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Article

Effects of Different Geographical Aspects and Ontogenetic Variability on Total Hypericin Content of *Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L.

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Abstract: The present study was conducted to determine the total hypericin contents of *Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L. species which are naturally distributed in the flora of Siirt province, Turkey. Hypericin contents of *Hypericum* species grown in different geographical aspects (North, South, East, and West), and it was measured at different harvest times (full blooming and post blooming period). In the current study, it has been determined that total hypericin content varies considerably according to aspects, plant developmental stages (ontogenetic variance), and species. According to species x aspect interaction, the highest total hypericin content was recorded from the west aspect (3.13 mg/g) in *Hypericum triquetrifolium*, while, the lowest hypericin content was also obtained from the west aspect (1.22 mg/g) in *Hypericum scabrum*. When the highest total hypericin content was analyzed according to aspect x species x harvest time interaction, the highest total hypericin content was produced from *Hypericum triquetrifolium* at the harvest of west aspect with 5.28 mg/g, while the minimum amount of hypericin was obtained from the same aspect in *Hypericum scabrum* with 0.50 mg/g. In species x harvest time interaction, the highest total hypericin content was obtained from the full bloom (3.10 mg/g) harvest in *Hypericum triquetrifolium*, while the lowest hypericin was obtained from the full bloom (1.26 mg/g) harvest in *Hypericum scabrum*. The data suggest that the average total hypericin content was 2.26 mg/g in *Hypericum triquetrifolium* and 1.28 mg/g in *Hypericum scabrum*.

Keywords: *Hypericum triquetrifolium*; *Hypericum scabrum*; aspect; hypericin

1 Introduction

Hypericum triquetrifolium Turra. and *Hypericum scabrum* L. species are belonging to the *Hypericum* genus, which is of great medicinal and economic value due to the anti-viral, anti-depressive and anti-cancer properties. *Hypericum* is a genus with 484 species in the Clusiaceae (=Guttiferae) family and is found naturally in the tropics and subtropics regions of the world (except Antarctica) including Asia, Europe, Africa, Siberia, North America, and Cyprus. The species of *Hypericum* are perennial herbaceous or shrub plants. Turkey is considered an essential gene center of *Hypericum* species [1]. Turkey has

96 *Hypericum* species of which 46 are endemics. The most common *Hypericum* species in Turkey are *Hypericum perforatum* L., *Hypericum trigqetrifolium* Turra, *Hypericum calycinum*, *Hypericum empetrifolium* Willd., *Hypericum scabrum* L. and *Hypericum tedrapetum* Fries [2–5].

This species has been used as a healing agent for centuries in the treatment of nerve diseases, stomach ailments, joint inflammation, wound and burn, and some skin diseases [6]. Yellow flowers of the plants are soaked in olive oil and kept under the sunlight until the oil color turns red, and the ointment obtained is used to cure wounds and burns [3]. As well as, the well-known therapeutic properties, the *Hypericum* has been the subject of different legends in the past. The plant was considered as the symbol of the Sun God in the Paganistic ages due to the golden flowers. The oil obtained by distilling the leaves and flowers of the plants was used for both good and evil in Greek mythology. The *Hypericum* was also believed to keep evil spirits away in Ancient Greece [7].

The metabolites of *Hypericum* species were determined to contain anti-inflammatory, anti-ulcer, wound-healing, antibacterial, antidepressant, sedative, anti-cancer, antimalarial, antiviral, anti-inflammatory, antioxidant, antimicrobial, intravenous pressure reducing, diuretic, cardiotonic, antifungal and antiseptic effects. The plants belonging to the *Hypericum* genus are also used to cure diabetes, chronic rheumatism, gastrointestinal diseases, liver-biliary disorders, jaundice, bronchitis, diarrhea, dysentery, throat infections, colds, and to suppress worms [3,6,8].

