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The chemical composition of the essential oils of aerial parts of *Artemisia campestris* ssp. *campestris,* collected from ten different locations in Lithuania is detailed in this paper. The major component in all the oils was caryophyllene oxide (8.5-38.8%), whereas compounds with the caryophyllane skeleton ranged from 10.2 to 44.5%. Other representative constituents were germacrene D (≤15.0%), humulene epoxide II (≤8.1%), β-ylangene (≤7.7%), spathulenol (≤6.8%), β-elemene (≤6.8%), β-caryophyllene (≤6.2%), junenol (≤6.1%) and α- or β-pinene (≤5.5%). Eighty-seven compounds were identified, comprising 73.6-92.3% of the oils. The chemical composition was highly variable depending on the sample location.

Toxicity of *A. campestris* oils was determined using the brine shrimp (*Artemia* sp.) assay. LC_{50} values ranging to 20 µg/mL were obtained for three of the oils after 24 hours of exposure. Data of this test revealed that *A. campestris* ssp. *campestris* essential oils with dominant caryophyllene oxide are notably toxic.

Keywords: *Artemisia campestris* ssp. *campestris*, essential oil, toxicity, brine shrimp lethality test, β-caryophyllene, caryophyllene oxide, β-elemene, β-ylangene, humulene epoxide II, junenol.

Artemisia (with up to 500 species) is one of the largest and most widely distributed genus of the Asteraceae. *A. campestris* (tribe Anthemideae, *Dracunculus* section), commonly known as field wormwood or field sagewort, is a perennial plant that is found in temperate regions throughout the northern hemisphere. Field wormwood, common for most parts of Europe, prefers open sites in light (sandy) and medium (loamy) soils. The species is polymorphous and is divided into several subspecies and forms. The plant is known as a medicinal herb with anthelmintic, antiseptic, cholagogue, deobstruent, emmenagogue, stomachic and tonic properties.

Flavonoids and terpenoids are the main secondary metabolites of the genus *Artemisia* [1]. Previous studies of the chemical composition of essential oils of *A. campestris* from different countries gave contrasting results (Table 1, [2-13]). However, the authors worked on plant material from various locations with many environmental variables, and in some of these studies, the plant parts used were not clearly specified and/or the subspecies was not indicated. Moreover, even for the same subspecies, i.e. *A. campestris* ssp. *glutinosa*, oil compositions varied from one study to another [2-5,12]. In addition, variation in the essential oil composition may occur depending on the phenological stage. This parameter was not always specified in the previous reports.

Furthermore, the literature shows the lack of research concerning the ethno-pharmacology, volatile oil composition and antibacterial activity of *A. campestris* from Lithuania. Thus the aim of this study was to determine the chemical composition and toxicity of the essential oils from the aerial parts of *A. campestris* ssp. *campestris* from various Lithuanian locations. All samples were collected at the full flowering to compare oil compositions of plants at the same phenological stage.

Table 1: Major constituents (%) of *Artemisia campestris* essential oils from various countries, according to the literature.

