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Product Quality and Safety

Ontogenetic and diurnal variations of essential oil content of *Hypericum montbretii* Spach, cultivated in Kazdağı (Edremit/Balıkesir), Turkey

C. Paşa[1]*, E. Esendal[2], T. Kiliç[3]

[1]Department of Plant and Animal Production, Altinoluk Vocational School, Balikesir University, Medicinal and Aromatical Plants Programme, Altinoluk, Edremit, Balikesir, Turkey
[2]Department of Field Crops, Faculty of Agriculture, Namik Kemal University, Tekirdağ, Turkey
[3]Faculty of Necatibey Education, Balikesir University, Balikesir, Turkey

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Abstract. *This research was carried out to determine the Hypericum montbretii Spach types essential oil content and composition, and its diurnal variations (9.00 am, 12.00 am and 16.00 pm) during the growing season of 2012. The plant was cultivated in the area of Mount Ida (Balikesir-Edremit), Turkey. The oil composition was determined with GC-MS. During the plant developmental stages the highest quantity of essential oil content (0.30-0.39%) was found at the full plant flowering stage and the lowest one at fresh fruiting stage (0.03-0.04%). Diurnal fluctuation in essential oils of whole plant was also observed – higher were the levels at noon (12.00 am) – 0.04-0.39% compared to the morning (9.00 am) and in the afternoon (16.00 pm). The oils consisted mainly of: at the vegetative stage - germacrene-D (6.9-11.7 %), gamma-Gurjunene (6.1-11.6%), 2-methyl octane (3.8-4.9%), δ-cadinol (2.7-4.8%) and phytol (2.9-4.5%); at the flowering stage - α-pinene (26.4-28.0%), undecane (14.1-16.0%), β-pinene (12.8-14.3%), delta-cadinene (6.8-8.0%) and caryophyllene (4.9-6.0%); at the fresh fruiting stage - amorphene (6.8-8.2%), β- caryophyllene (4.1-5.3%), delta-cadinene (5.0-5.5%) and α-cadinene (4.3-6.1%).*

Keywords: *Hypericum montbretii* Spach, diurnal variations, developmental stages, essential oil, β-pinene

Introduction

The genus *Hypericum* contains approximately 400 different species of annuals, perennials, shrubs and small trees, ranging from very small perennials to trees. The species of this genus have been used as healing agent due to their various medicinal properties for the last two hundred years (Dias et al., 1998). Hypericum species are also used as sedatives, antiseptics and antispasmodics in Turkish folk medicine under the names “kantaron, peygamber çiçeği, kılıçotu, kan otu, kuzu kıran and binbirdelikotu” (Baytop 1999). *Hypericum* genus is represented in Turkey by 89 species, of which 43 are endemic (Davis 1988).

Hypericum montbretti Spach (Guttiferae, Clusiaceae) is a perennial herb widely distributed in northern Turkey as well as in the Balkans, Syria and Georgia. Naphthoadiantrones (hypericin, pseudohypericin, etc.), phloroglucinol derivatives (hyperforin, adhyperforin), flavonoids (rutin, quercetin, quercitrin, etc.), xanthones, biflavonoids, chlorogenic acid and volatile compounds have been determined in *H. montbretti* extracts (Cirak et al., 2008; Öztürk et al., 2009; Can et al., 2011).

The methanolic extract of the aerial parts of *Hypericum* species has been reported to contain many bioactive compounds, namely the naphthodianthrones, hypericin and pseudohypericin, the phloroglucinol, derivatives hyperforin and adhyperforin, flavonoids, essential oils, xanthones, tannins, procyonids and other water-soluble components that possess a wide array of biological properties (Cirak et al., 2011). The essential oil components of the *Hypericum* species are composed of hydrocarbons, monoterpenes (α-pinene, β-pinene) and sesquiterpenes (β-caryophyllene, caryophylleneoxide) (Sköld et al., 2006). *Hypericum* montbretii Spach species: α-pinene, undecane, germacrene-D, β-pinene, carvacrol, caryophyllene, β-caryophyllene and δ-cadinene and gamma-Gurjunene components have been identified (Erken et al., 2001).

The purpose of the present study was to determinate the components of essential oil content and its ontogenetic variations during a growing season at *Hypericum montbretii* Spach species, cultivated in Mount Ida, Balikesir-Edremit, Turkey.

Material and methods

Object of study

The object of study was *Hypericum montbretii* Spach, which was collected at different stages of plant development from Edremit district of Balikesir province, Turkey, between April and August of 2012. The soil of the trial area was sandy with pH value - 6.9 and content of organic matter - 6.8%, sand - 68%, silt - 24% and clay - 8%. On the place where the trial was reflected the mean air temperature was 20.4ºC, mean rainfall - 28.1 mm and relative humidity - 60.7%.

