

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 α -thujone (1.1–10.9%), β -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, α - and β -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 α -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 (α -pinene, α -thujone, terpinen-4-ol, β -caryophyllene, neryl isobutanoate) to 4.4 (thymol, (*E*)- α -cadinol).

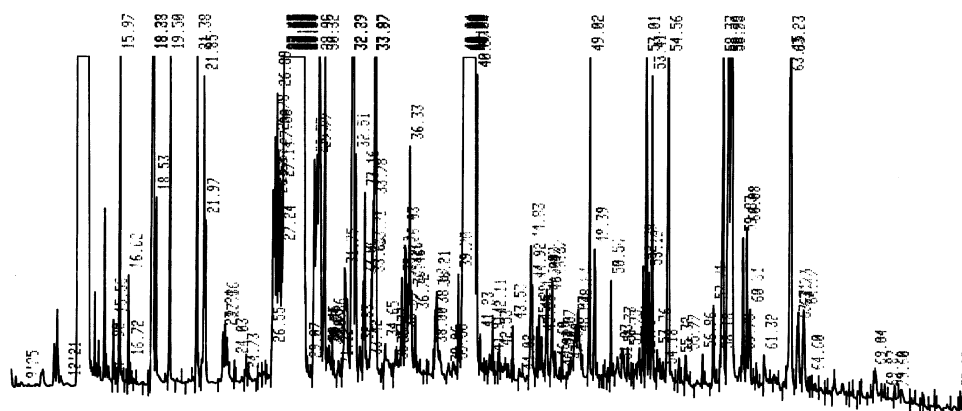


Fig. 1. Chromatogram of the essential oil from the common wormwood (*Artemisia absinthium* L.) on NB-30 capillary column (sample 4 from Estonia).

Table 1. Composition of the essential oil from *Artemisia absinthium* L. (boldface designates the principal components)

| Compound | RI NB-30 | RI NB-20M | Range, % | Mean, % | SD <i>n</i> = 19 | Variation coefficient |
|------------------------------------------------------|----------|-----------|-----------------|-------------|------------------|-----------------------|
| Tricyclene | 916 | 1010 | 0–0.7 | 0.08 | 0.17 | 2.13 |
| α -Thujene ^{MS} | 920 | 1017 | 0–1.5 | 0.29 | 0.40 | 1.39 |
| α -Pinene ^{MS} | 930 | 1015 | 0–1.5 | 0.54 | 0.37 | 0.68 |
| Camphene | 943 | 1055 | 0–1.0 | 0.24 | 0.25 | 1.04 |
| Sabinene^{MS} | 969 | 1118 | 0–30.1 | 7.82 | 9.14 | 1.17 |
| β -Pinene ^{MS} | 970 | 1095 | 0–1.8 | 0.27 | 0.41 | 1.54 |
| Myrcene^{MS} | 986 | 1152 | 0.1–38.9 | 8.38 | 11.74 | 1.40 |
| α-Phellandrene^{MS} | 999 | 1164 | 0–7.2 | 0.90 | 1.87 | 2.08 |
| α -Terpinene ^{MS} | 1011 | 1169 | 0–0.6 | 0.20 | 0.24 | 1.21 |
| <i>p</i>-Cymene^{MS} | 1015 | 1263 | 0.2–9.6 | 2.29 | 2.59 | 1.13 |
| 1,8-Cineole^{MS} | 1022 | 1206 | 0.1–18.0 | 2.01 | 4.12 | 2.05 |
| Limonene ^{MS} | 1024 | 1192 | 0–0.9 | 0.22 | 0.21 | 0.95 |
| (<i>Z</i>)- β -Ocimene | 1030 | 1220 | 0–0.4 | 0.09 | 0.12 | 1.29 |
| Artemisia ketone | 1040 | 1344 | 0–14.9 | 1.00 | 3.38 | 3.39 |
| γ -Terpinene ^{MS} | 1050 | 1237 | 0–4.2 | 0.59 | 0.96 | 1.64 |
| (<i>E</i>)-Sabinene hydrate ^{MS} | 1058 | 1465 | 0–0.3 | 0.10 | 0.11 | 1.16 |
| (<i>Z</i>)-Linalool oxide | 1056 | 1425 | 0–0.9 | 0.10 | 0.22 | 2.28 |
| (<i>E</i>)-Linalool oxide | 1076 | 1453 | 0–0.4 | 0.06 | 0.12 | 1.84 |
| Terpinolene ^{MS} | 1080 | 1270 | 0–0.5 | 0.09 | 0.13 | 1.43 |
| α-Thujone^{MS} | 1087 | 1418 | 1.1–10.9 | 4.16 | 2.74 | 0.66 |
| Linalool^{MS} | 1089 | 1554 | | | | |
| β-Thujone^{MS} | 1100 | 1438 | 0.1–64.6 | 8.73 | 15.98 | 1.83 |
| <i>cis</i> -Epoxyocimene | 1111 | 1452 | 0–2.3 | 0.26 | 0.57 | 2.17 |
| <i>trans</i>-Epoxyocimene | 1115 | 1468 | 0.1–59.7 | 5.01 | 14.13 | 2.82 |
| <i>trans</i> -Sabinol ^{MS} | 1126 | 1557 | 0–2.7 | 0.76 | 0.93 | 1.22 |
| <i>cis</i> -Sabinol ^{MS} | 1128 | 1628 | 0–3.5 | 0.61 | 0.85 | 1.41 |
| <i>cis</i> -Verbenol | 1132 | 1718 | 0–1.0 | 0.29 | 0.34 | 1.17 |