A. campestris subspecies	Organ	Extract	Main components, (%)	Place of origin	Reference
ssp. *glutinosa*	not specified	EO	*ar*-curcumene, caryophyllene oxide, *p*-cymene, β-pinene and germacrene D	Italy	2
ssp. *glutinosa*	flowers and leaves		β-pinene (6.9-57.2), germacrene D (0.4-28.6), bicyclogermacrene (1.0-14.5) and myrcene (1.7-11.2)	Italy	3
ssp. *glutinosa*	aerial parts	EO	γ-terpinene (≤46.5), capillene (33.1), 1-phenyl-2,4-pentadiyne (≤29.7) and spathulenol (≤11.3)	France	4
ssp. *glutinosa* even if ns	aerial parts	EO	(*Z,E*)-farnesol (10.3), cedrol (5.4) and verbenone (3.8)	Algeria	5
ssp. *campestris* and *borealis*	aerial parts	EO	α-pinene (≤16.5), β-pinene (≤10.7), caryophyllene oxide (≤18.2), spathulenol (≤18.7), *epi*-cubenol (≤14.2) and 1,8-cineole (≤19.2)	North west Italy	1, 6
ssp. *maritima Archangelis**	aerial parts ?	EO	β-pinene (17.8), cadin-4-en-7-ol (16.4), γ-terpinene (8.7), (*Z*)-β-ocimene (7.4), aromadendrene (6.7)	Portugal	7
ns	leaf	EO	β-pinene (24.0-49.8), α-pinene (5.9-12.5), *p*-cymene (3.4-9.4), limonene (4.9-9.3), spathulenol (1.2-8.9), γ-terpinene (2.0-6.5), eudesmol (1.0-6.4) and (*Z*)-β-ocimene (0.2-5.5)	Southern Tunisia	8
ns		vapor	α-pinene (41.0), β-pinene (29.7), limonene (6.4) and sabinene (4.5)	Southern Ural	9
ns	leaf	EO	β-pinene (24.2-27.9), *p*-cymene (17.4-22.3) and α-pinene (4.1-11.0)	South eastern Tunisia	10
ns	aerial parts	EO	spathulenol (9.2), β-pinene (9.1), α-pinene (3.4), limonene (2.5), germacrene D (3.3), 4-hydroxy-9-*epi*-β-caryophyllene (3.0)	Serbia	11
var. *glutinosa* Gay ex Bess	aerial parts	EO	β-pinene (41.0), *p*-cymene (9.9), α-terpinene (7.9), limonene (6.5) and myrcene (4.1)	Southern Tunisia	12
ns	flowers, leaves and stems, separately	EO	α-pinene (23.9, 23.0, 29.2) and spathulenol (23.9, 15.8, 29.2) in the flower, leaf and stem oils, respectively; bicyclogermacrene (12.0) in the flower and β-pinene (12.6) in the leaf oil	Iran	13

EO-essential oils, ns-name of subspecies not indicated, * also referred to as *Artemisia crithmifolia* L.

1. Chemical composition of Lithuanian *Artemisia campestris* essential oils: The composition of the essential oils of *A. campestris* collected from 10 populations in different parts of Lithuania (east, west and south of the country) and rich in caryophyllene oxide is presented in Table 2. The major component in all the oils was caryophyllene oxide (8.5-38.8%), while the amount of compounds with a caryophyllane skeleton (caryophyllene, its oxide and caryophylla-4(12),8(13)-dien-5α-ol) ranged from 10.2 to 44.5%.

In four samples (A, E, F and K) out of ten, germacrene D (9.4-15.0%) was the second main constituent. In the other six oils, four compounds were identified as the second principal component, i.e. caryophyllene (5.7-6.2% in oil samples H and J), β-pinene (4.9% in sample C), spathulenol (5.7-6.8% in B and D) and humulene epoxide II (8.1% in G).

The third most dominant components were found to be α- or β-pinene (4.5-5.5% in A, C and D), β-ylangene (7.7% in sample E), germacrene D (4.9-7.5% in G, H and J), humulene epoxide II (5.3% in B), junenol (6.1% in F) and β-elemene (6.8% in K). Caryophyllene oxide, germacrene D, α- or β-pinene and spathulenol have been previously determined in appreciable amounts in field wormwood oils from other countries (Table 1), while humulene epoxide II, junenol, β-elemene and β-ylangene have not been mentioned before among the main constituents of these oils. Bicyclogermacrene was only determined in minor amounts in this study, while it was a major constituent (up to 14.5%) in *A. campestris* oils from Italy and Iran [3,13].

Eighty-seven identified constituents (one of them tentatively) comprised 73.6-92.3% of the total oils. Chemical analysis showed a multi-component composition of the essential oils of Lithuanian field wormwood. The main fraction was sesquiterpenoids (49.9-79.3%), where amounts of sesquiterpene hydrocarbons and oxygenated sesquiterpenes varied from 16.1 to 51.2% and from 25.0 to 52.6%, respectively. Monoterpene hydrocarbons accounted for only 5.4-19.7%.

2. Toxic activity of *A. campestris* essential oils against *Artemia* sp. nauplii: Three oils obtained from the studied plant materials were chosen for tests of toxic activity. The test showed that lethality (LC_{50}) of brine shrimp larvae was 15-20 μg/mL (16.8, 19.5 and 14.9 μg/mL for samples A, H and K, respectively). The essential oils of *A. campestris* containing 12.1%, 22.1% and 10.2% of caryophyllene oxide, and 9.4%, 5.8% and 9.9% of germacrene D, respectively were toxic enough to kill the shrimps, despite the fact that the extremely toxic ketones (such as α- and β-thujone or artemisia

Table 2: Main constituents (with quantity over 5%) of essential oils, rich in caryophyllene oxide, of *Artemisia campestris* from Lithuania (2003-2007).