Sampling and sample preparation

Ontogenetic sampling corresponded with different date for *Hypericum montbretii* Spach shoots with leaves harvested at the vegetative stage. At the full flowering stage, only shoots with fully opened flowers were harvested. At the fresh fruiting stage, the shoots which had green capsules were harvested. The plant materials were dried at room temperature (20ºC). Dried plant sample (50g) was subjected to hydro distillation for 6h using a Clevenger type apparatus for determining the oil content.

Oil composition and analyses

The oil composition was determined with GC-MS. GC-MS analyses were conducted in the TUBITAK (MAM). GC-MS conditions: helium was used as carrier gas at a constant flow rate of

* e-mail: cenk_pasa1903@hotmail.com

1mL/min in which 1µL of sample was injected. The GC temperature program was set as follows: 50ºC hold for 5 min, ramp to 250ºC at 5ºC/min and hold for 10min. The temperature of the MS transfer line was set at 220ºC. Using scan mode a mass range from 50 to 650 m/z. The column used was DB-5 30 m x 0.25 mmID x 0.25 µm. The Thermo Scientific TSQ GC-MS/MS was used in this study. Database of GC-MS device in TUBITAK Chemistry Research Laboratory was used.

Statistics

Statistical analysis was subjected to Analysis of Variance using MSTAT software program. The DUNCAN test was applied to the results using the same program.

Results and discussion

Total essential oils content in *Hypericum montbretii* Spach during ontogenetic development was higher in the full flowering stage (0.30-0.39%), followed by vegetative stage (0.19-0.28%) and fresh fruiting stage (0.03-0.04%) (Table 1). The differences among essential oils values during the different developmental stages were significant and statistically proven at P<0.01. One-way tendency of change in the essential oils content during the different hours of the day was observed. In all developmental stages the oils values were higher at noon (12.00 am) – 0.04-0.39% compared with the morning (9.00 am) – 0.03-0.30% and the afternoon (16.00 pm) – 0.03-0.34%.

Table 1. Total essential oils content (%) and changes during collecting times of the day and development stages of *Hypericum montbretii* Spach

Diurnal collecting times**	Developmental stages***			
	Vegetative, %	Full flowering, %	Fresh fruiting, %	Mean[1], %
09:00 am	0.28[c]	0.30[c]	0.03[g]	0.20[ab]
12:00 am	0.21[d]	0.39[a*3]	0.04[f]	0.21[a]
16:00 pm	0.19[de]	0.34[b]	0.03[g]	0.19[b]
Mean[2]	0.22[b]	0.34[a]	0.03[c]	0.20

*Diurnal x developmental int.: (p<0.01); **Diurnal: (p<0.01); ***Developmental stages: (p<0.01);
LSDmean1: 0.092; LSDmean2: 0.043; LSDint.3: 0.0189;
There is no statistically significant difference (P>0.05) between figures including the same letters in the columns.

Investigations of ontogenetic variation of secondary metabolites have been made over several, e.g. alkaloid changes during fruit development in *Papaver somniferum* (Miriom and Pfeifer, 1959) and *Conium maculatum* (Fairbairn and Challen, 1959). Also, it was found that essential oil changed during the course of ontogenesis in *Hypericum perforatum* (Schwob et al., 2004), as well as the artemisinin level during phonological cycle of *Artemisia annua* (Gupta et al., 2002) and foliar monoterpenoid variation in *Umbellularia californica* in seedlings, saplings and adult tree stages. Chemical concentrations vary considerably during the course of ontogenesis in a medicinal plant, not only the concentrations of plant chemicals fluctuate through the season, but they can also be short-lived and experience rapid turnover (Smith et al., 1996).

The identity, the retention time and percent composition of the essential oils content from *Hypericum montbretii* Spach is listed in Table 2. As can be seen, the studied oils were resolved into 65 components at the vegetative, full flowering and fresh stage, respectively.