Table 1. Continued

| Compound | RI NB-30 | RI NB-20M | Range, % | Mean, % | SD <i>n</i> = 19 | Variation coefficient |
|----------------------------------------------------|-------------|--------------|-----------------|--------------|---------------------|--------------------------|
| <i>trans</i>-Verbenol ^{MS} | 1152 | 1740 | 0–11.7 | 2.11 | 3.47 | 1.64 |
| Borneol ^{MS} | 1157 | 1700 | 0–1.6 | 0.29 | 0.54 | 1.86 |
| Terpinen-4-ol ^{MS} | 1166 | 1604 | 0–2.5 | 1.26 | 0.67 | 0.53 |
| Myrtenal | 1171 | 1635 | 0–2.1 | 0.23 | 0.48 | 2.11 |
| α -Terpineol ^{MS} | 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 ^{MS} | 1217 | 1806 | 0–4.0 | 1.08 | 1.24 | 1.14 |
| (<i>E</i>)-Crysantenyl acetate | 1220 | 1802 | 0–1.8 | 0.20 | 0.43 | 2.15 |
| Neral | 1220 | 1645 | | | | |
| Carvone ^{MS} | 1222 | 1730 | 0–18.5 | 1.43 | 4.21 | 2.94 |
| β -Citronellol | 1222 | 1800 | | | | |
| (<i>Z</i>)-Anethole | 1233 | | 0–0.4 | 0.05 | 0.11 | 2.29 |
| Geraniol ^{MS} | 1240 | 1857 | 0–1.3 | 0.20 | 0.36 | 1.77 |
| Linalyl acetate | 1242 | 1557 | | | | |
| (<i>Z</i>)-Crysantenyl acetate ^{MS} | 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 ^{MS} | 1264 | 1834 | 0–7.1 | 1.00 | 1.62 | 1.62 |
| (<i>E</i>)-Sabinyl acetate ^{MS} | 1273 | 1667 | 0–70.5 | 11.39 | 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 |
| α -Terpinyl acetate | 1333 | 1693 | 0–0.5 | 0.05 | 0.14 | 3.02 |
| Neryl acetate ^{MS} | 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 ^{MS} | 1371 | 1754 | 0–0.4 | 0.13 | 0.16 | 1.23 |
| α -Copaene | 1373 | 1473 | 0–0.7 | 0.13 | 0.21 | 1.64 |
| β -Bourbonene | 1381 | 1500 | 0–0.9 | 0.09 | 0.21 | 2.49 |
| β -Elemene | 1388 | 1608 | 0–0.4 | 0.08 | 0.13 | 1.62 |
| Terpinyl propionate | 1398 | | 0–3.4 | 0.55 | 0.95 | 1.72 |
| α -Ionone | 1405 | 1840 | 0–0.4 | 0.09 | 0.15 | 1.62 |
| Neryl propionate ^{MS} | 1408 | 1758 | 0–1.6 | 0.34 | 0.49 | 1.43 |
| (<i>E</i>)- β -Caryophyllene ^{MS} | 1417 | 1584 | 0–2.1 | 0.89 | 0.60 | 0.68 |
| Aromadendrene | 1441 | | 0–0.4 | 0.04 | 0.11 | 2.56 |
| α -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 |
| β -Ionone | 1464 | 1930 | 0–0.6 | 0.05 | 0.15 | 2.86 |
| Curcumene ^{MS} | 1470 | 1682 | 0–7.0 | 1.59 | 1.58 | 0.99 |
