CHEMIJA. 2009. Vol. 20. No. 3. P. 190–194

Analysis of the chemical composition of flower essential oils from *Arnica montana* of Lithuanian origin

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The chemical composition of essential oils of *Arnica montana* L. was investigated by GC / MS. *A. montana* is a rare and protected plant, so herb material for analysis was obtained from plants cultivated from seeds of collected in natural habitats of Lithuania. Fifty-six compounds (two of them tentatively identified) comprised 70% of the total oil content. Most of the constituents were found in minor quantities (up to 1%), while two major compounds were geranyl linalool (14.7%) and an unknown compound (13.8%). Aliphatic hydrocarbons and their derivatives comprised 11.3%, while farnesene derivatives up to 9% of the total oil. Lithuanian arnica volatile oil composition differed from that of oils of the plants from other countries.

Key words: *Arnica montana* L., Asteraceae, flowers, essential oil composition, GC / MS, geranyl linalool, farnesene derivatives

INTRODUCTION

The genus Arnica (Asteraceae family, tribe Senecioneae) comprises about 30 species. Two of them – *A. montana* and *A. chamissonis* – grow in Lithuania [1]. *Arnica chamissonis* is introduced and cultivated in our country, while *A. montana* is growing spontaneously.

Arnica montana (also commonly called leopard's bane root) is a perennial and an alpine herb native to mountains, originated from the high mountains of the Alps and the Carpathians.

The arnica plant is up to 60 cm height, has a bright yellow or orange, daisy-like flower that blooms in June–July, sometimes until the middle of August. It grows in moist, sandy, acid soils with an adequate drainage, mostly in pinewoods or mixed forests, sometimes in meadows (in mat-grass community), likes full sun. *A. montana* is not an easily recognizable plant, people very often confuse it with *Hypochoeris maculata*, *Scorzonera humilis*, *Crepis paludosa*, *Leontodon autumnale*, *Inula britanica* or even *Calendula officinalis* and / or with other plants from Asteraceae family [2].

A. montana is a medicinal plant and is widely used as a herbal remedy. For healing purposes, different plant parts such as inflorescences (Flores Arnicae), rhizomes (Rhizoma Arnicae), roots (Radix Arnicae) and leaves (Folia Arnicae) are collected. More than 100 drug preparations contain this plant as the main ingredient. Also, the plant is popular in cosmetic and liqueur industry. Preparations made from flowering heads have been used in homeopathic medicine for hundreds of years. Its flowers contain volatile oils, terpenoids, sesquiterpene lactones, flavonoids, bitter principals with antiseptic, antifungal, antimicrobial and antibiotic activities.

The most active components of all the plant are sequiterpene lactones which are known to reduce inflammation and decrease pain (this process works by stimulating white blood cell activity), show antioxidant [3] and cytotoxic effects [4]. Arnica is known to stimulate blood circulation and can raise blood pressure, especially in the coronary arteries; it is a tonic to the nervous system. It has strong anti-bacterial and anti-inflammatory properties that can reduce pain and swelling [5], improve wound healing; it is used in gynaecology. Other active principals are essential oils, flavonoids, inulin, polysaccharides, carotenoids and tannins. The most common external uses for this herb are arthritis, burns, ulcers, eczema and acne. Arnica is also mentioned as a good first aid salve for sport injuries and discomforts. The internal use of arnica is not recommended; its preparations are not used on open wounds.

Arnica is a protected plant in Europe. Due to its intensive collection from nature, it is now as a rare and endangered species in the wild. *A. montana* is a critically endangered species in Belgium, Bosnia, Croatia and Luxemburg, endangered in Belarus and the Netherlands, vulnerable in Estonia, Germany, Hungary, Latvia, Lithuania, Portugal and Romania, and nearly threatened in Denmark and Norway [6]. The plant is also included in the Red Book of Lithuania. The decrease of populations is mostly considered to be taking place as a result

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of environmental changes (land reclamation, use of fertilizers and atmospheric acidification), habitat destruction and over-exploitation for medicinal row material which is picked mainly from the wild [7].

