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RESEARCH ARTICLE

Morphogenetic changes in essential oil composition of *Hypericum perforatum* during the course of ontogenesis

Alessandra Bertoli[1], Cüneyt Cirak[2], Michele Leonardi[1], Fatih Seyis[3], and Luisa Pistelli[1]

[1]*Department of Bioorganic Chemistry and Biopharmaceutics, University of Pisa, Pisa, Italy,* [2]*The Vocational High School of Bafra, University of Ondokuz Mayıs, Samsun, Turkey, and* [3]*Faculty of Agriculture, Department of Agronomy, University of Bozok, Yozgat, Turkey*

Abstract

Context: In the past few years, an increasing interest in the volatile secondary metabolites of *Hypericum perforatum* L. (Guttiferae) has been arising.

Objective: The present study is a contribution to better understand the relationship between the morphological variations and volatile composition during the phenological cycle.

Materials and methods: Leaves at the stages of vegetative, floral budding, flowering and green capsule, buds, full opened flowers and green capsules were assayed for essential oil (EO) components by gas chromatography-flame ionization detector (GC-FID) and GC-mass spectrometry (MS).

Results: Significant amounts of sesquiterpenes (oxygenated 26–50% and hydrocarbons 20–40%) and oxygenated hydrocarbons (13–38%) characterized the all analyzed samples showing peculiar fluctuations during the seven phenological stages. Although monoterpenes were present in much lower amounts (monoterpene hydrocarbons 0.4–6%; oxygenated monoterpenes 0.8–6%) they were considered also important discrimination for several stages. The green capsules and the full opened flowers collected at flowering stage were clearly distinguished in terms of EO compositions from the other samples.

Discussion: For the first time, the EO composition of Turkish wild *Hypericum perforatum* was monitored by the hydrodistillation of different plant organs collected at different seven stages in order to point out the modification of target volatiles related to each phenological step.

Conclusions: Based on the EO composition monitored during these seven morphological stages by GC-MS, principal component analysis and cluster analysis, significant metabolite modifications were observed during the phenological cycle which involved the levels of specific volatile target compounds belonging to the chemical classes of hydrocarbons, monoterpenes and sesquiterpenes.

Keywords: Cluster analysis, essential oil, GC-MS, *Hypericum perforatum*, PCA, phenological cycle

Introduction

Hypericum perforatum L. (Guttiferae) (St. John's wort) is a well-known traditional medicinal plant that has been used for centuries for the treatment of several diseases, such as skin lesions, eczema, burns, and microbial, inflammatory, and psychological disorders (Sánchez-Mateo et al., 2002; Toker et al., 2006). The crude extract of *H. perforatum* is now widely used in Europe as a drug for the treatment of depression (Greeson et al., 2001). Proven photodynamic, antiviral, antiretroviral, and antitumoral activities of *Hypericum* extracts also suggests using of this plant in acquired immune deficiency syndrome and cancer treatments (Vlietinck et al., 1998; Guedes and Eriksson, 2005).

H. perforatum contains a number of secondary metabolites from different classes namely naphthodianthrones, phloroglucinols, flavonoids, phenylpropanes, essential oils (EOs), amino acids, xanthones, tannins, procyanidins and other water-soluble components (Greeson et al., 2001). Furthermore, the species is especially rich,

Address for Correspondence: Cüneyt Cirak, The Vocational High School of Bafra, University of Ondokuz Mayıs, 55400, Bafra, Samsun, Turkey. Telephone: +90–362-5426763, Fax: +90–362-5426761. E-mail: cuneytc@omu.edu.tr

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as are other members of the genus *Hypericum*, in volatile compounds (Mathis and Ourisson, 1964; Mimica-Dukic et al., 1998; Gudzic et al., 2001; Petrakis et al., 2005; Saroglou et al., 2007).

Although the ecological importance of volatile compounds in the direct interaction with the habitat, and the contribution of EO to the bioactivities of *Hypericum* spp. have been studied (Couladis et al., 2003; Cakir et al., 2005), only a few studies on the volatile chemistry of *H. perforatum* have been undertaken due to the low amount of EO (Radusiene et al., 2005). In addition, it should be noted that consistent results have not been reported (Gudzic et al., 2001; Schwob et al., 2002; Azizi, 2008; Cirak et al., 2010). In fact, the plant material studied in previous reports were from various locations where very different ecological conditions are prevalent and in most of those studies, the plant parts used and the phenological stages in which sampling was performed, were not clearly specified. Although ontogenetic changes in EO composition in wild (Schwob et al., 2004) and field grown (Azizi, 2008) *H. perforatum* plants were previously reported, to our knowledge, there is no report on the organ-dependence of this variation.

As few and variable data have been reported on the EOs variations related to the plant organ-development cycle, the present study aims to give a contribution to determine the links between EO composition and different plant organs considering the course of ontogenesis in *H. perforatum* var. *perforatum* of Turkish origin.

Materials and methods

Brief description of plant material

The plant material was described in our previous study (Cirak et al., 2010). The species were identified by Dr. Hasan Korkmaz, Faculty of Science and Art, Department of Biology, University of 19 Mayis, Samsun, Turkey. Voucher specimen was deposited in the herbarium of Ondokuz Mayis University Faculty of Agriculture (OMUZF # 61/10).

Experimental procedures

The plant material was collected in a dry grassland within the Çakallı district of Samsun, Turkey (41°04' N; 36°01' E; 470 m above sea level) between April and October of 2007 at different stages of plant development. The mean temperature during the sampling period was 18.5°C, and the precipitation sum 450 mm. The sampling site was not grazed or mown during the plant gathering period. The material represented 30 randomly gathered plants in four phenological stages: vegetative, floral budding, full flowering and fresh fruiting. Newly emerged shoots (4–6 weeks old-age) with leaves were harvested at the vegetative stage. For the floral budding stage, only shoots with floral buds were selected. At the full flowering stage, only shoots with full opened flowers were harvested. At the fresh fruiting stage, the shoots which had green capsules were harvested. The top ⅔ of the plant was harvested between 12:00 am and 13:00 pm. After collected, plant materials were dissected into floral, leaf and stem tissues, dried at room temperature (20 ± 2°C) and subsequently assayed for volatile constituents.