| γ -Muurolene | 1472 | 1685 | 0–1.4 | 0.16 | 0.41 | 2.51 |
| Germacrene D | 1474 | 1702 | | | | |
| Neryl isobutanoate ^{MS} | 1476 | 1766 | 0–3.2 | 0.89 | 1.00 | 1.13 |
| Geranyl isobutanoate | 1482 | 1770 | 0–1.6 | 0.59 | 0.52 | 0.89 |
| α -Muurolene | 1494 | 1720 | 0–1.1 | 0.21 | 0.33 | 1.58 |
| Neryl butanoate ^{MS} | 1498 | 1857 | 0.1–13.9 | 2.48 | 3.08 | 1.24 |
| γ -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 |
|------------------------------------------------------------------------------------------|-------------|--------------|---------------------|-------------|---------------------|--------------------------|
| δ -Cadinene | 1518 | 1745 | 0–0.5 | 0.05 | 0.13 | 2.48 |
| Geranyl butanoate ^{MS} | 1523 | 1877 | 0–0.9 | 0.18 | 0.27 | 1.52 |
| Curcumene compound ^{MS} 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 |
| Neryl 2-methyl- butanoate^{MS} | 1562 | 1874 | 0.1–9.2 | 2.88 | 2.63 | 0.91 |
| Neryl 3-methyl- butanoate^{MS} | 1566 | 1876 | 0.4–7.3 | 3.14 | 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 ^{MS} | 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 |
| Cubanol* | 1610 | 2090 | 0–0.9 | 0.06 | 0.21 | 3.39 |
| Neryl valeriate ^{MS} | 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 ^{MS} | 1642 | | 0–2.5 | 0.34 | 0.65 | 1.93 |
| (<i>E</i>)- α -Cadinol | 1644 | 2190 | 0–3.2 | 0.17 | 0.73 | 4.36 |
| (<i>Z</i>)- α -Cadinol | 1647 | 2224 | 0–1.8 | 0.45 | 0.57 | 1.26 |
| Farnesol* | 1653 | 2242 | 0–3.0 | 0.32 | 0.77 | 2.43 |
| α -Santanol* | 1660 | | 0–1.3 | 0.11 | 0.31 | 2.84 |
| α-Bisabolol | 1670 | 2193 | 0–7.5 | 0.71 | 1.72 | 2.41 |
| <i>n</i> -Heptadecane | 1700 | 1700 | 0–0.8 | 0.15 | 0.22 | 1.44 |
| Chamazulene^{MS} | 1710 | 2380 | 0–6.6 | 0.80 | 1.51 | 1.89 |
| Dihydrochamazulene* | 1732 | 2350 | 0–4.9 | 0.31 | 1.12 | 3.67 |
| Curcumene compound ^{MS} 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 ^{MS} m/z: 119, 132, 41, 69, 145, 105, 159, 227 | 1938 | 2930 | 0–4.3 | 0.78 | 0.98 | 1.25 |
| Curcumene compound ^{MS} m/z: 119, 132, 105, 41, 55, 45, 91, 159, 185, 241 | 2000 | | 0–2.5 | 0.53 | 0.68 | 1.29 |
| Curcumene compound ^{MS} 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) | | | |