Compound	RI [a]	A	B	C	D	E	F	G	H	J	K	Interval
α-Pinene	939	5.3	t	4.5	4.0	6.8	1.1	5.6	1.3	1.5	0.7	t-6.8
β-Pinene	975	3.9	4.3	4.9	5.5	2.3	0.5	0.3	0.2	t	0.3	t-5.5
cis-Pinane	986	1.0	3.6	0.8	1.5	1.2	1.5	6.0	3.3	3.2	1.9	0.8-6.0
β-Elemene	1391	3.2	t	0.3	0.9	0.7	1.3		0.7	0.1	6.8	0-6.8
β-Caryophyllene	1419	3.0	2.0	2.7	1.7	1.6	2.8	6.1	6.2	5.7	4.9	1.6-6.2
β-Ylangene	1420	1.8	1.0	1.5	0.7	7.7	3.1		1.0		6.0	0-7.7
γ-Curcumene+Acoradiene	1480		5.7			4.6	0.8	5.1	1.5	3.1		0-5.7
Germacrene D	1485	9.4	3.5	3.8	3.7	10.1	15.0	7.5	5.8	4.9	9.9	3.5-15.0
Spathulenol	1578	0.9	6.8	4.5	5.7							0-6.8
Caryophyllene oxide	1583	12.1	19.7	14.5	8.5	16.0	18.7	22.0	22.1	38.8	10.2	8.5-38.8
Humulene epoxide II	1608	3.7	5.3	0.7	2.5	2.9	5.2	8.1	5.6	4.1	2.4	0.7-8.1
Junenol	1619	1.6		1.4			6.1	2.9	0.8		4.6	0-6.1
Sum of main constituents (%)		45.9	51.9	39.6	34.7	53.9	56.1	63.6	48.5	61.4	47.7	34.7-63.6
Total ([b] including all compounds with quantity ≤5.0%)		75.2	73.6	79.3	80.1	81.2	85.4	92.3	84.3	81.0	92.0	73.6-92.3
Compounds with caryophyllane skeleton [b]		15.4	21.7	17.2	11.8	17.6	21.5	30.0	28.3	44.5	15.1	11.8-44.5
Monoterpene hydrocarbons [b]		17.1	9.7	15.6	19.7	15.9	5.4	13.3	10.8	8.1	6.8	5.4-19.7
Sesquiterpene hydrocarbons [b]		27.2	16.1	24.7	21.1	34.1	33.0	31.4	25.7	18.4	51.2	16.1-51.2
Oxygenated sesquiterpenes [b]		25.0	42.2	33.2	28.8	26.6	40.8	39.3	40.4	52.6	28.1	25.0-52.6

A-K indicate harvesting localities, in eastern Lithuania: Trakai district, Streva (A, 2003) and Vilnius district, Vievis (B, 2007); in western Lithuania: Palanga city, Sventoji (C, 2005), Klaipeda district, Plikiai (D, 2005), Palanga city, Butinge (E, 2007), Silute district, Pagegiai (F, 2007), Klaipeda district, Karkle (G, 2007), Kretinga city (H, 2007), Klaipeda city (J, 2007) and in south Lithuania: Druskininkai city, Latezeris (K, 2007).

[a]RI- retention index on nonpolar column DB-5; t-traces (≤0.05%).

[b]Compounds with quantity ≤5.0%:

0-0.5%: α-thujene, camphene, sabinene, *p*-cymene, 6-camphenone, *allo*-ocimene, *neo-allo*-ocimene, *trans*-verbenol, camphor, verbenone, α-cubebene, citronellyl acetate, neryl acetate, geranyl acetate, β-bourbonene, aromadendrene, *cis*-muurola-4(14),4-diene, α-cadinene, nonadecane and eicosane;

up to 1.5%: α-terpinene, β-phellandrene, (*Z*)-β-ocimene, benzene acetaldehyde, (*E*)-β-ocimene, γ-terpinene, terpinolene, linalool, α-campholenal, *trans*-pinocarveol, terpinen-4-ol, α-terpineol, myrtenol, 4(*Z*)-decen-1-ol, *trans*-sabinyl acetate, terpinyl acetate, *ar*-curcumene, bicyclogermacrene, γ-cadinene, *trans*-cadina-1(2),4-diene, α-calacorene, β-copaen-4-α-ol, *trans*-β-elemenone, *epi*-α-muurolol and phytol;

