

## Composition of the essential oil of *Artemisia absinthium* L. of different geographical origin

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**Abstract.** Variations in the essential oil composition of *Artemisia absinthium* L. obtained from different geographical areas of Europe were determined using capillary gas chromatographic and mass spectrometric analysis methods. The oils from air-dried wormwood were obtained in yields of 0.1–1.1%. The *Absinthii herba* grown in Estonia corresponded to the EP standards in the aspect of the essential oil contents. A total of 107 components were identified, representing over 85% of the total yield of oil. The principal components in the oils were sabinene (0.9–30.1%), myrcene (0.1–38.9%), 1,8-cineole (0.1–18.0%), artemisia ketone (0–14.9%), linalool and  $\alpha$ -thujone (1.1–10.9%),  $\beta$ -thujone (0.1–64.6%), *trans*-epoxyocimene (0.1–59.7%), *trans*-verbenol (0–11.7%), carvone (0–18.5%), (*E*)-sabinyl acetate (0–70.5%), curcumene (0–7.0%), neryl butyrate (0.1–13.9%), neryl 2-methylbutanoate (0.1–9.2%), neryl 3-methylbutanoate (0.4–7.3%), and chamazulene (0–6.6%). Monoterpenes were predominant (44.0–67.9%) in the oils from Scotland, Estonia (2000, 2002), Moldova, and Hungary. In the other oils studied oxygenated monoterpenes (41.2–93.9%) were found to predominate. The highest content of oxygenated sesquiterpenes (11.9–29.8%) was found in the oils from Italy, Latvia, Lithuania, and Germany. Armenian oil contained more chamazulene (6.6%) than the other samples studied (0–2.1%). Four chemotypes were found to be characteristic of *A. absinthium* growing in Europe: sabinene and myrcene rich oil,  $\alpha$ - and  $\beta$ -thujone rich oil, epoxyocimene rich oil, and (*E*)-sabinyl acetate rich oil. Some mixed chemotypes were also found.

**Key words:** *Artemisia absinthium* L., Compositae, wormwood, essential oil, geographical origin, chemotypes.

### INTRODUCTION

*Artemisia absinthium* L. (wormwood) is a perennial undershrub growing naturally in Europe, North America, and Asia. It is widely used in folk medicine. The volatile oil distilled from dried leaves and flowers is used in fragrance

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compounding and in some external analgesics [1–3]. The composition of the essential oil from *A. absinthium* has been the object of several studies [1–12], especially for its contents of such compounds as thujone isomers and chamazulene with pharmacodynamic properties. *cis*-Epoxyocimene and *trans*-sabinene hydrate were found in the oil of *A. absinthium* in high quantities by Chialva et al. [6, 7]. Several chemotypes were detected in these works: *cis*-epoxyocimene and  $\alpha$ -thujone chemotypes (plants from Italy), chrysantenyl acetate and sabinyl acetate chemotypes (plants from France), and a mixed chemotype (plants from Italy, Siberia, and Romania).

According to the *European Pharmacopoeia* [13], the crude drug of wormwood (*Absinthii herba*) consists of the basal leaves or slightly leaf, flowering tops, or of a mixture of these dried, whole or cut organs of *A. absinthium*. It contains not less than 2 mL/kg (~0.2%) of essential oil, calculated with reference to the dried drug. The essential oil (content 0.2–1.5%) varies considerably in composition [14].

An overdose of alcoholic preparations or essential oil may cause disturbances of the central nervous system, which can lead to convulsions and ultimately to unconsciousness and death. Thujone isomers are responsible for the hallucinogenic and toxic effect of wormwood oil and absinthe, and therefore thujones rich chemotypes of wormwood are not appreciated. They are neurotoxic, and the side effects include epileptic fits and long-lasting psychiatric disturbances. The use of essential oil and the content of thujone in foods and beverages are either strictly regulated or prohibited [14, 15]. The content of thujones in the essential oils of wormwood is up to 35% [16].

The essential oil of wormwood growing wild in Estonia has not been analysed by capillary gas chromatographic and gas chromatographic–mass spectrometric methods before. In this work a comparative study of the essential oil composition of wormwood samples obtained from retail pharmacies of Estonia and other European countries was carried out and the chemotypes of wormwood oil were determined.