Sample preparation

Air-dried plant samples (200 g) were hydrodistilled by Clevenger apparatus for 2 h. The EO were diluted in *n*-hexane (HPLC solvent grade, 10%) and injected in GC-FID (injection volume 1 μl) and GC-mass spectrometry (MS) (injection volume 0.1 μl).

Gas chromatography-FID analysis

GC analyses were accomplished by HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 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 nitrogen (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl; The identification of the components was performed, for both the columns, by the comparison of their retention times with those of pure authentic samples and by mean of their linear retention indexes (LRI) relative to a series of *n*-hydrocarbons.

Gas chromatography-MS analysis

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m × 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 to 240°C at 3°C/min; carrier gas helium at 1 ml/min; triplicate injections of 0.2 ml (10% hexane solution); split ratio 1:30. Identification of the constituents was based on the comparison of the retention times with those of authentic samples, comparing their LRI relative to a series of *n*-hydrocarbons and computer matching by both two commercial data base (NIST 98 and ADAMS) and a home-made library mass spectra built up from pure substances, components of known oils and MS literature data (Stenhagen et al., 1974; Massada, 1976; Swigar and Silverstein, 1981; Connolly and Hill, 1991; Adams 1995). Moreover, the molecular weights of the all identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas. The GC-MS results are shown in Tables 1 and 2.

Statistical analysis

Principal component analysis (PAC) was carried out using the statistical software package SPSS Version 13.0. This analysis is the two-dimensional visualization of the position of investigated exemplars relative to each other. The principal components (PCs) represent the axes which are the orthogonal projection for the values representing the highest possible variances, in this case the first and second PCs.

Table 1. GC-MS analyses of the essential oils of Turkish *H. perforatum* samples collected in different phenological stages (1–7)[&]

Sample		1	2	3	4	5	6	7							
Oil yield (%, v/w)		0.44	0.35	0.25	0.61	0.04	0.53	0.07							
Compounds	LRI*[1]	Mean*[2]	SD*[3]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
(*E*)-2-Hexenal	856	1.40	0.03	1.75	0.02	0.13	0.01	0.96	0.04	0.19	0.01	0.26	0.01	0.20	0.01
(*E*)-2-Hexenol	860	2.00	0.26	4.69	0.98	0.97	0.09	0.51	0.06	1.89	0.78	1.72	0.47	4.23	1.02
2-Methyl-octane		0.28	0.11	0.41	0.13	0.17	0.05	0.11	0.03	0.06	0.02			0.11	0.02
n-Nonane	900	0.24	0.00	0.55	0.09	0.14	0.03	0.07	0.02	0.20	0.03	0.10	0.01	0.26	0.04
n-Heptanal										0.09	0.01	0.41	0.05	0.63	0.07
α-Thujene		0.17	0.0.4	0.21	0.02	0.10	0.02			0.91	0.04				
α-Pinene	941			0.11	0.01	0.29	0.01	0.13	0.05	0.69	0.02	1.60	0.03	4.14	0.01
Camphene	954	0.17	0.03	0.19	0.03	0.11	0.02	0.11	0.03	0.11	0.03	0.05	0.02	0.09	0.02
(*Z*)-2-Heptenal	960									0.03	0.01			0.11	0.03
Benzaldehyde	968	0.50	0.07	0.66	0.10	0.28	0.01		0.17	0.26	0.05	0.24	0.05	0.44	0.07
Sabinene	977									0.12	0.04	0.27	0.05	0.73	0.08
β-Pinene	984	tr		tr						tr		0.01	0.01		
6-Methyl-5-hepten-2-one[#]	985	0.27	0.01	0.38	0.02	0.15	0.01	0.30	0.03	0.27	0.04	0.38	0.06	0.73	0.08
Myrcene	991	0.02	0.01	tr		0.08	0.01	tr		tr				tr	
n-Octanal	1001	0.19	0.02							0.06	0.01			0.08	0.01
3-Cyclohex-1-enyl-prop-2-enal[#]	1002	0.31	0.08	0.17	0.03			0.09	0.02	0.13	0.04	0.12	0.06	0.48	0.08
α-Terpinene	1018									0.04	0.02	0.07	0.03	0.05	0.01
p-Cimene	1028	0.16	0.01	0.19	0.01	0.22	0.02	0.14	0.01	0.16	0.02	0.20	0.01	0.24	0.01
Limonene	1031			0.20	0.05			0.10	0.03	0.37	0.08	0.13	0.03	0.20	0.09
1,8-Cineol	1033									0.07	0.01			0.07	0.01
cis-β-Ocimene				0.09	0.02							0.14	0.04		
trans-β-Ocimene	1059									0.07	0.02			0.08	0.02
Benzene acetaldeide	1169			0.09	0.03							0.14	0.01		
γ-Terpinene	1062									0.02		0.09		0.12	0.02
(*E*)-2-Octenal	1064	0.47	0.02	0.55	0.03	0.32	0.07	0.24	0.04	0.84	0.05	0.79	0.02	0.12	0.02
cis-Linalool oxide	1076	0.19	0.02	0.21	0.06			0.31	0.07	0.16	0.04	0.50	0.08	0.75	0.02
trans-Linalool oxide	1088			0.09	0.01			0.14	0.01	0.07	0.05	0.34	0.05	0.35	0.03
p-Cymenene[#]	1089									0.06	0.02			0.13	0.03
n-Undecane	1100	0.59	0.08	0.50	0.05	0.26	0.01	0.20	0.04	0.40	0.04	0.27	0.07	0.24	0.05
Linalool	1001	1.13	0.05	0.72	0.06	0.12	0.01	0.57	0.08	0.41	0.05	0.85	0.10	0.95	0.08
n-Nonanal	1104	0.41	0.02	0.46	0.03	0.15	0.01	0.50	0.01	0.34	0.01	0.40	0.02	0.44	0.01
α-Capholenal	1125									0.10	0.02	0.12	0.01	0.20	0.03
trans-Pinocarveol	1147									0.10	0.02	0.08	0.02	0.15	0.03
cis-Verbenol	1154									0.04	0.01	0.06	0.01	0.04	0.02
Camphor	1156									0.05	0.01	0.08	0.02	0.04	0.01
(*Z*)-2-Nonenal	1148			0.09	0.02			0.07	0.01	0.11	0.06	0.10	0.02	0.13	0.03

Table 1. continued on next page

Table 1. Continued.

