BIOLOGICAL ACTIVE COMPOUNDS FROM ROOTS OF *VALERIANA OFFICINALIS* L. CULTIVATED IN SOUTH-EASTERN REGION OF POLAND

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Abstract

The presented studies concern the determination of content and chemical composition of essential oil from plants of the *Valeriana officinalis* L. ('Lubelski' common valerian) cultivar grown in the south-eastern region of Poland, as well as the contents of valerenic acids, protein and ash in the discussed raw material. In the examined oil, the presence of 71 compounds was determined, and the predominant compound was bornyl acetate (15.42%), followed by elemol (8.01%), β-gurjunene (6.20%) and camphene (5.43%). The contents of sesquiterpene – valerenic and acetoxy valerenic acids in the examined valerian raw material was respectively: 0.0519 and 0.0677%. The total valerenic acid contents (0.1196%) are comparable to other Polish cultivars.

Rezumat

Studiul de față prezintă determinarea conținutului și a compoziției chimice a uleiului volatil din *Valeriana officinalis*, plante recoltate din sud-estul Polonieie, precum și conținutul în acizi valerianici, proteine și cenușă a materialului vegetal analizat. În uleiul esențial au fost puși în evidență 71 de compuși, cel mai abundent fiind acetatul de bornil (15,42%), urmat de elemol (8,01%), β-gurjunene (6.20%) și camfen (5,43%). Conținutul în sesquiterpene valerianice și acizi acetoxi valerianici în materialul vegetal studiat a fost de 0.0519% respectiv 0.0677%. Conținutul total în acizi valerianici (0.1196%) este comparabil cu plante obținute în alte regiuni din Polonia.

Keywords: valerian essential oil, valerenic acids, bornyl acetate.

Introduction

The genus common valerian of *Valerianaceae* family is distinguished by a large number of species, sub-species and local ecotypes, which are morphologically and chemically differentiated [5, 6, 8]. The most important species that finds its application in European therapeutics is common valerian (*Valeriana officinalis* L.), for which different chemotypes are described [18, 22]. The root of common valerian has been long known as a sedative, especially recommended in the states of nervous hyperexcitability, insomnia and anxiety [5, 7]. Sleep disorders occur in about 20-40% of the population of Western European countries, and the occurrence of insomnia or problems with falling asleep increase with age

[14]. The main biologically active compounds in valerian include valepotriate, essential oil, sesquiterpenes, flavonoids, phenolic acids, as well as lignins, and so far therapeutic properties of that raw material have not been ascribed to any particular group of compounds [10, 14]. The valerian root contains about 0.5% of valepotriates, compounds with triester character and with iridoid skeleton. The valerian essential oil, occurring in the raw material in variable amounts, contains terpenes, derivatives of guaials, borneol derivatives, valeric acid, valerenal and its composition depends upon the origin of raw material and the technology of obtaining the oil [23]. Most of pharmacopoeias give the contents of 0.5% for the essential oil and 0.17% for valerenic acid and its derivatives [5].

The essential oil and sesquiterpene acid contents in valerian root are highly genetically and ontogenetically determined [18, 24], but they are also affected by agrotechnical and climatic factors [2, 12, 15, 17, 22, 23]. Anti-anxiety and anti-oxidation activity of valerian extract is most probably related to the presence of valerenic acid [8, 10]. The studies reveal that the extract must be standardized due to total contents of valerenic acids, that is the total amount of valerenic and acetoxy valerenic acid [10]. The biological value of herbal materials is also connected with the presence of nutritional components, including proteins and mineral compounds. One of the indicators of food products quality assessment is the content of ash. The determination of this component's content constitutes a basis for estimating the contents of mineral compounds, including elements indispensable for the normal functioning of human organism. The essential oil and valerenic acid seem to be the most important active components of common valerian raw material, significant in the process of its standardization. The presented studies concern the determination of content and chemical composition of common valerian essential oil from plants grown in the south-eastern region of Poland, as well as the contents of valerenic acids, protein and ash in the studied raw material.

Materials and Methods

Plant material

The roots of 'Lubelski' common valerian cultivar were obtained from the herbalist plantation situated in the region of Michów rural administrative district (51°31'26''N, 22°18'47''E), province of Lublin. The grown cultivar 'Lubelski', which belongs to the species of wide-leafed common valerian (*Valeriana officinalis* L. var. *latifolia*), is distinguished by large number of roots with appropriate diameter and weight, as well as high concentration of essential oil [3]. The valerian roots were harvested on the

20th day of October 2012, then the material was dried in the thermal drying store (35°C), and after drying, on sieves, the residuals were crumbled. The valerian root weight (g) was determined before and after drying, on the basis of a randomly selected sample of 40 plants.