Different bioactive secondary metabolites (sesquiterpene lactones, acids, oligosaccharides, oligofructosides, etc.) of *A. montana* have been investigated [8–14], while data on arnica volatile oils are very limited, despite that essential oils of the plant make a contribution to all plant medicinal properties. The aim of the study was to elucidate the chemical composition of the flower oil of arnica plants of Lithuanian origin.

EXPERIMENTAL

A. montana flowers were collected at the flowering stage from the Botanical Garden of Vilnius in 2004. Plant material was dried at room temperature (~20 °C) and crushed. The volatile fraction was isolated by hydrodistillation for 2 h with a Clevenger type apparatus; a mixture of hexane and diethyl ether (1 : 1) was used as a collecting solvent. Two repetitions of distillation were done. The yield of the volatile fraction was less than 0.1% (v/w) on the dry weight basis.

GC / MS analyses were performed using an HP 5890II chromatograph interfaced with an HP 5971 mass spectrometer (ionization voltage 70 eV, m/z scan rage 35–350 Da, scan time 0.6 s) and equipped with a Sil B CB capillary column (30 m × 0.32 mm, film thickness 0.25 μm). The oven temperature was kept at 60 °C for 2 min, then programmed from 60° to 160 °C at a rate of 5 °C/min, kept for 1 min and then increased up to 280 °C at the rate 10 °C/min and kept at the final temperature for 3 min, using He as a carrier gas (1.0 ml/min, split ratio 1 : 20). The injector and detector temperatures were 250 °C.

The percentage composition of the oils was computed from GC peak areas without correction factors. The qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data in the literature [15] and computer mass spectra libraries (Wiley and NBS 54K). Three repetitions of the analysis were performed.

RESULTS AND DISCUSSION

A. montana, a rare plant, grows only in the southern and eastern parts of Lithuania and is almost absolutely absent in the other parts of the country. Scientists from the Institute of Botany investigated the natural populations of arnica in Lithuania [16]. Field surveys were conducted in 42 subpopulations, where the distance between the northermost and southermost points of the arnica distribution was 121 km, and between the easternmost and westernmost points (139 km) [16]. Because arnica is rare, does not form colonies and is a protected plant in Lithuania, herb material for essential oil investigations for this study was obtained from cultivated plants (seeds collected in natural habitats).

According to the various literature data, the quantity of essential oils differs in various parts of *A. montana*, reaching 0.04–0.14% in flowers, 0.5–6.3% in rhizomes and 1.7–3.7% in roots. In our study, the yield of essential oil from arnica flowers was low – less than 0.1%.

Data on the chemical composition of volatile oils (as the average value of three repetitions) are presented in Table,

