Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2024.052790

ARTICLE

Variation in the Composition of the Essential Oil of Commercial *Salvia officinalis* L. Leaves Samples from Different Countries

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Received: 15 April 2024 Accepted: 19 July 2024 Published: 30 August 2024

ABSTRACT

Salvia officinalis L. (Lamiaceae) leaves and its essential oil is used for mouth and throat disorders, skin disorders, minor wounds, and gastrointestinal disorders, and is widely used worldwide. The research aimed to conduct a comparative study of the composition of *S. officinalis* essential oils from commercial samples, and their main chemotypes. The volatile constituents from *S. officinalis* leaves were investigated using gas chromatography (GC). The commercial samples of sage leaves were obtained from retail pharmacies in nine mainly European countries. The yield of essential oil in *S. officinalis* commercial leaves was between 10.0 and 24.8 mL/kg. The principal components (>5%) among the main identified 25 compounds were 1,8-cineole (8.3%–45.3%), α-thujone (3.0%–34.0%), camphor (11.3%–29.3%), β-thujone (1.5%–12.9%), viridiflorol (1.1%–10.4%), camphene (2.6%–7.1%), and α-pinene (1.3%–5.8%). In seven (Estonia, England, France, Hungary, Belgium, Ukraine, Georgia) samples α-thujone dominated. Four samples (Estonia, Georgia, England, Hungary) belong to the most common chemotype α-thujone > camphor > 1,8-cineole. Eight chemotypes of *S. officinalis* essential oils have been found. Toxic thujones are widespread compounds among them.

KEYWORDS

Sage; terpenoids; chemotypes; thujone; toxicity

1 Introduction

The genus *Salvia* is the largest genus in the Lamiaceae family, including over 900 species spread all over the world. All organs of the *Salvia* plants contain essential oils (EO), the main components of which are cyclic, acyclic, and aromatic monoterpenoids with the predominance of one or several components [1,2]. *Salvia officinalis* L. (Common sage, Lamiaceae) leaves are used for diseases of the throat and mouth disorders, minor wounds, skin disorders, and gastrointestinal disorders. Sage has been shown to have significant antibacterial and anti-inflammatory effects [3]. *Salvia officinalis* essential oil has been

implemented to treat diseases like the respiratory, digestive and nervous systems, heart and blood circulation, endocrine, and metabolic diseases. In addition, sage EO has been shown to have antioxidant, carminative, antispasmodic, antiseptic, and astringent properties [4–6].

The herbal drug of European Pharmacopoeia *Salvia officinalis folium* contains more than 15 ml/kg of EO for the whole leaves and not less than 10 mL/kg for the cut raw material in calculation to the anhydrous drug [7,8]. *Salvia officinalis* EO content has been varied from 0.1% to 2.8% [9–14]. In aerial parts of *S. officinalis* more than 120 components of the EO have been discovered. The raw material contains up to 3% EO, the dominant components of which are monoterpenoids: 1,8-cineole (1%–15%), camphor (5%–20%), α-thujone (10%–60%) and β-thujone (4%–36%); sesquiterpenes: β-caryophyllene, α-humulene, and viridiflorol [7]. In addition, borneol, pinene, camphor, elemene, ledene, were found in the *Salvia* EO [15–20].

Its pharmacological activity largely depends on the composition of the EO, which is inherent in the chemotype of the plant. Adapting to various environmental conditions, sage *S. officinalis* synthesizes different groups of biologically active substances that help it survive, forming stable characteristics of the chemical composition of the plant, the so-called chemotypes. First of all, adaptive substances are represented by terpenoids and phenolic compounds. EO has a very variable composition depending on the harvesting time, genetics, climate, seasonality, environment, and other factors [21–24]. Phenolic substances, amino acids, and monosaccharides in the composition of *S. officinalis* were also studied [2,25,26].

The effect of drought on the accumulation of cineole, α-thujone, β-thujone, and camphor in the sage leaves was established (the content of monoterpenes in plants that received a sufficient amount of moisture was compared with those that were in conditions of limited water supply—70% of the optimal). Studies have shown that in arid conditions, sage leaves accumulate a significantly higher concentration of monoterpenes (approximately 33%) than those plants cultivated under optimal irrigation conditions [27].