Essential oil isolation

The essential oil content in raw material was determined through distillation with water vapor [9]. 40 g of dried and freshly grounded raw material was placed in a 2 L flask and imbibed with 500 mL of redistilled water with the addition of 0.50 mL of xylene. Distillation was conducted for 4 hours with the speed of about 3 mL/min. The analysis was performed in triplicate. The obtained oil was stored for further chemical determinations in a dark glass container, at the temperature of 10°C.

Essential oil analysis

The quantitative and qualitative composition of valerian essential oil was determined by means of GC-MS method, using a gas chromatograph Varian Chrompack CP-3800 with weight detector (4000 GC-MS/MS). The temperature of 50°C was applied for 1 min, and then 250°C for 10 min, with the speed of 4°C/min. The VF-5ms column was used (an equivalent of DB-5) (J&W, USA); the carrier gas was helium (He), with steady flow of 0.5 mL/min; sample injector: 250°C, division 1:100. We assessed 1 μL of the solution (10 μL of the sample in 1000 μL of hexane). Varian 4000 MS/MS detector was applied, the recording range: 40-1000 m/z, scanning speed: 0.8 sec *per* scan. Kovats's retention indices (non-isothermal retention index) were determined on the basis of a series of C_{10}-C_{40} alkanes [26]. The content of a component in mg of the analyzed oil sample was determined by comparing with internal standards. Compounds were identified on the basis of authors' own retention indices and literature [1].

Analysis of sesquiterpene acids

The content of valerenic ($C_{15}H_{22}O_2$) and acetoxy valerenic ($C_{17}H_{24}O_4$) acids was determined by means of HPLC method. The ground plant material was subjected to extraction with the use of methanol. The extract was analysed by HPLC [9] and at the same time the chromatographic analysis was conducted with addition of an internal standard (1,8 dihydroxyantrachinone).

Raw protein and total ash contents

Dried valerian raw material was assessed for protein and total ash contents. Raw protein content was determined using Kjeldahl's method: having mineralized the sample, distilled and titrated ammonia to change the colour (pH = 4.3), we multiplied the determined total nitrogen quantity by coefficient 6.25. Total ash quantity was determined by weight method [19],

after grinding a 2 g sample and burning it at the temperature of 550±25°C to constant weight.

Results and Discussion

Essential oil content and chemical composition

Through distillation with water vapour we obtained from the examined plant material a yellowish essential oil with a characteristic valerian scent, with the yeld of 2.06% (Table I).

Table I

Essential oil content and composition of valerian roots

No	Component	LRI	Peak area (%)
1	α-Thujene	924	0.07±0.00
2	Thuja-2,4(10)-diene	927	0.06±0.00
3	α-Pinene	935	1.40±0.06
4	α-Fenchene	950	2.22±0.22
5	Camphene	952	5.43±0.25
6	Sabinene	977	0.23±0.01
7	β-Pinene	981	1.13±0.05
8	β-Phellandrene	1037	3.04±0.20
9	Isopentyl-2-methyl butanoate	1125	0.06±0.02
10	Isopenthyl isovalerate	1127	0.06±0.05
11	Terpinen-4-ol	1202	0.29±0.06
12	Myrtenol	1240	0.06±0.00
13	Bornyl acetate	1292	15.42±0.38
14	cis-Pinocarvyl acetate	1305	0.15±0.01
15	Myrtenyl acetate	1334	3.90±0.04
16	δ-Elemene	1341	1.32±0.12
17	N.i.	1355	0.51±0.12
18	Terpinyl acetate	1359	0.68±0.08
19	Longicyclene	1377	1.00±0.01
20	α-Copaene	1383	0.45±0.03
21	β-Panasinsene	1388	0.22±0.02
22	β-Elemene	1396	0.63±0.01
23	β-Longipinene	1402	0.21±0.02
24	α-Funebrene	1406	Tr
25	Longifolene	1415	3.07±0.00
26	α-Gurjunene	1420	Tr
27	E-Caryophyllene	1429	3.82±0.04
28	N.i.	1433	Tr
29	β- Gurjunene	1440	6.20±0.05
30	Aromadendrene	1444	0.06±0.01
31	Thujopsadiene	1447	1.69±0.03
32	9-epi-(E)-Caryophyllene	1460	4.77±0.01
33	α-Acoradiene	1466	0.16±0.00
34	γ- Gurjunene	1470	3.62±0.04
35	N.i.	1479	Tr
36	γ-Curcumene	1488	1.32±0.13
37	Germacrene D	1491	2.50±0.29
38	γ-Amorphene	1496	0.25±0.02