lower than 3.0%: 1,3-dimethyl benzene, myrcene, γ-terpineol, δ-elemene, α-copaene, (*E*)-β-ionone, α-zingiberene, *trans*-β-guaiene, α-muurolene, (*E*, *E*)-α-farnesene, δ-cadinene, (*E*)-nerolidol, caryophylla-4(12),8(13)-dien-5α-ol, germacra-4(15),5,10(14)-trien-1-α-ol and (*6R*, *7R*)-bisabolone;

up to 5.0%: limonene, bornyl acetate, β-copaene, (*Z*)-β-farnesene, α-humulene, (*E*)-β-farnesene, β-selinene, *trans*-muurola-4(14),5-diene, (*Z*)-nerolidol, unknown 1, salvial-4(14)-en-1-one, *epi*-α-cadinol, α-cadinol and 3-thujopsanone.

Unknown 1(RI-1580): M^+ 220, 107(100), 135(69), 91(57), 41(37), 79(34), 69 (21), 119(21), 204(16).

ketone) characteristic for *Artemisia* species, were not detected in the investigated oils. The strong toxicity of *A. campestris* evaluated by us can justify why the plant is used in Lithuanian folk medicine, but not as a food or spice.

According to the literature data [14-16], essential oils of other plant species possessing caryophyllene oxide as a major constituent are toxic. Volatile oils of *Acroptilon repens*, containing 36.6% of caryopyllene oxide and 10% of caryophyllene inhibited the growth of Gram-positive bacteria [14]. This sesquiterpene oxide is toxic to ants and inhibits growth of ant-associated fungi [15]. The toxicity of essential oils isolated from *Artemisia* species and containing caryophyllene oxide as a major component showed a high mortality to granary weevil [16]. However, not only compounds present in the greatest proportions are responsible for the total oil activity. The influence of the less abundant constituents and synergetic effects might also be considered.

Experimental

Plant material and oil isolation: The aerial parts (~20 cm, 15-100g) of individuals from various *A. campestris* ssp. *campestris* populations were collected at full flowering stage in July-August (2003, 2005 and 2007) from ten different localities in Lithuania. Voucher specimens were deposited in the herbaria of the Institute of Botany (BILAS), Vilnius and Vytautas Magnus University, and Kaunas Botanical Garden, and their numbers are: A-68906, B-68919, C-68913, D-68914, E-SS 657, F-SS 658, G-SS 659, H-68918, J-68911 and K-SS 660. Plant material was dried at room temperature (20-25°C). Essential oils of the air-dried aerial parts (leaves and inflorescences) were prepared by hydrodistillation for 2 h using a Clevenger-type apparatus and a mixture of *n*-pentane and diethyl ether (1:1) as a collecting solvent. Pure oils of yellow-grey color were obtained from 80-100g of dry material. Yields ranged from 0.03 to 0.08 %, v/w on a dry mass basis.

GC-MS analysis: Analyses were performed using an HP 5890 chromatograph interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV, scan time 0.6 s, scan range 35-400 Da) and equipped with a capillary column DB-5 (50 m × 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 to 160°C at a rate of 5°C/min, held for 1 min, then increased to 250°C at a rate 10°C/min and finally isothermal at 250°C for 3 min, using He as a carrier gas (1.0 mL/min), split 1:40. Injector and detector temperatures were 250°C. Qualitative analysis was based on a comparison of retention times, indexes and MS with the corresponding data in the literature [17], by co-injection of some terpene references, and from computer MS libraries (Wiley and NBS 54K).

Toxicity test: Toxicity of 3 of the *A. campestris* oils (A, H and K, plant material from different parts of Lithuania) was tested *in vivo*, using brine shrimp *Artemia* sp. (larvae) [18]. The eggs of the shrimps hatch within 48 h to provide larvae (nauplii) in sea water (31g/L sea salt) at 20-25°C. Then, different concentrations of field wormwood essential oils dissolved in dimethyl sulfoxide (DMSO) were added, and survivors were counted after 24 h. Lethality (LC_{50}) of nauplii was calculated (n=4, with 95% confidence interval). A control test was done with DMSO.

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