## EXPERIMENTAL

### Materials

Plant materials (commercial *Absinthii herba*) were obtained from retail pharmacies of different European countries in 2000 (Estonia, France), 2001 (Hungary, Belgium, Estonia, Russia), 2002 (Estonia, Greece, Ukraine), 2003 (Scotland, Armenia, Moldova, Estonia), and 2004 (Estonia, Latvia, Lithuania, Italy, Spain, and Germany). Taxonomic identification of the plants was carried out in the Institute of Pharmacy of the University of Tartu.

### Isolation of essential oil

Essential oil was isolated from dried wormwood herb by the distillation method described in the *European Pharmacopoeia* (EP) [13] using 50 g of cut drug, a 1000 mL round-bottomed flask, and 500 mL distilled water as the distillation liquid. Xylene (0.5 mL in a graduated tube) was added to take up the essential oil. The distillation time was 3 h at a rate of 2–3 mL/min.

### Capillary gas chromatography

The essential oil extracts were analysed using a Chrom-5 chromatograph with FID on two fused silica capillary columns (50 m × 0.20 mm) with two stationary phases: nonpolar poly(dimethylsiloxane) (NB-30, Nordion, Finland) and polar poly(ethylene glycol) (NB-20M, Nordion, Finland). The film thickness of both stationary phases was 0.25 µm. The carrier gas was helium with the split ratio of 1 : 150, and a flow rate of 20–25 cm/s was applied. The temperature program was from 50 to 250 °C at 2 °C/min, and the injector temperature was 200 °C.

The identification of the oil components was accomplished by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data bank, and literature data. The results obtained were confirmed by gas chromatography–mass spectrometry (GC–MS).

The percentage composition of the oils was calculated in peak areas (nonpolar column) applying a normalization method without using correction factors. The relative standard deviation of percentages of oil components of three repeated GC analyses of single oil did not exceed 5%.

### GC–MS analysis

The MS analysis was carried out on a Hitachi M-80 B gas chromatograph double focusing mass spectrometer using an AT-5 poly(5%-phenyl-95% dimethylsiloxane) (30 m × 0.32 mm, film thickness 0.30 µm) fused silica capillary column from Alltech. The column temperature was at 70 °C for 2 min and then 70–290 °C at 2 °C/min.

## RESULTS AND DISCUSSION

The complex nature of the essential oil from common wormwood (Sample 4 from Estonia) is demonstrated in the chromatogram (Fig. 1). Retention indices (RI) on two columns, concentration range, mean %, standard deviation (SD), and variation coefficients of each component are presented in Table 1. In the wormwood oils studied 107 compounds were identified, representing more than 85% of the total oil. As it is seen from Table 1, considerable qualitative and quantitative differences exist between the wormwood samples from different geographical origins. Variation coefficients ranged from 0.5–0.7 ( $\alpha$ -pinene,  $\alpha$ -thujone, terpinen-4-ol,  $\beta$ -caryophyllene, neryl isobutanoate) to 4.4 (thymol, (*E*)- $\alpha$ -cadinol).

