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VOLATILES CONSTITUENTS FROM THE LEAVES, FLOWERS AND STEMS OF *CENTAUREA VLACHORUM* HARTVIG (*ASTERACEAE*), GROWING WILD IN ALBANIA

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Abstract

Centaurea vlachorum Hartvig is a rare endemic species known from only two mountains, Milea and Aftia of Northern Pindos, but in 2009 it was reported to occur also in Albania too. *Centaurea* species have been used in folk medicine, against menstrual disorders, vaginal candidiasis, as laxative, tonic, diuretic, expectorant and stimulant for liver and gallbladder function. Recently, some *Centaurea* species have been used in cosmetic preparations. So far, the volatile components of the aerial parts of *C. vlachorum* have not been analyzed.

The volatile constituents of the aerial parts (flowers, leaves and stems) of *C. vlachorum* were obtained by hydrodistillation and analyzed by gas chromatography-mass spectroscopy (GC-MS).

The volatiles of their components were represented mainly of oxygenated sesquiterpenes (63.1%, 61.3% and 46.9%) for leaves, flowers and stems, respectively. The major compounds resulted as follows: for the leaves - caryophyllene oxide (18.2%), spathulenol (15.6%), (E)-nerolidol (7.8%) and humulene epoxide II (7.5%), for the flowers - caryophyllene oxide (26.7%), spathulenol (15.7%), n-heneicosane (9.3%), and methyl linoleate (8.1%), and for the stems the major constituents were caryophyllene oxide (11.9%), spathulenol (9.5%), 13-epi-malool oxide (7.9%) and n-heneicosane (6.0%). Caryophyllene oxide was the most abundant constituent among all in the ratios of 11.9%, 18.2% and 26.7% from stems, leaves and flowers, respectively.

C. vlachorum is a completely unexploited plant, because of its rareness and inaccessibility, as it is restricted to high-altitude Albanian and Greek mountains. Our results reveal the first evidence for the volatile oil composition of leaves, stems and flowers of this species, and contribute to understanding its chemical profile. More investigations are in progress from our research group concerning the study of its non-volatile secondary metabolites.

***Key words**: Centaurea vlachorum Hartvig, Volatile oils, GC-MS.*

1. Introduction

The large polymorphous genus *Centaurea* (*Asteraceae*, *Cardueae*) comprises about 500 species, and it includes grassy plants, from annual to perennial, rarely suffruticose. Since antiquity, many *Centaurea* species have been used in folk medicine, menstrual disorders, vaginal candidiasis, as laxative, tonic, diuretic, expectorant and stimulant for liver and gallbladder function. Recently, some *Centaurea* species have been used in cosmetic preparations (Blumenthal [1]).

Centaurea vlachorum is a rare endemic species known from only two mountains, Milea and Aftia of Northern Pindos (Hartvig [2]), but in 2009 Shuka and Tan reported its presence in Albania, too (Shuka *et al.,* [3]). It has always been found associated with serpentine and can therefore be considered as a serpentine endemic. It is found near Lura Lake region at altitudes of 1600 - 2000 m, and the populations were local but abundant and luxuriant.

Since its description, the systematic placement of the species has been intriguing. According the botanists' *C. vlachorum* is very rare and its position in section *Jacea*

is still intriguing, but Hartvig described that it's close with two species, *C. Rhaetica* Moritzi and *C. prutensis* Thuill. To the best of our knowledge, there are no reports in the literature concerning the chemical profile of *C. vlachorum*. In continuation of our research efforts on this genus (Lazari *et. al.,* [4 and 5]), we report here the chemical analysis of the volatile oils from the aerial parts of *C. vlachorum*.

2. Materials and Methods

2.1 Plants and essential oils

The aerial parts were collected during its flowering season from Lura lake region, in early July 2011. The plant was taxonomically identified by Prof. Shuka L. (Faculty of Natural Sciences, Tirana, Albania) and a voucher specimen was deposited in the herbarium of the Faculty of Natural Sciences, Tirana, under the code Shuka 2017-2020 (TIR). The plant material was separated into: leaves, stems, flowers and seeds before air-drying.

2.2 Distillation and analysis of the volatile fraction

Air-dried leaves, flowers and stems were cut in small pieces, and 50 g of each sample were submitted to hydrodistillation for 2 hours using a Clevenger-type apparatus. The volatiles were trapped in 5 mL GC grade n-pentane, according to a standard procedure described in European Pharmacopoeia and dried over anhydrous sodium sulphate and kept in closed, air-tight Pyrex containers at 4 ^{0}C. Essential oil yield was expressed in mL 100^{-1} g d.w. The composition of the volatile constituents was established by GC-MS analyses.