One of the key terpenes in the *S. officinalis* EO is thujone, whose contents due to its toxicity should be regulated. Thujone is a neurotoxic terpen and chemotypes with its low content should be preferred. The amount of thujone has to be specified in the given product and its daily exposure has to be below 6.0 mg [8]. The *S. officinalis* aerial parts have been used in traditional medicine and cookery for centuries. Its leaves are approved for use in the European Union as a coloring, category N_2, with preliminary restrictions on the content of α- and β-thujones in the product (0.5 mg/kg) [28]. In the USA, Sage leaves are permitted for use in food and are recognized as safe (21 CFR 182.10 and 182.20) [29].

Also, *S. officinalis* leaves contain diterpene bitter principles, triterpenes, steroids, rosmarinic acid (up to 3.3%), flavonoids, and tannins [3,21,30–32].

Previously we studied the content of *S. officinalis* EO from several countries [33]. The purpose of this work is to determine the EO composition in commercial samples of *S. officinalis* leaves from nine countries to establish the variability of the content of their components and to identify possible chemotypes of this species with a focus on toxic thujone.

2 Material and Methods

2.1 Materials

The *S. officinalis* L. leaves were obtained as commercial samples from retail pharmacies or health shops in different countries: Austria (AUT), Belgium (BEL), England (ENG), Estonia (EST), France (FRA), Georgia (GEO), Greece (GRC), Hungary (HUN), and Ukraine (UKR) from 2007 to 2020. We used the samples only of local production, which usually are grown by local farms. All the samples were marked accordingly. They were stored at a room temperature (22 ± 2°C) in their commercial packaging and analyzed as soon as possible after acquisition within four months, and all had a valid “best before” date

when studied. The EO from the dried raw materials (20.0 g for a one experiment) were obtained using the method of distillation according to the European Pharmacopoeia requirements [8]. The EO were analyzed as soon as possible after the distillation, but not later than within 1–2 days. They were collected into glass vials for chromatography and were kept in a freezer (−17 ± 2°C).

2.2 Capillary Gas Chromatography

GC analysis was carried out using a Chrom-5 chromatograph (Laboratorni Pristroe Prague, Czech Republic) with FID on two fused silica capillary columns with a bonded stationary phase: poly(5%-diphenyl-95%-dimethyl) siloxane SPB-5 (30 m × 0.25 mm, Supelco) and polyethyleneglycol SW-10 (30 m × 0.25 mm, Supelco). Film thickness of both stationary phases was 0.25 μm. Carrier gas was helium with a split ratio 1:150, and the flow rate 35–40 (SPB-5) and 30–35 (SW-10) cm/s was applied. The temperature was from 50°C to 250°C at 2°C/min, and the injector temperature was 200°C. A Hewlett-Packard Model 3390A integrator was used for data processing.

The identification of the EO components was carried out by comparing their retention indices (RI) using as standards *n*-alkanes C6–C24, on two columns with the RI values of reference standards, both our RI data bank and literature data [5,8,9]. GC/MS confirmed the results obtained. The percentage composition of the EOs was established in peak areas (nonpolar column) using the normalization method without correction factors. The relative standard deviation of percentages of EO components of three repeated GC analyses of a single oil sample didn't exceed 5% [20,30–32].

3 Results and Discussion

The identified constituents in the leaves EO of the nine *S. officinalis* samples from different countriesare gained in Table 1. The EO yields in the studied samples were 10.0–24.8 mL/kg (Table 2), which corresponded in all cases to the minimum standard (10 mL/kg) of European Pharmacopoeia for the cut drug [8].