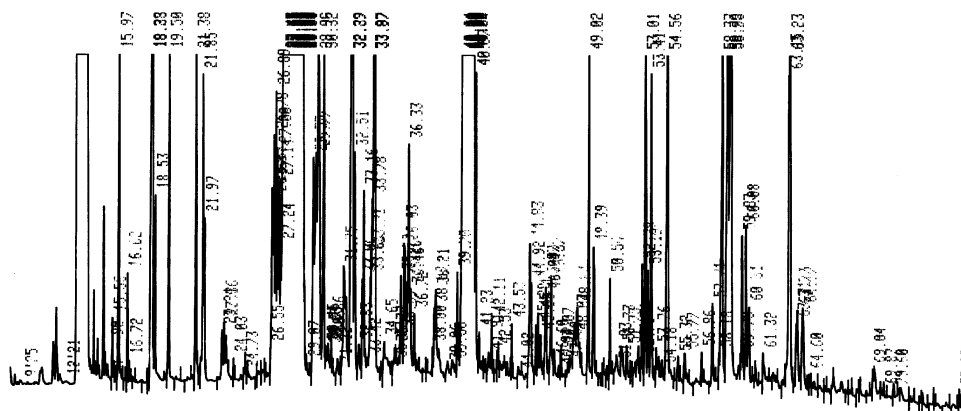


Table 1. Continued

Compound	RI NB-30	RI NB-20M	Range, %	Mean, %	SD <i>n</i> = 19	Variation coefficient
<b><i>trans</i>-Verbenol</b> <sup>MS</sup>	1152	1740	<b>0–11.7</b>	<b>2.11</b>	3.47	1.64
Borneol <sup>MS</sup>	1157	1700	0–1.6	0.29	0.54	1.86
Terpinen-4-ol <sup>MS</sup>	1166	1604	0–2.5	1.26	0.67	0.53
Myrtenal	1171	1635	0–2.1	0.23	0.48	2.11
$\alpha$ -Terpineol <sup>MS</sup>	1177	1703	0–1.1	0.35	0.31	0.87
Myrtenol	1180	1796	0–0.8	0.12	0.22	1.86
Verbenone	1182					
Nerol <sup>MS</sup>	1217	1806	0–4.0	1.08	1.24	1.14
( <i>E</i> )-Crysanthenyl acetate	1220	1802	0–1.8	0.20	0.43	2.15
Neral	1220	1645				
<b>Carvone</b> <sup>MS</sup>	1222	1730	<b>0–18.5</b>	<b>1.43</b>	4.21	2.94
$\beta$ -Citronellol	1222	1800	0–0.4	0.05	0.11	2.29
( <i>Z</i> )-Anethole	1233					
Geraniol <sup>MS</sup>	1240	1857	0–1.3	0.20	0.36	1.77
Linalyl acetate	1242	1557				
( <i>Z</i> )-Crysanthenyl acetate <sup>MS</sup>	1248	1805	0–1.7	0.35	0.54	1.52
Perillaldehyde	1250	1790	0–0.4	0.04	0.10	2.32
Geranial	1258	1730	0–0.3	0.03	0.07	2.37
( <i>E</i> )-Anethole <sup>MS</sup>	1264	1834	0–7.1	1.00	1.62	1.62
<b>(<i>E</i>)-Sabinyl acetate</b> <sup>MS</sup>	1273	1667	<b>0–70.5</b>	<b>11.39</b>	17.64	1.48
Thymol	1274	2193	0–10.2	0.54	2.34	4.36
( <i>Z</i> )-Sabinyl acetate	1276	1652	0–0.6	0.08	0.19	2.21
Carvacrol	1286	2220	0–9.7	0.56	2.22	3.97
$\alpha$ -Terpinyl acetate	1333	1693	0–0.5	0.05	0.14	3.02
Neryl acetate <sup>MS</sup>	1345	1724	0–0.6	0.21	0.21	1.00
Decanoic acid	1362	2280	0–0.4	0.05	0.11	2.27
Geranyl acetate <sup>MS</sup>	1371	1754	0–0.4	0.13	0.16	1.23
$\alpha$ -Copaene	1373	1473	0–0.7	0.13	0.21	1.64
$\beta$ -Bourbonene	1381	1500	0–0.9	0.09	0.21	2.49
$\beta$ -Elemene	1388	1608	0–0.4	0.08	0.13	1.62
Terpinyl propionate	1398		0–3.4	0.55	0.95	1.72
$\alpha$ -Ionone	1405	1840	0–0.4	0.09	0.15	1.62
Neryl propionate <sup>MS</sup>	1408	1758	0–1.6	0.34	0.49	1.43
( <i>E</i> )- $\beta$ -Caryophyllene <sup>MS</sup>	1417	1584	0–2.1	0.89	0.60	0.68
Aromadendrene	1441		0–0.4	0.04	0.11	2.56
$\alpha$ -Humulene	1450	1655	0–0.9	0.14	0.17	1.25
Geranyl propionate	1452	1770	0–0.9	0.10	0.25	2.62
$\beta$ -Ionone	1464	1930	0–0.6	0.05	0.15	2.86
<b>Curcumene</b> <sup>MS</sup>	1470	1682	<b>0–7.0</b>	<b>1.59</b>	1.58	0.99
$\gamma$ -Muurolene	1472	1685	0–1.4	0.16	0.41	2.51
Germacrene D	1474	1702				
Neryl isobutanoate <sup>MS</sup>	1476	1766	0–3.2	0.89	1.00	1.13
Geranyl isobutanoate	1482	1770	0–1.6	0.59	0.52	0.89
$\alpha$ -Muurolene	1494	1720	0–1.1	0.21	0.33	1.58
<b>Neryl butanoate</b> <sup>MS</sup>	1498	1857	<b>0.1–13.9</b>	<b>2.48</b>	3.08	1.24
$\gamma$ -Cadinene	1500	1743	0–1.3	0.19	0.33	1.75

