprotective, anti-obesity, hepatoprotective and reproductive role, antiaging, and immunomodulatory properties [52,53]. The compound has also been effective in animal studies in alleviating anxiety and depression [54].

The chemotype of the plant from the UK was determined by anethole (25.64%), carvacrol (22.98%), hexahydrofarnesyl acetone (8.99%) and phthalic acid (6.03%). Hexahydrofarnesyl has been identified as having antibacterial and antifungal effects [55].

The EO of the plant, which grows in Greece, contains a large amount of palmitic acid (35.02%), carvacrol (18.49%), anethole (6.41%), a small amount of hexahydrofarnesyl acetone (3.89%) and carvone (3.04%). The composition of the most similar sample from Greece was the Polish plant, which contained palmitic acid (>20%), linalool (>8%), anethole (>5%) and carvone (>3%) as the main components [38].

The main components of the EO of the plant from Hungary were palmitic acid (20.00%), 1-octen-3-ol (7.76%), phytol (7.01%), anethole (6.8%), L-carvone (5.82%), piperitone (4.98%), and hexahydrofarnesyl acetone (4.79%). Phytol has the potential to treat anxiety, depression and insomnia [56]. In an animal study, -octen-3-ol exerts toxicity via disruption of dopamine homeostasis and may represent a naturally occurring environmental agent involved in parkinsonism. Moreover, it provides possible insights into reported movement disorders associated with human exposure to fungi and their volatile organic compounds [57].

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The EO of the plant from Germany was characterised by the content of carvacrol (11.51%), α-curcumene (8.04%), (E)-β-ionone (7.54%), anethole (6.48%) and D-limonene (5.43%). α-Curcumene has shown potential as an inhibitor of cancer cell growth [58].

Plants from Ukraine also differed significantly from each other. The EO of the Ukrainian industrial variety Ukraine 2 contained α-curcumene (16.76%), L-bornyl acetate (15.86%), 1-octen-3-ol (6.04%), hexahydrofarnesyl acetone (5.96%) and (E)-β-ionone (5.41%). L-Bornyl acetate has promising pharmacological properties, especially anti-inflammatory and immunomodulatory effects [59].

On the other hand, the EO of the plant collected in the wild Ukraine 1 contained the naphthalene series sesquiterpene γ-muurolene (48.82%) as the main component, which was found in other samples in insignificant quantities (from 0.02% to 0.5%) and was absent in the industrial sample from Ukraine. The dominant substances were also α-curcumene (14.78%) and L-bornyl acetate (7.43%). It has been suggested that γ-muurolene may be responsible for antimicrobial activity against *Bacillus subtilis* and *Candida tropicalis* (including clinical strains) of *Piper ovatum* Vahl EO [60]. Previously published data indicate a significant content of α-curcumene (6.00%) in the EO from the leaves of Moroccan plants [37].

Based on literary data, the EO of the plant grown in Italy also had a completely different composition, with citral (45.5%) and isobornyl formate (44.4%) as the main components [39]. The EO of a plant from Iran contained 4-(1-methylethyl)-benzyl alcohol safranal (53.8%), eucalyptol (7.44%) and thymol (7.30%) as the main components [28].

The chemical markers for the studied chemotypes of *V. officinalis* EOs are the terpenoids *o*-cymene, *p*-cymene, L-carvone, thymol, carvacrol, α-curcumin, hexahydrofarnesylacetone, phytol, (E)-β-ionone and phenylpropene anethole.

Correlation links of EOs showed a high degree of affinity (similarity) of chemotypes (Table 3).

The location of the EO samples in the continuum under study is presented in descending order of their similarity to the continuum: UK > Estonia 2 > Greece > Hungary > Germany > Estonia 1 >USA > Ukraine 2 >Ukraine 1.

The chemotype from UK demonstrated the greatest affinity to the continuum under study, it has the highest levels of similarity - 85.17% with the chemotype from Greece, 69.42% with the chemotype from the USA, 68.24% with the chemotype from Estonia (2), 58.67% with the chemotype from Germany and 58.60% with the chemotype from Hungary.

Table 3. Interrelations of essential oil samples of *V. officinalis* based on the correlation matrix of component composition.