Hypericin has been reported to induce antidepressant and anxiolytic (anxiety and fear) effects. In a few studies on the mechanism of action, it was also recorded effective against the occurrence of restlessness and hallucinations. A small dose of Hypericin is suggested as a tonic and stimulant (provides regular functioning of the internal glands) against mental depression. It has also reported to show a strong photodynamic effect on tumor cells and viruses (complexed agents) [2]. Hypericin and pseudohypericin are highly effective against lipid-encircled or non-lipid DNA and RNA viruses, and these substances are effective in preventing infections caused by a large number of viruses and the spread of HIV. It was reported that hypericin and hyperforin, one of the metabolites contained in *Hypericum* sp., prevent cancer formation and development by causing programmed cell death in different cancer cell lines [6].

Hypericum triquetrifolium Turra., one of the species was investigated in the current study, has a wide distribution in Siirt, Diyarbakır, Bitlis, Şanlıurfa, Kahramanmaraş, Elazığ, Kilis, Çanakkale, Istanbul, Amasya, Tokat, İzmir, Manisa, Ankara, Istanbul, Izmir, Antalya, Mersin and Hatay provinces of Turkey [9] (Fig. 1). The stem length of *Hypericum triquetrifolium* Turra. are generally between 15–55 cm and the plant is upright or reclined, widely branched, and usually pyramid-shaped. The leaves are approximately 3–20 mm long and have varying shapes of triangular-lanceolate or linear-oblong (linear-rectangular shape). Sepals are oblong to ovate-oblong shaped. Petals are 5 to 7 mm long and have no black spots, or spots can be only on one side. The capsules are 3–5 mm long, ovate shaped, with a longitudinal secretion channel and sometimes have a small number of lateral vesicles (water-filled bubble) [5].

Hypericum scabrum L, the second species examined in the study, is a perennial herbaceous plant that is found on rocky hills, with 40–50 cm height (sometimes 10–60 cm) and blooms between May to August [10]. *Hypericum scabrum* L. which is quite common in Turkey is previously reported in Siirt, Hakkari, Van, Gaziantep,

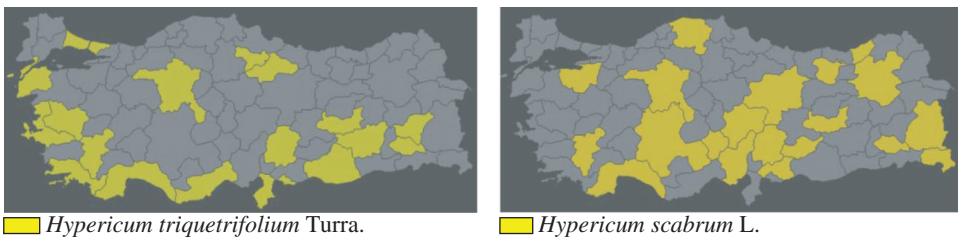

Figure 1: Distribution of *Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L. in Turkey [9]

Kahramanmaraş, Elazığ, Erzurum, Sivas, Adıyaman, Adana, Bursa (Uludağ at 1650 m), Antalya, Denizli, Ankara, Konya, Niğde, Kayseri (Erciyes Mountain), Kastamonu, Gümüşhane and Rize provinces [11] (Fig. 1).

According to Ceylan et al. [12] and references therein hypericin rate of a drug is influenced by the cutting height as well as the harvest time. Similarly, according to Kaçar et al. [13] and references therein the difference in hypericin concentration is not only related to the characteristics of a genotype but also depends on the environmental conditions, plant growth stage, plant parts analyzed, harvest time, drying method and storage conditions. These factors are effective in all medicinal and aromatic plants. The production of secondary metabolites in medicinal and aromatic plants are influenced by the environmental conditions as well as the genetic factors [14–16]. Therefore, this study aimed to determine the potential of using *Hypericum* sp. as a natural product for different purposes, and to determine the total hypericin content of two different *Hypericum* species that grow naturally in the flora of Siirt. In the present study, we report our investigations, the effects of ontogenetic variability, and different aspect on hypericin contents of *Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L. species were examined.

