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Full Length Research Paper

Effects of morphogenetic and diurnal variability on the hypericin content in St. John's Wort (*Hypericum perforatum* L.)

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This research was conducted to investigate effects of morphogenetic and diurnal variability on the hypericin content of St. John's Wort (*Hypericum perforatum* L.) populations originating from Turkey for maximum utilization of the active substance in plants. During 2002 and 2003, field trials were conducted at Uludag University, Faculty of Agriculture, Department of Field Crops, Bursa, Turkey. Samples of bud, flower and capsule of *H. perforatum* L. populations were collected in the second year of cultivation (2003). Factorial arrangements of three *H. perforatum* L. populations (Bursa, Edirne, Izmir), three part of plant (bud, flower, capsule) and six collection h (8:00, 10:00 a.m; 12:00 (noon), 2:00, 4:00, 6:00 p.m) were evaluated in a completely randomized block design with three replications. Hypericin content (%) in parts of *H. perforatum* L. populations was determined according to DAC (1986). Consequently, the content of hypericin in the examined populations varied from 0.260% in Bursa to 0.283% in Izmir. Evaluating plant parts revealed that the hypericin content both in floral parts (0.309%) and buds (0.308%) were higher than capsule tissues (0.208%) for all populations. When collection hours were examined for the hypericin content of plants, the highest content was recorded at 10:00 a.m (0.279%) and the lowest value at 4:00 p.m (0.272%) and 6:00 p.m (0.272%). As a result, this study showed that the highest hypericin ratio was determined in flowers and buds generally collected between 8:00 and 10:00 a.m within a day for examined *Hypericum* populations.

Key words: St. John's Wort, *Hypericum perforatum* L., morphogenetic variability, diurnal variability, hypericin content.

INTRODUCTION

Hypericum perforatum L, also known as St. John's Wort, is a herbaceous perennial plant belonging to the *Clusiaceae* family (Upton et al., 1997). *H. perforatum* L. (St. John's Wort) is widespread in Mediterrenean region, Europe, Asia, North and South Africa, Western and Eastern North America (Jensen et al., 1995). Although it was known from ancient Greek and Roman times, St. John's Wort has recently become a widely popular herbal remedy due to its antidepressive effects (Linde et al., 1996; DeSmet and Mohen, 1996).

Hypericum contains numerous compounds with documented biological activity groups that have stimulat-ed the most interesting compounds including the naphthodianthrones hypericin and pseudohypericin, a broad range of flavonoids quercetin, quercitrin, amentoflavone and hyperin, the phloroglucinols hyperforin and adhyperforin, the essential oil and xanthones (Upton et al., 1997). From a pharmacological point of view, the hypericins are the most interesting compounds of *H. perforatum* L. (Patocka, 2003). Because of their use for mild depression treatment, clinical trials suggest that hypericin is the primary biologically active compound (Lavie et al., 1995; Linde et al., 1996). The naphtodianthrones, hypericin and pseudohypericin are known to contribute to the antidepressant action of this species, and most of *Hypericum* phytomedicines are currently standardized according to their hypericin content (Briskin, 2000). Hypericin also

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Table 1. Climatic data for the experimental field during the growing period of *H. perforatum* L.

Month	Mean temperature (°C)			Rainfall (mm)			Relative humidity (%)		
	Long term	2002	2003	Long term	2002	2003	Long term	2002	2003
1	5.5	3.2	8.6	88.8	62.3	65.3	74.8	65.4	68.3
2	6.2	8.9	2.7	77.5	42.7	106.2	72.5	67.2	71.2
3	8.3	10.3	4.4	69.8	87.9	33.1	71.7	71.3	64.0
4	12.9	11.5	9.9	62.9	126.5	112.1	69.9	76.0	70.4
5	17.8	17.3	18.8	50.0	50.5	45.7	69.2	67.9	67.7
6	22.1	23.0	23.8	30.4	25.2	2.4	61.1	62.1	54.8
7	24.5	26.7	25.3	24.0	49.9	-	58.8	64.4	64.4
8	24.1	24.6	25.6	18.9	31.1	-	60.4	65.2	65.2
9	20.1	20.8	19.2	40.1	67.2	66.9	65.8	70.3	70.3
10	15.6	15.8	16.6	60.4	119.3	125.1	72.1	75.2	75.2
11	12.3	10.7	10.1	76.3	67.9	64.5	74.4	72.6	77.3
12	7.6	5.0	6.2	99.9	28.8	91.0	74.2	67.0	71.8
Total	-	-	-	699.0	759.3	712.3	-	-	-
Mean	14.8	14.8	14.3	-	-	-	68.7	68.7	68.4

shows a significant antiviral and antiretroviral activity (Meruelo et al., 1988; Vlietinck et al., 1998).