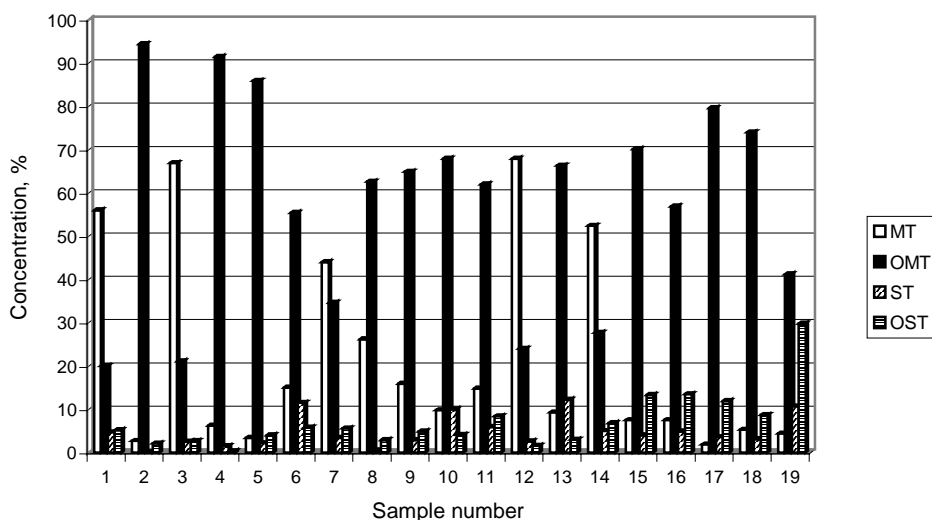
Table 1. Continued

Compound	RI NB-30	RI NB-20M	Range, %	Mean, %	SD <i>n</i> = 19	Variation coefficient
$\delta$ -Cadinene	1518	1745	0–0.5	0.05	0.13	2.48
Geranyl butanoate <sup>MS</sup>	1523	1877	0–0.9	0.18	0.27	1.52
Curcumene compound <sup>MS</sup> m/z: 186, 157, 142, 171	1546		0–1.1	0.09	0.29	3.08
Germacrene B	1550	1830	0–0.3	0.04	0.08	1.99
( <i>E</i> )-Nerolidol	1552	2040	0–0.8	0.21	0.24	1.15
<b>Neryl 2-methyl- butanoate<sup>MS</sup></b>	1562	1874	<b>0.1–9.2</b>	<b>2.88</b>	2.63	0.91
<b>Neryl-3-methyl- butanoate<sup>MS</sup></b>	1566	1876	<b>0.4–7.3</b>	<b>3.14</b>	2.24	0.71
Spathylenol	1568	2126				
Caryophyllene oxide	1570	1974	0–2.9	0.88	0.93	1.06
Viridiflorol	1581	2080	0–2.2	0.32	0.58	1.83
Geranyl isovaleriate	1585		0–2.3	0.49	0.52	1.04
Geranyl isovaleriate <sup>MS</sup>	1590	1877	0–4.9	0.91	1.26	1.39
Dodecanal*	1600	2100	0–0.9	0.17	0.30	1.70
Ledol*	1604	2100	0–0.6	0.17	0.19	1.14
Cubenol*	1610	2090	0–0.9	0.06	0.21	3.39
Neryl valeriate <sup>MS</sup>	1618	1974	0–2.8	0.43	0.71	1.64
T-Muurolol	1640	2180	0–5.0	0.73	1.36	1.85
Geranyl valeriate <sup>MS</sup>	1642		0–2.5	0.34	0.65	1.93
( <i>E</i> )- $\alpha$ -Cadinol	1644	2190	0–3.2	0.17	0.73	4.36
( <i>Z</i> )- $\alpha$ -Cadinol	1647	2224	0–1.8	0.45	0.57	1.26
Farnesol*	1653	2242	0–3.0	0.32	0.77	2.43
$\alpha$ -Santanol*	1660		0–1.3	0.11	0.31	2.84
<b><math>\alpha</math>-Bisabolol</b>	1670	2193	<b>0–7.5</b>	<b>0.71</b>	1.72	2.41
<i>n</i> -Heptadecane	1700	1700	0–0.8	0.15	0.22	1.44
<b>Chamazulene<sup>MS</sup></b>	1710	2380	<b>0–6.6</b>	<b>0.80</b>	1.51	1.89
Dihydrochamazulene*	1732	2350	0–4.9	0.31	1.12	3.67
Curcumene compound <sup>MS</sup> m/z: 186, 157, 143, 142, 141, 171	1830		0–2.9	0.35	0.71	2.02
Valerenic acid	1850	2305	0–4.7	0.37	1.10	2.98
<i>n</i> -Nonadecane	1900	1900	0–0.7	0.11	0.20	1.83
Curcumene compound <sup>MS</sup> m/z: 119, 132, 41, 69, 145, 105, 159, 227	1938	2930	0–4.3	0.78	0.98	1.25
Curcumene compound <sup>MS</sup> m/z: 119, 132, 105, 41, 55, 45, 91, 159, 185, 241	2000		0–2.5	0.53	0.68	1.29
Curcumene compound <sup>MS</sup> m/z: 119, 132, 105, 145, 41, 55, 81	2005		0–4.2	1.07	1.30	1.21
<i>n</i> -Heneicosane	2100	2100	0–0.9	0.14	0.23	1.65
<i>n</i> -Tricosane	2300	2300		0.11	0.29	2.77
Yield, % v/dry wt			0.1–0.8 (EP method)			

