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Essential Oil Composition and *in vivo* Volatiles Emission by Different Parts of *Coleostephus myconis* Capitula

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The essential oil obtained by hydrodistillation of the flowering capitula of *Coleostephus myconis* (syn. *Chrysanthemum myconis*) was constituted almost exclusively of oxygenated sesquiterpenes (85.8%). The main constituent was T-cadinol (66.2%), followed by valeranone (8.2%), germacrene D (6.0%) and α-cadinol (4.6%). By mean of the SPME technique, the volatiles emitted *in vivo* by the whole capitula and by tubular and ligulate florets have been identified. Many differences were evidenced among the different organs and with respect to the essential oil

Keywords: *Coleostephus myconis*, Asteraceae, essential oil, *in vivo* volatile emission, T-cadinol.

In Italy, the genus *Coleostephus* (Asteraceae) is represented by only *C. myconis* (L.) Cass. (syn. *Chrysanthemum myconis* L.; *Myconia myconis* Briq. et Cavill.). Here it grows from Liguria to Calabria, Sicily, Sardinia and smaller islands [1], mainly on cultivated and abandoned fields, preferably on siliceous soil.

This study is part of phytochemical investigations of the flora of Caprione Promontory [2-4], in Montemarcello-Magra Natural Regional Park (Eastern Liguria, Italy) [5-11]. The Park protects a large district of La Spezia province, including the Vara and Magra rivers (the latter in the Liguria part only) and the Promontory of Caprione, a hill system that separates the Gulf of La Spezia from the plain of the Magra River. This is an important area from historic, cultural and natural points of view.

This paper deals with the composition of the essential oil obtained from the flowering aerial parts of *Coleostephus myconis* and the volatiles emitted *in vivo* by the whole capitula and by the isolated tubular and ligulate florets. To the best of our knowledge, no previous studies of the volatiles from this species have been reported in the literature.

The essential oil yield was 0.1% (w/w) and its composition is reported in Table 1. Altogether, 70 compounds were identified in the essential oil and *in vivo* volatile emissions, accounting for 85.3% to 98.0% of the whole oil. The essential oil of the capitula was characterized by the sole presence of sesquiterpene derivatives (93.2%). Among these, hydrocarbons accounted for 7.4%, while the remaining ones (85.8%) were oxygenated compounds. The main constituent was T-cadinol (66.2%), followed by valeranone (8.2%), germacrene D (6.0%), α-cadinol and α-bisabolol (both 4.6%).

The SPME investigation evidenced a precise emission pattern, with marked differences between tubular and ligulate florets (Table 1). In tubular florets the main volatile was T-cadinol (26.8%), whereas in the ligulate ones, (*E*)- β-farnesene (35.0%) was the major sesquiterpene released.

Among the volatiles emitted *in vivo*, as opposed to those from the essential oil, many monoterpenes (23.4%) were detected, both hydrocarbon (17.5%) and oxygenated derivatives, the major hydrocarbons being α-pinene (7.4%) and camphene (8.1%). Despite their high number (13 compounds), oxygenated monoterpenes formed only 5.9%. Furthermore, some non-terpene compounds were also present, such as straight-chain alkanes, alcohols, aldehydes, ketones and esters. This particular emission pattern could be developed by the plant for pollination purposes. It is well known that the color of a flower is the first and

Table 1: Composition of the essential oil of *Coleostephus myconis* and *in vivo* volatile emission of different flower parts.