Sample		1	2	3	4	5	6	7							
Oil yield (%, v/w)		0.44	0.35	0.25	0.61	0.04	0.53	0.07							
Compounds	LRI*[1]	Mean*[2]	SD*[3]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Isoborneol	1162											0.03	0.01	0.03	0.01
(*E*)-2-Nonenal	1163			0.09	0.02					0.07	0.01	0.10	0.05	0.30	0.09
trans-Pinocamphone	1167			0.18	0.06					0.05	0.01	0.09	0.02	0.07	0.01
Borneol	1169			0.09	0.01			0.17	0.05	0.15	0.04	0.23	0.03	0.24	0.03
Pinocampheol	1170					0.29	0.04	0.18	0.06	0.15	0.04	0.12	0.04	0.09	0.03
Unknown										0.08	0.03	0.11	0.03	0.14	0.02
Terpinen-4-ol	1180	0.17	0.07	0.16	0.01	0.15	0.09	0.15	0.06	0.19	0.08	0.28	0.02	0.29	0.05
p-Cymen-8-ol	1183							0.07	0.02	0.06	0.01	0.15	0.04	0.09	0.04
Methylsalicylate	1189			0.11	0.04					0.03	0.01	0.06	0.04	0.07	0.03
α-Terpineol	1190	0.41	0.01	0.35	0.01	0.29	0.02	0.29	0.06	0.22	0.01	0.41	0.01	0.50	0.01
Safranal	1202			0.11	0.03			0.09	0.04	0.08	0.02	0.11	0.06	0.09	0.02
Decanal	1202	0.09	0.00	0.13	0.02			0.08	0.04	0.08	0.03	0.06	0.01	0.11	0.05
Verbenone	1205			0.11	0.01			0.12	0.01	0.07	0.02	0.19	0.06	0.09	0.03
Unknown		0.30	0.05	0.28	0.06			0.19	0.04	0.15	0.03	0.16	0.03	0.12	0.03
trans-Carveol	1218											0.18	0.01	0.17	0.03
cis-Carveol	1231											0.09	0.02	0.06	0.02
Cuminaldheyde	1242											0.05	0.01	0.05	0.02
Carvone	1248													0.06	0.02
Geraniol	1254	0.09	0.01	0.08	0.01							0.14	0.06	0.08	0.02
Carvenone[#]	1259			0.07	0.01							0.05	0.01	0.05	0.01
(*E*)-2-Decenal	1261	0.32	0.11	0.34	0.08	0.39	0.09	0.33	0.05	0.63	0.05	0.83	0.09	1.11	0.22
Nonanoic acid	1271			0.09	0.01									0.10	0.01
Carvone oxide	1276													0.14	0.03
2-Undecanone	1293											0.06	0.02	0.09	0.04
cis-Carveol	1297													0.05	0.01
n-Tridecane	1300	0.18	0.07	0.17	0.04	0.21	0.02	0.15	0.03	0.20	0.05	0.22	0.05	0.43	0.10
(*E*,*E*)-2,4-Decadienal	1304	0.10	0.01											0.09	0.04
α-Cubebene	1351	0.12	0.01	0.12	0.03	0.14	0.01	0.10	0.01	0.16	0.01	0.21	0.08	0.24	0.02
Unknown												0.15	0.03	0.23	0.02
α-Longipinene	1352	0.10	0.01	0.10	0.01	0.12	0.01	0.12	0.04	0.18	0.04	0.11	0.05	0.19	0.01
Cyclosativene	1368											0.12	0.06	0.25	0.02
α-Ylangene	1372	0.37	0.02	0.26	0.04	0.30	0.04	0.21	0.01	0.33	0.01	0.33	0.06	0.59	0.02
α-Copaene	1377	0.92	0.03	0.87	0.04	1.02	0.18	0.70	0.10	0.89	0.11	1.19	0.31	2.02	0.59
(*E*)-β-Damascenone	1381											0.33	0.03		
Unknown										0.32	0.04				
β-Bourbonene	1386	0.20	0.02	0.21	0.03	0.30	0.02	0.25	0.04	0.31	0.01	0.18	0.07	0.42	0.05
β-Cubebene	1390			0.08	0.02					0.15		0.42		0.13	

Table 1. continued on next page

Table 1. Continued.

