

Chemical composition of essential oils of *Artemisia absinthium* L. (wormwood) growing wild in Vilnius

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Plants of *Artemisia absinthium* L. were collected in six localities during 1999–2002. Ten essential oils were produced by hydrodistillation and analyzed by gas chromatography – mass spectrometry. The first major constituent of the oils was thujone (cis+trans: 11.2–36.3%, 7 samples) or trans-sabinyl acetate (20.0–36.0%, 3 oils). The second main constituent was trans-sabinyl acetate (9.8–22.1%, 6 oils), thujone (10.2–12.5%, 2 samples), trans-sabinene hydrate (11.0, one oil) or cis-chrysanthenyl acetate (11.1%, one sample). The third dominant component in 7 samples was β -pinene (4.3–10.4%) and in other three oils were myrcene (3.9%), 1,8-cineole (7.1%) and trans-sabinyl acetate (8.8%). One oil of trans-sabinyl acetate (31.0%) chemotype contained only traces of thujone, while another nine samples including 8.8–36.0% of sabinyl acetate consisted of 11.2–36.3% of thujones. The largest part of the essential oils was formed by oxygenated monoterpenes (47.1–66.7%). Compounds with sabinane carbon skeleton made up 33.9–61.0%. Eighty-four identified constituents comprised 70.5–91.3% of the oils.

Key words: *Artemisia absinthium* L., Asteraceae, essential oil composition, β -pinene, trans-sabinyl hydrate, cis-thujone, trans-thujone, cis-chrysanthenyl acetate, trans-sabinyl acetate

INTRODUCTION

Artemisia absinthium L. (wormwood) is widespread in Lithuania [1]. The authors of [2] proposed that the local wormwood plants biosynthesized some biologically active compounds.

A. absinthium plants produced several chemotypes of essential oils in different countries [3–6]. The main chemotype of wormwood essential oils was thujone. The plants and their parts from Croatia contained notable amounts of (Z)-6,7-epoxyocimene (syn. (Z)-6,7-myroxide) besides the first major constituent trans-thujone [6]. Some volatile oils from North Italy [3], France [3, 6] and Spain [4, 5] did not contain thujones. The predominant components of the above oils were (Z)-6,7-epoxyocimene and/or (Z)-chrysanthenyl acetate. Two chemotypes were determined in Spain: one oil contained both the above compounds as the main ones, but another oil was characterized by (Z)-6,7-epoxyocimene with minor amounts of other constituents [4, 5]. (Z)-Chrysanthenol was the major component in oils from Central France [3]. Sabinyl acetate prevailed in some oils from different countries [3, 4]. High levels of thujanol and thujyl acetate (60–70%), myrcene ($\leq 35\%$), camphor and 1,8-cineole were also deter-

mined in essential oils [3]. Thujones, trans-sabinyl acetate, cis-chrysanthenyl acetate and cis-epoxyocimene are the most common constituents in wormwood essential oils [3–6].

A. absinthium extracts and essential oils are used for healing various diseases [2, 3, 7, 8]. Anthelmintic, antibacterial, antifungal, insect repellent, narcotic, digestive, tonic and other bioactivities are characteristic of preparations from wormwood plants. Their stimulant property is dependent on bitter substances as artabsin (sesquiterpene lactone) and absinthin (dimer of sesquiterpene lactones) present in plant extracts [3].

Wormwood essential oil components 1,8-cineole, cis(α)- and trans (β)-thujones help people to withstand cold and other hardships of the Himalayan region [3]. Higher amounts of the above compounds are toxic [9, 10]. Seizures may be caused by 1,8-cineole and thujones for chronic users. Also, thujones may evoke dementia. The wormwood plants producing volatile oils of thujone type were used for Absinths production, which was banned for its toxicity and hallucinogenic effects [3, 9, 10]. The German Spirit law (29 Oct.1991) allowed up to 10 mg thujone/litre in drinks [10].

Large amounts of thujone ($\leq 79.4\%$) and/or 1,8-cineole ($\leq 48.4\%$) were contained in essential oils of wild *Tanacetum vulgare* L. (tansy) in some localities

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of Lithuania [11, 12]. Cultivated *Salvia officinalis* (sage) produced lower quantities of thujone ($\leq 21.4\%$) and 1,8-cineole ($\leq 8.2\%$) [13].

This study presents the chemical composition of essential oils from aerial parts of *Artemisia absinthium* L. collected in six habitats of Vilnius. The content of thujones varied from traces to 36.3% of the oil.

