Biologia Futura

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Factors affecting the variation of bioactive compounds in *Hypericum* species

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DOI: 10.1556/019.70.2019.25

The genus *Hypericum* (*Hypericaceae*) consists of 484 species from 36 sections with worldwide distribution in different areas. Turkey is considered as hot spot for diversity of *Hypericum* genus. Despite numerous publications, *Hypericum* species still attracted considerable scientific interest due to pharmaceutically relevant secondary metabolites: naphthodianthrones, acylphloroglucinol derivatives, phenolic acids, flavonoid glycosides, biflavonoids, and some other valuable constituents. Phytochemical investigations carried out on different *Hypericum* species provided highly heterogeneous results. The content of bioactive compounds varies significantly due to many internal and external factors, including plant organs, phenological stage, genetic profile, environmental abiotic and biotic factors, such as growing site, light, temperature, radiation, soil drought and salinity, pathogens, and herbivores attack. The variations in content of bioactive compounds in plants are regarded as the main problem in the standardization of *Hypericum*-derived pharmaceuticals and dietary supplements. The review discusses the main factors contributing to the variations of bioactive compounds and what kind of modulations can increase quality of *Hypericum* raw material.

Original Article

Cite this article: Cirak C, and Radusiene J. 2019. Factors affecting the variation of bioactive compounds in *Hypericum* species. *Biol. Fut. 70*, 198–209.

Received: 14 December 2018
Accepted: 19 July 2019

Keywords:
abiotic and biotic factors, variation of bioactive compounds, hyperforin, hypericin, *Hypericum* species

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INTRODUCTION

Hypericum (*Hypericaceae*) is one of the 100 largest genera including 22% of angiosperm diversity (Carine & Christenhusz, 2010) and consists of 484 species from 36 sections (Crockett & Robson, 2011). *Hypericum* species are well-recognized healing agents in folk medicine due to their various pharmaceutical properties. Despite the large number of *Hypericum* species, only *H. perforatum* L. has been searched deeply to date and its herbal preparations are greatly used as a remedy for the treatment of mild to moderate depression (Fiebich et al., 2011). Turkey is an important extensity center of *Hypericum* species and Guner et al. (2012) have recently reported the presence of 96 *Hypericum* species in Turkish flora, 46 of which are endemic. *Hypericum* species have been used in Turkish traditional medicine under the names "peygamber çiçeği, kantaron, kuzukıran, kanotu, kılıçotu and binbirdelik otu" as wound healing, antiseptics, sedatives, and antispasmodics.

The healing properties of *Hypericum* plants have prompted investigations of their secondary metabolites and biological activities, which have been mainly attributed to phytochemical groups as naphthodianthrones and phloroglucinol derivatives, phenolic compounds, and essential oils (Zhao et al., 2015). Among the chemical constituents, hypericin and hyperforin were indicated to be synergistically responsible for the antidepressant activity of *Hypericum* extracts (Kasper et al., 2010, Ramalhete et al., 2016). Antitumor, antiangiogenic (Martinez-Poveda et al., 2005; Rothley et al., 2009; Schiavone et al., 2014), and neuroprotective (Ma et al., 2018) effects were also induced by hyperforin and its derivatives. Extendedly investigated hypericin has been reported to possess antiviral, photodynamic (Shih et al., 2018), antitumor (Kim et al., 2018), and antibacterial activities (Rodriguez-Amigo et al., 2015). Although hyperforin and hypericin have been reported to promote mainly to the bioactivities of *Hypericum* extracts, several other constituents as phenolic acids and flavonoids have also made a significant contribution to the antidepressant (Tusevski et al., 2018), antimicrobial (Zhao et al., 2010), antioxidant (Alia et al., 2006; Oztürk et al., 2009), and neuroprotective (Silva et al., 2008) activities. Wound-healing activity of *Hypericum* extracts has been attributed to their accumulation of essential oils.