Hypericin and pseudohypericin may function as defensive allelochemicals in St. John's Wort (Fields et al., 1990). Hypericin is found in dark glandular structures visible located on the epidermal surface of leaves, flowers and to some extend the stems of plants (Fields et al., 1990; Curtis and Lerstern, 1991; Fornasiero et al., 1998; Crompton et al., 1988; Sirvent et al., 2002)

The formation of secondary metabolites is typical of the plants depend on both genetic and environmental factors. Besides climatic conditions of the growing site, particularly edaphic factors also play an important role in variation of these secondary metabolites (Franz, 1983; Palevitch, 1987). Variations of hypericin that derivate naphthodianthrones depend on not only genetic character-ristics but also environmental factors, plant development stage, plant parts, harvest time, processing and storage methods (Bombardelli and Morazzoni, 1995; Büter et al., 1998; Jensen et al., 1995; Palevitch, 1991; Upton et al., 1997; Büter and Büter, 2002). Sirvent et al. (2002) stated that further studies are needed to investigate the relationships between the hypericin content and these factors.

The objective of this study was to asses the effects of plant parts (bud, flower and capsule) and collecting hour on hypericin content of three *H. perforatum* L. populations obtained from different regions of Turkey.

MATERIALS AND METHODS

During 2002 and 2003, three populations of *H. perforatum* L. (Bursa, Edirne, Izmir) were cultivated on the experimental field of Uludag University, Faculty of Agriculture, Department of Field Crops, Bursa (40° 11' N, 29° 04' E). The soil was clay loam, slightly alkaline and rich in phosphorus and potassium containing 1.8% organic matter. Long-term average of annual total precipitation is 699 mm, mean temperature for the whole year is 14.3°C and relative humidity is 68.7%. In 2002 total precipitation 759.3 mm year $^{-1}$, mean temperature for the whole year was 14.8°C and relative humidity was 68.7 %; in 2003 total precipitation 712.3 mm year $^{-1}$, mean temperature for the whole year was 14.3°C and relative humidity was 68.4%. The weather conditions during the experi-mental period are presented in Table 1.

The populations were propagated by seeds and thus they were regarded as populations. *Hypericum* seedlings were pre-grown in the green house during February to May, 2002 and transferred to the field on May 20/21, 2002. Seedlings were planted in 40 x 20 cm distances. Total 60 kg/ha nitrogen and 60 kg/ha P_2O_5 were applied in spring and autum of each year. During the vegetation period, plots were irrigated as needed.

Samples of bud, flower and capsule of *H. perforatum* L. populations were collected at different collection h (8:00, 10:00 a.m; 12:00 (noon), 2:00, 4:00, 6:00 p.m) within a day in the second year (2003) of cultivation. The *Hypericum* plants could not reach their final size and morphology before the second year of cultivation. Similar to our observations, Büter et al. (1998) also stated that in the first year plants were smaller with less main shoots and less flowers resulting in considerably lower total plant yields. Moreover Osinska and Weglarz (2001) reported that the optimal time for harvest appeared to be the full blooming stage, especially in the second year of vegetation as far as the mass of herb and the content of investigated compounds are concerned. Similarly, Kordana and Zalecki (1997) reported that in the second year of cultivation yields of raw material, increased by 100% and hypericin content increased by 50%, compared with the first year. The relatively high hypericin content in the second year was possibly due to the higher proportion of flowers, leaves on the top drug herbage analysed. Therefore, in the present study, the observation and measurements were obtained from the plants of second year (2003).

Each replicate comprised a bulk sample of five individual plants. From each plant, 25 buds at onset of flowering (closed buds with yellow petals already visible), flower at full flowering (fully opened flowers) and capsule at seed formation (brown capsules) were removed and combined in order to obtain one extract per population, plant part and collection hour. Plant samples of *H. perforatum* L. were collected in 30.05.2003 for buds, 06.06.2003 for flowers and 27.06.2003 for capsules. Finally, harvested parts of plant were

Table 2. Effect of different populations, different parts of plant and their interactions on hypericin content (%) in *H. perforatum* L.

