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Micromorphological Traits and Essential Oil Contents of the Aerial Parts of *Valeriana tuberosa* L.

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***Abstract*—The chemical composition of the essential oil obtained by hydrodistillation of the aerial parts of *Valeriana tuberosa* was characterized by GC and GC/MS. The main components in the oil were iso-valerianic acid (17.2%) and the geranil-isovalerate (12.2%). Overall, forty constituents were detected in the essential oil representing 94.1% of the total oil composition. Two types of glandular trichomes: peltate (one basal epidermal cell, one short stalk cell and a small head) and long glandular trichomes (one basal epidermal cell, one elongated stalk cell) were observed on leaf of *V. tuberosa*.**

Index Terms*—isovaleric acid, essential oil, GC-MS, glandular trichomes, *Valeriana tuberosa

I. Introduction

Valeriana tuberosa L. (Valerianaceae) is perennial plant, well-known in folk medicine due to numerous biological effects. The plant grows natively in southern and central Europe in most of the Iberian Peninsula, the Mediterranean region to the Caucasus, southwest Asia and in North Africa The plant is common in the Mediterranean region, occurring from Portugal to Greece, Spain, Corsica, Italy and Sicily Flowering period of *V. tuberosa* is from May to August. The plant grows in a humid to sub-arid Mediterranean climates [1], [2].

Herbal drugs play an important role in health care programs especially in developing countries. Important biological activities of *V. tuberosa* made this plant very popular in traditional madicine. The infusion of dried roots in water is used as antispasmodic and sedative drug, facilitates sleep disorders, anxiety and gastrointestinal spasms, and facilitates bowel movement and kidney problems [1]. The crude drug *Valerianae* radix and the valerian-derived phytomedicines are used as mild sedatives. The pharmacological activity of the *Valerianae* radix has been attributed to two major groups of constituents, the valepotriates and the sesquiterpenes [3], [4]. Despite the positive effects of this species to human health, the existing literature data dealing with the composition of essential oil within the genus *Valeriana* are very scarce.

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The types and distribution of trichomes are of great interest for taxonomic investigations. Glandular trichomes are the site of essential oil biosynthesis, secretion and accumulation and they have been investigated by many authors [5]-[7]. According to our knowledge, glandular trichomes of *V. tuberosa* are not described. With this background, results of this work will improve knowledge concerning genus *Valeriana* and improve standardization of this plant material to be used as a medicine. Here we report chemical composition of the essential oil from the aerial parts (stems, leaves) of *V. tuberosa* and types of leaf glandular trichomes.

II. Material and Methods

A. Plant material

Randomly selected aerial parts of *V. tuberosa* were collected from wild-growing populations at the locality Malačka (coordinates by Gauss-Kruger: X=5606932; Y=4827102; altitude=523 m), Kozjak Mountain (near the city of Split, Croatia) in the April, 2011. Voucher specimens are deposited in the herbarium at the Department of Biology, Faculty of Science, University of Split, Croatia [No.FNSST 2011: 15].

B. Gas-chromatography and mass spectrometry (GC, GC-MS)

Dried aerial parts of plant material (stems, leaves) were subjected to hydrodistillation for 3 h in Clavenger type apparatus. Gas chromatography analyses were performed on gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with flame ionization detector (FID) and mass spectrometer (MS) (model 2100T; Varian Inc.), non-polar capillary column VF-5MS and polar CP Wax 52. The individual peaks were identified by comparison of their retention indices, and/or authentic samples, as well as by comparing their mass spectra with literature data [8].

C. Micromorphological traits

Light microscopically investigations of glandular trichomes were performed on hand-cut cross sections of fixed material, using a Opton Axioskop MC63A.

III. Results and Discussion

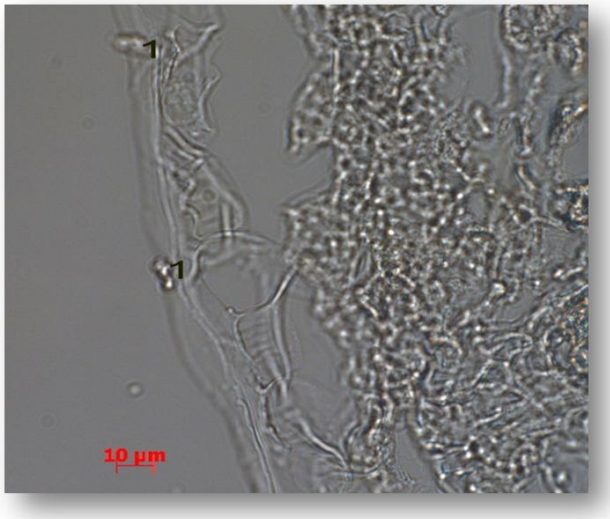
The essential oil isolated by hydrodistillation from the aerial parts of *V. tuberosa* has been analysed by GC and GC/MS. The chemical compositon of the essential oil, the percentage of identified components and their retention indices are given in Table 1, where all the components are

arranged in order of their elution from the VF-5ms capillary column. A total of forty components have been identified in the essential oil by comparison of their mass spectra with Wiley and NIST library data accounting for 94.1 % of the total composition. The total yield of oil was 0.7%, based on dry weight of samples.