<sup>MS</sup> – identification by GC/MS; \* – tentatively identified.

The main components, whose content in oils could be over 6%, were sabinene (0.9–30.1%), myrcene (0.1–38.9%),  $\alpha$ -phellandrene (0–7.2%), *p*-cymene (0.2–9.6%), 1,8-cineole (0.1–18.0%), artemisia ketone (0–14.9%), linalool and  $\alpha$ -thujone (1.1–10.9%),  $\beta$ -thujone (0.1–64.6%), *trans*-epoxyocimene (0.1–59.7%), *trans*-verbenol (0–11.7%), carvone (0–18.5%), (*E*)-anethole (0–7.1%), (*E*)-sabinyl acetate (0–70.5%), thymol (0–10.2), carvacrol (0–9.7%), curcumene (0–7.0%), neryl butyrate (0.1–13.9%), neryl 2-methylbutanoate and spathulenol (0.1–9.2%), neryl 3-methylbutanoate (0.4–7.3%),  $\alpha$ -bisabolol (0–7.5%), and chamazulene (0–6.6%). Some compounds with curcumene structure (Table 1), not earlier reported, were identified in wormwood samples studied using MS data.

The oil composition of five wormwood samples from Estonia was quite different. Monoterpenes (Fig. 2) predominated in samples 1 and 3. High amounts of sabinene and myrcene (21.2% and 25.6%) were characteristic of these samples (Table 2). Comparatively large contents of sabinene and myrcene (9.2–38.9%) were also found in the oils from wormwood growing in Hungary, Scotland, and Moldova (samples 7, 12, and 14). Sample 5 from Estonia was rich in (*E*)-sabinyl acetate (70.5%), in sample 2 from Estonia *trans*-epoxyocimene (59.7%) and (*E*)-sabinyl acetate (23.6%), and in sample 4  $\beta$ -thujone (64.6%) and (*E*)-sabinyl acetate (18.2%) predominated. (*E*)-Sabinyl acetate rich oil was characteristic of samples from Armenia (34.2%), Latvia (23.6%), Belgium (18.6%), and Lithuania (13.7%) too. In the samples from Greece, Spain, Ukraine, France, and Italy



**Fig. 2.** Variation in the concentrations of the component groups of wormwood (*Artemisia absinthium* L.) oils isolated from plants of different European countries. MT – monoterpenes, OMT – oxygenated monoterpenes, ST – sesquiterpenes, OST – oxygenated sesquiterpenes. 1 – Estonia (2000), 2 – Estonia (2001), 3 – Estonia (2002), 4 – Estonia (2003), 5 – Estonia (2004), 6 – France (2000), 7 – Hungary (2001), 8 – Belgium (2001), 9 – Russia (2001), 10 – Greece (2002), 11 – Ukraine (2002), 12 – Scotland (2003), 13 – Armenia (2003), 14 – Moldova (2003), 15 – Latvia (2004), 16 – Lithuania (2004), 17 – Italy (2004), 18 – Spain (2004), 19 – Germany (2004).