Parts of plant	Populations		
	Bursa	Edirne	Izmir
Bud	0.308 b*	0.309 b	0.308 b
Flower	0.306 b	0.312 a	0.308 b
Capsule	0.166 e	0.226 d	0.233 c
Mean	0.260 b	0.282 a	0.283 a

*Means with the same letter are not significantly different at p=0.05.
LSD 0.05 for population means: 0.001144.
LSD 0.05 for population x plant parts: 0.001982.

Table 3. Effect of different parts of plant, collection hour with in a day and their interactions on hypericin content in *H. perforatum* L.

Collection hour	Parts of plant		
	Bud	Flower	Capsule
8:00 a.m	0.309 b*	0.310 a	0.207 e
10:00 a.m	0.306 b	0.310 a	0.222 c
12:00 p.m (noon)	0.308 b	0.308 b	0.213 d
2:00 p.m	0.309 b	0.307 b	0.206 e
4:00 p.m	0.310 a	0.309 b	0.198 f
6:00 p.m	0.307 b	0.307 b	0.203 e
Mean of Plant Parts	0.308 a	0.309 a	0.208 b

*Means with the same letter are not significantly different at p=0.05.
LSD 0.05 for Parts of Plant: 0.001144.
LSD 0.05 for Parts of Plant x Collection Hour: 0.002803.

air-dried in a closed room at 25 - 30°C and stored at room temperature at dark conditions.

Extraction of hypericin

Hypericin content (%) in parts of *H. perforatum* L. populations was determined according to DAC (1986). The parts of harvested five individual plants were well homogenized in order to eliminate the variability between plants. Air dried plant material was powderized by a coffe mill. A 1 g powder was Soxhlett-extracted with chloroform. The extract was evaporated to dryness and the dried powder was then extracted with methanol. The hypericin content in methanol extracts was determined by a spectrophotometer at 590 nm.

Statistical analyses

Factorial arrangements of three *H. perforatum* L. populations (Bursa, Edirne, Izmir), three part of plant (bud, flower, capsule) and six collection hours (8:00, 10:00 a.m; 12:00 (noon), 2:00, 4:00, 6:00 p.m) were evaluated in a completely randomized block design with three replications. All data were subjected to analysis of variance for each character using MSTAT-C (version 2.1, Michigan State University, 1991) and MINITAB (University of Texas at Austin) software. For mean seperation, the F-protected least significant difference (LSD) was calculated at the 0.05 probability level according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Hypericin contents of *H. perforatum* L. populations, different parts of plant, different collection hours and their interactions were presented in Tables 2, 3 and 4. Significant differences were detected in the mean concentrations of hypericin of investigated factors and their interactions.

The content of hypericin in the examined populations varied from 0.260% in Bursa to 0.283% in Izmir. The average hypericin content of Izmir population (0.283%) was found to be similar to those of Edirne (0.282%) (Table 2).

Evaluating parts of plant, it was also found that hypericin content in both floral parts (0.309%) and buds (0.308%) were higher than that of capsule tissues (0.208%) for all populations (Table 3). Regarding interactions between population and parts of plant, the distribution of the average amounts of hypericin in buds were similar to all populations. Significantly more hypericin (0.312%) were found in the flowers of Edirne population (Table 2). Also, hypericin content of capsules was found lower in Bursa population (0.166%) than those in other populations. Thus, the lowest hypericin content was determined in Bursa population (0.260%) (Table 2). Earlier investigations of *H. perforatum* L. in different countries indicated a higher hypericin concentration in the flowers compared to the other parts of plant (Cellarova et al., 1994; Schütt, 1996; Constantine and Karchesy, 1998; Tekelova et al., 2000; Southwell and Bourke, 2001; Sirvent et al., 2002).

Previous studies showed that hypericin contents varied among different parts of plant, ranging from 0.003 - 0.609% in flowers (Constantine and Karchesy, 1998; Melikian et al., 1998; Campbell et al., 1992; Büter et al., 1998; Sirvent et al., 2002; Poutaraud and Girardin, 2004), 0.215% in flower and bud (Southwell and Campbell, 1991), 0.03% in leaves+stems+flowers (Constantine and Karchesy, 1998), 0.038-0.045% in top leaves; 0.029-0.035% in bottom leaves; 0.012-0.0115% in side stem; 0.004% in main stem; 0.073-0.077% in capsules (Southwell and Campbell, 1991; Campbell et al., 1992). It has been reported that dried flowers can yield as 1.8% hypericin (Upton et al., 1997). As a result of these studies, hypericin concentrations generally declined towards from flowers to capsules. Repcak and Martonfi (1997) found that hypericin and pseudohypericin were located mainly in the petals and in the stamina of *Hypericum*. This finding may explain the progressive decrease of dianthrone content in older flowers, as their stamina has lost anthers and developing fruits have only rests of the corona. Similarly, the petals of small buds represent a smaller portion of their weight. The content of hypericin increased from the first bud phases to flowers just opened, then decreased from over the blooming to unripe

Table 4. Effect of different populations, collection hour with in a day and their interactions on hypericin content in *H. perforatum* L.