Table 2. Variation of essential oils content of *Hypericum montbretii* Spach within a day during the ontogenetic development (%)

KI*	RT**	Compounds	Vegetative stage			Full flowering stage			Fresh fruiting stage		
			09:00 am	12:00 am	16:00 pm	09:00 am	12:00 am	16:00 pm	09:00 am	12:00 am	16:00 pm
861	11.78	2-methyl octane	4.9	4.0	3.8	-	-	-	0.8	1.0	0.6
900	12.99	Nonane	1.6	1.2	1.6	-	-	-	-	-	-
939	14.25	α-pinene	3.4	5.8	4.5	27.7	28.0	26.4	1.5	1.9	1.3
953	14.85	Camphene	0.8	1.0	0.8	-	-	-	-	-	-
979	15.77	β-pinene	3.7	4.8	3.4	14.3	13.5	12.8	2.6	2.2	2.3
991	16.01	β-myrcene	2.0	0.8	1.0	-	-	-	-	-	-
1000	16.36	Decane	0.9	0.7	0.6	-	-	-	-	-	-
1017	16.75	α-terpinene	0.5	0.5	0.4	-	-	-	-	-	-
1025	17.22	σ-cymene	0.6	0.7	0.6	-	-	-	-	-	-
1029	17.40	Limonene	1.9	0.8	1.1	0.5	0.4	0.7	0.8	1.1	0.5
1037	17.48	β- ocimene(Z)	1.0	0.7	0.7	-	-	-	-	-	-

1050	17.84	β- ocimene(E)	1.1	0.7	0.6	-	-	-	-	-	-
1060	18.29	τ-terpinene	0.7	0.6	0.7	-	-	-	-	-	-
1063	18.39	2-methyl decane	0.7	0.6	0.7	-	-	-	-	-	-
1099	19.51	Undecane	0.9	0.8	0.7	16.0	15.4	14.1	0.6	0.8	0.5
1122	20.20	Fenchol, exo-	0.9	0.8	0.8	-	-	-	-	-	-
1141	20.96	Cis-verbenol	3.0	0.7	1.7	-	-	-	-	-	-
1165	21.77	1-borneol	0.6	0.5	0.8	-	-	-	-	-	-
1177	21.98	4-terpineol	0.9	0.3	1.0	-	-	-	-	-	-
1189	22.35	α-terpineol	0.7	1.1	0.9	3.0	2.8	2.9	3.3	2.9	2.4
1290	25.06	Thymol	0.5	0.5	0.3	-	-	-	-	-	-
1351	26.54	α-cubebene	0.7	0.6	0.7	-	-	-	-	-	-
1375	27.19	α-ylangene	0.6	0.6	0.5	-	-	-	-	-	-
1377	27.34	α-Copaene	0.8	0.7	0.9	3.9	2.8	3.0	1.8	2.0	1.3
1387	27.62	Dodecanal	0.7	0.9	0.8	0.8	1.1	1.0	0.8	1.3	0.6
1419	28.52	Caryophyllene	2.0	1.3	1.1	6.0	5.4	4.9	0.9	1.1	0.7
		β-caryophyllene	2.0	2.3	1.7	3.2	3.0	2.4	4.8	4.1	5.3
1430	28.73	β-copaene	0.7	0.9	1.0	1.5	1.4	1.1	2.2	0.9	1.5
1441	29.01	Aromadendrene	3.3	1.4	2.1	2.0	2.6	2.7	1.0	1.4	1.1
1455	29.40	α-humulene	2.0	1.5	1.7	0.6	1.1	1.1	1.5	2.0	1.3
1477	29.53	gama-Gurjunene	11.6	6.1	9.0	1.8	2.1	3.0	0.8	1.0	0.5
1480	29.81	τ-muurolene	6.6	2.8	4.3	7.9	9.1	6.4	1.1	1.0	0.9
1485	29.90	Amorphene	1.0	0.7	0.8	0.6	1.0	1.1	8.2	7.5	6.8
1485	30.05	Germacrene D	11.7	6.9	8.4	3.7	4.2	3.0	1.0	1.1	0.6
1496	30.23	gama-amorphene	3.2	2.9	2.3	6.0	5.4	4.2	4.2	3.9	3.9
1496	30.35	Valencene	1.7	1.3	1.2	4.6	4.8	4.1	5.1	4.1	4.1
1514	30.48	gama-cadinene	1.1	1.0	0.9	5.5	6.0	6.1	5.2	4.8	4.5
1523	30.74	delta-cadinene	3.8	3.4	2.9	8.0	7.2	6.8	5.5	5.1	5.0
1539	30.82	α-cadinene	4.4	4.2	3.6	3.1	2.8	3.5	4.3	5.2	6.1
1540	30.92	Calamenene	1.2	1.1	0.9	0.6	1.1	1.5	1.0	1.1	1.6
1536	31.17	α-bisabolene	0.7	0.8	0.8	2.1	1.9	2.2	0.4	0.5	0.3
1556	31.27	Nerolidol	0.7	1.1	0.5	0.7	1.1	1.0	0.6	0.4	0.5
1566	31.39	α-calacorene	0.5	0.6	0.5	0.6	0.9	1.0	0.5	0.6	0.5
1567	31.69	Dodecanoic acid	0.9	1.0	0.9	5.5	7.1	6.4	-	-	-
1570	31.94	Cis-3-hexenyl benzoate	0.8	0.6	0.7	7.6	7.2	7.4	0.6	0.8	0.5
1578	32.28	Spathulenol	3.6	2.6	1.8	0.9	1.2	1.0	2.8	3.3	3.0
1585	32.52	Globulol	1.8	1.3	2.0	2.1	1.8	2.0	4.1	4.0	3.7
1593	32.73	Viridiflorol	0.7	0.5	0.7	4.7	3.5	4.2	-	-	-
1619	33.12	Cubenol|<1,10-di-epi->	0.9	0.4	0.7	7.9	6.8	5.9	0.9	0.7	0.5
1635	33.41	Cubenol	1.8	1.6	1.2	0.7	1.1	1.0	1.1	1.0	0.9
1644	33.57	δ-cadinol	4.5	4.8	2.7	0.6	0.9	1.1	5.0	4.2	4.4
1647	33.75	tau-muurolol	1.9	1.7	1.2	-	-	-	4.1	5.5	4.0
1657	34.04	α-cadinol	2.5	2.1	1.7	-	-	-	-	-	-
1664	34.14	Caryophylla-3(15), 7-dienol(6) I	0.8	0.3	0.6	0.7	0.5	0.5	0.8	0.5	0.3
1682	34.39	Caryophylla-3(15), 7-dienol(6) II	0.7	0.4	0.4	0.6	0.5	0.6	0.9	1.1	0.7
1748	35.93	Tetradecanoic acid	1.0	0.6	0.6	-	-	-	-	-	-
1876	36.55	Hexadecanol	0.9	0.5	0.5	0.2	0.3	0.2	-	-	-
1910	37.72	Palustrol	0.6	0.4	0.4	0.8	0.7	0.8	-	-	-
1922	40.02	Hexadecanoic acid	0.8	1.1	0.9	0.3	0.4	0.6	0.5	0.2	0.6