β-Elemene	1392	0.27	0.01	0.17	0.02	0.17	0.03	0.13	0.02	0.17	0.03	0.17	0.06	0.21	0.05
Sample		1	2	3	4	5	6	7							
Oil yield (%, v/w)		0.44	0.35	0.25	0.61	0.04	0.53	0.07							
Compounds	LRI*[1]	Mean*[2]	SD*[3]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
β-Longipinene	1401	0.09	0.03	0.08	0.02	0.12	0.01	0.06	0.03	0.17	0.02	0.17	0.03	0.21	0.05
Isocaryphyllene[#]	1404	0.23	0.06	0.17	0.01	0.17	0.03	0.13	0.05	0.17	0.03	0.17	0.03	0.21	0.04
α-Gurujene	1409			0.08	0.01	0.11	0.03	0.04	0.03	0.10	0.01	0.08	0.01	0.19	0.03
Unknown		0.37	0.07	0.49	0.08	0.32	0.09	0.12	0.04	0.22	0.07	0.04	0.01	0.11	0.01
α-Cedrene	1141	0.36	0.01	0.49	0.06	0.32	0.10	0.12	0.07	0.23	0.01	0.03	0.04	0.10	0.06
β-Caryophyllene	1420	3.44	0.43	3.26	0.59	4.56	0.75	3.49	0.81	4.20	0.82	7.10	1.12	7.47	1.35
β-Cedrene	1418	0.49	0.11	0.58	0.02	0.59	0.09	0.40	0.06	0.49	0.01	0.42	0.08	0.47	0.03
β-Gurjunene	1432	0.49	0.04	0.48	0.03	0.56	0.09	0.45	0.07	0.49	0.08	0.59	0.13	0.87	0.04
Aromadendrene	1440	0.32	0.11	0.33	0.05	0.42	0.06	0.29	0.02	0.34	0.01	0.38	0.08	0.65	0.03
Isopentyl benzoate	1441			0.46	0.06	0.87	0.22	0.44	0.05	0.53	0.01	0.93	0.02		
Unknown		0.10	0.01	0.09	0.04	0.14	0.01			0.12	0.03				
α-Himachalene[#]	1446	0.58	0.07	0.81	0.03	0.97	0.10	0.73	0.05	1.24	0.11	0.97	0.16	1.32	0.02
(*E*)-β-Farnesene	1455	0.77	0.05	0.74	0.25	1.13	0.07	0.81	0.33	1.20	0.23	2.25	0.05	2.21	0.06
α-Humulene	1457	0.18	0.01	0.18	0.04	0.24	0.02	0.18	0.05	0.27	0.01	0.38	0.10	0.42	0.05
Alloaromadendrene	1461	0.33	0.02	0.34	0.02	0.49	0.12	0.30	0.04	0.52	0.01	0.44	0.03	0.56	0.03
cis-Muurolα–4(14),5-diene[#]	1463	0.12	0.05	0.10	0.02	0.17	0.01	0.09	0.03	0.13	0.06	0.11	0.05	0.16	0.04
α-Acoradiene[#]	1466	0.12	0.05	0.19	0.05	0.17	0.03	0.17	0.03	0.20	0.01	0.19	0.09	0.21	0.06
Drima-7.9(11)-diene[#]	1469	0.24	0.03	0.17	0.00	0.28	0.03	0.16	0.01	0.23	0.04	0.24	0.02	0.52	0.01
γ-Muurolene	1479	2.47	0.85	2.78	0.96	3.40	1.15	3.10	1.09	2.79	1.05	3.74	1.10	4.70	1.08
γ-Curcumene	1480											0.82	0.08		
Dodecanol		12.04	1.35	12.0	0.87	5.55	0.98	3.56	0.56	4.05	0.34	1.01	0.78	0.77	0.34
(*E*)-β-Ionone	1484									0.73	0.01	0.57	0.06	0.56	0.09
Germacrene D	1482	0.13	0.02	0.09	0.03	0.14	0.12	0.06	0.01	0.73	0.03	0.26	0.10	0.37	0.06
β-Selinene	1486	4.68	0.04	3.79	0.35	5.70	0.35	3.56	0.67	3.72	0.90	4.96	0.95	8.53	1.12
γ-Amorphene	1491	0.54	0.08	0.52	0.09	0.58	0.07	0.55	0.10	0.58	0.12	0.79	0.10	0.89	0.11
α-Selinene	1496	3.72	0.09	2.64	0.17	4.33	0.76	2.39	0.22	3.04	0.03	3.79	0.05	7.50	0.08
α-Muurolene	1499	0.42	0.05	0.49	0.03	0.53	0.01	0.49	0.11	0.79	0.05	0.66	0.11	0.83	0.10
(*E*,*E*)-α-Farnesene	1503									0.08	0.01	0.16	0.11	0.09	0.01
cis-γ-Cadinene	1511	1.31	0.52	1.55	0.29	1.92	0.50	1.87	0.37	1.88	0.33	2.41	1.07	2.43	0.91
γ-Cadinene	1524	1.63	0.17	1.35	0.14	1.91	0.13	1.58	0.19	1.67	0.14	2.62	0.24	2.96	0.31
β-Sesquiphellandrene	1525			0.46	0.04	0.54	0.06	1.62	0.23	1.11	0.10	0.63	0.06	0.71	
trans-Calamenene[#]	1528			0.13	0.04	0.17	0.06			0.26	0.08	0.16	0.03		
Unknown										0.12	0.04	0.05	0.05		
trans-Cadina-1(2).4-diene[#]	1538	0.21	0.02	0.25	0.05	0.17	0.01	0.15	0.02	0.18	0.05	0.10	0.03		

Table 1. continued on next page

Table 1. Continued.