^{MS} – 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%), α -phellandrene (0–7.2%), *p*-cymene (0.2–9.6%), 1,8-cineole (0.1–18.0%), artemisia ketone (0–14.9%), linalool and α -thujone (1.1–10.9%), β -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%), α -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 β -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

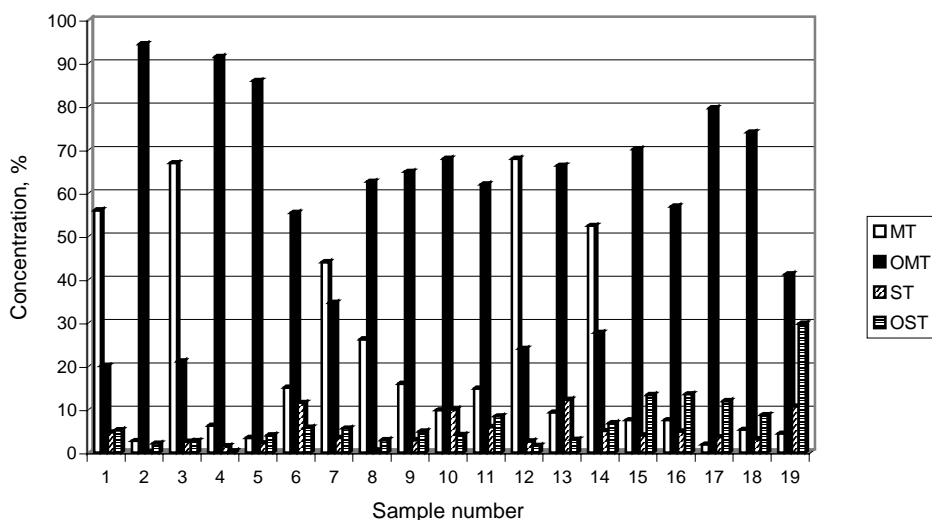


Fig. 2. Variation in the concentrations of the component groups of wormwood (*Artemisia absinthium* L.) oils isolated from plants of different European countries. MT – monoterpenes, OMT – oxygenated monoterpenes, ST – sesquiterpenes, OST – oxygenated sesquiterpenes. 1 – Estonia (2000), 2 – Estonia (2001), 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+ α -thujone | β -Thujone | Epoxyocimenes | Sabinyl acetate | Curcumene structures | Neryl butanoate | Neryl-3-methyl butanoate | Essential oil |
|------------|----------------|-------------|-------------|-------------|--------------------------------|------------------|---------------|-----------------|----------------------|-----------------|--------------------------|---------------|
| 1 | Estonia | 21.2 | 25.6 | 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 | 59.7 | 23.6 | 0.1 | 0.5 | 0.9 | 0.4 |
| 3 | Estonia | 25.3 | 29.9 | 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 | 64.6 | 0.2 | 18.2 | 0.1 | 0.1 | 0.5 | 0.8 |
| 5 | Estonia | 1.7 | 0.2 | 0.3 | 2.5 | 2.3 | 0.7 | 70.5 | 0.3 | 1.4 | 0.4 | 1.1 |
| 6 | France | 3.6 | 5.0 | 0.8 | 10.3 | 2.0 | 0.4 | 0.3 | 11.3 | 13.9 | 7.3 | 0.7 |
| | France [7] | 2.7 | 0.8 | n.d. | 1.8 | 0.4 | 48.9 | n.d. | n.d. | 1.0 | 1.0 | 1.6 |
| | France [7] | 0.8 | 0.5 | 0.2 | 0.8 | 0.7 | 0.1 | 84.5 | 0.3 | 0.2 | 0.2 | |
| 7 | Hungary | 18.1 | 17.7 | 0.5 | 4.2 | 4.5 | 3.0 | n.d. | 2.6 | 3.3 | 2.0 | 0.3 |