Country	Estonia 1	Estonia 2	UK	Greece	USA	Germany	Hungary	Ukraine 1	Ukraine 2
	Pairwise similarity coefficient, %								
Estonia 1	100,00	95,93	55,95	43,16	39,46	31,28	60,87	-6,39	-9,52
Estonia 2	95,93	100,00	68,24	45,83	54,56	37,31	65,47	-3,67	-3,87
UK	55,95	68,24	100,00	85,17	69,42	58,67	58,60	-3,37	-2,09
Greece	43,16	45,83	85,17	100,00	54,33	65,39	46,28	-4,40	-2,09
USA	39,46	54,56	69,42	54,33	100,00	48,22	37,94	-7,31	-5,60
Germany	31,28	37,31	58,67	65,39	48,22	100,00	37,93	6,07	27,92
Hungary	60,87	65,47	58,60	46,28	37,94	37,93	100,00	-1,93	12,69
Ukraine 1	-6,39	-3,67	-3,37	-4,40	-7,31	6,07	-1,93	100,00	26,98
Ukraine 2	-9,52	-3,87	-2,09	-2,94	-5,60	27,92	12,69	26,98	100,00

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Chemotypes Estonia 1 and Estonia 2, which have an affinity of 95.93% between themselves, consistently demonstrate medium (from 30% to 50%) and high affinity (more than 50%) to most samples and no affinity to samples from Ukraine.

For two chemotypes, Ukraine 1 and Ukraine 2, no affinity was noted with most samples, except for a weak level of affinity for the sample from Germany at 6.07% and 27.92%, respectively, and for Ukraine 2, a weak affinity was noted for the sample from Hungary at 12.69%.

The degree of relatedness of the studied essential oils of *V. officinalis* from different countries is presented in Figure 3.

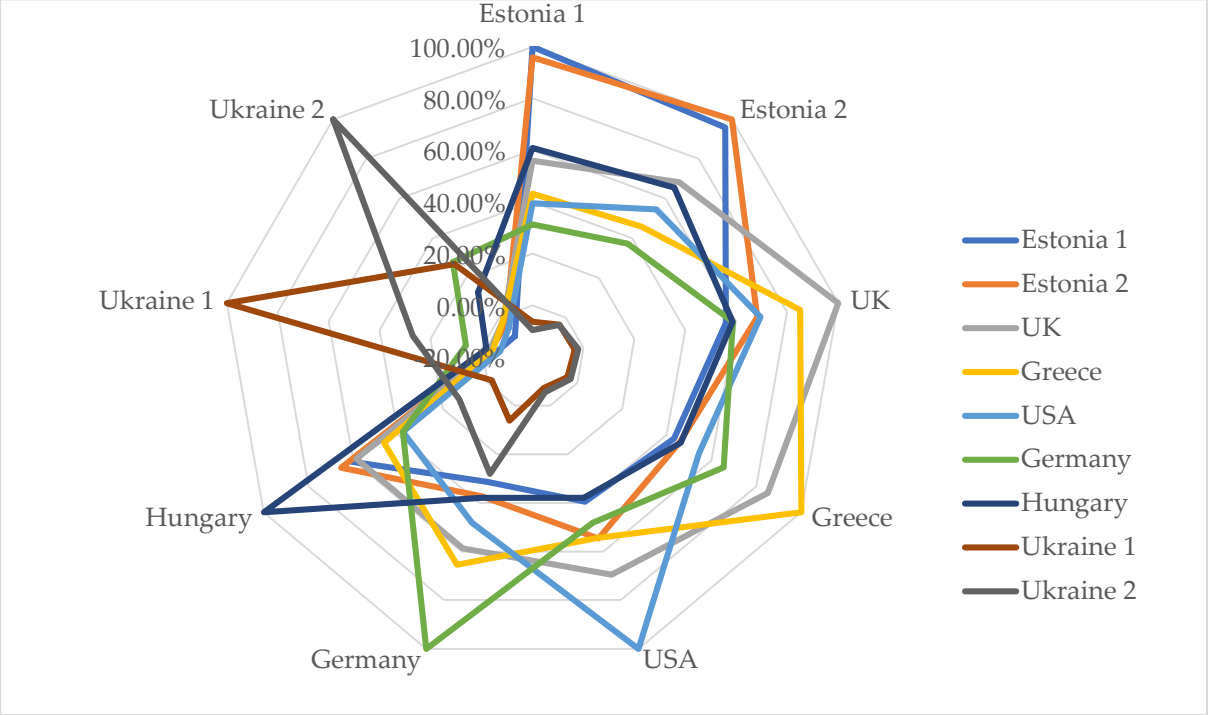

Figure 3. The degree of affinity of the studied essential oils of *V. officinalis*, %.