Unknown				0.22	0.05	0.19	0.06	0.21	0.03	0.37	0.06				
Sample		1	2	3	4	5	6	7							
Oil yield (%, v/w)		0.44	0.35	0.25	0.61	0.04	0.53	0.07							
Compounds	LRI*[1]	Mean*[2]	SD*[3]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
α-Cadinene	1540	0.22	0.03	0.26	0.15	0.30	0.04	0.34	0.02	0.39	0.05	0.48	0.06	0.39	0.01
α-Calacorene	1546	0.52	0.06	0.48	0.13	0.49	0.06	0.48	0.03	0.74	0.10	0.51	0.01	0.51	0.04
Unknown		0.30	0.08	0.51	0.09	0.36	0.04	0.44	0.02	0.47	0.03	0.44	0.05	0.32	0.06
Oxigenated sesquiterpene[#]	0.60	0.12	0.54	0.07	0.49	0.10	041	0.04	0.39	0.03	0.22	0.04	0.19	0.05	
trans-Nerolidol	1563	1.20	0.26	1.21	0.56	1.23	0.45	1.79	0.76	1.83	0.38	2.43	0.87	1.12	0.77
Dodecanoic acid				0.41	0.05	0.40	0.07	0.71	0.06	0.73	0.23	0.31	0.09	0.34	0.05
(*Z*)-3-Hexenal-benzoate[#]	1567			0.39	0.08	0.24	0.07	0.43	0.05	0.27	0.03	0.45	0.04	0.24	0.05
Unknown						0.36	0.04					0.70	0.02		
Spathulenol	1577	3.62	0.96	2.90	0.77	4.58	0.91	3.94	0.86	2.52	0.79	2.25	0.85	1.01	0.95
Caryophyllene oxide	1582	8.51	1.08	8.56	1.13	11.13	1.34	10.96	0.94	7.98	0.99	14.35	2.22	8.80	1.22
Globulol	1585									0.09	0.01				
Viridiflorol	1593	0.32	0.13	0.70	0.04	0.27	0.04	0.69	0.03	0.57	0.02	0.57	0.04	0.35	0.01
n-Hexadecane	1600	0.17	0.09	0.13	0.05	0.17	0.05	0.17	0.04	0.17		0.20		0.30	
Oxygenated sesquiterpene	0.49	0.02	0. 86	0.08	0.19	0.01	0.23	0.02	0.25	0.09	0.17	0.01	0.15	0.06	
guaiol	1601	1.56	0.35			0.88	0.04	0.57	0.06						
Humulene epoxide II	1609	1.18	0.03	0.67	0.07	0.59	0.03	0.69	0.07	0.65	0.04	0.40	0.02	0.52	0.06
β-Himachalene oxide II[#]	1616			0.55	0.04	0.51	0.03	0.41	0.05	0.51	0.03	0.30	0.02	0.24	0.01
1.10-di-*epi*-cubenol[#]	1618	0.34	0.01	0.37	0.01	0.31	0.03	0.44	0.10	0.20	0.01	0.18		0.35	0.03
Humulane-1.6-dien-3-ol[#]	1624	1.11	0.02	0.67	0.04	0.58	0.08	0.69	0.04	0.65	0.03	0.40	0.06	0.51	0.12
γ-Eudesmol	1625	0.28	0.03	0.42	0.07	0.21	0.01	0.24	0.05	0.17	0.05	0.11	0.02		
1-*epi*-cubenol[#]	1629	0.34	0.01	0.37	0.01	0.31	0.03	0.44	0.10	0.20	0.01	0.18		0.35	0.03
Benzophenone	1628			0.31	0.05					0.23	0.04				
cis -cadin-4-en-7-ol[#]	1637	0.53	0.02	0.49	0.03	0.17	0.08	0.59	0.02	0.57	0.02	0.16	0.03	0.26	0.08
Epi-α-muurolol[#]	1639	1.85	0.95	2.64	0.73	6.11	0.95	6.29	0.78	3.76	0.46	4.23	0.83	0.30	0.07
τ-Cadinol	1640	1.01	0.79	1.48	0.66	1.18	0.62	2.22	0.32	1.65	0.48	1.80	0.43	1.07	0.18
Cubenol	1641	0.50	0.12	0.55	0.09									0.20	0.02
τ-Muurolol	1643	0.88	0.18	1.09	0.45	1.01	0.09	1.17	0.75	1.91	0.91			0.32	0.06
α-Muurolol	1647	0.34	0.05	0.31	0.02	0.14	0.02	0.61	0.01	0.45	0.02	0.13	0.03		
β-Eudesmol	1652					0.12	0.02			0.41	0.07				
α-Cadinol	1653	1.00	0.10	1.49	0.42	1.18	0.39	2.22	0.29	1.65	0.32	1.80	0.74	1.07	0.39
Himachalol	1654	0.83	0.09	1.09	0.54	1.02	0.33	1.17	0.28	1.92	0.21			0.30	0.07
α-Eudesmol	1655	0.57	0.04	0.43	0.09	0.57	0.10	0.53	0.11	1.10	0.98			1.06	0.46
Unknown										1.12	0.56			0.64	0.08
Selina-11-en-6-a-ol[#]	1660	3.50	0.24	1.08	0.46	0.70	0.19	0.66	0.11	1.75	0.76	1.57	0.11	0.80	0.02
Oxygenated sesquiterpene	0.57	0.10	0.44	0.06	0.57	0.04	0.54	0.12	1.10	0.54	1.11	0.39	1.10	0.29	
Oxygenated sesquiterpene									0.41						

Table 1. continued on next page

Table 1. Continued.

