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A RESEARCH ON DETERMINATION OF ONTOGENETIC AND DIURNAL VARIATION OF ESSENTIAL OIL CONTENT AND COMPOSITION IN *Hypericum kazdaghensis* GROWING WILD IN IDA

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Abstract

The research carries out the determination in Hypericum kazdaghensis types of components essential oil content for growing season in Mount Ida (Turkey-Balikesir-Edremit) in 2012. Moreover the diurnal and ontogenetic variations were investigated.
In this paper we determine that change in essential oils of whole plant within a day during the course of ontogenetic did not follow the same trend in H. kazdaghensis. 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 at full flowering 0.26% and the lowest level fresh stage is 0.02%. We obtained the six-four components from aerial parts of H. kazdaghensis at the vegetative, full flowering and fresh stage. In addition, we determined that the oils consisted of mainly calamene (29.4%), germacrene-D (20.1%), gurjunene-gama (14.8%), tau-muurool (9.0%); cubenol (6.0%) and δ -cadinol (6.0%). at the vegetative stage. Finally we determined that the oils consisted calamene (16.5%), gurjunene-gama (12.8%), germacrene-D (10.9%) and α-cadinene (7.9%) at the fresh fruiting stage.

Key words*: Hypericum kazdaghensis, essential oil content, calamine, germacrene-D.*

INTRODUCTION

Hypericum L. (*Hypericaceae*) is a large genus of herbs or shrubs, which grown in temperate regions of the world (Campbell and Delfosse, 1984). The genus *Hypericum* contains 469 species that have been classified into 36 taxonomic sections by the most recent count (Crockett, 2010). *Hypericum* species are also used as sedatives, antiseptics and antispasmodics in Turkish folk medicine (Baytop, 1999). Turkey is an important place for *Hypericum* species. The *Hypericum* genus, a member of the *Hypericaceae* family is represented in Turkey by 96 species of which 43 are endemic (Cirak et al., 2006; Aslan, 2012).
Morphologically, *Hypericum* species are characterized by the presence of different kinds of secretory tissues including light glands, dark glands and secretary canals. These secretory structures are sites of synthesis and accumulation of biologically active substances and their localizations are different depending on plant tissue (Cicracelli et al., 2001). Therefore, organ-dependence of phenolic compounds has an important role to understand the underlying sources of variation in phenolic contents of *Hypericum* species (Ayan et al., 2007).
The research carries out the determination in *Hypericum kazdaghensis* types of components essential oil content for growing season in Mount Ida (Turkey-Balikesir-Edremit) in 2012. Moreover, the diurnal and ontogenetic variations were investigated.

MATERIALS AND METHODS

Hypericum kazdaghensis 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, pH value (6.9), organic matter (6.8%), sand (68%), silt (24%) and clay (8%). On the place where the trial was reflected mean temperature is 20.4 ºC, mean rainfall is 28.1 mm and relative humidity is 60.7 % in

2012. Collections were done three times a day (9.00 am; 12.00 am and 16.00 pm) for each development stages. Ontogenetic sampling corresponded with different date for *Hypericum kazdaghensis* shoots with leaves were 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 materials (50 g each *Hypericum kazdaghensis*) was subjected to hydro distillation for 6 h using a Clevenger type apparatus for determining the oil content. 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 1 mL/min. 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 10 min. The temperature of the MS transfer line was set at 220ºC. Using scan mode a mass range from 50 to 650 *m/z*. Used column, DB-5 30 m x 0.25 mm ID x0.25 µm. The Thermo Scientific TSQ GC-MS/MS was used in this study.

RESULTS AND DISCUSSIONS

Results of this study reveal that Diurnal and ontogenetic variations significantly affected ($p<0.01$) essential oils. The differences between that means were compared by Duncan's multiple range test (Duncan's test). They are shown Table 1.

Table 1. Diurnal collecting times and developmental stages of *Hypericum kazdaghensis*

Diurnal Collecting Times	Developmental Stages			
	Vej. Stage	Full Flow.	Fresh Fruit	Mean
09:00 am	0.19	0.23	0.02	0.15
12:00 am	0.17	**0.29**	0.02	**0.16**
16:00 pm	0.16	0.26	0.02	0.15
Mean	0.17	**0.26**	0.02	0.15

Change in essential oils of whole plant within a day during the course of ontogenetic did not follow the same trend in *Hypericum kazdaghensis*. Essential oils in whole plant increased during flower ontogenesis and reached their highest level at full flowering. Then it decreased at the full flowering stage. The highest level at full flowering 0.26% and the lowest level fresh stage is 0.02%. The difference among essential developmental stages was found significant ($p<0.01$). Diurnal fluctuation in essential oils of whole plant was also observed for *Hypericum kazdaghensis* and it was highest (0.29%) at 12:00 pm (Table 1).