No	Component	LRI	Peak area (%)
39	Viridiflorene	1501	0.66±0.03
40	Bicyclogermacrene	1506	4.61±0.07
41	δ-Amorphene	1514	0.09±0.01
42	β-Curcumene	1521	0.10±0.03
43	γ-Cadinene	1524	0.06±0.01
44	δ- Cadinene	1528	1.14±0.00
45	Kessane	1542	0.69±0.05
46	α-Cadinene	1549	0.08±0.07
47	cis-Sesquisabinene hydrate	1556	1.24±0.03
48	Germacrene B	1572	0.92±0.03
49	Elemol	1585	8.01±0.21
50	Spathulenol	1594	1.66±0.04
51	Caryophyllene oxide	1603	0.23±0.00
52	Globulol	1612	0.08±0.01
53	N.i.	1617	0.05±0.02
54	Viridiflorol	1622	0.27±0.07
55	Khusimone	1651	0.84±0.24
56	Eremoligenol	1662	0.49±0.12
57	epi-α-Muurolol	1674	0.44±0.05
58	Cubenol	1679	0.79±0.07
59	epoxy-allo Alloaromadendrene	1693	0.24±0.08
60	14-hydroxy-9-epi(E)-Caryophyllene	1702	Tr
61	Germacrone	1731	4.57±0.02
62	N.i.	1743	0.08±0.00
63	14-hydroxy-α-Humulene	1755	Tr
64	Aristolone	1786	0.08±0.00
65	hydroxy-α-Muurolene	1790	0.07±0.02
66	8-Cedren-13-ol acetate	1815	0.21±0.02
67	8-α-Acetoxyelemol	1820	3.71±0.04
68	Khusinol acetate	1841	0.20±0.00
69	Z-Lanceol acetate	1859	0.06±0.00
70	8S,14-Cedranediol	1918	2.01±0.00
71	N.i.	2069	0.09±0.00
	Total	99.78%	
	Essential oil content (%)	2.06±0.10	

Notes: LRI, linear retention index as determined on a GC-5MS column using the homologous series of n-alkanes; N. i. – not identified; Tr, trace amount <0.05%

The determined essential oil quantity was high [9] and proved a good quality of the raw material. The studies by Raal et al. [22] demonstrated that the roots of valerian grown in Estonia contain from 0.28 to 1.16% of oil. The raw material from Lithuania in turn, had an average content of oil of 0.55% [2]. Comparing productivity and essential oil contents in the roots of various valerian genotypes [20], indicated the Polish cultivar Munka, as the richest in essential oil (1.19%). Most authors emphasize a high variability in valerian essential oil contents, which is caused by genetic, ontogenetic and agrotechnical factors. Valerian roots may contain from 0.8 to 1.3% of oil, depending on raw material harvesting phase [24], and from 0.2 to 4.9%, depending on the method of extraction [23]. Besides, Morteza et al. [17]

found increased oil content in valerian roots from 1.00 to 1.69%, as sowing term was delayed and decreased content of that substance from 1.74 to 1.06%, as plant density increased. The results of the foregoing study confirm the above theses. In the examined oil the presence of 71 compounds was determined, 65 of which were identified (Table I, Figure 1).

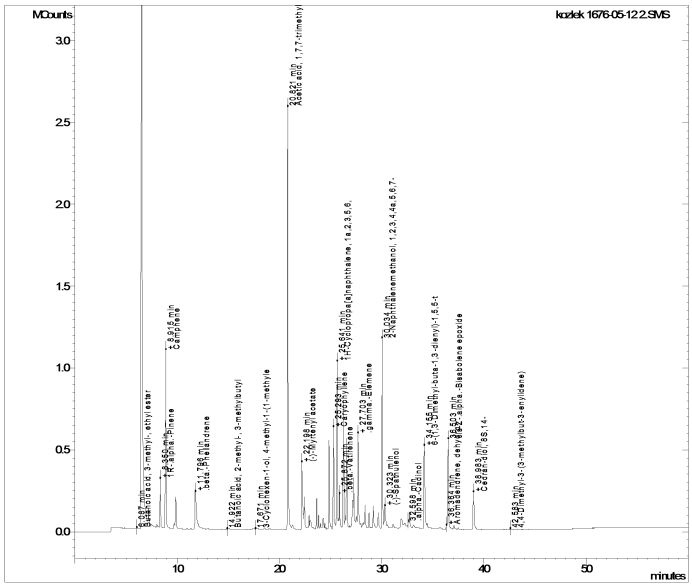

Figure 1
GC-MS chromatogram of the valerian essential oil

The predominant compound in the essential oil obtained from valerian roots of 'Lubelski' cultivar was bornyl acetate (15.42%). Besides, the following were present in the oil in larger quantities: elemol (8.01%), β-gurjunene (6.20%), camphene (5.43%), 9-epi-E-caryophyllene (4.77%), bicyclogermacrene (4.61%), germacrone (4.57), myrthenyl acetate (3.90%), *E*-caryophyllene (3.82%), 8-α-acetoxyelemol (3.71%) and γ-gurjunene (3.62%); 5 compounds occurred in trace amounts. Valerian essential oil composition is different in particular species and cultivars [5, 22]. Usually the predominant oil component of *Valeriana officinalis* ssp. *officinalis* is bornyl acetate (10.6-15.4%) [2, 15, 23].