β-Bisabolol	1672	1.01	0.25	1.48	0.34	1.18	0.13	2.22	0.22	1.68	0.38	1.80	0.27	1.06	0.21
Sample		1	2	3	4	5	6	7							
Oil yield (%, v/w)		0.44	0.35	0.25	0.61	0.04	0.53	0.07							
Compounds	LRI*[1]	Mean*[2]	SD*[3]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cadalene[#]	1677	0.83	0.06	0.99	0.09	1.07	0.06	1.74	0.12	1.89	0.25	1.81	0.23	0.86	0.09
n-Tetradecanol	1677	14.94	1.20	7.17	1.09	11.87	0.99	10.85	0.89	5.10	0.45	1.71	0.56	0.18	0.03
α-Bisabolol	1684	0.55	0.04	0.51	0.05	0.51	0.18	0.78	0.10	0.35	0.02	0.22	0.12	0.05	0.14
n-Heptadecane	1700			0.12	0.03	0.07	0.02	0.16	0.03	0.14	0.02	0.15	0.01	0.11	0.02
α-Bisabolol oxide A	1748	0.30	0.05	0.78	0.07	0.11	0.02	0.12	0.05	0.08	0.03	0.03	0.12	0.05	0.03
Oxygenated sesquitepenes		0.08	0.00	0.07	0.02	0.05	0.04	0.10	0.02	0.13	0.03			0.06	0.02
Benzyl benzoate	1760	0.34	0.08	0.28	0.12	0.49	0.02	0.48	0.01	0.40	0.04	0.33	0.11	0.06	0.02
Unknown		0.72	0.12	0.67	0.01	0.59	0.03	0.73	0.04	0.29	0.04	0.13	0.03		
Trimethylpentadecanone[a]	1843	1.20	0.12	1.73	0.10	1.96	0.34	2.24	0.23	2.87	0.76	2.59	0.59	1.29	0.38
n-Hexadecanol	1876	1.30	0.16	1.03	0.27	1.04	0.21	1.59	0.19	0.45	0.07	0.09	0.02		
n-Eicosane	2100	0.26	0.06	0.14	0.01	0.17	0.03	0.36	0.03	0.96	0.06	1.26	0.01	0.78	0.06
Oxygenated hydrocarbon[#]		1.25	0.18	0.81	0.07	0.68	0.06	1.70	0.23	0.21	0.07	0.08	0.02		
n-Docosane	2300	0.05	0.01			0.05	0.01	0.04	0.01	0.11	0.03	0.16	0.05	0.04	0.02
n-Tricosane	2300							0.24	0.02					0.24	0.05
n-Tetracosane	2400							0.05		0.03	0.01	0.05	0.01		
n-Pentacosane	2500	0.53	0.06	0.26	0.05			0.38	0.06	0.13	0.02	0.14	0.03	0.07	0.01
Total		99.96		99.98		98.53		99.97		97.69		99.78		99.70	

*[1]Linear retention indexes on (DB-5 column).

*[2]Normalized quantitative data for volatile compounds were obtained without considering calibration factors (i.e., $F = 1.00$ for all compounds); percentage values are means of three replicates.

tr< 0.05%.

[#]Tentative identification.

[&]1-leaf at vegetative stage, 2-leaf at floral budding, 3-leaf at full flowering, 4-leaf at fruiting, 5-floral buds, 6-full opened flowers, 7-green capsules.

[a]Correct isomer was not identified.

Table 2. Typical chemical classes of volatile constituents in the essential oils of Turkish *H. perforatum* samples collected in different phenological stages (1–7)*.

Sample	1	2	3	4	5	6	7
Chemical classes							
Monoterpene hydrocarbons	2.30	2.28	1.24	1.93	2.40	2.55	2.58
Oxygenated hydrocarbons	37.67	33.39	25.15	24.03	19.46	12.81	12.85
Monoterpenes	0.52	0.99	0.80	0.48	2.55	2.56	5.78
Oxygenated mononoterpenes	1.99	2.17	0.85	2.09	1.97	4.12	4.67
Sesquiterpenes hydrocarbons	26.42	25.59	33.60	26.76	32.02	40.15	50.39
Oxygenated sequiterpenes	28.77	31.63	33.73	40.17	33.31	33.34	19.85

*1-leaf at vegetative stage, 2-leaf at floral budding, 3-leaf at full flowering, 4-leaf at fruiting, 5-floral buds, 6-full opened flowers, 7-green capsules.

The obtained data were used to create scatter plot diagrams (Backhaus et al., 1989). Therefore a factor analysis was performed, where each variable was used to calculate relationships between variable and investigated factors. Based on the obtained data also a dendogramme (cluster) was created (Backhaus et al., 1989) showing the relationship of investigated samples regarding their EO composition.

Results and discussion

Significant differences in the EOs yield were observed during the seven phenological stages of the selected plant material. The analyzed samples were collected from different plant organs of the same species *H. perforatum* var. *perforatum* during its phonological cycle: leaves at the stages of vegetative, floral budding, flowering and green capsule; buds, full opened flowers and green capsules. The highest and comparable values in terms of the EO yields were obtained for the leaf at vegetative stage (1), leaf at fruiting (4), and full opened flowers (6) (0.44, 0.61, and 0.53% v/w dry plant, respectively). The lowest yield resulted from the floral buds (5) and green capsules (7) (0.04 and 0.07% v/w dry plants, respectively) (Table 1).

Few data have been reported in the literature on the EO variations related to the plant development cycle in *Hypericum* spp. Changes in the content and EO composition during ontogenesis in *H. perforatum* from wild French populations (Schwob et al., 2004) and field-cultivated plants (Azizi, 2008) were previously reported. However, morphogenetic sampling was not performed in these studies. The present work represents the first report investigating organ-dependence phenologic fluctuations in the EOs content and components in wild *H. perforatum* plants growing in Turkey. Such a numerous specific phenological stages (1–7) were related to the EOs production in wild *H. perforatum* for the first time. Comparing with the few previous data (Schwob et al., 2004; Azizi, 2008), the present results confirmed that the EO yield tends to be the highest at full flowering stage (Table 1).

The complete GC-MS fingerprint of the volatile constituents detected in each hydrodistilled EO are reported in Table 1. The same compounds are grouped in the main chemical classes and submitted in Table 2. The PC values calculated regarding the EO constituents of collected samples are shown in Table 3. PC1 corresponds to 41.48% of the total variation and PC2 corresponds to 22.28% (totally 63.76%). The obtained scatter plot using these first and second PCs is shown in Figure 1. Based on the analysis GC-MS results and the obtained scatter plot, it can be concluded that remarkable differences characterized the EOs composition during phenological cycle. In fact, samples collected at different vegetative stages could be clearly differentiated from samples collected at more generative ones.

Table 3. Cumulative values of calculated principal components for *H. perforatum* plants collected in different stages of plant phenology.