Despite numerous studies and publications, *Hypericum* species are of high-priority research due to the increasing pharmacological significance and commercial value of *Hyperici herba*, officially obtained from *H. perforatum* (Stojanovic et al., 2013). Thus, a number of *Hypericum* species from different countries of the world, such as Brazil (França et al., 2013), Greece (Mathioudaki et al., 2018), Italy (Mandrone et al., 2017), Tunisia (Hosni et al., 2017), Serbia, Montenegro (Zdunic et al., 2017), Portugal (Nogueira et al., 2008), Lithuania (Bagdonaite et al., 2010), Jordan (AI-Rifaee et al., 2010), Iran (Pirbalouti et al., 2014), Peru (Ccana-Ccapatinta et al., 2014), and Turkey (Cirak et al., 2016a, 2016b, 2017a, 2017b) have been investigated in respect to the presence of chemical ingredients that give health benefits.

The previous results indicated evident differences referring to accumulation levels of these phytochemicals among different species of *Hypericum* from various sections (Cirak et al., 2016b); different accessions of the same species from diversified geographic origins (Cirak et al., 2015a; Nogueira et al., 2008), different ontogenetic phases of the same species (Abreu et al., 2004; Cirak et al., 2014b, 2014c) and even among the individuals, cultured under the same controlled environment (Bruni & Sacchetti, 2009) or regenerated from the same *in vitro* culture (Ayan et al., 2005). Based on the results, it is not possible to define the exact patterns of synthesis of the main secondary metabolites within and among species of *Hypericum* genus. Importantly, the data presented in most studies are variable due to different extraction and analysis approaches and are hardly comparable. The chemical heterogeneity of *Hypericum* species has a remarkable effect on the bioactivity of plant extracts and poses the problem of standardizing of end herbal-derived products (Costa et al., 2016). As a result, the issues on the efficacy and safety of *Hypericum*-derived pharmaceuticals and dietary supplements have risen. In this context, up to 17-fold and 13-fold differences in hypericin and pseudohypericin amounts, respectively, are reported in several *Hypericum*-derived commercial products (Murthy et al., 2014). The first step to be taken to solve this problem is to clarify the main reasons underlying the huge variation in the content and composition of bioactive compounds in *Hypericum* spp. Thus, based mainly on our previous results and data of other authors, the aim of this study is to discuss the main internal and external impacts on chemical variation to gain a deeper understanding of the regulation of the accumulation of important secondary metabolites in *Hypericum* species raw materials. In this study, we present our review of relevant data including the past three decades by screening main international online databases, namely, ASABE Technical Library, CAB Abstracts, CAB International E-Books, ProQuest Dissertations and Theses Global, Science Direct, Scopus, Taylor & Francis Online Journals, Springer E-Book Collection, Web of Science, and JSTOR (Journal Storage).

PLANT ORGAN DEPENDENCE

Plants of the genus *Hypericum* are qualified by different types of secretory vesicles, namely translucent glands, black nodules, and secretory canals (Lotocka & Osinska, 2010). Among *Hypericum* metabolites, hypericin was reported to accumulate only in the dark glands of plant aerial parts (Fig. 1; Lu et al., 2001; Robson, 2001). In our previous research, we have detected a positive correlation between dark glands number and hypericin content in the leaves of *H. perforatum*, *H. avicullariifolium* Jaup. and Spach subsp. *depilatum* (Freyn and Bornm.), Robson var. *depilatum*, *H. pruinatum* Boiss. and Bal. (Cirak et al., 2006b), and *H. lydium* Boiss (Cirak, 2006). Several authors reported that the lack of hypericin was concerned with the absence of dark glands in the corresponding *Hypericum* species (Crockett & Robson, 2011; Nor et al., 2008). It was also shown that the amount of hypericin was negatively correlated with the leaf area of *H. perforatum* (Cirak et al., 2007d).