Table 1: Composition of nine essential oils of *Salvia officinalis* leaves from different countries

Compound	RI		Range, %	Mean, %, n = 9	Variation coefficient
	SPB-5	SW-10			
α-thujene	922	1024	tr.–0.4	0.14	0.92
α-pinene	929	1022	1.3–6.4	4.48	0.34
Camphene	942	1067	2.6–6.8	5.07	0.26
β-pinene	970	1106	0.3–4.9	1.76	0.79
Myrcene	988	1165	0.7–4.2	1.24	0.90
α-terpinene	1012	1177	0.1–0.5	0.18	0.67
p-cymene	1019	1264	0.3–1.7	0.72	0.63
1,8-cineole	1026	1210	8.3–45.3	15.15	0.76
(Z)-β-ocimene	1034	1236	tr.–0.4	0.10	1.22
γ-terpinene	1053	1242	0.1–0.7	0.27	0.69
Terpinolene	1083	1274	0.1–0.5	0,21	0.60
α-thujone	1104	1419	3.0–34.0	20.92	0.39
β-thujone	1114	1435	1.5–11.6	6.95	0.47

(Continued)

Table 1 (continued)

Compound	RI		Range, %	Mean, %, n = 9	Variation coefficient
	SPB-5	SW-10			
Camphor	1138	1502	11.3–29.3	17.48	0.30
Borneol	1162	1694	1.8–5.0	3.16	0.43
Terpinen-4-ol	1172	1606	0.1–0.6	0.33	0.47
α-terpineol	1188	1704	0.1–0.7	0.22	0.84
Myrtenol	1195	1785	tr.–0.4	0.06	2.25
Bornyl acetate	1284	1573	0.1–2.7	1.53	0.51
Thymol	1293	2186	tr.–0.1	0.04	1.19
(E)-β-caryophyllene	1410	1575	tr.–4.9	1.98	0.74
α-humulene	1443	1654	0.4–6.4	2.60	0.77
Spathulenol	1566	2110	tr.–0.2	0.05	1.31
Caryophyllene oxide	1568	1954	tr.–0.9	0.42	0.73
Viridiflorol	1582	2072	1.1–10.4	5.56	0.50
In total			80.1–93.6	90.74	

Notes: tr.: traces (<0.05%). Bold–>1%. The mean % of 9 samples; RI values are given accordingly to the sample 1.

Table 2: Predominant components of nine essential oils of *Salvia officinalis* from different countries analyzed by GC-MS

Compound	Estonia	France	Hungary	Belgium	England	Greece	Ukraine	Georgia	Austria
	Content of essential oils, %								
α-pinene	**5.1**	**1.3**	**5.8**	**5.1**	**6.4**	**5.1**	**3.7**	**4.6**	**3.3**
Camphene	**5.2**	**2.6**	**5.1**	**6.8**	**5.5**	**5.9**	**3.6**	**4.7**	**6.3**
β-pinene	**2.5**	**1.2**	**2.4**	0.3	**1.6**	**4.9**	**1.6**	**1.0**	0.4
Myrcene	0.9	**1.0**	0.8	0.9	**1.1**	**4.2**	0.7	0.8	0.8
p-cymene	**1.7**	0.4	0.7	**1.0**	0.3	0.6	0.5	**1.0**	0.3
1,8-cineole	**11.7**	**8.7**	**13.0**	**8.3**	**10.2**	**45.3**	**13.7**	**13.0**	**12.5**
α-thujone	**23.9**	**34.0**	**18.6**	**19.6**	**24.1**	**3.0**	**22.4**	**23.8**	**18.9**
β-thujone	**6.3**	**9.4**	**6.6**	**5.4**	**5.2**	**1.5**	**11.6**	**11.4**	**5.2**
Camphor	**19.3**	**19.2**	**13.7**	**19.2**	**16.4**	**11.3**	**12.9**	**16.1**	**29.3**
Borneol	**1.8**	**2.3**	**5.0**	**2.0**	**4.9**	**1.6**	**3.0**	**3.2**	**4.7**
Bornyl acetate	**2.1**	**1.0**	**1.2**	**1.7**	**2.1**	**0.1**	**1.9**	**1.0**	**2.7**
(E)-β-caryophyllene	Tr.	**2.2**	**2.9**	**1.1**	**2.4**	**4.9**	**2.7**	0.8	0.9
α-humulene	**5.3**	**2.6**	**2.6**	**1.4**	**6.4**	0.4	**2.1**	0.8	**1.8**
Viridiflorol	**4.0**	**3.9**	**8.3**	**10.4**	**4.9**	**1.1**	**7.9**	**4.9**	**4.7**
Content of essential oil (mL/kg)	16.4	24.8	10.0	15.0	13.7	22.1	21.2	11.0	16.4

Notes: Bold–>1%; all symbols are individual.