Investigations of ontogenetic variation of secondary metabolites have been made over several, eg. alkaloid changes during fruit development in *Papaver somniferum* (Miriom and Pfeifer, 1959) and *Conium maculatum* (Fairbairn and Challen, 1959). Also essential oil changes during the course of ontogenesis in *Hypericum perforatum* (Schwob et al., 2004), changes of artemisinin 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).

This compositional trend which is characterized by an increase of the oil complexity during the plant development suggests that numerous metabolic pathways were elicited in the *Hypericum triquetrifolium* secondary metabolism (Schwob et al., 2004). The major constituents of the oil were 3-methyl nonane (10.5-43.5%), carvacrol (0.2-7.6%), caryophyllene (10.4-32.9%), Germacrene-D (2.9-13.6%), α-pinene (2.7-17.6%) and Caryophyllene oxide (1.4-10.8%).

Table 2. Variation of essential oils content of *Hypericum kazdaghensis* within a day during the course of ontogenetic (%)

KI	RT	Compounds	Vej. Stages / 09.00 am	Vej.Stages / 12.00 am	Vej. Stages/ 16:00 pm	Full Flow./ 09:00 am	Full Flow./ 12:00 am	Full Flow./ 16:00 pm	Fresh Fruit/ 09.00 am	Fresh Fruit/ 12.00 am	Fresh Fruit/ 16.00 pm
861	11.78	2-methyl octane	3.1	3.0	2.1	-	-	-	2.3	2.2	4.8
939	14.25	α-pinene	4.0	3.1	3.0	-	-	-	6.0	5.3	1.1
971	15.41	3-methyl nonane	0.7	0.7	0.8	-	-	-	0.7	0.7	0.9
991	16.01	β-myrcene	0.6	0.4	0.4	-	-	-	0.6	0.7	0.7
1025	17.22	σ-cymene	0.8	0.5	0.4	-	-	-	0.7	0.6	0.7
1037	17.48	β- ocimene(Z)	0.7	0.8	0.9	-	-	-	0.7	0.7	0.9
1060	18.29	τ-terpinene	1.1	1.2	0.8	-	-	-	1.0	1.0	0.5
1097	19.46	β-linalool	0.9	1.4	0.9	-	-	-	0.6	1.2	0.3
1122	20.20	Fenchol, exo-	1.0	0.9	0.7	-	-	-	0.3	0.7	1.2
1141	20.96	Cis-verbenol	2.4	1.6	1.8	1.0	0.9	1.1	1.7	2.3	0.7
1177	21.98	4-terpineol	0.7	0.8	0.9	5.8	6.1	6.0	0.7	1.0	0.4
1194	22.52	Myrtenol	0.7	0.7	1.0	1.3	0.5	0.9	0.5	0.7	0.4
1253	24.13	Piperitone	0.5	0.5	0.4	4.2	3.4	3.8	0.4	0.6	0.6
1290	25.06	Thymol	0.5	0.4	0.6	3.3	2.6	2.9	0.3	0.4	0.7
1338	26.18	τ-elemene	0.7	0.9	1.0	0.9	0.4	0.9	0.7	0.7	0.8
1351	26.54	α-cubebene	0.8	0.6	0.9	0.8	0.9	0.8	0.9	0.8	0.6
1375	27.19	α-ylangene	0.9	1.2	0.8	4.0	3.8	3.5	0.6	0.6	4.0
1377	27.34	α-Copaene	5.8	2.4	4.0	1.4	0.6	1.1	5.1	4.0	2.8
1387	27.62	Dodecanal	2.4	1.6	1.9	0.8	0.5	0.5	3.4	2.0	0.9
1418	28.45	β-cedrene	2.0	1.3	1.4	6.0	5.8	6.0	1.8	1.1	0.2
		β-caryophyllene	1.8	2.1	1.5	4.0	3.1	3.5	0.7	0.4	0.7
1430	28.73	β-copaene	1.2	1.1	1.0	18.6	16.7	17.4	1.2	1.5	2.2
1441	29.01	Aromadendrene	1.4	1.5	1.3	4.9	2.8	3.2	4.3	2.9	2.8
1455	29.40	α-humulene	2.4	1.7	1.4	4.0	2.6	3.0	4.3	2.4	3.2
1477	29.53	Gurjunene-gama	14.8	10.0	16.0	0.9	0.3	0.8	12.8	7.3	8.2
1480	29.81	τ-muurolene	6.1	3.8	5.0	1.8	2.9	2.5	3.8	2.8	1.2
1485	29.90	Amorphene	0.9	0.9	0.9	4.1	4.2	4.0	0.9	0.6	3.0
1485	30.05	Germacrene D	20.1	12.1	17.1	16.0	16.4	15.7	10.9	7.2	13.2
1496	30.23	gama-amorphene	4.2	3.8	4.0	0.5	0.4	0.5	3.3	2.8	3.0
1496	30.35	Valencene	1.8	1.3	1.5	0.5	0.6	0.8	3.8	3.9	3.2
1514	30.48	gama-cadinene	1.1	0.9	1.0	1.5	1.2	1.1	0.8	0.2	1.1
1523	30.74	delta-cadinene	3.2	3.0	2.4	1.4	1.3	1.3	2.6	1.5	3.2
1539	30.82	α-cadinene	7.2	6.1	6.0	4.1	4.5	4.0	7.1	5.9	7.9
1540	30.92	Calamenene	29.4	30.1	25.3	6.0	6.8	5.5	16.5	11.3	9.7

