An investigation on the determination of diurnal and ontogenetic variabilities of essential oil content and composition in Hypericum triquetrifolium Turra (Hypericaceae)

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Abstract: Hypericum genus includes especially species used as depression and wound healing in alternative and modern medicine. In this study, Hypericum triquetrifolium which grows naturally in western of Turkey (Kazdağ Mount / Edremit Balikesir) were investigated essential oil content, essential oil composition, diurnal and ontogenetic variabilities. The diurnal and ontogenetic variabilities of the species were first studied. Essential oils in part of the aerial plant raised during flower ontogenesis and achieved the highest level at full flowering and decreased at the fresh fruiting phase. The highest level at full flowering is 0.30% while the lowest level of fresh fruiting is 0.09%. As a result of the study, 27 components were detected from the aboveground parts of H. triquetrifolium at the before flowering, beginning of flowering and full flowering stages. The major components were obtained caryophyllene (32.9%) and caryophyllene oxide (10.8%) at the before the flowering stage, 3-methyl nonane (17.1%) and caryophyllene (14.9%) at the flowering stage and 3-methyl nonane (43.5%) and α-pinene (17.6%) at the fresh fruiting stage.

Keywords: Diurnal variability - Essential oil components - Hypericum triquetrifolium - Ontogenetic variability.

INTRODUCTION

Hypericum L. is an important member of Hypericaceae family and is a large genus of herbs or shrubs included medicinal species as Hypericum perforatum Turra which distributed in temperate areas of the world (Ciccarelli et al. 2001). The Hypericum contains about 500 species by the most recent count that has been classified into 36 taxonomic sections (Crockett & Robson 2010). The Hypericum is represented in Turkey by 96 species (106 taxa) of which 48 are endemic (Aslan et al. 2012). Hypericum species are morphologically characterized by the presence of secretory tissues including glands which to be dark or light-coloured on the stem, leaves and flowers (Campbell & Delfosse 1984, Ciccarelli et al. 2001, Cirak et al. 2006, Ayan et al. 2007).

Hypericum species mostly are showing the distribution South Europe, Africa, Cyprus, Syria, Syrian Iraq, Iran, Njery and Turkey. It is a perennial plant that grows in open dry, sandy ground and mesophytic areas as habitat in Turkey (Robson 1967).

Hypericum taxa have traditionally been used in the treatment of wounds, burns and stomachic diseases by local peoples in Turkey (Baytop 1999, Selvi & Pasa 2011). In addition it has been ethnomedical used in the world as antiseptic, antimicrobial, sedative, antihistaminic, antioxidant, and cytotoxic (Conforti et al. 2002, Couladias et al. 2002, Bertolli et al. 2003, Kizil et al. 2004, Pistelli et al. 2005).

In this study, the determination of diurnal and ontogenetic variabilities of essential oil content and composition in H. triquetrifolium distributed in naturally Turkey were investigated. The diurnal and ontogenetic variabilities of this species were first studied. With study, the amount of essential oil contained the plant; in
which part of the plant, during which development period and at what times of the day and the essential character of essential oils will be determined. In addition, this study is important for the determination of harvest time in Western Anatolia conditions for *H. triquetrifolium*.

**MATERIALS AND METHODS**

*Hypericum triquetrifolium* was gathered at different stages of plant development from Kazdağ (Balıkesir) in between April and August. The collected locality is Turkey, B1 Balıkesir: Edremit, Kazdağ Mount (Ida Mount), Şahindere canyon, Olive grove fields, 39° 35' 23.67" N, 26° 50' 39.49" E, 125 m, 12.04.2016, (SV 1655). General habitus of *H. triquetrifolium* is figure 1.

![General habitus of *Hypericum triquetrifolium* Turra.](image)

For ontogenetic variability, herb samples were taken at different growth stages (before flowering, beginning of flowering and full flowering). At the same time, herb samples were taken at different times of day (09.00 am, 12.00 pm, 4.00 pm) for diurnal variability. Flower samples were taken for essential oil rate and its components were investigated. At the beginning of the flowering process, the shoots which had green bud were harvested. Again, at the full flowering process, only shoots with fully opened flowers were harvested. The plant drugs were dried at room temperature (21ºC) and later oil content of plant parts (50 g each sample) were determined.

The oil composition was identified by GC-MS and GC-MS analyzes were carried out in TUBITAK (MAM). Helium was used as carrier gas at a constant flow rate of 1 ml/min and 1 µl of the sample was injected.

The GC temperature program was set as follows; 50ºC, stand for 5 minutes, increase to 250ºC at 5ºC / min and stand for 10 minutes. The temperature of the MS transfer line was set at 220ºC. This study was used by the Thermo Scientific TSQ GC-MS/MS.

The soil structure of the area gathering of the samples was ecologically sandy, [sand (69%), silt (23%) and clay (7%)]. pH value (6.8) and organic matter (6.9%). The average temperature of the place where the trial is 22.2°C, mean rainfall 26.9 mm and relative humidity 61.1% in 2016.