| 8 | Belgium | 9.3 | 5.4 | 3.9 | 3.8 | 3.5 | 0.5 | 18.6 | 1.1 | 2.9 | 1.5 | 0.7 |
| 9 | Russia | 9.3 | 0.8 | 0.6 | 1.9 | 1.7 | 22.1 | tr. | 2.9 | 4.9 | 5.5 | 0.3 |
| | Siberia I [7] | 0.4 | 2.1 | n.d. | 2.7 | 0.6 | n.d. | 31.5 | 1.7 | 7.9 | 9.1 | 0.3 |
| | Siberia II [7] | 1.4 | 3.6 | 0.2 | 12.7 | 7.6 | 1.8 | 7.9 | 1.0 | 7.0 | 6.6 | 1.2 |
| 10 | Greece | 3.0 | 2.9 | 0.3 | 4.5 | 38.7 | 0.8 | 0.9 | 3.0 | 2.5 | 3.7 | 0.3 |
| 11 | Ukraine | 5.1 | 5.9 | 0.2 | 5.4 | 6.3 | 0.4 | 4.9 | 7.8 | 3.8 | 7.3 | 0.4 |
| 12 | Scotland | 30.1 | 18.0 | 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 | 6.1 | 3.1 | 0.4 | 34.2 | 5.7 | 1.8 | 3.5 | 0.1 |
| 14 | Moldova | 9.2 | 38.9 | 0.1 | 3.0 | 0.4 | 0.6 | 5.7 | 2.4 | 2.0 | 3.0 | 0.2 |
| 15 | Latvia | 3.4 | 2.8 | 4.1 | 5.8 | 6.2 | 0.6 | 23.6 | 9.0 | 0.6 | 4.7 | 0.4 |
| 16 | Lithuania | 2.7 | 2.5 | 3.6 | 4.1 | 4.6 | 1.1 | 13.7 | 6.3 | 0.5 | 4.1 | 0.2 |
| 17 | Italy | 0.2 | 0.1 | 0.3 | 1.9 | 12.3 | 3.6 | 11.4 | 7.1 | 2.4 | 2.6 | 0.2 |
| | Italy [7] | 4.0 | 3.2 | 0.2 | 0.6 | n.d. | 56.6 | n.d. | 0.4 | 0.3 | 0.3 | 0.8 |
| | Italy [7] | 6.3 | 1.4 | 0.3 | 2.2 | 40.6 | 23.1 | 0.9 | n.d. | 1.0 | 1.2 | 0.5 |
| | Italy [7] | 2.2 | 2.1 | 0.1 | 1.1 | 5.2 | 33.7 | 11.5 | 0.3 | 0.9 | 0.7 | 0.5 |
| 18 | Spain | tr. | 0.1 | 18.0 | 10.9 | 6.2 | 1.3 | 0.2 | 1.4 | 0.1 | 5.9 | 0.1 |
| 19 | Germany | 0.6 | 0.3 | 3.4 | 1.1 | 0.4 | 1.2 | n.d. | 8.9 | 2.7 | 3.8 | 0.3 |

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

α - and β -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% α -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, Edinburg, 2004, 209–210.
16. Evans, W. C. *Trease and Evans' Pharmacognosy*. 15th ed. Saunders, Edinburg, 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%), β -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-, α - ning β -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.