2 Material and Method

2.1 Location of the Experiment

The research was conducted in 2016 on *Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L., which have a natural distribution in the flora of Siirt, Turkey. In the identification of these species, [17–22] were used for the species identification.

The study area is located between 37°96 north latitude and 41°85 east longitude with an altitude of 580 m above sea level (Fig. 2).

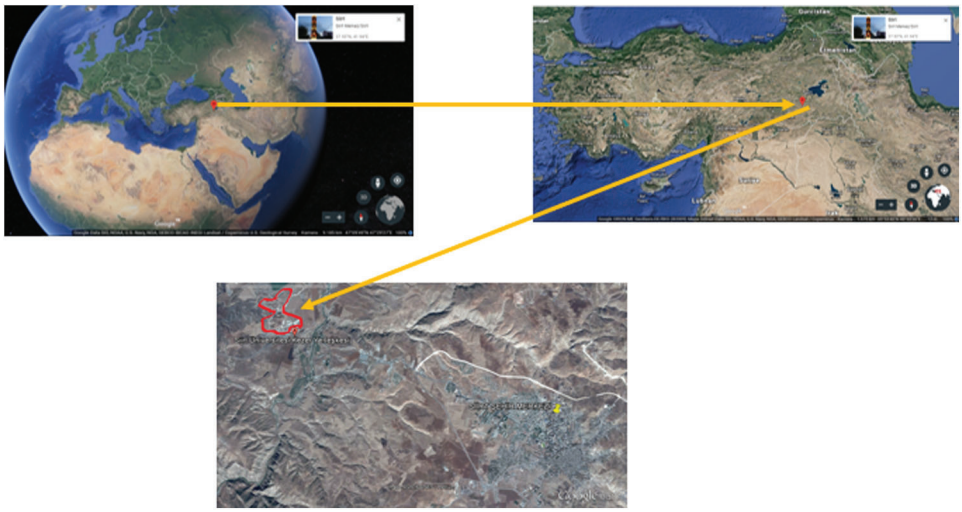

Figure 2: Satellite image for the location of the study area

2.2 Soil Characteristics and Meteorological Information during Growth Seasons

The average temperature (°C), average relative humidity (mm) and total precipitation (mm) of the study area for 2016 and long term (1938–2015) were given in Tab. 1. The monthly average temperature of the research year (2016) had some differences compared to the average of long term data, and the average minimum and the maximum temperatures indicate a warmer season compared to the long term averages (Tab. 1). The monthly average relative humidity and total monthly precipitation values did not differ significantly compared to the long-term averages. Monthly total precipitation values were higher than the

Table 1: Some climatical data of studied area for 2016 and long-term averages (1938–2015)

Years	2016					Long Term				
Months	Mean Temperature (°C)			Mean Humidity (%)	Total Precipitation (mm)	Mean Temperature (°C)			Mean Humidity (%)	Total Precipitation (mm)
	Mean.	Max.	Min.			Mean	Max.	Min.		
April	16.6	26.5	4.2	47.5	104.2	14.4	19.1	8.9	57.9	96.0
May	19.9	30.6	8.0	48.9	63.1	20.0	25.2	13.5	48.5	44.6
June	26.5	38.4	14.8	32.7	8.6	27.0	32.1	18.9	32.3	9.3
July	31.4	41.6	20.6	24.5	1.6	31.2	36.9	23.4	26.9	1.2
August	32.3	41.8	22.4	20.5	0.9	30.1	36.8	23.1	21.6	0.9
September	25.0	36.3	12.4	29.8	0.0	25.1	32.2	18.7	12.9	4.9
MEAN	**25.28**	**35.87**	**13.73**	**33.99**	**29.73**	**24.63**	**30.38**	**17.76**	**33.36**	**26.15**

Reference: Siirt Meteorology Stations Records, Provincial Meteorology Directorate, Siirt.

long-term averages. The average highest temperature during the field studies in 2016 was recorded in August (41.8°C) while the lowest value (4.2°C) was in April.