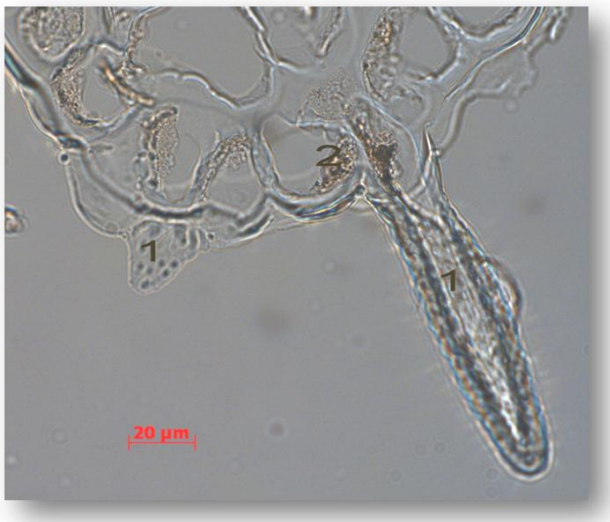
The essential oil composition of *V. tuberosa* was characterized by a high percentage of sesquiterpenes and monoterpenes (Table 1). The most abundant component in the composition of essential oil was isovaleric acid (17.2%) (Table 1). Isovaleric acid was also present as typical constituent of *Valeriana officinalis* roots from Estonia [9]. Among sesquiterpenes (42.8%), hydrocarbons (35.4%) were detected in higher percentages than oxygenated ones (7.4%) (Table 1). The most abundant component among this class was sesquiterpene geranyl isovalerate (12.2%) and oxygenated sesquiterpene caryophyllene oxide (7.7%) (Table 1). Among the monoterpene fraction (25.8%), limonene (4.7%) was the most abundant (Table 1). Literature data dealing with the chemical composition of essential oil of *V. tuberosa* are very scarce. Fokialakis *et al.* (2002) [4] found that the major constituents of the essential oil of the roots were trans-caryophyllene (72.7%), caryophyllene oxide (10.9%) and camphene (5.8%), of the stems and leaves were phytol (16.2%), eicosanoic acid methyl ester (15.0%), docosanoic acid methyl ester (13.5%) and of the inflorescences were trans, trans-α-farnesene (13.3%), 1,4-dimethoxy benzene (11.3%) and 6,10,14-trimethyl-2-pentadecanone (8.9%). The oil of *V. tuberosa* described by Fokialakis *et al.* (2002) [4] and also in our study completely lacked the characteristic kessane sesquiterpenes.

Oil biosynthesis and accumulation of essential oil of oil-bearing plants have been localized in secretory cells of glandular trichomes. Morphological, anatomical and biochemical characteristics of plant material are used in standardization of plant material. In this study the structure of glandular trichomes of this species is described. The presence and structure of trichomes as seen in transverse section of leaves can be used as the distinguishing features. According to Payne's plant hair terminology [10] two types of glandular trichomes: peltate (one basal epidermal cell, one short stalk cell and a small head) (Fig. 1a) and long glandular trichomes (one basal epidermal cell, one elongated stalk cell) (Fig. 1b) were observed on leaf, using light microscopy. Colombo *et al*. [11] divided capitate trichomes of *Primula* species into two types based on the dimension of stalk: long stalked capitate trichomes of *P. albenensis* and *P. auricula* and short-stalked capitate trichomes of *P. farinosa* and *P. halleri*. On the leaf of *V. tuberosa* long glandular trichomes contain elongated stalk cell whereas in peltate trichomes stalk cell is short and often invisible (Fig. 1). Subcuticular space and epidermal cells are often filled with essential oil (Fig. 1b).

With the aim to expand and deepen the current knowledge of genus *Valeriana*, phytochemical and micromorphological traits of *V. tuberosa* were investigated in this study.



a)



b)

Figure1. Light microscope micrographs of the different trichome types on the leaf of *Valeriana tuberosa*. Peltate trichomes (a) and long glandular trichomes (b).
1-glandular trichome; 2- epidermal cells filled with essential oil

Table 1. Phytochemical composition (%), identification and major groups of chemical components (%) of essential oil of *Valeriana tuberosa* L.