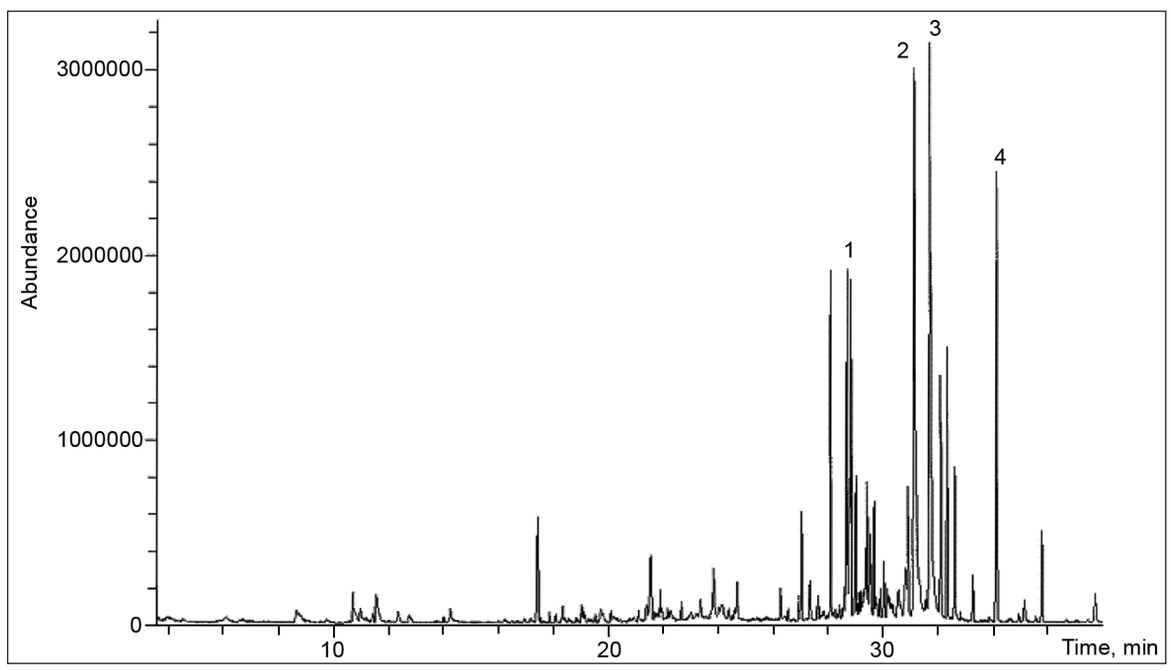

Fig. 1. Chromatogram of *Arnica montana* essential oil. Major constituents: *1* – unknown 1; *2* – (Z, Z)-geranyl linalool ?; *3* – unknown 2 and *4* – docosane

Table. **Chemical composition (%) of *Arnica montana* essential oil**

Compound	RI	A (n = 3)	SD	Compound	RI	A (n = 3)	SD
α-Thujene	930	0.1	0.14	5-oxy-Isobornyl isobutanoate	1604	0.3	0.42
n-Undecane	1100	0.2	0.28	Humulene epoxide II	1608	0.3	0.07
n-Nonanal	1100	0.2	0.28	epi-α-Muurolol	1642	0.2	0.21
trans-β-Terpineol	1163	0.1	0.14	α-Cadinol	1654	0.6	0.21
Nonanol	1169	0.1	0.07	α-Bisabolol	1685	0.6	0.28
Terpinen-4-ol	1177	0.1	0.14	Amorpha-4,9-dien-2-ol	1700	0.4	0.49
Myrtenal	1195	1.0	0	n-Heptadecane	1700	0.3	0.14
Myrtenol	1195	0.4	0.07	(2Z, 6E)-Farnesol	1701	t	0
n-Dodecane	1200	0.2	0.07	Pentadecanal	1710	0.2	0.14
n-Decanal	1201	1.0	0	(2E, 6Z)-Farnesol	1713	0.2	0.07
Thymol, methyl ether	1235	0.3	0	1-Hexadecanal	1715	0.3	0.14
trans-Sabinyl acetate	1290	0.1	0	6, 10, 14-trimethyl-2-Pentadecanone	1720	1.6	0.21
Thymol	1290	0.4	0.07	(2Z, 6E)-Farnesol	1723	0.4	0.14
β-Caryophyllene	1419	1.9	0.14	(2E, 6E)-Farnesol	1743	4.6	0.30
2,5-dimetoxy-p-Cymene	1426	0.1	0	β-Bisabolenal	1769	0.4	0.21
α-trans-Bergamotene	1434	0.2	0	β-Bisabolenol	1789	0.8	0.70
α-Humulene	1454	0.4	0.07	n-Octadecane	1800	0.8	0.35
(E)-β-Fernesene	1456	0.1	0.07	(2Z, 6E)-Farnesyl acetate	1822	2.1	0.07
Germacrene D	1485	0.4	0.07	Unknown 1	1830	4.5	0.28
Neryl isobutanoate	1491	0.1	0.07	(2E, 6E)-Farnesyl acetate	1846	1.7	0.49
n-Pentadecane	1500	0.1	0.14	(Z, Z)-Geranyl linalool ?	1961	14.7	0.57
(E,E)-α-Farnesene + β-Bisabolene	1505	0.3	0.07	Unknown 2	1980	13.8	0.92
(Z)-Nerolidol	1532	0.3	0	(Z, E)-Geranyl linalool ?	1998	3.2	0.07
(E)-Nerolodol	1563	0.2	0.21	n-Eicosane	2000	4.5	2.05
Spathulenol	1578	0.3	0.35	n-Heneicosane	2100	0.9	0.57
Neryl isovalerate	1583	0.3	0.35	Docosane	2200	3.0	3.54
Caryophyllene oxide	1583	1.6	0.07	Total		70.0	3.04
Salvial-4(14)-en-1-one	1594	0.2	0.10	Farnesene derivatives		9.0	
n-Hexadecane	1600	0.2	0.14	Aliphatic hydrocarbons and their derivatives		11.3	