2.3 Experimental Design, Treatments, Plant Materials, and Management Practices

The samplings were taken at 4 replications according to the split plots divided into randomized blocks experimental design. The species (*Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L.) constituted the main plots, different aspect of collected area (north, south, east and west) were the sub-plots, and the harvest time (full bloom and bloom/fruiting stages) based on the different growth stages of the plant were the sub-subplots. Plant samples were collected in full blooming and post-blooming stages (initiation of fruit setting). Aspects were determined based on the peak of hilly areas in the Kezer Region. Samples were collected from the east, west, north, and south aspects of these hilly areas.

2.4 Data and Their Collection Procedure

The plant materials were ground into fine powder for the hypericin analysis, and 1.0 g of dried powder plant material was weighed and placed into the bag. The extraction was carried out using chloroform in the soxhlet apparatus until the bags were colorless, then the bags were removed from the apparatus and dried. The samples were once more placed into the soxhlet apparatus and extraction was continued with methanol. After the methanol remaining at the top of the extraction became completely white, the solution was transferred to a 100 ml flask to determine the adsorption at 590 nm wavelength, and the hypericin content was calculated with the following equation:

$$\%\ \text{Hypericin (mg)} = \frac{\text{E.590}}{718} \times \frac{1}{\frac{\text{Sample weight (g)}}{100\ \text{ml}}} \times 100$$

In the equation; E590 is the value recorded at the 590 nm wavelength in the spectrophotometer; 718 refers to the extraction coefficient [23].

2.5 Statistical Analysis

The data obtained in the study were subjected to variance analysis (ANOVA) according to the Split Plots Divided into Randomized Blocks Experimental Design and grouped by the LSD test. All statistical analysis

were carried out using the JMP Statistical Analysis Software (JMP®, Version 7.0 SAS Institute Inc., Cary, NC, 1989–2019).

3 Results and Discussion

It was recorded the effects of species, aspect, harvest time, aspect × species, species × harvest time, species × aspect × harvest time interactions on hypericin contents of *Hypericum triquetrifolium*, and *Hypericum scabrum* were statistically significant ($p < 0.01$). The effects of four aspects (north, east, south, and west) and two different harvest times on total hypericin contents (mg g^{-1}) of *Hypericum triquetrifolium* Turra. and *Hypericum scabrum* L., are given in Tab. 2.

Table 2: Effects of different aspect and harvest times on total hypericin (mg g^{-1}) contents of *Hypericum triquetrifolium* and *Hypericum scabrum*

Aspect	Species	Harvest time		Mean
		Full Blooming Period	Post-Blooming Period	
North	*Hypericum triquetrifolium*	0.98*l*	4.11*b*	2.55$^{\mathbf{c}}$
	Hypericum scabrum	0.95*m*	1.71*f*	1.33$^{\mathbf{e}}$
Mean values for Aspect × Harvest Time		0.97	2.90	1.93
East	*Hypericum triquetrifolium*	3.61*c*	2.60*d*	3.10$^{\mathbf{b}}$
	Hypericum scabrum	1.41*h*	1.21*j*	1.31$^{\mathbf{f}}$
Mean values for Aspect × Harvest Time		2.51	1.90	2.20
South	*Hypericum triquetrifolium*	2.55*e*	0.60*o*	1.57$^{\mathbf{d}}$
	Hypericum scabrum	1.35*ı*	1.18*k*	1.27$^{\mathbf{g}}$
Mean values for Aspect × Harvest Time		1.95	0.89	1.42
West	*Hypericum triquetrifolium*	5.28*a*	0.99*l*	3.13$^{\mathbf{a}}$
	Hypericum scabrum	1.50*g*	0.93*n*	1.22$^{\mathbf{h}}$
Mean values for Aspect × Harvest Time		3.39	0.96	2.18
Mean for Harvest Time		2.20A	1.67B	1.94
	Species	Harvest time		Mean
		Full Blooming Period	Full-Blooming Period	
	Hypericum triquetrifolium	3.10^{a}	2.07^{b}	2.26***A***
	Hypericum scabrum	1.30^{c}	1.26^{d}	1.28***B***
	Mean for Harvest Time	2.20**A**	1.67A	1.94
	CV	0.57		
	$LSD_{0.01}$	Means followed by different letters in species (LSD = 0.009; uppercase bold italic letters), aspect (0.009), harvest time (0.006; uppercase letters), aspect × species (0.13; lowercase bold letters), species × harvest time (0.008; lowercase letters), species × aspect × harvest time (0.02; lowercase italic letters) are different according to LSD values.		