The differences between these tools were compared with Duncan's multi-class test (Duncan's test) and presented in table 1. The differences between that means were compared by Duncan’s test (Duncan’s multiple range test) and given in table 1.

**RESULTS AND DISCUSSION**

Total essential oils content in *H. triquetrifolium* during ontogenetic development was higher in full flowering stage (0.20–0.30 %), followed by before flowering stage (0.25–0.28 %) and fresh fruiting stage (0.09–0.12 %) (Table 1). The results of this study showed that diurnal and ontogenetic variability were significantly affected by essential oils (p <0.01).
Table 1. Total essential oils content (%) and changes during collecting times of the day and development stages of Hypericum triquetrifolium Turra.

| Diurnal Collecting Times | Before flowering 09:00 am | Before flowering 12:00 am | Before flowering 4:00 pm | Full flowering 09:00 am | Full flowering 12:00 am | Full flowering 4:00 pm | Fresh fruiting 09:00 am | Fresh fruiting 12:00 am | Fresh fruiting 4:00 pm |
|--------------------------|---------------------------|---------------------------|--------------------------|-------------------------|------------------------|-------------------------|------------------------|------------------------|------------------------|
| Before flowering         | 0.28 b                    | 0.20 e                    | 0.10 g                   | 0.19 b                  |                        |                         |                        |                        |                        |
| Full flowering           | 0.25 cd                   | 0.23 d                    | 0.09 h                   | 0.19 b                  |                        |                         |                        |                        |                        |
| Fresh fruiting           | 0.26 a                    | 0.24 b                    | 0.10 c                   | 0.20                    |                        |                         |                        |                        |                        |

Note: There is no statistically significant difference (p > 0.05) between figures including the same letters in the columns.

Table 2. Variability of essential oil content of H. triquetrifolium within a day during the course of ontogenetic (%).

| Compounds          | Before flowering 09:00 am | Before flowering 12:00 am | Before flowering 4:00 pm | Full flowering 09:00 am | Full flowering 12:00 am | Full flowering 4:00 pm | Fresh fruiting 09:00 am | Fresh fruiting 12:00 am | Fresh fruiting 4:00 pm |
|--------------------|---------------------------|---------------------------|--------------------------|-------------------------|------------------------|-------------------------|------------------------|------------------------|------------------------|
| α-pinene           | 3.8                       | 2.7                       | 3.5                      | 4.5                     | 4.7                    | 4.7                     | 17.6                   | 16.4                   | 16.5                   |
| 3-methyl nonane     | 11.6                      | 10.5                      | 13.0                     | 16.4                    | 16.9                   | 17.1                    | 43.5                   | 42.4                   | 40.7                   |
| β-pinene           | 1.2                       | 1.4                       | 1.3                      | 1.2                     | 1.4                    | 1.7                     | 1.6                    | 1.4                    | 2.0                    |
| α-phellandrene     | 0.2                       | 0.2                       | 0.4                      | 0.5                     | 0.3                    | 0.5                     | 0.8                    | 0.7                    | 0.9                    |
| p-Cymene           | -                         | -                         | 0.1                      | 0.1                     | 0.1                    | -                       | -                      | 0.1                    | 0.1                    |
| Limonene           | 0.5                       | 0.2                       | 0.3                      | 0.6                     | 0.4                    | 0.2                     | 1.2                    | 1.7                    | 1.5                    |
| β-Ocimene          | -                         | -                         | 0.4                      | 0.2                     | 0.2                    | 0.8                     | 0.4                    | 0.7                    | 0.7                    |
| 2-methyl decane    | 0.1                       | 0.2                       | 0.2                      | 0.2                     | 4.1                    | 3.0                     | 4.6                    | 4.9                    | 5.0                    |
| Undecane           | 0.8                       | 1.1                       | 1.4                      | 1.3                     | 2.5                    | 2.8                     | 3.8                    | 3.2                    | 4.0                    |
| Carvacrol          | 7.1                       | 5.9                       | 7.6                      | 1.2                     | 0.7                    | 0.4                     | 0.2                    | -                      | -                      |
| Copaene            | 3.2                       | 2.8                       | 2.4                      | 3.3                     | 3.8                    | 4.9                     | 0.5                    | 0.7                    | 0.5                    |
| β-bergamotene      | 0.4                       | 0.3                       | 0.2                      | 0.2                     | 0.3                    | 0.3                     | -                      | 0.1                    | 0.2                    |
| Caryophyllene      | 32.9                      | 30.6                      | 28.4                     | 14.9                    | 14.2                   | 12.4                    | 10.4                   | 11.8                   | 11.8                   |
| β-gurjunene        | 0.5                       | 0.5                       | 0.7                      | 0.9                     | 0.7                    | 0.6                     | 0.1                    | 0.2                    | 0.5                    |
| α-humulene         | 3.2                       | 2.9                       | 3.1                      | 2.9                     | 2.8                    | 2.4                     | 1.7                    | 1.9                    | 1.5                    |
| Aromadendrene      | 1.8                       | 1.6                       | 1.6                      | 0.3                     | 0.9                    | 1.5                     | 0.1                    | 0.1                    | 0.3                    |
| α-amorphene        | 2.4                       | 2.8                       | 3.1                      | 2.8                     | 3.1                    | 4.2                     | 0.2                    | 0.7                    | 1.4                    |
| Germacrene-D        | 7.9                       | 7.6                       | 7.6                      | 13.6                    | 12.6                   | 10.4                    | 3.1                    | 2.9                    | 3.0                    |
| α-terpinene        | 1.3                       | 1.1                       | 1.5                      | 0.8                     | 0.5                    | 0.7                     | 1.1                    | 0.6                    | 0.9                    |
| α-elemol           | 0.9                       | 1.1                       | 1.0                      | 0.7                     | 1.1                    | 1.4                     | 0.4                    | 0.1                    | 0.6                    |
| Gamma-cadinene     | 1.1                       | 1.0                       | 1.7                      | 1.2                     | 1.8                    | 2.3                     | 1.3                    | 1.4                    | 1.4                    |
| α-Cadinene         | 4.0                       | 3.7                       | 4.4                      | 4.1                     | 4.9                    | 3.7                     | 1.3                    | 1.0                    | 1.1                    |
| Caryophyllene oxide| 10.8                      | 10.5                      | 10.2                     | 9.1                     | 8.0                    | 7.2                     | 1.5                    | 1.4                    | 1.8                    |
| α-cadinol          | 0.8                       | 0.9                       | 0.9                      | 0.4                     | 0.3                    | 0.4                     | 0.2                    | 0.1                    | -                      |
| Valeranone         | 0.7                       | 0.8                       | 0.8                      | 0.3                     | 0.2                    | 0.3                     | 0.1                    | -                      | -                      |
| Bisabolol          | 1.1                       | 1.1                       | 1.3                      | 0.5                     | 0.7                    | 0.6                     | -                      | -                      | 0.1                    |
| Phytol             | 1.0                       | 1.1                       | 1.4                      | 0.5                     | 0.5                    | 0.7                     | 0.1                    | 0.1                    | -                      |