1944	42.84	Phytol	4.5	3.7	2.9	-	-	-	0.4	0.9	0.6
2099	46.09	Heneicosane	0.6	0.7	0.6	0.3	0.5	0.4	0.2	0.4	0.2
2504	49.22	Pentacosane	0.9	0.9	0.8	-	-	-	0.4	0.5	0.3
2706	52.18	Heptacosane	1.0	0.8	0.8	0.5	0.6	0.5	0.6	0.5	0.4
2797	54.71	Octacosane	1.7	1.9	1.3	-	-	-	-	-	-
2902	56.02	Nonacosane	1.6	0.9	1.0	0.3	0.2	0.2	0.8	0.4	0.5

*KI: Kovats Index; **RT: Retention Time

At the vegetative stage, the oils consisted mainly of germacrene-D (6.9-11.7%), gamma-Gurjunene (6.1-11.6%), 2-methyl octane (3.8-4.9%), δ-cadinol (2.7-4.8%) and phytol (2.9-4.5%). At the flowering stage the oils consisted mainly of α-pinene (26.4-28.0%), undecane (14.1-16.0%), β-pinene (12.8-14.3%), delta-cadinene (6.8-8.0%) and caryophyllene (4.9-6.0%). At the fresh fruiting stage the oils consisted mainly of amorphene (6.8-8.2%), β- caryophyllene (4.1-5.3%), delta-cadinene (5.0-5.5%) and α-cadinene (4.3-6.1%).

The essential oil components of the Hypericum species are composed of hydrocarbons, monoterpenes (α-pinene, β-pinene) and sesquiterpenes (β-caryophyllene, caryophylleneoxide) (Sköld et al., 2006). *Hypericum montbretii* Spach species: α-pinene (25.7%), undecane (4.8%), germacrene-D (5.9%), β-pinene (18.8%), carvacrol (22.0%), caryophyllene, β-caryophyllene (5.7%), δ-cadinene (4.9 %) and gamma-Gurjunene (8.6%) components have been identified (Erken et al., 2001). Hypericum species have changed in essential oil contents and components according to changes in geographical conditions (Touafek et al., 2005; Nogueira et al., 2008).

Conclusion

Ontogenetic and diurnal variations were applied on Hypericum montbretii Spach and 67 essential oil components were detected. The main components of the oil of *Hypericum montbretii* Spach aerial parts were α-pinene, undecane, β-pinene, β- caryophyllene and germacrene-D. Essential oils in whole plant increased during flower ontogenesis and reached their highest level at full flowering. Then it decreased at the fresh fruiting stage. The highest level was at full flowering stage 0.39% (12:00 am) and the lowest one - at fresh stage 0.03% (09:00 am-16.00 pm).

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