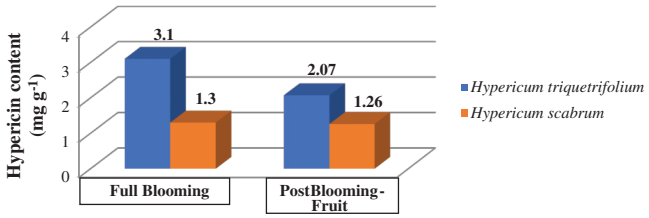

Figure 3: Total Hypericin content *Hypericum triquetrifolium* and *Hypericum scabrum* at different harvest times

Results indicated that mean total hypericin contents of *Hypericum triquetrifolium* and *Hypericum scabrum* were 2.26 and 1.28 mg g^{-1} respectively, in the harvest periods (Fig. 3 and Tab. 2).

The total hypericin contents of plant samples collected in different aspects was varied between 1.42 and 2.20 mg g^{-1} (Tab. 2 and Fig. 4). The mean total hypericin content ranged from 1.22 to 3.13 mg g^{-1} in the aspect × species interaction. The highest total hypericin content was obtained from the west aspect in *Hypericum triquetrifolium* and the lowest hypericin contents were also obtained from the plants collected in the west aspect in *Hypericum scabrum*.

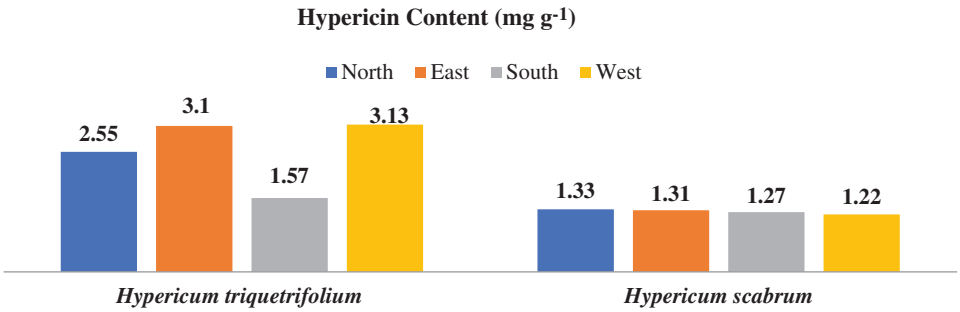

Figure 4: Total Hypericin content of *H.triquetrifolium* and *H.scabrum* at different aspects

Data related to the highest total hypericin content in the aspect x harvest time interaction was produced from the plants that were harvested from the west aspect (3.39 mg g^{-1}) while the lowest hypericin content (0.89 mg g^{-1}) was found in the south aspect (Tab. 2).

On the other hand, the highest total hypericin content in species x harvest time interaction was observed in *Hypericum triquetrifolium* (3.10 mg g^{-1}) from the plants harvested in the full blooming period, and the lowest hypericin content (1.26 mg g^{-1}) was obtained in *Hypericum scabrum* from the plants harvested in post blooming period. However, the highest total hypericin content (5.28 mg g^{-1}) in the aspect × species × harvest time interaction was recorded in *Hypericum triquetrifolium* in the west aspect at the full blooming harvest period, and the lowest hypericin content (0.50 mg g^{-1}) was obtained in *Hypericum scabrum* species in the west aspect at the full blooming harvest period (Fig. 5 and Tab. 2).