MATERIALS AND METHODS

Artemisia absinthium L. plants were dried at room temperature (20–25 °C). Voucher specimens were deposited in the Herbarium of the Institute of Botany (BILAS), Vilnius, Lithuania. Numbers of growing localities: A-65243, B-65245, C-65244, D-65246, E-65255, F-65245a.

The essential oils were prepared by hydrodistillation for 2 h using a mixture of hexane and ether as a collecting organic solvent.

GC/MS analyses were performed using a chromatograph interfaced with an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with a CP-Sil 8 CB capillary column (50 m \times 0.32 mm i. d., film thickness 0.25 μm). The oven temperature was held at 60 °C for 2 min, then programmed from 60 °C to 160 °C with the increase rate of 5 °C min^{-1} , held for 1 min, then programmed from 160 to 250 °C at the rate of 10 °C min^{-1} , and isothermal at 250 °C for 5 min, using He as the carrier gas (1.0 mL/min). The injector and detector temperatures were 250 °C.

Qualitative analysis was based on a comparison of retention times, retention indexes and mass spectra with corresponding data in the literature [14] and computer mass spectra libraries (Wiley and NBS 54K).

RESULTS AND DISCUSSION

Essential oils of *Artemisia absinthium* L. collected from six habitats of Vilnius city were examined. Thujones were the first or the second prevailing component of volatile oils in five (A–E) growth localities (Table 1). The oil of habitat F contained only traces of it. The plants were collected during three years (1999–2001) in A and B localities. The volatile oils produced in the little habitat B were of thujone chemotype. The sum of cis (α)- and trans (β)-thujones varied from 23.7 to 33.8%. The relation of thujone isomers varied more markedly than their sum. The percentage of cis-thujone changed from 4.2 to 23.5% and of trans-thujone from 0.2 to 29.6%. The content of the second major constituent, trans-sabinyl acetate, made up 9.8–19.1%. The differences in essential oil composition of wormwood plants collected in the large habitat A during

three years were more marked than that in the small locality B. The close amounts of two major constituents permitted to attribute these oils to mixed chemotype. The amount of thujones was lower in the oils of habitat A (11.2–19.4%) than in samples from locality B (23.7–33.8%). The first predominant component thujone was in two oils and trans-sabinyl acetate in one sample from habitat A. The second main constituent in oils from the above locality was thujone or trans-sabinyl acetate or trans-sabinene hydrate. Variations of essential oil composition might be caused by the fact that wormwood plants had been collected in different parts of the large growing area of habitat A.

The volatile oils produced by plants from habitats C and D were of thujone type containing 36.0–36.3% of the major constituent, trans-sabinyl acetate (12.4–22.1%) was the second main component in the above localities as in habitat B, while this compound (20.0–31.0%) was the first predominant constituent in the oils (of trans-sabinyl acetate type) from localities E and F. The oil of E contained 10.2% of thujones (the second main component), while the sample from locality F included only traces of it. The second major compound in the oil of locality F was cis-chrysanthenyl acetate (11.1%).

Six out of ten investigated essential oils contained thujone as the first major constituent and trans-sabinyl acetate as the second one. An opposite position of the above compounds was determined in another two oils. Two oils where the second position was occupied by trans-sabinyl hydrate and cis-chrysanthenol acetate differed from the above eight oils. The third main component was β -pinene in seven oils and in the other three samples it was trans-sabinyl acetate (A-1999), 1,8-cineole (B-1999) and myrcene (B-2001).

All essential oils under study contained some unknown compounds. The mass spectra of three unknown (Table 1, unknown 1, 3, 4) constituents were similar to the corresponding spectra of unknowns produced by wormwood in Spanish Pyrenees [4]. The main m/z (115, 128, 142, 143, 156, 171, 186) of the above unknowns are characteristic of sesquiterpenoids containing an aromatic cycle joint by two carbon atoms with simple carbon cycles of six or seven atoms. Unknowns 1, 3, and 4 (MW 186) are compounds with 14 carbon and 18 hydrogen atoms. The same quantity of carbon atoms is in chamazulene, and the mass spectra of the unknown compound 5 was nearly the same as of chamazulene [14]. The above compound might have conjugated two aromatic cycles as of chamazulene with four methyl groups displayed at different carbon atoms. The unknown 5 could be biosynthesized from compounds with a cedrane carbon skeleton. Three cederenes (cedrane skeleton) with a different location of double bonds were determined in the essential