**Table 2.** Content of the principal components and essential oil (%) of the common wormwood (*Artemisia absinthium* L.) of different geographical origin

Sample No.	Origin	Sabinene	Myrcene	1,8-Cineole	Linolool+ $\alpha$ -thujone	$\beta$ -Thujone	Epoxyocimenes	Sabinyl acetate	Curcumene structures	Neryl butanoate	Neryl-3-methyl butanoate	Essential oil
1	Estonia	<b>21.2</b>	<b>25.6</b>	0.6	1.7	4.1	1.3	0.4	5.5	0.8	0.4	0.8
2	Estonia	1.4	0.2	0.1	1.1	1.3	<b>59.7</b>	<b>23.6</b>	0.1	0.5	0.9	0.4
3	Estonia	<b>25.3</b>	<b>29.9</b>	0.4	3.4	0.1	1.4	0.2	2.7	2.5	0.9	0.5
4	Estonia	3.5	0.8	0.4	2.4	<b>64.6</b>	0.2	<b>18.2</b>	0.1	0.1	0.5	0.8
5	Estonia	1.7	0.2	0.3	2.5	2.3	0.7	<b>70.5</b>	0.3	1.4	0.4	1.1
6	France	3.6	5.0	0.8	<b>10.3</b>	2.0	0.4	0.3	<b>11.3</b>	<b>13.9</b>	<b>7.3</b>	0.7
	France [7]	2.7	0.8	n.d.	1.8	0.4	<b>48.9</b>	n.d.	n.d.	1.0	1.0	1.6
	France [7]	0.8	0.5	0.2	0.8	0.7	0.1	<b>84.5</b>	0.3	0.2	0.2	
7	Hungary	<b>18.1</b>	<b>17.7</b>	0.5	4.2	4.5	3.0	n.d.	2.6	3.3	2.0	0.3
8	Belgium	9.3	5.4	<b>3.9</b>	3.8	3.5	0.5	<b>18.6</b>	1.1	2.9	1.5	0.7
9	Russia	9.3	0.8	0.6	1.9	1.7	<b>22.1</b>	tr.	2.9	<b>4.9</b>	<b>5.5</b>	0.3
	Siberia I [7]	0.4	2.1	n.d.	2.7	0.6	n.d.	<b>31.5</b>	1.7	<b>7.9</b>	<b>9.1</b>	0.3
	Siberia II [7]	1.4	3.6	0.2	<b>12.7</b>	<b>7.6</b>	1.8	<b>7.9</b>	1.0	<b>7.0</b>	<b>6.6</b>	1.2
10	Greece	3.0	2.9	0.3	<b>4.5</b>	<b>38.7</b>	0.8	0.9	3.0	2.5	3.7	0.3
11	Ukraine	<b>5.1</b>	<b>5.9</b>	0.2	<b>5.4</b>	<b>6.3</b>	0.4	4.9	<b>7.8</b>	3.8	<b>7.3</b>	0.4
12	Scotland	<b>30.1</b>	<b>18.0</b>	0.2	5.0	3.5	0.8	0.2	2.1	0.5	1.7	0.8
13	Armenia	0.9	2.2	0.4	<b>6.1</b>	3.1	0.4	<b>34.2</b>	<b>5.7</b>	1.8	3.5	0.1
14	Moldova	<b>9.2</b>	<b>38.9</b>	0.1	3.0	0.4	0.6	5.7	2.4	2.0	3.0	0.2
15	Latvia	3.4	2.8	<b>4.1</b>	<b>5.8</b>	<b>6.2</b>	0.6	<b>23.6</b>	<b>9.0</b>	0.6	<b>4.7</b>	0.4
16	Lithuania	2.7	2.5	3.6	4.1	4.6	1.1	<b>13.7</b>	<b>6.3</b>	0.5	<b>4.1</b>	0.2
17	Italy	0.2	0.1	0.3	1.9	<b>12.3</b>	3.6	<b>11.4</b>	<b>7.1</b>	2.4	2.6	0.2
	Italy [7]	4.0	3.2	0.2	0.6	n.d.	<b>56.6</b>	n.d.	0.4	0.3	0.3	0.8
	Italy [7]	6.3	1.4	0.3	2.2	<b>40.6</b>	<b>23.1</b>	0.9	n.d.	1.0	1.2	0.5
	Italy [7]	2.2	2.1	0.1	1.1	<b>5.2</b>	<b>33.7</b>	<b>11.5</b>	0.3	0.9	0.7	0.5
18	Spain	tr.	0.1	<b>18.0</b>	<b>10.9</b>	<b>6.2</b>	1.3	0.2	1.4	0.1	<b>5.9</b>	0.1
19	Germany	0.6	0.3	<b>3.4</b>	1.1	0.4	1.2	n.d.	<b>8.9</b>	2.7	<b>3.8</b>	0.3

tr. – trace (<0.05%); n.d. – not determined.