Compounds	l.r.i.	essential oil	SPME		
			whole capitula	tubular florets	ligulate florets
(*E*)-3-Hexen-1-ol	851	–	1.2	–[c]	–
(*E*)-2-Hexen-1-ol	862	–	–	0.3	–
2,6-Dimethyl-1-heptene	866	–	–	–	0.3
α-Pinene	941	–	7.4	0.5	0.4
α-Fenchene	953	tr[d]	–	–	–
Camphene	955	–	8.1	2.2	18.9
Sabinene	978	–	0.2	–	0.1
1-Octen-3-ol	980	–	–	0.8	–
β-Pinene	982	–	0.9	–	0.6
3-Octanone	988	–	0.4	0.5	0.6
(*E*)-3-Hexen-1-ol acetate	1004	–	–	0.3	–
3-Methyl-4-penten-1-ol acetate	1005	–	0.8	–	0.2
(*E*)-2-Hexen-1-ol acetate	1016	–	–	–	0.2
Limonene	1033	–	0.5	0.5	0.7
1,8-Cineole	1035	–	0.9	0.7	3.4
Phenyl acetaldehyde	1045	–	–	–	0.1
cis-Sabinene hydrate	1070	–	0.2	–	0.1
Terpinolene	1088	–	–	0.3	–
trans-Sabinene hydrate	1099	–	0.2	–	0.1
Nonanal	1104	–	–	–	2.3
1-Octen-3-ol acetate	1114	–	5.9	3.6	–
β-Thujone	1116	–	1.2	0.3	0.1
trans-Pinocarveol	1141	–	1.6	1.4	–
cis-Verbenol	1142	–	0.2	–	–
Camphor	1145	–	0.3	–	–
(*E*)-2-Nonenal	1162	–	–	0.3	–
Pinocarvone	1165	–	–	0.8	–
Borneol	1167	–	0.2	–	–
Isopinocamphone	1175	–	0.2	1.1	–
4-Terpineol	1179	–	0.3	–	–
Decanal	1202	–	–	–	0.5
trans-Carveol	1219	–	0.3	–	–
Nerol	1228	–	0.2	–	–
cis-3-Hexenyl isovalerate	1238	–	0.6	–	–
Hexyl-3-methyl butanoate	1244	–	0.2	–	–
n-Tridecane	1300	–	–	0.2	–
Neryl acetate	1365	–	0.3	0.3	–
Cyclosativene	1370	–	0.4	0.4	–
α-Copaene	1376	–	1.1	1.4	0.8
β-Cubebene	1390	–	–	–	0.1
β-Elemene	1391	–	0.3	0.6	0.1
n-Tetradecane	1400	–	–	0.3	0.1
α-Gurjunene	1409	–	–	0.3	–
β-Caryophyllene	1418	tr	14.6	2.8	12.4
β-Gurjunene	1432	–	–	0.4	0.4
α-Guaiene	1439	–	–	0.3	–
Isoamyl benzoate	1441	–	–	0.3	–
(*E*)-Geranyl acetone	1453	–	–	–	0.2
α-Humulene	1456	–	0.2	–	0.2
(*E*)- β-Farnesene	1458	0.5	15.8	14.5	35.0
Alloaromadendrene	1461	–	4.2	5.0	3.4
α-Acoradiene	1464	–	–	0.6	–
Germacrene D	1481	6.0	3.9	4.6	12.2
Viridiflorene	1495	0.9	0.7	–	–
α-Muurolene	1499	–	0.3	0.6	0.2
(*E*,*E*)- α-Farnesene	1507	–	0.8	1.3	0.5
β-Bisabolene	1509	–	–	–	0.1
δ-Cadinene	1511	tr	0.8	2.3	0.5
trans- γ-Cadinene	1513	–	–	7.6	–
cis- γ-Cadinene	1524	tr	5.0	–	3.2
Spathulenol	1577	tr	–	–	–
Caryophyllene oxide	1583	1.3	0.6	0.4	–
Guaiol	1596	–	–	0.4	–
T-Cadinol	1642	66.2	3.1	26.8	0.1
α-Muurolol	1647	0.9	–	–	–
α-Cadinol	1655	4.6	–	1.2	–
Valeranone	1675	8.2	–	–	–
α-Bisabolol	1685	4.6	–	–	–
n-Heptadecane	1700	–	0.6	0.5	–

[A] %obtained by FID peak-area normalization;[b] Linear retention indices (HP-5 column); [c] – not detected; [d] tr < 0.1%.

foremost cue for a pollinator's attraction, but the scent of a flower also plays a major role in attracting pollinating insects. Distinctive volatile compounds could allow insects both to recognize specific host plants and to assess the amount of rewards in a flower; furthermore, odors can act both at long distances as attraction cues and at short distances as orientation cues among different parts of the flower or among different flowers [19-23].