Note: *Kovats Index; **Retention Time
Chemical concentrations vary considerably during 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).

Our study, 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 %). Most of them have been previously reported in the essential oil of H. triquetrifolium (Petrakis et al. 2005, Cirak et al. 2006, Hosni et al. 2011).

From a compositional standpoint, the chemical composition of H. triquetrifolium essential oils from different locations has been reported. Petrakis et al. (2005) studied the essential oil of Greece specimens and found that α-pinene, n-nonane, β-caryophyllene, 3-methylnonane and 2-methyloctane in phenological stage. Hosni et al. (2011) studied phenological variability of secondary metabolites from H. triquetrifolium and found that β-caryophyllene, α-pinene, n-nonane, 2-methyloctane, germacrene-D and n-octane. Bertolli et al. (2003) were investigated of volatile constituents of leaves and flowers of H. triquetrifolium in Italy and analyses results showed the major compounds of the leaf and flowers essential oils that were the α-pinene, β-pinene, β-caryophyllene, n-nonane, sabine, myrcene, caryophyllene oxide and germacrene-D.

Variability of essential oils content of H. triquetrifolium within a day during the course of ontogenetic is listed table 2. As can be seen in table 2 the studied oils were resolved into 27 components at the before flowering, full flowering and fresh fruiting stage respectively.

At the before flowering, the oils consisted mainly of caryophyllene (32.9%), caryophyllene oxide (10.8%), 3-methyl nonane (10.5%), carvacrol (7.6%); germacrene-D (7.9%), α-cadinene (4.4%) and α-humulene (3.2%).

At the full flowering stage, the oils consisted mainly of α-pinene (5.8%), 3-methyl nonane (17.1%), copaene (4.9%), caryophyllene (14.9%), α-amorphene (4.2%), germacrene-D (13.6%), α-cadinene (5.7%) and caryophyllene oxide (9.1%). At the fresh fruiting stage oils consisted mainly of α-pinene (17.6%), 3-methyl nonane (43.5%), 2-methyl decane (5.0%), undecane (4.0%), caryophyllene (11.8%) and germacrene-D (3.1%).

The diurnal variability of H. triquetrifolium has not been reported so far. However, same studies with Hypericum taxa by various authors have been described H. triquetrifolium (Schwob et al. 2004, Cirak et al. 2011, Hosni et al. 2011, Pasa 2013).

CONCLUSION
Ontogenetic and diurnal variabilities were applied on H. triquetrifolium and 27 essential oil components were detected. The major components of the oil of H. triquetrifolium aerial parts were caryophyllene and caryophyllene oxide.

The essential oils of the above-ground parts of the plant increased during flowering ontogenesis and reached the highest level in full flowering. Afterwards, it decreased at the beginning of the flowering phase. The highest level at full flowering 0.30% and the lowest level fresh fruiting 0.09%.

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