Component	RI[a]	RI[b]	%	IM
Monoterpene hydrocarbons			**11**	
α-Pinene	938	-	0.2	RI, MS, S
Camphene	962	-	0.2	RI, MS
Myrcene	992	<1200	0.3	RI, MS
α-Terpinene	1016	1251	0.9	RI, MS
p-Cymene	1021	1268	1.7	RI, MS
Limonen	1032	1204	4.7	RI, MS, S
(Z)-β-Ocimene	1052	1218	1.6	RI, MS
γ-Terpinene	1057	1255	0.9	RI, MS
allo-Ocimene	1128	1351	0.5	RI, MS
Oxygenated monoterpenes			**14.8**	
1,8-cineole	1026	1208	0.5	RI, MS
Sabinene hydrate	1065	1474	0.8	RI, MS
Linalool	1097	1548	1.9	RI, MS, S
Camphor	1143	1499	0.6	RI, MS
Borneol	1176	1719	0.9	RI, MS, S
Terpinen-4-ol	1184	1611	1.1	RI, MS
α -Terpineol	1186	1646	1.9	RI, MS
Myrtenol	1194	1782	0.8	RI, MS
Nerol	1227	1795	1.7	RI, MS
Thymol, methyl ether	1230	1587	0.9	RI, MS
Carvacrol, methyl ether	1241	1598	0.7	RI, MS
Geraniol	1249	1835	1.7	RI, MS
Carvacrol	1298	2239	1.1	RI, MS, S
Neryl acetate	1358	1692	0.2	RI, MS
Sesquiterpene hydrocarbons			**35.4**	
β-Caryophyllene	1424	1585	2.5	RI, MS, S
(Z)-β -Farnesene	1454	1639	5.6	RI, MS
α -Humulene	1456	1654	2.8	RI, MS
allo-Aromadendrene	1465	1662	1.9	RI, MS
Viridiflorene	1496	1695	1.9	RI, MS
δ-Cadinene	1517	1745	0.3	RI, MS
β-Elemene	1389	1589	2.4	RI, MS
Germacrene D	1484	1692	3.5	RI, MS
Bicyclogermacrene	1500	1728	2.3	RI, MS
Geranyl isovalerate	1606	1905	12.2	RI, MS
Oxygenated sesquiterpenes			**7.4**	
Spathulenol	1578	2101	0.2	RI, MS, S
Caryophyllene oxide	1582	1955	7.2	RI, MS, S
Aliphatic compounds			**25.5**	
isovaleric acid	839	1686	17.2	RI, MS
1-Octen-3-ol	974		0.2	RI, MS
n-Nonanal	1101	1391	0.4	RI, MS
Hexadecanoic acid	1962	-	3.2	RI, MS
Docosane	2200	2200	4.5	RI, MS, S
Total identified (%)			**94.1%**	

Retention indices determined relative to a series of *n*-alkanes ($C_8 - C_{40}$) on the non-polar capillary column VF5-ms (RI^a) and polar column CP Wax 52 (RI^b); IM, Identification Method: RI, comparison of RIs with those listed in a homemade library, reported in the literature [8], and/or authentic samples; MS, comparison of mass spectra with those in the mass spectral libraries NIST02 and Wiley 7; S, co-injection with reference compounds

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REFERENCES

[1] H. Rankou, A. Ouhammou, M. Taler, and G. Martin, *Valeriana tuberosa*. The IUCN red list of threatened species 2015: e.T53785801A53798747.http://dx.doi.org/10.2305/IUCN.UK.2015-4. RLTS.T53785801A53798747.en. Downloaded on 07 March 2016.

[2] R. Domac, Flora of Croatia: Manual For Determination of Plants, Školska knjiga, Zagreb, Croatia, 2002.

[3] J. Bellakhdar, R. Claisse, J. Fleurentin, and C. Younos. Repertory of standard herbal drugs in the *Morrocan pharmacopoea*. *Journal of Ethnopharmacology*, 1991, 35: 123-143.

[4] N. Fokialakis, P. Magiatis, and S. Mitaku, Essential Oil Constituents of *Valeriana italica* and *Valeriana tuberosa.* Stereochemical and Conformational Study of 15-Acetoxyvaleranone. *Verlag der Zeitschrift für Naturforschung*, 2002, 57c(c): 791-796.

[5] C. R. Metcalfe, and L. Chalk, Anatomy of the dicotyledons. Oxford Univ. Press, Oxford,1972, pp. 292–297.

[6] H. D. Behnke, Plant trichomes – structure and ultrastructure. In Biology and chemistry of plant trichomes. Rodriguez E, Healey PL, Metha I. (Eds). Plenum, New York, 1984, 1–21.

[7] E. Werker, U. Ravid, and E. Putievsky, Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. Israel Journal of Botany, 1985, 34, pp. 31–45.

[8] R. P. Adams, Identification of essential oil components by gas chromatography and mass spectroscopy. 4th Edition, Allured Publ. Corporation, Carol Stream, IL. 2007.

[9] A. Raal, A. Orav, E. Arak, T. Kailas, and M. Müürisepp, Variation in the composition of the essential oil of *Valeriana officinalis* L. roots from Estonia, Proceedings of the Estonian Academy of Sciences, Chemistry, 2007, 56, 2, 67–74.

[10] W. W. Payne, A glossary of plant hair terminology. Brittonia, 1978, 30, 239–255.

[11] P. S. Colombo, G. Flamini, M. S. Christodoulou, G. Rodondi, S. Vitalini, D. Passarella, and G. Fico, Farinose alpine Primula species: phytochemical and morphological investigations, *Phytochemistry*, 2014, 98, 151-159.

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