When the species are evaluated separately, the highest total hypericin content in *Hypericum triquetrifolium* was recorded in the west aspect at full blooming harvest period with 5.28 mg g^{-1}, while the lowest hypericin content (0.60 mg g^{-1}) was recorded in the south aspect at the bloom-fruit harvest period (Fig. 5). The highest total hypericin content (1.71 mg g^{-1}) in the *Hypericum scabrum* was recorded from plants harvested in the north aspect, while the lowest hypericin content (0.50 mg g^{-1}) was produced from the plants harvested in the west aspect at the full blooming period (Fig. 5).

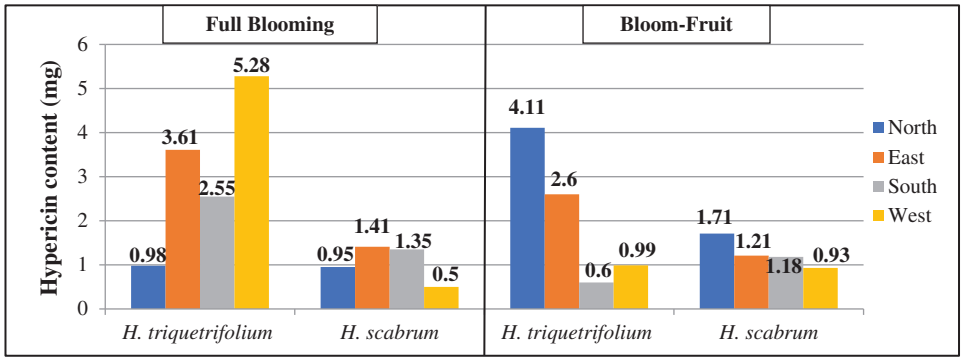

Figure 5: Total hypericin contents of *Hypericum triquetrifolium* and *Hypericum scabrum* depending on different aspects and harvest times

The total hypericin content in *Hypericum triquetrifolium* reported by Other researchers has had a large variability. The hypericin content was reported by Ayan et al. [24] between 0.253 and 4,54 mg g^{-1}, Hosni et al. [25] between 0.29 and 1,2 mg g^{-1}, and Çamaş et al. [26] between 1,53 and 4,80 mg g^{-1}. In contrast to the above reports, Smelcerovic et al. [27] reported higher total hypericin content (7,05 mg g^{-1}) in *Hypericum triquetrifolium*. The mean total hypericin content in Siirt ecological conditions was 2.26 mg g^{-1}, which was higher than the hypericin content has been observed in most studies.

The total hypericin content in *Hypericum scabrum* reported by Smelcerovic et al. [27] was 0.11 mg g^{-1}, by Ayan et al. [28] was between 0 and 0.262 mg g^{-1} and by Ayan et al. [29] was between 0.215 and 1,37 mg g^{-1}. In this study, the mean total hypericin content in *Hypericum scabrum* was 1.28 mg g^{-1}, which was higher than the other studies. In addition, total hypericin content in different *Hypericum* species was reported between 0.01 and 21,0 mg g^{-1} [27,30,31]. The total hypericin content obtained from both *Hypericum* species discussed in the study were similar to previous studies. Additionally, as a result of the studies conducted in different plants [32–35] it has been reported that the care has a significant effect on the quality and yield criteria in plants and these results were in accordance with our study.

4 Conclusion and Recommendations

Results of the current observation revealed that the total hypericin content varies significantly depending on the species, the plant growth stages (ontogenetic variability), and aspects. The findings of the study reveal the importance of the information on the Hypericum species, and the most appropriate time to harvest from the nature. In addition, significant effects of different aspects on total hypericin content were also considered. Natural populations of most *Hypericum* species, collected for medicinal purposes from natural flora, particularly the endemics are rapidly decreasing. Therefore, investigations on conservation and agricultural production possibilities of *Hypericum* species are urgently desired. Therefore, it may be recommended that the total hypericin content in *Hypericum* species is significantly affected not only by species and ontogenetic variability (from the harvest period) but also influenced by the aspect that the plants are grown.

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