Although Asteraceae species are a significant component of almost all terrestrial ecosystems, the pollination biology of relatively few taxa has been examined in detail [24]. Asteraceae are pollinated by many insect orders, such as Hemiptera, Coleoptera, Lepidoptera, Hymenoptera and Diptera [25]. However, because of the strong structure and the flat shape of their head inflorescence, beetles are the most frequent visiting insects [26]. Among the main volatiles emitted by the florets, camphene is an attractant of many beetles, such as *Thanasimus* sp. [27], and β-caryophyllene is an attractant of Lepidoptera, i.e. *Diachasmimorpha longicaudata* [28]. Hymenoptera, such as *Andrena erigeniae* [29], are attracted by *(E,E)*-α-farnesene. Another Hymenoptera attractant seems to be germacrene D [30]. Finally, T-cadinol has beetle-attraction properties [31]. This large presence of possible semiochemicals in *Coleostephus myconis* is in good agreement with the generalist nature of this family that lacks specialized pollinators [32]. Their different distribution in the inflorescence could provide orientation cues to pollinators, even if visual signals, such as color and simultaneous presence of disk and ray florets [33] cannot be neglected. Furthermore, the role of minor constituents cannot be *a priori* ignored because most semiochemicals have effects at extremely low concentration.

One of the major constituent of the essential oil, valeranone, is endowed with several pharmacological effects, such as antispasmodic activity [34], antiulcerogenic and weak hypotensive and tranquilizing action [35]. Furthermore, it seems to be one of the possible CNS active principles of valerian [36]. These findings could permit the oil to be considered as a candidate in aromatherapy as a sedative drug.

In addition, because of its quite high content of T-cadinol (66.2%), the oil could represent a valid source of this sesquiterpene, an important molecule used for immunotherapy of cancer [37]. This molecule also has moderate antimycobacterial properties and strong activity against filamentous fungi and *Culex quinquefasciatus* larvae [38], as well as calcium-antagonistic properties [39].

Experimental

Plant material: The aerial parts of *C. myconis* were collected at the full blooming stage, during June 2009, in the locality of Cima Terroni (Montemarcello municipality, La Spezia Province), at 250 m above sea level, on a slope facing south-south west, on siliceous soil (Falda di Montemarcello, Unità di Massa), inside an *Olea europaea* plantation. A voucher specimen has been deposited in the Herbarium Horti Botanici Pisani as Nuove Acquisizioni N. 9341 *Coleostephus myconis*/17.

Extraction and analysis: A few hours after collection, about 300 g of flowering aerial parts were coarsely cut and immediately hydrodistilled in a Clevenger-like apparatus for 2 h.

GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and DB-5 capillary columns (30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5°C/min up to 220°C; injector and detector temperatures 250°C; carrier gas helium (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 μL). The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by mean of their linear retention indices (l.r.i.) relative to a series of *n*-hydrocarbons.

GC-EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10% *n*-hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of their retention times with those of authentic samples, comparing their linear retention indices relative to a series of *n*-hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS) and home-made library MS built up from pure substances and components of known oils and MS literature data [12-18]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

Volatiles emitted *in vivo* were sampled by mean of SPME using a Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 μm). The headspace of 3 whole living capitula, or of some tubular flowers, or some ligulate flowers inserted into a 25 mL glass conical flask and in a 4 mL glass septum vial, respectively was sampled. All the samples were obtained from living plants immediately inserted in the glass container and allowed to equilibrate for 30 mins. The inflorescence was collected at full blooming, cut a few mm below the calyx, and the pedicels were wrapped in aluminum foil to minimize water loss. After the equilibration time, the fiber was exposed to the headspace for 20 mins at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC-MS system, operating under the same conditions as above, except that the splitless injection mode was used and the injector temperature was 250°C.

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