High variation coefficients of the predominant compounds (>1) demonstrated that their content strongly differs from samples to samples. Low variation coefficients (0.56–0.75) are typical for *p*-cymene, α-terpinene, γ-terpinene, terpinolene, (E)-β-caryophyllene and caryophyllene oxide. Trace amounts (<0.05%) of α-thujene, (E)-β-caryophyllene, and caryophyllene oxide were detected in one sample, (Z)-β-ocimene in three samples, myrtenol in seven samples, and thymol and spathulenol in five samples of the studied EOs.

Twenty-five compounds, representing 80.1%–93.6% of the total EO, were identified in the nine studied sage EO. Such a rather large range presented in Table 1 indicates variability in the EO composition of *S. officinalis*. All identified components have been previously found in the *S. officinalis* EO [17,19,22,23]. In all the studied samples, monoterpenoids dominate (70.1%–85.4%), much less sesquiterpenoids (6.9%–13.6%) and the least aromatic compounds (0.3%–1.7%). All EOs are characterized by a strong negative correlation (r = −0.88) between the content of monoterpenoids and sesquiterpenoids (Fig. 1), where symbols are individual. To perform scatter plots (or correlation fields), the application package Statistica of Microsoft Excel was used.

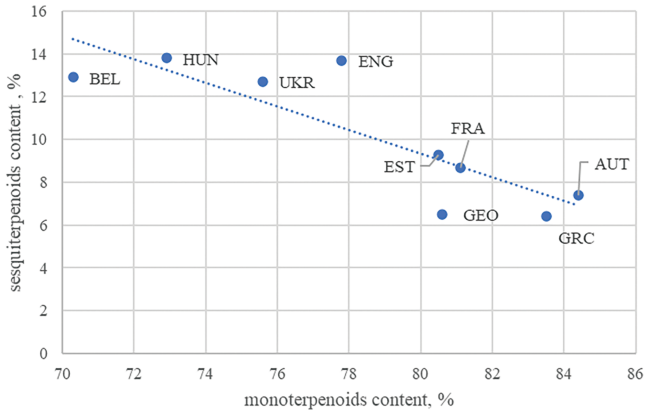

Figure 1: Correlation of monoterpenoids and sesquiterpenoids content in nine commercial samples of *Salvia officinalis* essential oil

The amount of α-thujone (18.0%–43.0%) and β-thujone (3.0%–8.5%), 1,8-cineole (5.5%–13.0%), bornyl acetate (≤2.5%), camphene (1.5%–7.0%) and camphor (4.5%–24.5%), α-humulene (≤12.0%), α-pinene (1.0%–6.5%), limonene (0.5%–3.0%), and linalool+linalyl acetate (≤1.0%) in EOs for medicinal uses are regulated by ISO 9909:1997 [34]. This standard covers EO production methods, quality requirements and evaluation criteria to ensure its purity and potency. The requirements for the content of α-pinene, camphene and α-humulene are met by all the studied samples (Table 2). The content of 1,8-cineole is significantly higher than the upper limit of normalization in the EO sample from Greece (45.3%) and slightly higher in Ukraine (13.7%). At the same time, the sample of oil from Greece differs from others in its low content of α-thujone (3%) and β-thujone (1.5%), which is significantly below the lower limit of normalization of the content of these compounds. The content of β-thujone exceeds the upper limit of normalization in EO samples from France, Ukraine, and Georgia and is 9.4%, 11.6%, and 11.4%, respectively. In the sample of EO from Austria, the content of camphor and bornyl acetate is above the norm—29.3% and 2.7%, respectively. Limonene, linalool, and linalyl acetate are absent in the studied samples.