$\alpha$ - and  $\beta$ -thujones were found as principal components (4.5–38.7%). A high content of epoxyocimenes (22.1%) was found in the sample from Russia.

Chialva et al. [7] studied 19 samples of *A. absinthium* from Italy, France, Romania, and Siberia (harvest year 1979–1981). They divided these samples into four chemotypes: sabinyl acetate rich oil, epoxyocimenes rich oil, chrysantenyl acetate rich oil, and thujones rich oil. The content of sabinene and myrcene in these samples was small (0.1–6.3%) (Table 2). The chrysantenyl acetate chemotype of *A. absinthium* was not found in the present work (harvest year 2000–2004); the content of chrysantenyl acetate in the studied wormwood oils was 0–1.8%. The sabinene and myrcene rich chemotype was detected in five wormwood samples. Comparison of our results with literature data showed that the



wormwood samples from other European countries also vary greatly as was observed with the samples from Estonia.

Differently from the other oils, the Italian wormwood oil contained high amounts of carvone (18.5%), thymol (10.8%), and carvacrol (9.7%). The highest content of neryl butanoate (13.9%) and compounds with curcumene structure (11.3%) was identified in the sample from France. The highest 1,8-cineole content (18.0%) was characteristic of the sample from Spain; the highest content of *trans*-verbenol was found in the samples from Latvia and Lithuania (9.2% and 11.7%, respectively).

A high content of oxygenated sesquiterpenes (11.9–29.8%) characterized the samples from Italy, Latvia, Lithuania, and Germany (Fig. 2). The principal compounds in these groups were curcumene oxygenated compounds (2.2–7.4%). The oil from German wormwood contained 7.5%  $\alpha$ -bisabolol. Only Armenian wormwood oil was rich in chamazulene (6.6%), in the other oils the chamazulene content was 0–2.1%.

The oils were obtained in the yields of 0.1–1.1% (Table 2), which usually corresponds to literature data [14] and mainly corresponded to the EP standard (not less than 0.2%) [13]. Only samples from Armenia and Spain contain essential oil below the EP standard (both 0.1%). The oil yields were higher (0.8–1.1%) in samples from Estonia (Nos 1, 4, 5), also the crude drug of wormwood from Scotland was rather rich in essential oil (0.8%).

## CONCLUSIONS

Three chemotypes of wormwood oil, mentioned earlier in the literature [7], were found in the studied samples: thujones rich oil, sabinene acetate rich oil, and epoxyocimenes rich oil. In addition, we found a chemotype of *A. absinthium* in which oil monoterpenes sabinene and myrcene were predominant from Estonia, Scotland, Moldova, and Hungary. This chemotype of wormwood has not been distinguished in the literature earlier. Some mixed chemotypes were also found. Some compounds with curcumene structure, not earlier reported, were identified in wormwood samples studied using MS data. The *Absinthii herba* grown in Estonia corresponds to the EP standards in the aspect of the essential oil contents.