Table 1. Chemical composition of the essential oils of *Artemisia absinthium* L. from Vilnius city

Compound	RI	A			B			C	D	E	F
		1999	2000	2001	1999	2000	2001	2000	2000	2002	2001
Tricyclene	926	0.1	tr.		tr.			tr.	tr.	0.2	
α -Thujene	931	0.9	0.2	0.2	0.2	tr.	tr.		tr.	0.4	tr.
α -Pinene	939	0.3	4.3	4.4	tr.	2.2	0.1	2.0	0.2	0.6	4.2
Sabinene	976	4.0	3.8	1.0	2.2	3.1	1.5	1.2	1.6	2.5	3.5
β-Pinene	980	1.6	8.0	10.4	5.4	9.0	1.9	4.3	6.4	5.4	10.2
Myrcene	991	4.2	1.0	3.3	0.4	0.1	3.9	tr.	tr.	2.2	6.0
β -Phelandrene	1005	1.0	tr.		tr.		0.1	tr.		tr.	
α -Terpinene	1018	0.3	0.4	0.2	0.2	0.1	0.1	tr.	tr.	0.1	tr.
p-Cymene	1026	0.4	0.2		1.6		0.3	0.2	0.2	tr.	
1,8-Cineole	1033	3.2	1.4	1.0	7.1	1.2	0.6	2.2	0.9	3.7	5.0
(Z)- β -Ocimene	1040	0.1	tr.		0.3		0.3				
γ -Terpinene	1062	1.0	0.8	0.5	tr.	0.4	0.3	0.5	0.4	tr.	0.1
cis-Sabinene hydrate	1068	0.3					0.1	tr.		tr.	
Terpinolene	1088	0.3	0.2		tr.		0.1	tr.	tr.	0.1	tr.
trans-Sabinene hydrate	1097	11.0				tr.	0.1	tr.	tr.	tr.	
Linalool	1098	4.0	2.0	2.7	tr.	1.1	4.7	0.1	0.8	1.5	2.8
cis-Thujone	1102	3.2	9.4	5.5	10.5	4.2	23.5	13.4	5.6	5.7	
trans-Thujone	1114	8.0	10.0	7.0	17.7	29.6	0.2	22.6	30.7	4.5	tr.
endo-2-Norborneol acetate	1125	1.4			4.0			tr.			
iso-3-Thujanol	1133	0.1	0.1		0.3		0.1		tr.	0.1	
trans-Sabinol	1140	1.9	2.8	1.3	0.4	0.5	0.1	6.4	1.0	0.7	0.4
trans-Verbenol	1144	0.1	tr.	tr.	0.2						
Pinocarvone	1162	0.2		tr.	0.1		0.2			tr.	tr.
cis-Chrysanthenol	1162	0.3			0.5	tr.	tr.	tr.	0.8	4.1	0.5
Lavandulol	1166	0.2	0.1	tr.	0.2	tr.	0.1	0.3	0.2		tr.
Terpinen-4-ol	1177	1.3	1.8	1.0	1.6	1.3	0.8	1.1	0.8	0.9	
α -Terpineol	1189	0.6	0.3	0.2	0.4	0.2	0.2	0.4	0.2	0.9	0.1
cis-Piperitol	1193	0.1		0.2	0.2					0.2	
trans-Carveol	1217	0.1								0.3	
γ -Isogeraniol	1222	0.2			0.1					0.2	
Nerol	1228	0.3	0.2	tr.		0.1		0.3	0.4	0.1	tr.
n-Hexyl 2-methyl butyrate	1234	0.2	0.2	0.1	0.2	0.1			0.3	0.1	
trans-Chrysanthenyl acetate	1235							0.2		0.9	0.7
Neral	1240	0.2		0.5	0.2	tr.				tr.	
Geraniol	1255	0.2	0.3	tr.	0.2			0.2	0.1		
cis-Chrysanthenyl acetate	1262	0.2		tr.	0.1				0.5	0.2	11.1
Perilla aldehyde	1271		0.3	0.1				tr.	0.2		
trans-Sabiny acetate	1291	8.8	17.7	36.0	9.8	19.1	10.8	12.4	22.1	20.0	31.0
Isoamyl benzyl ether	1310	0.1	0.2	0.1	tr.		tr.	0.1		0.1	
Hexyl tiglate	1331	0.1	0.1		0.3	0.1		0.1			0.1
Eugenol	1356	0.1	0.2	tr.	0.3	0.1		tr.	tr.	0.2	tr.
Neryl acetate	1365	0.3	0.4	0.1	0.2	0.3	0.1			0.4	
α -Copaene	1376	0.1			0.1				0.1		
Geranyl acetate	1383	0.1			0.1					0.1	
β -Bourbonene	1384	0.2	tr.	0.1	0.4	0.1	0.2	0.1	0.1	0.2	tr.
cis-Jasmone	1394	0.2	0.2		0.1	tr.		0.1	0.1	0.2	
n-Tetradecane	1399	0.1			0.1	tr.					
Italicene	1401	0.1	0.1	0.1				0.1	0.1	0.1	tr.
cis-Caryophyllene	1404	2.4	1.7	1.9	2.3	1.3	1.4	1.7	1.6	1.9	1.8

