SHORT REPORT

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Toxic Activity and Chemical Composition of Lithuanian Wormwood (*Artemisia absinthium* L.) Essential Oils

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Abstract: Toxicity tests of wild wormwood essential oils were performed using the brine shrimp (*Artemia sp.*) test. Nauplii lethality (LC_{50}) ranged 15.7-31.9 µg/mL, depending on oil composition. The most toxic *A. absinthium* oils were found to be those containing appreciable amount of *trans*-sabinyl acetate (45.2%) and (*cis*+*trans*) thujones (12.3%), while other samples with equivalent amounts of sabinyl acetate, but without thujones were determined to be notably less toxic. Herb material for the tests was collected in Lithuania, the volatile oils were obtained by hydrodistillation from different plant organs (inflorescences and leaves) and analysed by GC-MS.

Keywords: *Artemisia absinthium*; essential oil composition; GC-MS; *trans*-sabinyl acetate; (*cis-trans*) thujones; (*Z*)-epoxy-ocimene; brine shrimp lethality.

1. Plant Source

Artemisia absinthium (wormwood, Asteraceae) is used as a medicinal remedy worldwide. The wormwood extracts possess numerous properties, such as antiprotozoal, cytotoxic, antimalarial, antipyretic, antiradical and antioxidant, antimicrobial, anthelmintic, antidepressant, neurotropic etc.

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This plant is widely used in the Lithuanian folk medicine mainly for stomachic and anthelmintic activity, also for healing gall-bladder and kidneys diseases, against insomnia.

2. Previous Studies

A long list of various biological activities, such as acaricidal, antifungal, antibacterial, antimicrobial, neurotropic etc. has been reported for wormwood essential oils [1-7]. Meanwhile, data on toxicity of wormwood oils are limited, so the aim of our study is to evaluate toxic activity of *A. absinthium* essential oils linked to the natural variations of composition of the oils from plants growing wild.

3. Present Study

The aerial parts of individuals from *Artemisia absinthium* L. populations were collected at full flowering stage (in August, 2008) in Western and South parts of Lithuania: A - Klaipeda district and B - Druskininkai, Latezeris. Voucher specimens were deposited in the herbarium (Institute of Botany (BILAS), Vilnius) with numbers: A-68929 and B-68930. The essential oils were obtained by hydrodistillation for 2 h using a Clevenger-type apparatus. Oils of dark orange-brown color were obtained and their yields ranged from 1.9 to 2.3% (v/w) on a dry weight basis.

GC/MS: Analyses by GC/MS were performed using a chromatograph HP 5890 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 5°C/min, held for 1 min, then heated from 160 °C to 250 °C at the 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 mass spectra with the corresponding data in the literature [8], co-injection of some terpenes references and computer mass spectra libraries (Wiley and NBS 54K).

GC/FID: An HP 5890 gas chromatograph, equipped with a FFAP column (30m × 0.25mm; 0.25μm) was used for quantitative analysis. The GC oven was programming from 70°C (10 min) to 250°C at a rate 3°C/min, using He as a carrier gas (0.7 mL/min). The injector and detector temperatures were 230 and 250°C, respectively.

Data on chemical composition of the different organs i.e. inflorescences and leaves of wormwood essential oils are presented in Table 1. Twenty-eight identified components (additionally, three unknown compounds) constituted up to 79.8-94.1% of total oil content. Monoterpenoids fraction was the major group of compounds in both inflorescence and leaf oils, comprising 55.2-84.7% of the oils. Amounts of oxygenated monoterpenes were higher in the inflorescence oils (≤70.7%) than that in leaves (≤60.9%). Compounds with sabinane skeleton were characteristic of all the oils. *Trans*-sabinyl acetate was the main constituent of all the oils. Thujones and (*Z*)-epoxy-ocimene were the second main compounds for Klaipeda and Druskininkai populations, respectively. The oils could be attributed to two different chemotypes: sabinyl acetate + thujones and sabinyl acetate + (*Z*)-epoxy-ocimene.

Toxicity test: Toxic activity of the wormwood oils was tested *in vivo*, using brine shrimp *Artemia sp.* (larvae) [9]. The eggs of shrimps hatch within 48 hours to provide larvae (nauplii) in sea water (31g/L sea salt) at 20-25°C. Then, different amounts of wormwood essential oils dissolved in dimethyl sulfoxide (DMSO) were added and survivors were counted after 24 hours. Lethality (LC_{50}) of nauplii was calculated (n=4, with 95% confidence interval). Control test was done with DMSO.

Test of wormwood essential oil toxicity has shown that lethality (LC_{50}) of brine shrimp larvae ranged between 15.7-31.9 μg/mL. Differences in the level of toxicity could be explained by the various quantities of main toxic constituents. The most toxic oil was defined as containing, in addition to the major constituents sabinyl acetate (45.2%) and thujones (12.3%), also appreciable amounts of myrcene (6.0%) and 1,8-cineole (5.3%).