RI – Retention index on a nonpolar CP-Sil 8CB column, t – traces (<0.05%), SD – standard deviation.

while a chromatogram of arnica essential oil is shown in Fig. 1. Fifty-six identified constituents, two of them tentatively and two geranyl linalool isomers with a query (mass spectrum of them is shown in Fig. 2) comprised 70% of the total oil content. Also, additionally about 5% of artifact, phthalate (from the environment), was identified in the oils. A large part of the determined compounds are present in small proportions (up to 1%). The main constituents were found to be geranyl linalool (?) and two unknown compounds, in amounts of 14.7% and 13.8%, respectively (Table). According to the similarities between the mass spectra of unknown compounds 1 and 2 (Fig. 2), it could be concluded that these constituents have a related chemical structure. Appreciable amounts of myrtenal (1.0%), caryophyllene (1.9%) and its oxide (1.6%), various isomers of farnesols, farnesyl acetates (total quantity of farnesene derivatives was 9.0%) and some aliphatic compounds (≤4.5%) were also determined in the oil. Aliphatic compounds and their derivatives comprised 11.3% of the total oil content; their origin could be the inflorescence surface waxes. In general, most constituents were oxygenated compounds (mostly alcohols).

The chemical profile of *A. montana* oils from Lithuania, determined in the study, differed from the data on the arnica volatile oils from other countries. Analysis of the essential oils (three samples) from flowers of *A. montana* grown on the Tara Mountains (in Serbia) has shown that the most abundant constituents were β-caryophyllene (31.5–34.6%), germacrene D (12.5–16.3%), trans-β-ionone (3.9–4.3%) and decanal (2.7–5.3%) [17]. The composition of essential oil of flowers, leaves and roots of arnica from Norway was investigated [18]. In the above study, the main constituents of flowers were found to be monoterpenes α-pinene (25.3%), β-myrcene (9.5%), p-cymene (8.7%) and the sesquiterpene germacrene D (8.1%). Appreciable and highest amounts of species-characteristic terpenoid phenols (thymol and its derivatives) were detected in the roots (the main constituent was thymol isobutyrate, 20.0%) [18]. The essential oil of commercial arnica contains as the main components