The main components in the nine studied EOs were 1,8-cineole (8.3%–45.3%), α-thujone (3.0%–34.0%), camphor (11.3%–29.3%), β-thujone (1.5%–11.6%), viridiflorol (1.1%–10.4%), camphene (2.6%–6.8%), α-pinene (1.3%–6.4%), borneol (1.8%–5.0%), β-pinene (0.3%–4.9%), (E)-β-caryophyllene (tr.–4.9%), myrcene (0.7%–4.2%), α-humulene (0.4%–6.4%), bornyl acetate (0.1%–2.7%) (Table 2).

In the seven studied EO samples from Estonia, England, France, Hungary, Belgium, Ukraine and Georgia α-thujone (18.6%–34.0%) is the main component. Previously scientific publications also indicate that α-thujone is the dominant component in *S. officinalis* EOs from Turkey [23], Bulgaria [14], Mexico and California [20], Georgia [35], Romania [19,36], Albania [37], Algeria [38], France and Hungary [33,36], Brazil [39], Ukraine, Belgium, Moldova, and Estonia [33]. High concentrations of β-thujone were reported in EO samples from Turkey [40], Sudan [41], Uzbekistan [42], Portugal and Czech Republic [37], but in the studied EOs there were less amount of it. The high concentrations of β-thujone were just observed in the EOs from Ukraine, Geogia and France. So, it is common for variations in these main chemical components in analyzes of EOs from the same plant species that were cultivated in different countries. Thujones are neurotoxic and their amount are key points in the standardization of the *S. officinalis* EO [43–45]. The European Union, the USA and other countries have restrictions on the content of α- and β-thujones in products [28,29]. In the USA the addition of pure thujone to food is prohibited and its content must be less than 6.0 mg per day [8]. Therefore, for farms cultivating medicinal plants, it is advisable to recommend *Salvia* spp. seeds from chemotypes with a low thujone content.

In the studied EO from Greece 1,8-cineole (45.3%) dominates. The dominance of 1,8-cineole in the essential oil from Greece is close to the literature data in EO samples from Jordan [9], Egypt [12], Albania [20], Iran [46], Greece [33], and Poland [47].

In the studied EO from Austria camphor (29.3%) is the predominant component. Previously the camphor dominance in *S. officinalis* EOs from Morocco [10,11], Tunisia [16], Romania [19] and Sudan [4] is confirmed.

A high content of camphor (19.2%–19.3%) was found in the EO samples from France, Estonia, and Belgium; 1,8-cineole (13.0%–13.7%)–samples from Hungary, Georgia and Ukraine; β-thujone (9.4%–11.6%)–samples from France, Ukraine and Georgia; α-pinene (5.1%–6.4%)–samples from England, Estonia, Hungary, Belgium and Greece; camphene (5.9%–6.8%)–samples from Austria, Belgium and Greece; β-pinene (2.4%–4.9%)–samples from Estonia, Hungary and Greece; mircene (4.2%)–sample from Greece; borneol (4.7%–5.0%)–samples from Hungary, England and Austria; bornyl acetate (2.1%–2.7%)–samples from Estonia, England and Austria; (E)-β-caryophyllene (2.7%–4.9%)–samples from Ukraine, Hungary and Greece; α-humulene (5.3%–6.4%)–samples from Estonia and England; viridiflorol (7.9%–10.4%)–samples from Hungary, Belgium and Ukraine. Viridiflorol was a principal compound in many samples published previously [48,49].

Our results indicate strong positive correlations between the content of α- and β-thujone ($r = 0.73$) (Fig. 2); between the content of 1,8-cineole and β-pinene ($r = 0.81$) (Fig. 3) and a negative correlation between 1,8-cineole and the sum of α- and β-thujone ($r = -0.82$) (Fig. 4). In these diagrams all symbols are individual. Having received the analysis diagram, we did not determine the value that was far from the totality of data and that needed to be removed. In biology and other natural sciences, a significant (strong) correlation is considered to be a value between 0.3 and 1.0 [50,51].