1,7-di-epi- β -Cedrene	1410	0.1		0.1	0.1	0.2			0.1		
β -Cedrene	1418	0.2	0.1	0.1	0.1						
α -Humulene	1454	0.5	0.4	0.3	0.5	0.2		0.2	0.3	0.5	0.5
β -Farnesene	1458		0.1			0.1	0.3	0.1		tr.	tr.
1,7-di-epi- α -Cedrene ?	1460	2.2			1.4						
Seychellene	1460										
Acoradiene	1464	1.4	0.4		tr.	0.6	0.7	0.8		0.1	
γ -Muurolene	1477					0.9		0.5	0.9		0.5
γ -Curcumene	1480		0.5	0.7	tr.			1.1	0.8	2.0	
ar-Curcumene-	1481	0.1		0.8							tr.
β -Selinene	1485		0.6	0.3		0.7	0.4		0.5		
Neryl isobutanoate	1491	0.1		0.6	tr.		0.8			0.9	0.4
Viridiflorene	1493	tr.		tr.						0.3	
α -Bisabolene	1504						0.7				0.1
Lavandulyl isovalerate	1510	0.4	tr.	1.3	0.5	0.1	2.2	tr.	0.1		0.1
Lavandulyl 2-methyl butanoate	1512	2.0	0.1	0.9	0.2	tr.	1.5	0.4	tr.	1.3	1.4
γ -Cadinene	1513		0.3							1.2	
Geranyl isobutanoate	1515			0.4			0.5	0.3		tr.	1.2
cis-Nerolidol	1534	0.2	0.5	0.3		0.9	2.1		0.9		2.3
Geranyl n-butyrate	1562	0.4	1.1	1.3	2.3	1.5	0.4	1.3	2.1	0.6	
Germacrene B	1556						0.7	0.3	tr.		tr.
β -Calacorene	1563	2.6	2.7	1.7	2.1	1.3		0.3	1.0	3.2	
trans-Nerolidol	1564	0.2	0.3			0.2	0.1	0.2		1.1	0.1
Caryophyllene oxide	1581	0.4	0.4		0.3	0.1	0.1	0.2	0.1	tr.	
Unknown 1	1605	0.2	2.8	2.0	0.7	1.8	2.2	2.0	2.1	0.9	2.4
Unknown 2	1624	0.8	1.7	0.5		0.3	0.5	0.3	0.4	0.3	0.1
Unknown 3	1630	0.5	0.5	0.4	0.5	1.2	1.1	1.0	0.2	2.6	1.4
Unknown 4	1635	0.6	0.2		1.1	0.4	0.9	0.4	0.2	1.1	0.6
Unknown 5	1640	0.2	0.3	0.2	0.4	0.3	0.3	0.2	0.2		
cis-Methyl jasmonate	1647	tr.		tr.	0.4	0.2	0.1		0.1		
Selin-11-en 4- α -ol	1652	0.1	1.5	0.2		0.5		0.3	1.0	0.6	0.3
α -epi-Bisabolol	1686	tr.	1.6			0.4	0.3	1.9	1.1		
(Z)- α -Atlantone	1713		0.2			0.3		0.2	tr.	0.2	tr.
Chamazulene	1725	0.9	1.4	0.5	1.3	0.6	0.7	0.8	0.7	0.4	
(Z)-Nuciferol ?	1758	1.7	1.5	0.3	2.0	1.1	1.2		0.8	0.6	tr.
Bisabolen-12-ol- β	1760	0.1	0.3		0.6	0.3	0.1	0.3	0.7		
(E)- α -Atlantone	1773		0.3			0.2	0.2	0.3		1.1	1.1
Nuciferol acetate	1835	1.6	1.2	1.0	3.0	1.2	1.6	0.9	0.6	1.4	1.4
Lanceol acetate	1860	3.0	1.7	2.5	3.9	1.4	3.5	tr.	1.1	4.3	2.1
Eicosane	2000	0.3	0.2	0.1		0.1	0.5	tr.	tr.	0.2	0.3
Total		84.9	91.3	94.4	89.4	91.3	75.5	84.3	91.4	83.7	93.8
Monoterpene hydrocarbons		14.2	18.9	20.0	10.3	14.9	8.6	8.2	8.8	11.5	24.0
Oxygenated monoterpenes		49.6	48.6	60.2	57.7	59.5	47.1	61.8	66.7	47.8	54.7
Sesquiterpene hydrocarbons		10.8	8.3	6.6	8.3	6.0	5.1	6.0	6.2	9.9	2.9
Oxygenated sesquiterpenes		7.3	9.5	4.3	9.8	6.6	9.2	4.3	6.3	9.3	7.3
Sabinene skeleton		38.2	44.0	51.0	41.1	56.5	36.4	56.0	61.0	33.9	34.9