The quantitative content and composition of the EO of *V. officinalis* vary significantly depending on the place of growth of the plant. Based on 72 analyzed components of the EO: terpenoids and phenolic compounds, several chemotypes of verbena officinalis were identified.

Chemotype (Ct) 1, Estonian (samples Estonia 1 and Estonia 2): carvone-anethole-DL-menthol-L-menthone-estragole-carvacrol.

Ct 2, UK: anethole-carvacrol-hexahydrofarnesyl acetone-phthalic acid.

Ct 3, Greece: carvacrol-anethole.

Ct 4, German: carvacrol-anethole-α-curcumene-(E)-β-ionone-D-limonene-*o*-cymene-*p*-cymene.

Ct 5, Hungary: anethole-L-carvone-piperitone-carvacrol-hexahydrofarnesyl acetone-phytol.

Ct 6, USA: anethole-carvacrol-*o*-cymene-*p*-cymene-humulene-asarone.

Ct 7, Ukraine 1: γ-muurolene-α-curcumene-L-bornyl acetate.

Ct 8, Ukraine 2: L-bornyl acetate-α-curcumene-(E)-β-ionone-hexahydrofarnesyl acetone-phytol.

Based on the above, it can be concluded that among the EOs of *V. officinalis* studied, oils of chemotypes 1–5 of European origin and 6 of American origin have the potential for use in the treatment of anxiety and depression.

New compounds not found before in *V. officinalis* EO [2,13,15,28,37–39]: hexanal, 1-hexanol, *p*-xylene, *(E)*-2-heptenal, *β*-myrcene, 2-pentyl-furan, *(Z)*-2-(2-pentenyl)furan, *(E,E)*-2,4-heptadienal, *(E)*-2-octenal, artemisia ketone, 1-octanol, nonanal, acetophenone, methyl salicylate, *(E)*-dihydrocarvone, decanal, *β*-citronellol, anisole, pulegone, *(E)*-2-decenal, *(E)*-cinnamaldehyde, menthyl acetate, *(E,E)*-2,4-decadienal, *α*-terpinyl acetate, eugenol, *n*-capric acid, L-β-bourbonene, methyleugenol, *(Z)*-β-

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copaene, *(E)*-geranylacetone, *(E)*-β-ionone, myristicin, cedrol, *α*-humulene epoxide II, *β*-asarone, selin-11-en-4-*α*-ol, ar-turmerone, asarone, apiol, ent-germacra-4(15),5,10(14)-trien-1β-ol, acorenone B, myristic acid, phenanthrene, phthalic acid, farnesyl acetone, methyl palmitate, dibutyl phthalate, methyl linolenate and hexacosane.

5. Conclusions

The quantitative content and composition of the essential oil of *V. officinalis* varies considerably depending on the place of growth and the chemotype of the plant. Based on 72 terpenoids and aromatic compounds analysed in the EO, 8 chemotypes of *V. officinalis* were identified. The chemotype from the UK showed the highest similarity to the continuum under study, in particular with chemotypes from Greece 85.17%, from the USA 69.42%, from Estonia (2) 68.24%, from Germany 58.67%, and from Hungary 58.60%. 49 compounds not previously mentioned in the scientific literature were identified in EOs as new for the species *V. officinalis*.

Author Contributions: Conceptualization, A.R., T.I., A.K. and O.K.; methodology, A.R., M.L., T.I., A.K., and O.K.; software, T.I., A.K., and A.R.; validation, A.R., M.L., T.I., A.G. and A.K.; formal analysis, A.R., M.L., G.D. and O.K.; investigation, A.R., M.L., G.D. and O.K.; resources, A.R., A.G. and O.K.; data curation, A.R., M.L., G.D. and O.K.; writing—original draft preparation, A.R., T.I., A.K. and O.K.; writing—review and editing, A.R., A.G., M.L., T.I., A.K. and O.K.; visualization, T.I. and A.K.; supervision, A.R.; project administration, A.R.; funding acquisition, A.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data supporting the results of this study can be obtained from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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