The statistics show a strong correlation between content of the biologically active substances and Pearson coefficients, which confirms this. The positive strong correlation is evidenced the conjugated biosynthesis and accumulation of these substances in *S. officinalis* leaves. Our research shows genotypic connections of these substances. There are some publications about correlations between terpenoids, phenolic compounds and ecological minds of production, that testify about their adaptation powers [52–55].

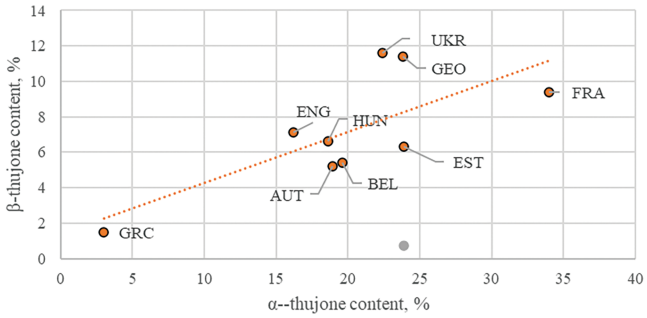

Figure 2: Correlation of α-thujone and β-thujone content in nine commercial samples of *Salvia officinalis* essential oil from different countries

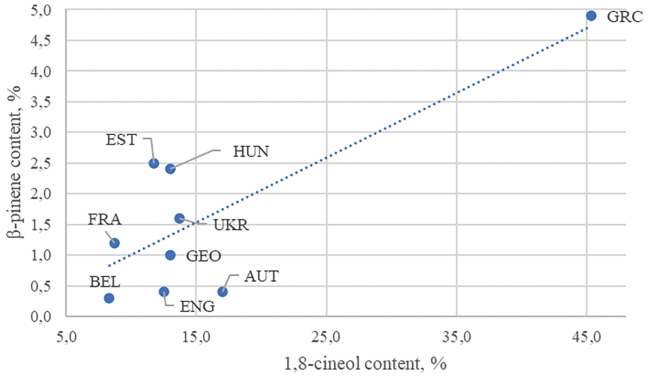

Figure 3: Correlation of β-pinene and 1,8-cineole content in nine commercial samples of *Salvia officinalis* essential oil from different countries

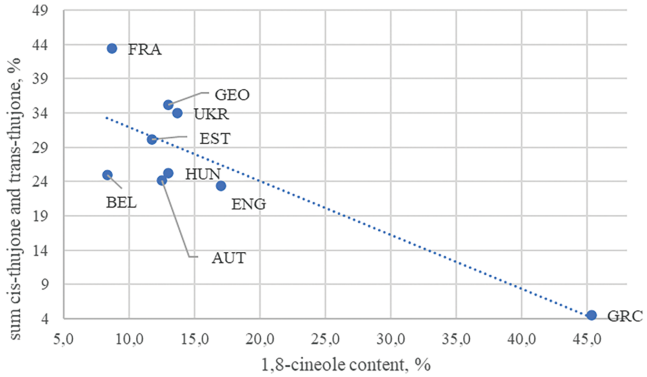

Figure 4: Correlation of 1,8-cineole and sum α-thujone and β-thujone content in nine commercial samples of *Salvia officinalis* essential oil

The results obtained by us (Table 2) show that if we take into account the content of four components, the samples we studied correspond to 8 chemotypes (CT): CT1 – α-thujone > camphor > 1,8-cineole > β-thujone (samples from Estonia and Georgia); CT2 – α-thujone > camphor > 1,8-cineole > α-humulene = α-pinene (sample from England); CT3 – α-thujone > camphor > 1,8-cineole > viridiflorol (sample from Hungary); CT4 – α-thujone > camphor > viridiflorol > 1,8-cineole (sample from Belgium);

CT5 – α-thujone > camphor > β-thujone > 1,8-cineole (sample from France); CT6 – α-thujone > 1,8-cineole > camphor > β-thujone (sample from Ukraine); CT7 – camphor > α-thujone > 1,8-cineole > camphene (sample from Austria); CT8 – 1,8-cineole > camphor > camphene > α-pinene (sample from Greece). Previously according to the content of dominant components, *S. officinalis* EOs can be divided into different chemotypes. Tucker and Maciarello described five groups of sage chemotypes based on four principal constituents: (1) camphor > α-thujone > 1,8-cineole > β-thujone; (2) camphor > α-thujone > β-thujone > 1,8-cineole; (3) β-thujone > camphor > 1,8-cineole > α-thujone; (4) 1,8-cineole > camphor > α-thujone > β-thujone; and (5) α-thujone > camphor > β-thujone > 1,8-cineole [32].