2.3 Gas chromatography-mass spectrometry

Essential oil analyses were performed on a Shimadzu GC-2010-GCMS-QP2010 system operating at 70 eV. This was equipped with a split/splitless injector (230 ^{0}C), and a fused silica HP-5 MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 *μm*). The temperature program was from 50 ^{0}C to 290 ^{0}C, at a rate of 4 ^{0}C/min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injection volume of each sample was 1 *μL*. Retention indices for all compounds were determined according to Van den Dool and Kratz (Van den Dool and Kratz [6]), using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 (Massada [7]), and by comparison of their retention indices with literature data (Adams [8]). Component relative concentrations were calculated based on GC peak areas without using correction factors. Essential oils were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

3. Results and Discussion

The volatile oils, with pale yellow color and viscous, yielded: 0.04%, 0.08% (w/w) and traces, for leaves, flowers and stems, respectively. Table 1 gives the percentage amounts of identified compounds of all the volatile oils as long as the percentage amounts of some group compounds in the same samples.

In total 25 compounds were identified, amounting to 74.9%, 88.4% and 77.1% of the total oils, of leaves, flowers and stems, respectively. All the oils were composed mainly of oxygenated sesquiterpenes, with caryophyllene oxide being the dominant compound (11.9%, 18.2% and 26.7% from stems, leaves and flowers, respectively), following by spathulenol (9.5%, 15.6% and 15.7% from stems, leaves and flowers, respectively) and (*E*)-nerolidol (5.1%, 7.8% and 7.2% from stems, leaves and flowers, respectively).

Methyl hexadecanoate and methyl linoleate were present in all volatile oils from the different tissues of *C. vlachorum,* being the dominant components of the fraction of fatty acid esters, mainly in leaves (6.2% and 8.1%, respectively). Furthermore, the hydrocarbon n-heneicosane was one of the major components of the volatile oil from flowers and stems (9.3% and 6.0%, respectively), while was absent from leaves. Manoyl oxide (3.9%) and 13-epi-manool oxide (7.9%) were identified as major diterpenes of the volatile oil from stems, while they were absent in the oil from leaves and flowers.

4. Conclusions

- *C. vlachorum* is a completely unexploited plant, because of its rareness and inaccessibility, as it is restricted to high-altitude Albanian and Greek mountains. Our results reveal the first evidence for the volatile oil composition of leaves, stems and flowers of this species, and contribute to understanding its chemical profile.

- More investigations are in progress from our research group concerning the study of its non-volatile secondary metabolites.

5. References

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Table 1. Composition of the volatiles oils of *C. vlachorum*: leaves (L), flowers (F) and stems (S)

Compounds [a]	AI [b]	L	F	S	ID [c]
n-Nonane	900	0.1	0.5	0.5	AI, MS
α-Copaene	1376	0.2	nd	nd	AI, MS
(E)-β-Damascenone	1385	0.7	nd	0.5	AI, MS
α-Ambrinol	1406	0.8	nd	0.4	AI, MS
β-Ionone	1487	2.6	nd	1.0	AI, MS
Germacene B	1552	nd	1.4	1.0	AI, MS
(E)-Nerolidol	1569	7.8	7.2	5.1	AI, MS
Longicamphenylone	1570	2.8	nd	nd	AI, MS
Spathulenol	1580	15.6	15.7	9.5	AI, MS
Caryophyllene oxide	1585	18.2	26.7	11.9	AI, MS, Co-GC
Salvial-4(14)-en-1-one	1596	5.6	1.9	3.8	AI, MS
(Z)-Sesquilavandulol	1607	1.3	nd	0.8	AI, MS
Humulene epoxide II	1611	7.5	2.8	5.5	AI, MS
trans-Isolongifolanone	1625	1.1	nd	0.7	AI, MS
Caryophylla-4(12),8(13)-dien-5a-ol	1637	1.1	nd	1.9	AI, MS
Heptan-2-one-6-methyl-6-(3-methylphenyl	1639	0.9	1.4	1.1	AI, MS
α-Santalol	1674	1.5	5.6	3.0	AI, MS
Mustakone	1681	1.9	nd	1.8	AI, MS
Germacra-4(15),5,10(14)-trien-1-α-ol	1689	0.6	nd	1.8	AI, MS
Methyl hexadecanoate	1930	1.3	6.2	4.2	AI, MS
Manool oxide	1995	nd	nd	3.9	AI, MS
13-epi-Manool oxide	2017	nd	nd	7.9	AI, MS
Methyl linoleate	2098	1.7	8.1	4.8	AI, MS
n-Heneicosane	2100	1.6	9.3	6.0	AI, MS
n-Tricosane	2300	nd	1.6	nd	AI, MS
Total		**74.9**	**88.4**	**77.1**	
Monoterpene Hydrocarbons		-	-	-	
Oxygenated Monoterpene		-	-	-	
Sesquiterpene Hydrocarbons		0.2	-	-	
Oxygenated Sesquiterpene		63.1	61.3	46.9	
Hydrocarbons		1.6	10.9	6.0	
Fatty acid esters		3.0	14.3	9.0	
Diterpenes		-	-	11.8	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b]AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c]Identification method: AI = Arithmetic Index, MS = Mass Spectrum, Co-GC=Coinjection with authentic compound, nd = non detected.

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