RI – Retention index on unpolar column CP-Sil 8CB, tr.-traces(< 0.05%)

Unknown 1 MW-186, (m/z): 157(100), 142(72), 143(70), 186(61), 143(70), 128(57), 115(46), 77(21), 91(21), 39(21)

Unknown 2 MW-220, (m/z): 135(100), 93(30), 41(29), 79(27), 67(18)

Unknown 3 MW-186, (m/z): 171(100), 186(75), 143(75), 129(60), 128(58), 140(43), 115(41)

Unknown 4 MW-186, (m/z): 171(100), 143(80), 186(76), 129(75), 128(74), 157(50), 91(41), 77(39)

Unknown 5 identical to chamazulene

oils under study (Table 1). Cedrenes might be converted to unknown derivatives. Chamazulene is produced from compounds with a quaiene carbon skeleton. Nine out of ten oils contained 0.5–1.4% of chamazulene (Table 1).

Monoterpenoids comprised a large part (55.7–80.2%) of wormwood oils in the study. Oxygenated monoterpenes (47.1–66.7%) formed about a half of oil content. Compounds with a sabinane carbon skeleton (thujene, sabinene, cis- and trans-sabinene hydrate, cis- and trans-thujone, iso-3-thujanol, trans-sabinol, trans-sabinyl acetate) made up 33.9–61.0% of total oils. Plants from locality D biosynthesized 61.0% compounds of the above skeleton. Three oils (A-2001, B-2000 and C-2000) contained >50.0% of compounds with a sabinane carbon skeleton. The sum of 89 constituents listed in Table 1 comprised 75.5–94.4% of the essential oils. Eighty-four identified compounds made 70.5–91.3% of oils.

CONCLUSIONS

The *Artemisia absinthium* L. plants collected from six habitats of Vilnius city biosynthesized essential oils of two chemotypes, thujone and trans-sabinyl acetate, according to the first major constituent. Several oils containing a close percentage of both major constituents might be attributed to the third mixed chemotype.

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VILNIUJE AUGANĖIO PELYNO (*ARTEMISIA ABSINTHIUM* L.) ETERINIŲ ALIEJŲ CHEMINĖ SUDĖTIS

Santrauka

Pelyno (karėiojo kiekio) antžeminė dalis surinkta dešiose augavietėse Vilniuje 1999–2002 m. Eteriniai aliejai, išgauti hidrodistiliacijos būdu, buvo analizuojami dujų chromatografijos–masių spektroskopijos metodu. Aštuoniasdešimt keturi identifikuoti junginiai sudarė 70,5–91,3% aliejų, vyraujant oksiduotų monoterpenų (47,1–66,7%) frakcijai. Pirmieji trys pagrindiniai komponentai galėtų būti tujonas (cis+trans), trans-sabinil acetatas, trans-sabineno hidratas, cis-chrisantenil acetatas, β-pinenas, mircenas ir 1,8-cineolis. Didžiausias kiekis tujonų (cis+trans) (11,2–36,3%) buvo nustatytas septyniuose mėginiuose, o trans-sabinil acetato (20,0–36,0%) – trijuose aliejuose.

Pelyno eteriniai aliejai pagal pirmąją vyraujančią komponentą priskirti tujono arba trans-sabinil acetato chemotipui, o turintieji panašų dūš junginių kiekį – mišriam tipui.