Jug-Dujaković et al. [39] divided sage leaves by chemotypes, based on the content of 8 main components (α-thujone, β-thujone, camphene, borneol and bornyl acetate, camphor, 1,8-cineole, β-pinene). The authors concluded that the first major component separates populations high in thujone from populations rich in camphor, while the second component separates populations rich in α-thujone from populations rich in β-thujone. They distinguish three chemotypes of *S. officinalis* populations: (A) α-thujone > camphor > 1,8-cineole > β-thujone; (B) β-thujone > α-thujone > camphor ≈ 1,8-cineole; and (C) camphor > α-thujone > 1,8-cineole > camphene ≈ borneol. The results of our research show that none of the studied EO samples can be attributed to the β-thujone chemotype.

Craft et al. [20] used the content of 26 EO components for cluster analysis and established the presence of 5 main sage chemotypes based on the content of two dominant compounds. They believe the most typical is the α-thujone > camphor > 1,8-cineole chemotype of sage. Of the samples studied by us, CT4 (samples from Estonia, Georgia, Hungary and England) correspond to this type.

In European countries, EO raw materials, in particular *S. officinalis*, are cultivated for the needs of industry (pharmaceutical, food, etc.,) and are usually supplied by specialized farms for the cultivation of medicinal herbs. This raw material is grown according to strictly regulated conditions (GACP) [56–59]. The collection period and cultivation conditions are regulated, so the seeds are the main and key factor that affect the quality of the raw material. It is usually standardized, thus the information about their chemotypes is especially important. The farmer is responsible for the quality of raw materials, for compliance with the regulatory document, but they, of course, do not analyse the EO composition, which was done in our work. Depending on the size of the country, the number of such farms may vary, but the issue of seed supply is not so varied. Therefore, taking this into account, the obtained data are of practical importance and will allow to make a targeted choice regarding chemotypes with low content of thujone and high concentration of other target terpenes.

4 Conclusions

The EO yields in the studied commercial sage leaves from nine countries corresponded to the minimum standard of European Pharmacopoeia for the cut drug. *S. officinalis* EOs were rich in thujones, camphor, 1,8-cineole, viridiflorol, α-humulene, camphene, and α-pinene. Toxic thujones are found in almost all analyzed samples. Based on these results eight chemotypes of *S. officinalis* were established. Considering the three components, the samples from Estonia, Georgia, Hungary, and England correspond to the most typical chemotype of 1,8-cineole, camphor, and α-thujone. The obtained results create prospects for purposeful choice of the chemotypes with low concentrations of toxic thujone and high content of other target terpenes.

Acknowledgement: The authors of the study thank all pharmacy students who helped to obtain commercial samples studied and performed hydrodistillations of EOs. The authors sincerely thank all the defenders who are fighting for the independence of Ukraine. The authors sincerely appreciate the support of the partners who stand with Ukraine.

Funding Statement: This work was carried out in the MSCA4 Ukraine project “Design and Development of 3D-Printed Medicines for Bioactive Materials of Ukrainian and Estonian Medicinal Plants Origin” (ID Number 1232466) and financed by the European Union.

Author Contributions: The authors confirm contribution to the paper as follows: study conception and design: Ain Raal, Anne Orav, Tetiana Ilina, Alla Kovalyova, Oleh Koshovyi; data collection: Anne Orav, Taras Koliadzhyn, Yuliia Avidzba; analysis and interpretation of results: Ain Raal, Anne Orav, Tetiana Ilina, Alla Kovalyova, Oleh Koshovyi; draft manuscript preparation: Ain Raal, Anne Orav, Tetiana Ilina, Alla Kovalyova, Oleh Koshovyi. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the author and/or corresponding author on reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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