Recently, hypericin, pseudohypericin and a proposed precursor of hypercins, and emodin were identified not only

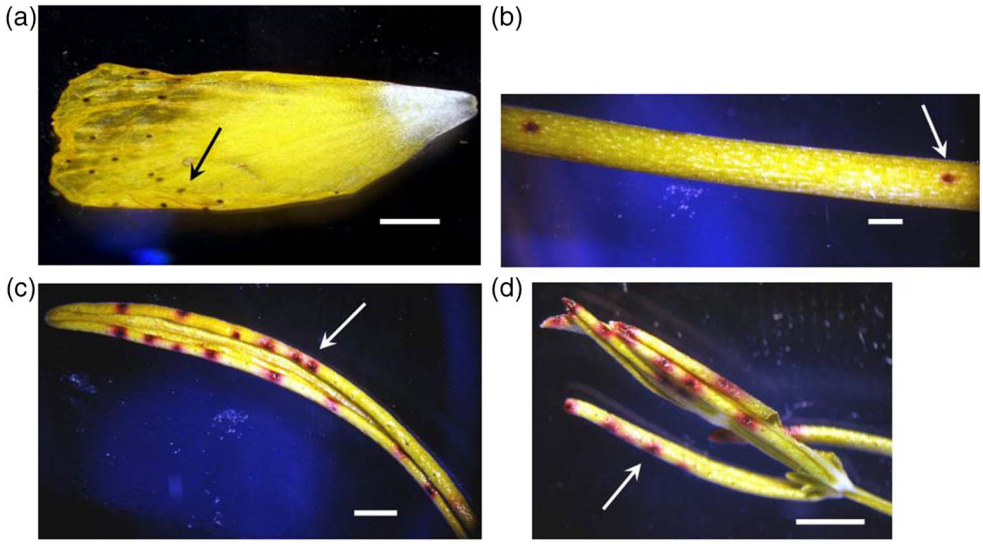

Fig. 1. Dark glands on petal (a), stem (b), and leaves (c, d) of *H. lydium* (photo by Cirak)

in dark glands, but also in translucent glands as well as in non-secretory tissues throughout the leaves of some *in vitro* cultured species from *Hypericum* section (Kucharíková et al., 2016). According to the chemotaxonomic surveys, hypericins are produced only in phylogenetically advanced clades of the genus as *Hypericum*, *Drosocarpium*, *Thasia*, *Adenosepalum* (Kitanov, 2001), *Olympia* (Cirak et al., 2016b), *Taeniocarpium*, and *Drosanthe* (Camas et al., 2014b), a result of adaptation to some selective factors. Similarly, essential oils are synthesized either in translucent glands or in secretory canals that may be localized in leaves, flowers including petals, sepals, and pistil (Fig. 2; Zhao et al., 2015). For example, Lotocka and Osinska (2010) reported significant differences in the content and composition of essential oil in leaves and flowers of *H. elegans* Steph. ex Willd., *H. inodorum* Willd., *H. olympicum* L., *H. forrestii* (Chitt.) N. Robson and two genotypes of *H. perforatum*. These species highly differed in localization and abundance of the secretory structures and the differences were reported by Zobayed et al. (2006) as the main reason for the variations of phytochemical levels in *Hypericum* plants. Secretory tissues, despite being present in the whole plant, are mostly located in leaves and reproductive organs, resulting in distinct organ dependence of secondary metabolites. The examples of organ-dependence differences of the pharmacologically important secondary compounds in 36 wild Turkish *Hypericum* species including subspecies were summarized in Table 1. The presented data have indicated that floral parts are unique organs for accumulation of hypericins and hyperforins in all *Hypericum* spp. Biflavonoids seem to be higher in leaves than in flowers for most of the species. Meanwhile, leaves of *Hypericum* species were exposed to a significant priority in the accumulation of chlorogenic, neochlorogenic, and dihydroxybenzoic acids compared to the inflorescences. As for the presented flavonoids, their maximum levels were provided either in flowers or in leaves, depending on the species.