Collection hour	Populations			Mean of collection hour
	Bursa	Edirne	Izmir	
8:00 a.m	0.266 d*	0.285 b	0.275 c	0.276 b
10:00 a.m	0.261 d	0.282 b	0.295 a	0.279 a
12:00 p.m (noon)	0.255 e	0.284 b	0.290 a	0.277 b
2:00 p.m	0.267 d	0.283 b	0.272 c	0.274 b
4:00 p.m	0.260 d	0.278 c	0.279 c	0.272 c
6:00 p.m	0.250 e	0.282 b	0.285 b	0.272 c

* Means with the same letter are not significantly different at p=0.05.
LSD 0.05 for Collection Hour: 0.001618.
LSD 0.05 for Population x Collection Hour: 0.002803.

fruits (Tekelova et al., 2000). Kitanov (2000) reported that the level of hypericins was very low before budding period, but increased rapidly and reached its maximum at the stage of budding and blossoming. Hypericin content gradually decreased towards ripening of the fruits and reached the same level as before budding.

When collection hours were examined for the hypericin content of plants, the highest content was recorded at 10:00 a.m (0.279%) and the lowest value at 4:00 p.m (0.272%) and 6:00 p.m (0.272%) (Table 4). Generally the higher hypericin contents were obtained from samples collected at 8:00 and 10:00 a.m compared to 12:00 (noon) and 6:00 p.m within a day (Table 4).

The accumulation level of hypericin may show variability (Pluhar et al., 2001). Differences between the studies can be explained by the different methods used, geographical and ecological factors, population variability, using herbarium or fresh plant materials and phases of plant collection and processing of the harvested plant material (Cellarova et al., 1994; Büter et al., 1998; Denke et al., 1999; Kitanov, 2000; Pluhar et al., 2001; Ayan et al., 2004). The size, number and chemical content of such glandular structures in plants can be influenced by a variety factors including nutrient availability (Mutikaninen and Walls, 1995; Guillet et al., 1997), light quality (Büter et al., 1998) and light intensity (Yamamaura et al., 1989; Upadhyaya and Furness, 1994; Guillet et al., 1997).

Martonfi and Repcak (1994) and Repcak and Martonfi (1997) pointed out that flower and its parts in medicinal and aromatic plants are the main organs that produced active principles in *H. perforatum* L. Therefore each treatment that changed the ratio of flower/herb affected active substances properties of St. John's Wort. Furthermore the extremely high hypericin content was possibly based on the higher proportion of flowers, leaves and fine stems (Pluhar et al., 2000). Only the 'flowering segment', is commonly harvested and processed for pharmaceutical use, since this segment contains the major part of the putative active compounds (Bomme, 1997; Southwell and Bourke, 2001; Pluhar et al., 2001).

The correct developmental stage, i.e., keeping the appropriate harvesting time, was imperative for the production of a *Hyperici* herba drug with a satisfying hypericin or hyperforin content (Büter and Büter, 2002). In earlier studies, different stages have been reported for the maximum hypericin concentrations. These stages were: the beginning of the blooming period (Kitanov, 1995), the loudding and blossoming period (Kitanov, 2000), the massive flower bud formation period (Kireeva et al., 1999, the late budding stage (Franke et al., 1999), the period that flowers just opened (Tekelova et al., 2000), the period that flowers were at anthesis (Walker et al., 2001), the flowering period or shortly before (British, 1976; ESCOP, 1996), the full flowering period (Kartnig et al., 1997; Pluhar and Zelnik, 1994; Seidler et al., 1999), the stage that flowering had almost finished, when fruiting capsules were forming (Southwell and Bourke, 2001), the stage at which equal ratios of buds and capsules are present and when primary flower turns into a gren capsule (Braunewell, 1991).

Our results demonstrate that in general, higher concentrations of hypericin were found in samples collected in Izmir and Edirne populations as compared to Bursa population. When these results are considered, it is possible to say that the highest hypericin ratio was determined in flowers and buds collected between 8:00 and 10:00 a.m within a day for examined *Hypericum* populations.

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