The predominance of that compound in valerian oil was found in genotypes from Estonia, France, Latvia, Lithuania, Moldavia and Russia [22]. Bornyl acetate is also one of the main components of essential oil obtained from the roots of *Valeriana officinalis* L. var. *latifolia* and *Valeriana hardwickii* Wall. (respectively: 22.55 and 11.2%) [6, 12]. The essential oil of Chinese valerian in turn, is characterized by a significantly lower content of bornyl acetate (6.73%) and as an important content of patchoulol (16.75%) and α-pinene (14.81%) [28]. Valerianol was predominant in the essential oil of common valerian plants from

Ukraine (18.2%), valerenal - in the oil of plants from France and Russia (respectively: 14.7 and 15.6%), and α-fenchen (28.3%) in the oil of Russian genotype [22]. The content and composition of common valerian essential oil undergoes not only genetic, but also environmental variability [12, 18, 22]. Growing conditions affect plant growth and raw material yield, as well as they modify the chemical composition of essential oil [17, 29]. The concentration of bornyl acetate in valerian essential oil was the highest (32.1%) in the case of flow system compared to aeroponic growing in a substratum or soil [29]. Besides, later sowing term and lower valerian plant density in plantation enhance the accumulation of oil camphene, bornyl acetate and valerenal [17]. The above relationships are most probably caused mainly by climatic factors. The fact that in the foregoing studies we obtained a high content of bornyl acetate (15.42 %), which is one of the most pharmacologically important components of valerian oil (15.42%) [14], might therefore be caused by genetic factors, but also connected with planting term and density of plants in the plantation [17].

Sesquiterpene acid contents

The contents of sesquiterpene – valerenic and acetoxy valerenic acids in the examined valerian raw material was respectively: 0.0519 and 0.0677% (Table II).

Table II

Characteristics of valerian roots

Weight of valerian roots (g)		Valerenic acid	Acetoxy valerenic acid	Total acids content	Crude protein	Total ash
		(%)			(% dry mass)	
551.2	134.5	0.0519	0.0677	0.1196	6.23	6.93

The contents obtained in the foregoing studies are not high [9], however, the total valerenic acid contents (0.1196%) are comparable to other Polish cultivars [24]. Valerenic acid contents in plants of *Valerianaceae* family range from 0.018 to 0.075 g *per* 100 g dry mass, depending on genotype [8]. The contents of the above mentioned compounds in the roots of common valerian is first of all dependent upon the harvest phase and growing conditions [8, 24], but it is not genotypically differentiated [24]. Regarding the quantity of raw material yield two valerian harvesting periods are recommended: late autumn in the first year of vegetation and early spring in the second year of vegetation. At the latter time, however, the lowest essential oil and sesquiterpene acid contents are found [24]. On the other hand, however, the level of valerenic acids can be very variable in particular years [4] and dependent upon genotype [8, 24]. Letchamo et al. [15] report that with the age of valerian plants the concentrations of valerenic acid, valerenal and α-humulene increase in their roots. Thus, most probably, larger amounts of sesquiterpene acids in the roots of 'Lubelski' valerian can be obtained in the second year of vegetation.

Weight of valerian roots and contents of protein and ash

The analysed material was characterized regarding the content of raw protein (N-total × 6.25) in the amount of 6.23% dry weight. The level of protein in valerian roots was higher than in some other medicinal plants [13, 21]. Accumulating protein by plants undergoes genetic, ontogenetic and environmental variability. The total contents of ash in the examined valerian raw material were on average 6.93% dry weight. That level did not exceed the recommended content [11], was comparable to the contents of ash in other medicinal plants [13, 16, 21, 27] and proved a good quality of the material. The studies of Stef et al. [25] reveal that valerian roots may contain even up to 10.07% of ash, which is most probably connected with the plant growing conditions (soil, fertilization, watering), but it can also result from a given genotype's predisposition for accumulating mineral components. On the basis of the above analysis the 'Lubelski' cultivar of valerian can be regarded as interesting also regarding the contents of protein and mineral components and important nutritional substances.

Conclusions

The roots of 'Lubelski' common valerian cultivar are of high biological value, due to appropriate contents of essential oil, valerian acids, ash and protein. The analysed raw material fully corresponds to the European standards, especially in the aspect of essential oil contents. Comparing the results of our studies with the achievements of other authors one can notice a high variability of biologically active substances of common valerian under the influence of environmental and developmental factors, as well as significant genetic determination of the chemical composition of the raw material.

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