Table 1. Main composition (over 1.0%) of inflorescence and leaf essential oils of *A. absinthium* from Lithuania

RI	Compounds	AF	AL	BF	BL
975	Sabinene	6.5	5.5	1.7	1.6
989	β-Pinene	1.5	3.5	2.0	3.0
990	Myrcene	6.0	2.0	4.6	3.8
1002	α-Phellandrene	t	t		t
1031	1,8-Cineole	5.3	4.9		
1037	(*Z*)-β-Ocimene	t	t	1.3	1.1
1096	Linalool	2.0	2.2	2.0	1.6
1102	*cis*-Thujone	7.0	6.5	t	
1114	*trans*-Thujone	5.3	4.6	t	
	(*cis*+*trans*)-Thujones	12.3	11.1	t	
1132	(*Z*)-Epoxy-ocimene	2.9	3.5	12.8	8.5
1142	*trans*-Sabinol	0.5	0.4	1.5	1.4
1164	*cis*-Chrysanthenol		t		
1177	Terpinen-4-ol	2.5	1.3	t	
1195	Myrtenol			1.6	1.2
1290	*trans*-Sabinyl acetate	45.2	37.5	40.5	33.0
1419	*trans*-Caryophyllene	1.5	1.7	1.8	2.3
1470	Acoradiene	1.0	2.0	0.9	1.2
1477	Geranyl propionate	0.3	0.5	1.7	2.0
1485	Germacrene D	0.2	0.6	1.9	2.6
1506	Unknown 1			0.4	2.9
1509	Lavandulyl isovalerate	1.1	0.8	2.4	2.3
1511	Lavandulyl 2-methyl butyrate	1.0	0.6	2.1	0.7
1515	Geranyl isobutanoate	1.1	0.4	0.4	0.5
1564	Geranyl butyrate			0.9	
1563	*trans*-Nerolidol	0.2			1.1
1583	Caryophyllene oxide	0.3		0.9	1.2
1605	Unknown 2	0.8	0.6	2.1	3.0
1615	Unknown 3	0.3	0.4	3.1	3.6
1731	Chamazulene	0.3	0.3	0.4	0.9
1756	Nuciferol acetate	0.5	1.2	1.8	
1860	Lanceol acetate	0.8	1.5	2.8	0.3
	Total	94.1	82.5	91.6	79.8
	Oxygenated monoterpenes	70.7	60.9	58.4	45.7
	Monoterpene hydrocarbons	14.0	11.0	9.6	9.5
	Monoterpenoids	84.7	71.9	68.0	55.2
	Compounds with sabinane skeleton	57.5	54.5	43. 7	36.0

RI-Retention indices on nonpolar column DB-5, t:- traces (≤0.05%). Inflorescences indicated by letter F, leaves - by L, growing localities indicated as follow: A - Klaipeda (Western Lithuania) and B - Druskininkai, Latezeris (South Lithuania).

Mass spectra of unknowns identified tentatively:

1: (M^+ 187): 157(100); 142(50); 186(43); 171(29); 141(29); 143(23); 128(19); 115(16); 156(16); 77(6); 187(6); 91(5); 51(3); 39(3); 102(1).

2: (M^+ 187): 157(100); 186(77); 143(60); 142(59); 128(47); 171(45); 141(45); 115(33); 129(32); 156(21); 77(12); 91(12); 187(11); 39(5); 41(5); 63(5); 105(4).

3: (M^+ 187): 171(100); 186(81); 143(64); 129(51); 128(49); 157(41); 142(40); 156(35); 115(34); 141(33); 91(25); 77(23); 79(16); 144(15); 172(15); 187(15); 93(13); 39(11); 41(11); 105(10); 65(9).

Since compounds present in the greatest proportions are mostly responsible for the greatest share of the total toxicity, the involvement of the less abundant constituents should also be considered. It is well known that thujone is a very toxic (mostly neurotoxic) compound, while sabinyl acetate is fetotoxic, a skin irritant and an abortifacient [10]. Other tested samples with equivalent amounts of sabinyl acetate but with appreciable amounts of (*Z*)-epoxy-ocimene (≤12.8%) and without thujones were found to be notably less toxic. Previously, it was evaluated that wormwood oils rich in (*Z*)-epoxy-ocimene have antimicrobial properties [3].

Table 2. Toxic activity of *A. absinthium* essential oils against *Artemia* sp. nauplii

Toxic activity value	AF	AL	BF	BL
LC_{50}, μg/mL	15.7	18.6	25.8	31.9
LC_{95}, μg/mL	44.2	56.9	91.2	150.3

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