Principal Component	Total	Cumulative %
1	41,48	41,48
2	22,28	63,76
3	11,88	75,64
4	10,48	86,12
5	7,40	93,52
6	6,48	100,00

The EOs constistuents of the green capsules allowed separating them clearly from the other samples. Similarly, the full opened flowers collected at flowering stage were different significantly compared with the other samples. The EOs obtained from leaves collected at the fruiting stage were similar to that of the floral buds. Additionally, no significant differences were observed in volatile constituents between the leaves hydrodistilled at floral budding and at the vegetative stages. The cluster analysis supports these results by dendogram in Figure 2. Results from the second PCA, performed using the relative percentages of the chemical classes revealed significant fluctuations in the content of monoterpens, oxygenated monoterpens and monoterpene hydrocarbons during the all phenological stages. These types of volatiles could be differentiated clearly from the oxygenated hydrocarbons, oxygenated sesquiterpenes and sesquiterpenes (Table 2; Figure 3) which represented the main volatile constituents of the samples harvested at each phenological stage. The produced first two PCs displayed 97. 44 % of the whole variation.

Several peculiar tendencies in specific variations of the typical volatiles were observed during the phenological cycle (Table 2):

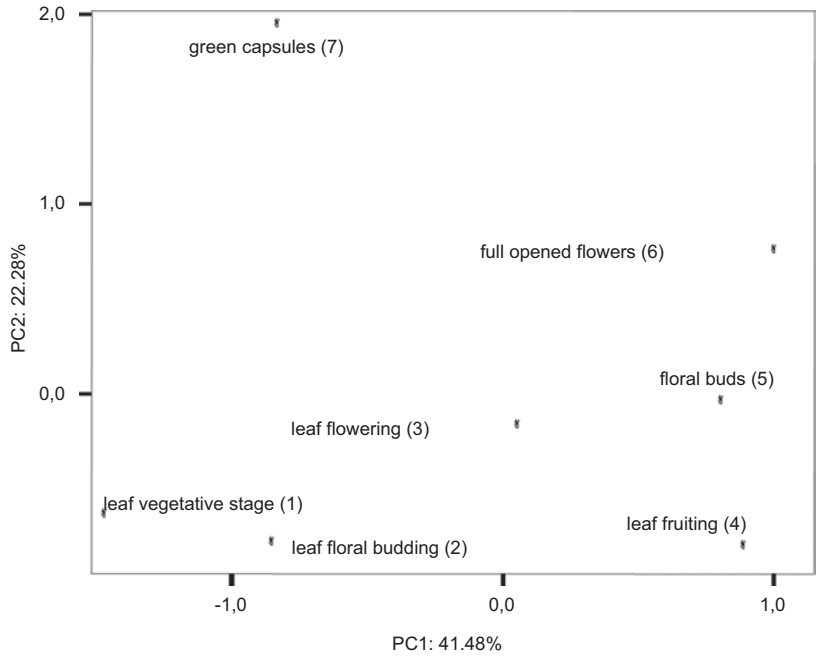

Figure 1. Scatter plot of different parts of *H. perforatum* plants, harvested at different stages of plant phenology.

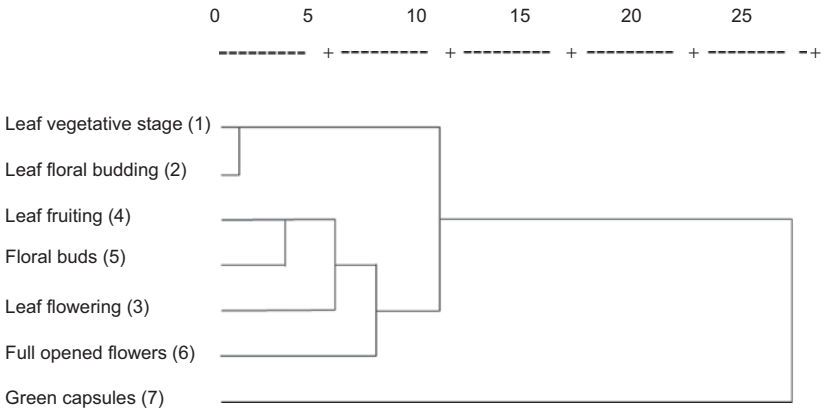

Figure 2. Results of cluster analysis of different parts of *H. perforatum* plants, harvested at different stages of plant phenology.

- Oxygenated derivatives of monoterpene hydrocarbons and sesquiterpenes showed high levels at each phenological stage;
- Oxygenated hydrocarbons showed a decrease from the first up to the last phenological stage;
- Oxygenated sesquiterpenes were the only volatiles which displayed the lowest production at the last phenological stage compared with the other constituents;
- Monoterpene hydrocarbons and sesquiterpene hydrocarbons showed higher percentages comparing the first and last phenological stages after increase/decrease fluctuations.

As reported in the literature, huge amounts of sesquiterpenes both as oxygenated and hydrocarbon sesquiterpenes are characteristic for the *H. perforatum* species native to different regions. In the present study, it was demonstrated that sequiterpenes are regularly produced in significant amounts (oxygenated 26–50% and hydrocarbons 20–40%, Table 2) in all of the seven phenological stages. The same situation was also true for oxygenated hydrocarbons ubiquitarious and abundant in the all analysed samples (13–38%, Table 2). However, even if these two classes of volatiles were the most representative, they showed an opposite trend during phenological cycle. The oxygenated hydrocarbons were predominant especially in the stages 1–2 (38–33%), while their production dropped at the flowering period and the formation of green capsules (3–7, 25–13%). To the contrary, the sesquiterpenes showed the highest levels during the two last stages (40–50%) and the lowest in the first steps (26%). Regarding the other chemical classes of volatile constituents detected in the selected *Hypericum* stages, they were also useful to relate the EOs composition and plant phenological variations. The monoterpenes (hydrocarbons 0.4–6%; oxygenateds 0.8–6%) and linear hydrocarbons (1–3%) were constantly produced at very low levels at the