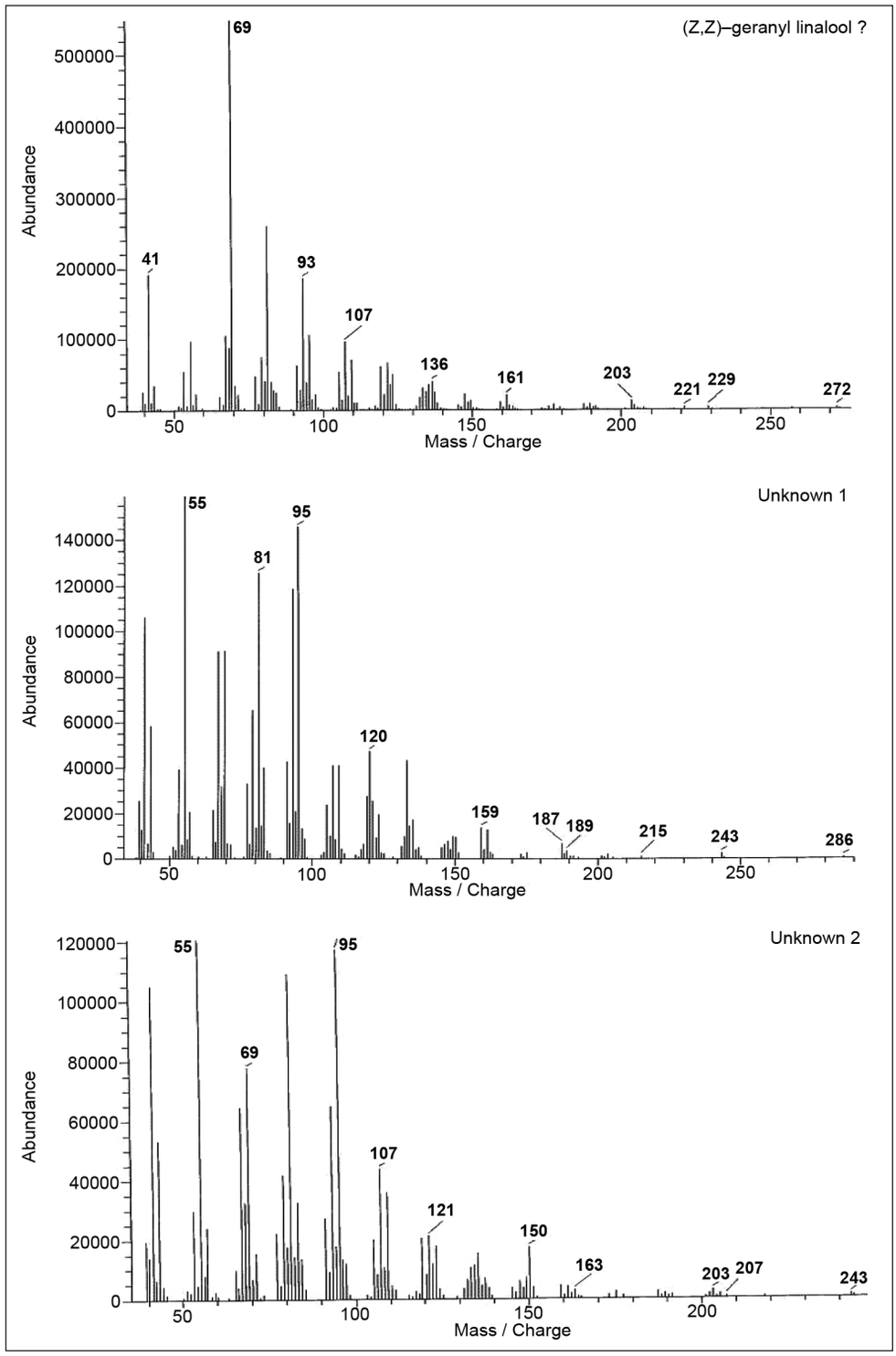

Fig. 2. Mass spectrum of unknown and main constituents

caryophyllene (70%) and eugenol (10%) [19]. Only about 50 kg per year of *A. montana* oil (distilled, absolute and concrete) produced in Croatia is presented to the world oil market, and the oil is little used in flavours.

Even a very limited number of investigations of *A. montana* volatile oils from different countries has shown the existence of their chemical polymorphism.

ACKNOWLEDGEMENTS

Authors thank Dr. J. Radušienė from the Institute of Botany for arnica raw material.

Received 20 March 2009
Accepted 17 April 2009

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LIETUVOJE AUGANČIOS KALNINĖS ARNIKOS (*ARNICA MONTANA* L.) ŽIEDŲ ETERINIŲ ALIEJŲ CHEMINĖS SUDĖTIES ANALIZĖ

S a n t r a u k a

Išanalizuoti kalninės arnikos žiedų eteriniai aliejai. Analizėms žaliava paimta iš Botanikos sode kultivuotų augalų iš laukinių sėklų, surinktų natūraliose Lietuvos augavietėse. Lakioji frakcija išskirta panaudojus hidrodistiliaciją Klevendžerio tipo aparatu kartu su ekstrakcija tirpikliais. Kiekybinė ir kokybinė analizė atlikta dujų chromatografijos–masių spektrometrijos metodu. Penkiasdešimt šeši junginiai sudarė 70 % viso aliejaus kiekio. Daugelio nustatytų junginių rasta nedideliais kiekiais (iki 1 %), o vyraujančių pagrindinių komponentų – geranilo linalolio (14,7 %) ir neidentifikuoto junginio (13,8 %). Alifatiniai angliavandeniliai ir jų dariniai sudarė 11,3 %, o farnezeno dariniai – iki 9 % aliejaus. Lietuviškos arnikos eterinio aliejaus tipas skiriasi nuo kitose šalyse augančių augalų.