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## REFERENCES

1. Montedoro, G. & Bertuccioli, M. The flavour of wines, vermouth and fortified wines In *Food Flavours, Part B. The Flavour of Beverages* (Morton, I. D. & MacLeod, A. J., eds). Elsevier, Amsterdam, 1986, 171–238.
2. Gambelunghe, C. & Melai, P. Absinthe: enjoying a new popularity among young people. *Forensic Sci. Int.*, 2002, **130**, 183–186.
3. Lachenmeier, D. W., Emmert, J., Kuballa, T. & Sartor, G. Thujone – cause of absinthism? *Forensic Sci. Int.*, 2006, **138**, 1–8.
4. Bertelli, D. J. & Crabtree, J. H. Naturally occurring fulvene hydrocarbons. *Tetrahedron*, 1968, **24**, 2079–2089.
5. Gregor, H. A new acetylenic ester from *Artemisia absinthium*. *Phytochemistry*, 1978, **17**, 806.
6. Chialva, F., Gabri, G., Liddle, P. A. P. & Ulian, F. Qualitative evaluation of aromatic herbs by direct headspace GC analysis. *J. High Resol. Chromatogr., Chromatogr. Commun.*, 1982, **5**, 181–188.
7. Chialva, F., Liddle, P. A. P. & Doglia, G. Chemotaxonomy of wormwood (*Artemisia absinthium* L.) I. Composition of the essential oil of several chemotypes. *Z. Lebensm. Unters. Forsch.*, 1983, **176**, 363–366.
8. Beauhaire, J., Fourrey, J.-L. & Guittet, E. Structure of absintholide a new guaianolide dimer of *Artemisia absinthium* L. *Tetrahedron Lett.*, 1984, **26**, 2751–2754.
9. Rücker, G., Manns, D. & Wilbert, S. Homoditerpene peroxides from *Artemisia absinthium*. *Phytochemistry*, 1991, **31**, 340–342.
10. Kennedy, A. I., Deans, S. G., Svoboda, K. P., Grey, A. I. & Waterman, P. G. Volatile oils from normal and transformed root of *Artemisia absinthium*. *Phytochemistry*, 1993, **32**, 1449–1451.
11. Lawrence, B. M. Progress in essential oils. *Perf. Flav.*, 1998, **23**, 39–50.
12. Chiasson, H., Belanger, A., Bostanian, N., Vincent, C. & Poliquin, A. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction. *J. Econ. Entomol.*, 2001, **94**, 167–171.
13. *European Pharmacopoeia*. 5th ed. Vol. 2. Council of Europe, Strasbourg, 2005, 2710–2711.
14. *E/S/C/O/P Monographs: The Scientific Foundation for Herbal Medicinal Products*. 2nd ed. Thieme, Stuttgart, 2003, 3–7.
15. Heinrich, M., Barnes, J., Gibbons, S. & Williamson, E. M. *Fundamentals of Pharmacognosy and Phytotherapy*. Churchill Livingstone, Edinburgh, 2004, 209–210.
16. Evans, W. C. *Trease and Evans' Pharmacognosy*. 15th ed. Saunders, Edinburgh, 2000, 256.

## Erinevatest geograafilistest paikadest pärit koirohu (*Artemisia absinthium* L.) eeterliku õli koostis

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Erinevatest Euroopa riikidest pärit koirohu (*Artemisia absinthium* L.) droogist eraldatud eeterliku õli koostis on uuritud kapillaargaasikromatograafia ja kromatomassispektromeetriliste meetoditega. Erinevate proovide põhikomponentide sisaldused varieeruvad suures ulatuses: sabineen (0,9–30,1%), mürtseen (0,1–38,9%), 1,8-tsineool (0,1–18,0%), *artemisia*-ketoon (0–14,9%),  $\beta$ -tujoon (0,1–64,6%), trans-epoksüotsimeen (0,1–59,7%), trans-verbenaol (0–11,7%), karvoon (0–18,5%), (E)-sabinüülatsetaat (0–70,5%), tümool (0–10,8%), nerüül-butanoaat (0,1–13,9%), nerüül-2-metüülbutanoaat (0,1–9,2%), nerüül-3-metüül-

butanoat (0,4–7,3%) ja hamasuleen (0–6,6%). Monoterpeenid (44,0–67,9%) domineerivad Šotimaalt, Moldovast, Ungarist ja Eestist pärit koirohu õlides. Ülejäänud proovides on leitud enim monoterpeenseid hapnikühendeid (41,2–93,9%). Kõige rohkem seskviterpeenseid hapnikühendeid (11,9–29,8%) sisaldavad Itaaliast, Lätist, Leedust ja Saksamaalt pärit koirohu õlid. Hamasuleeni sisaldus on suurim Armeenias pärit proovis (6,6%), ülejäänud proovides jääb see alla 2,1%. On leitud, et Euroopas kasvavale koirohule (*A. absinthium*) on iseloomulik neli eeterliku õli kemotüüpi: sabineeni- ning mürtseeni-,  $\alpha$ - ning  $\beta$ -tujooni-, epoksüotsimeeni- ja (E)-sabinüülsetaadirikas õli. Uuritud proovid sisaldavad 0,1–1,1% eeterlikku õli. Selle näitaja poolest vastavad Eestist kogutud koirohudroogid Euroopa farmakopöa nõuetele.