KI	RT	Compounds	Vej. Stages / 09.00 am	Vej.Stages / 12.00 am	Vej. Stages/ 16:00 pm	Full Flow./ 09:00 am	Full Flow./ 12:00 am	Full Flow./ 16:00 pm	Fresh Fruit/ 09.00 am	Fresh Fruit/ 12.00 am	Fresh Fruit/ 16.00 pm
1536	31.17	α-bisabolene	0.8	0.7	0.9	0.5	0.7	1.0	0.9	0.3	0.6
1556	31.27	Nerolidol	0.7	0.8	0.8	0.5	0.7	0.4	0.9	0.4	0.5
1567	31.69	Dodecanoic acid	0.6	0.9	1.1	1.5	1.3	1.0	0.9	0.7	0.5
1570	31.94	Cis-3-hexenyl benzoate	0.9	0.9	1.0	1.4	1.5	1.6	0.7	0.9	0.6
1576	32.11	Germacrene D-4-ol	0.8	0.8	0.7	-	-	-	0.9	0.8	0.8
1578	32.28	Spathulenol	2.3	1.6	1.3	2.4	2.8	2.2	5.2	2.8	4.0
1583	32.45	Caryophyllene oxide	2.1	0.9	2.2	-	-	-	4.0	2.5	2.3
1585	32.52	Globulol	1.0	1.3	1.0	1.3	1.5	1.1	0.8	0.4	0.7
1593	32.73	Viridiflorol	0.9	0.7	0.9	1.1	1.0	1.0	0.8	0.6	0.8
1608	32.97	Ledol	0.6	0.9	0.7	1.4	1.2	1.3	0.9	0.7	0.6
1619	33.12	Cubenol|<1,10-di-epi->	0.8	0.9	0.8	0.9	1.0	1.0	0.7	0.4	0.7
1635	33.41	Cubenol	6.0	4.3	4.1	8.0	8.9	7.8	5.0	4.0	4.0
1644	33.57	δ-cadinol	6.0	3.0	4.1	2.3	2.1	2.0	7.3	5.1	5.5
1647	33.75	tau-muurolol	9.0	8.1	6.3	4.2	3.8	3.1	9.0	7.7	7.8
1657	34.04	α-cadinol	5.1	5.0	4.0	6.1	5.8	4.7	5.3	5.3	5.3
1664	34.14	Caryophylla-3(15),7-dienol(6) I	1.1	0.9	1.1	1.8	1.7	1.4	1.4	0.9	1.6
1684	34.70	α-santalol	0.9	1.1	0.6	-	-	-	1.0	0.8	0.9
1748	35.93	Tetradecanoic acid	1.1	0.7	0.8	-	-	-	1.2	0.9	1.1
1769	36.42	Benzyl benzoate	0.5	1.4	1.3	-	-	-	0.8	0.7	0.6
1876	36.55	Hexadecanol	0.6	0.7	0.9	0.5	0.4	0.7	0.8	0.6	0.7
1910	37.72	Palustrol	0.8	0.9	0.8	0.7	0.8	0.4	1.2	0.8	0.9
1922	40.02	Hexadecanoic acid	1.0	1.2	0.9	0.9	1.1	0.4	1.0	0.9	1.0
1944	42.84	Phytol	3.8	4.1	3.4	3.4	4.0	2.7	7.0	5.1	5.0
2099	46.09	Heneicosane	1.1	0.9	1.1	0.9	1.1	0.6	2.0	1.9	2.1
2304	48.07	Tricosane	1.7	2.1	1.4	2.8	2.9	2.5	3.1	2.5	2.7
2504	49.22	Pentacosane	1.2	1.8	1.7	0.8	1.0	1.1	1.6	1.4	2.0
2706	52.18	Heptacosane	4.0	1.9	2.7	1.8	2.0	2.2	5.8	4.5	6.0
2902	56.02	Nonacosane	4.6	2.2	3.0	5.3	5.7	4.7	4.8	4.1	5.1
		α-selinene	3.0	3.8	3.4	-	-	-	0.8	0.9	0.4
		β-selinene	3.5	4.3	3.0	-	-	-	0.5	0.7	0.5