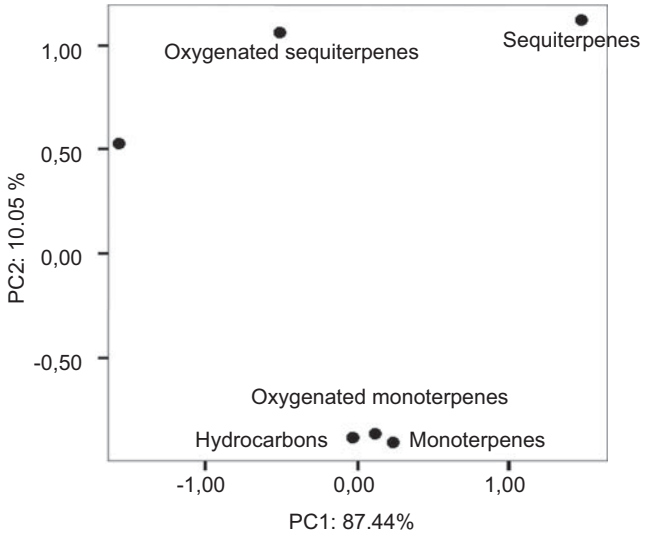

Figure 3. Scatter plot of main chemical classes in essential oil of *H. perforatum* plants, harvested at different stages of plant phenology.

all growth stages in comparison with the main constituents mentioned above.

These results are in contrast with previous data on Turkish *H. perforatum* which reported that α-pinene (50%) and carvacrol (22%) were identified as major constituents (Erken et al., 2001). Similarly α-pinene (61.7%) was reported again to be the major component as well as 3-carene (7.5%), β-caryophyllene (5.5%), myrcene (3.6%), cadalene (3.2%) and β-pinene (3.0%) for Turkish *H. perforatum* by Cakir et al. (1997).

However, in both studies, the specific ontogenetic stages were not established while sampling performed and only full flowering plants in wild were harvested. More importantly, *Hypericum* plants were collected in middle part of Anatolia by Erken et al. (2001) and in the southeastern part by Cakir et al. (1997). In our case, wild plant samples were harvested in the Black Sea region of Anatolia separated from that of Erken et al. (2001) by 350 km and that of Cakir et al. (1997) by 1200 km distances. Therefore, the different sampling procedures and great differences in habitats among previous studies and ours may be reason for the variations in EOs composition of the same species as already observed in our previous study (Cirak et al., 2010).

It is important to point out that a large variability in the EO composition of *H. perforatum* has generally due to the plant material origin in terms of not only the different countries but also the specific habitat of the collection site (Weyerstahl et al., 1995; Cakir et al., 1997; Mimica-Dukic et al., 1998; Gudzic et al., 2001). In fact, hydrocarbons and sesquiterpenes were dominant in EOs of *H. perforatum* especially from French and Serbian populations. However, *H. perforatum* collected from the region Barelic in Serbia was reported to contain important quantity of the monoterpene α-pinene (8.6%), but the same species from the Rujan mountains did not include α-pinene (Gudzic et al., 2001). On the other hand α- and β-pinenes have been indicated as the main constituents for EO of *H. perforatum*, native to Grece (Petrakis et al., 2005). However, it is important to remark that significant differences in the EO composition may be due to a combination of the specific variety and origin of *H. perforatum*. In fact, the major compounds in EO of *Hypericum perforatum* var. *angustifolium* collected in Italy (Sardinia) were 2-methyl octane (21.1%), germacrene D (17.6%) and α-pinene (15.8%) (Pintore et al., 2005).

French *H. perforatum* var. *angustifolium* samples were characterized by spathulenol (21.1%) and branched tetradecanol (9.1%) (Schwob et al., 2002). Besides, huge amounts of sequiterpenes were detected as the main components such as caryophyllene oxide, β-caryophyllene, spathulenol, β-funebrene, 1-dodecanol, and α-muurolene in EOs of *H. perforatum* var. *perforatum* from France population depending on the phenological stages in which sampling performed (Schwob et al., 2004). The results have confirmed those of our study in which *H. perforatum* var. *perforatum* was sampled and analyzed.

It is well-known that the EO of *H. perforatum* is synthesized in specific structures that may be localized in leaves, petals, sepals and pistil (Ciccarelli et al., 2001) which are not present at every stages of the developmental cycle. Schwob et al. (2004) considered one population of *H. perforatum* var. *perforatum* in one French location to study the presence of different types of secretory structures (translucent glands, black nodules, secretory canals) and the EOs production. In that study, the authors suggested that sesquiterpene variations during the phenological cycle should rather be analysed by taking into account also their oxygenated derivatives, as these molecules share closely metabolic pathways. However, monoterpenoids composition and the levels of aliphatic alcohols seemed to be more related to the phenological cycle than sesquiterpenes (Schwob et al., 2004).

Considering the different chemical classes of volatiles, the monoterpenoids were actually the less represented in the EOs composition among the group of terpenoids during phenological cycle in the wild *H. perforatum* collected in Turkey (Table 2). In fact, they increased especially at the last stage 6–7 where both monoterpenes and their oxygenated derivatives showed the highest production (5–6%, Table 2). Based on the morphological and metabolite modifications observed in French *H. perforatum* var. *perforatum* during its phenological cycle by Schwob et al. (2004), the same type of trends was observed for hydrocarbons, monoterpenes and sesquiterpenes during the seven stages of wild Turkish *H. perforatum*, analyzed in the present study. It is true that important qualitative–quantitative differences were pointed out between the EOs composition of French and Turkish *H. perforatum*. But, the present study confirms that the morphological modifications occurring during the different phenological stages have followed the same tendency in the variations of the same chemical classes

of volatile constituents for the same species native to a different country.

Conclusions

Different plant organs of wild Turkish *H. perforatum* var. *perforatum* were collected during seven stages of the phenological cycle: leaves at the stages of vegetative, floral budding, flowering and green capsule; buds, full opened flowers and green capsules of the plant. Based on the EOs composition monitored during these seven morphological stages by GC-MS, PCA and cluster analysis, significant metabolite modifications were observed during the phenological cycle which involved the levels of specific volatile target compounds belonging to the chemical classes of hydrocarbons, monoterpenes, and sesquiterpenes.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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