Most of them have been previously reported in the essential oil of *Hypericum triquetrifolium* (Bertoli et al., 2003; Petrakis et al., 2005; Hosni et al., 2011); *H.perforatum, H.tetrapterum, H. olympicum* (Pavlovic et al., 2006); *H. kazdaghensis; H. aucherii, H.perforatum* and *H. montbretii* (Pasa, 2013); *H. richerii* (Ferretti et al., 2005) and *H. hirsutum* (Gudzic et al., 2007).

For example, Petrokis et al. (2005) studied the essential oil of Greek specimens without specifying the phonological stage and found that 2-methyloctane, α-pinene, n-nonane, β-caryophyllene and 3-methylnonane; Hosni et al. (2011), β-caryophyllene, n-nonane, α-pinene, germacrene-D, n-octane and 2-methyloctane. Report from Italy showed that the n-nonane, β-pinene, β-caryophyllene, α-pinene, myrcene, sabinene, germacrene-D, Caryophyllene oxide were the major compounds of the leaf and flowers essential oils (Bertoli et al., 2003).

The identity, the retention index and percent composition of the essential oils content from *Hypericum kazdaghensis*. are listed Table 2. As can be seen the studied oils were resolved into 64 components at the vegetative, full flowering and fresh stage respectively.

At the vegetative stage, the oils consisted mainly of calamene (29.4%), germacrene-D (20.1%), gurjunene-gama (14.8%), tau-muurool (9.0%); cubenol (6.0%) and δ -cadinol (6.0%). At the flowering stage the oils consisted mainly of β-copaene (18.6%), germacrene-D (16.4%), cubenol (8.9%), 4-terpineol (6.1%) and calamine (4.5%). At the fresh fruiting stage flowering stage the oils consisted mainly of calamene (16.5%), gurjunene-gama (12.8%), germacrene-D (10.9%), α-cadinene (7.9%), cubenol (6.0%) and δ -cadinol (7.3%).

The effects of the diurnal variation on the essential oils composition *Hypericum kazdaghensis* have not been reported previously. Nevertheless, differences in the essentials composition of developmental stages have been described for the closely related species *H. perforatum* (Schwab et al., 2004), *H. aucherii, H.perforatum* and *H. montbretii* (Pasa, 2013), *Hypericum triquetrifolium* (Hosni et al., 2011).

CONCLUSIONS

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 at full flowering 0.26% and the lowest level fresh stage is 0.02%. We obtained the six-four components from aerial parts of *H. kazdaghensis* at the vegetative, full flowering and fresh stage.

In addition, we determined that the oils consisted of mainly calamene (29.4%), germacrene-D (20.1%), gurjunene-gama (14.8%), tau-muurool (9.0%); cubenol (6.0%) and δ-cadinol (6.0%). at the vegetative stage. Finally, we determined that the oils consisted calamene (16.5%), gurjunene-gama (12.8%), germacrene-D (10.9%) and α-cadinene (7.9%) at the fresh fruiting stage.

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