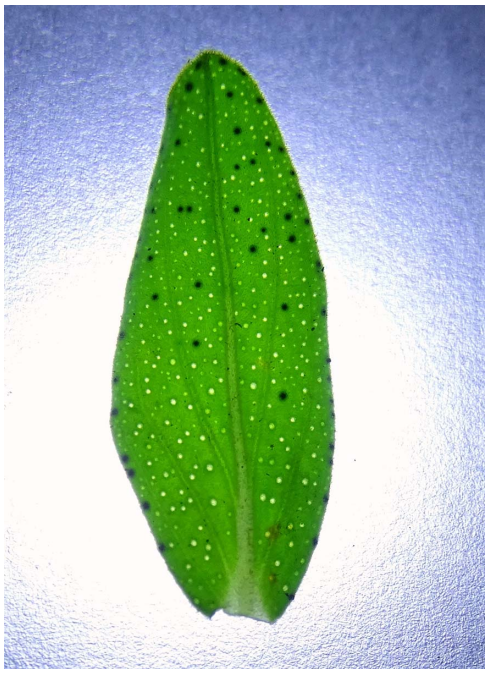

Fig. 2. Dark and transparent glands of *H. aviculariifolium* subsp. *depilatum* var. *depilatum* leaf (photo by Cirak C. and Bertoli A. 2013)

PHENOLOGY

The accumulation of bioactive secondary metabolites in different plant organs varied considerably during the seasonal development of plants. Peculiarly, the growth and development of reproductive tissues in *Hypericum* plants are pursued by speeding of secondary metabolism. The enhanced accumulation of hypericin, hyperforin, phenolic acids, amentoflavone, isoquercitrin, quercitrin, quercetin, avicularin, and catechins was reported during floral development for *H. perforatum* (Cirak et al., 2007b; Kazlauskas & Bagdonaite, 2004), *H. leptophyllum* L. (Seyis et al., 2016), *H. montbretii* Spach. (Cirak et al., 2008c), *H. avicularifolium* subsp. *depilatum* var. *depilatum*, *H. orientale* L. (Cirak et al., 2013), *H. origanifolium* Wild., *H. perfoliatum* (Cirak et al., 2007a, 2008a), *H. triquetrifolium* Turra (Cirak et al., 2014c), *H. pruinatum* (Cirak et al., 2015c), *H. scabrum* L. (Ayan et al., 2008), and *H. brasiliense* Choisy (Abreu et al., 2004). Similarly, the highest yields of essential oil were obtained in *H. perforatum* leaves at flowering and green capsules phases. However, the green capsules and the full opened flowers were different in the composition of essential oil (Bertoli et al., 2011). Schwob et al. (2004) reported that total number of compounds detected in essential oil of *H. perforatum* increased during plant ontogenesis.

Exceptionally, the highest levels of chlorogenic acid, hyperoside, and apigenin-7-O-glucoside accumulation in *H. montbretii* Spach. (Cirak et al., 2008c), *H. origanifolium* (Cirak et al., 2007b), *H. perfoliatum* (Cirak et al., 2007a), and *H. perforatum* (Cirak et al., 2007c) were found in vegetative phase, while the amount of these compounds significantly decreased with the advancement of plant growth. It was detected that the decrease in temperature and light intensity significantly decreased bioactive compounds accumulation during phenological development of *H. perforatum* (Radusiene et al., 2012). In addition, the relationship between the content of chemical compounds in the plant material of different *Hypericum* species and the harvesting time was mathematically formulated as $PC = [a + (b_1 \times S) + (b_2 \times L) + (b_3 \times R) + (b_4 \times S^2) + (b_5 \times (1/RP)]$, where PC is whole plant content of phenolic compound; S is phenolic content of stem; L is phenolic content of leaf; RP is phenolic content of reproductive parts; and a, b_1, b_2, b_3, b_4, and b_5 are coefficients. The results of these studies indicate that blooming is the most appropriate harvesting time to provide the highest level of secondary metabolites (Odabas et al., 2008, 2009b). The increasing accumulation of secondary metabolites such as hypericins and phenolic compounds during flowering can be attributed to reproductive adaptations within internal regulation of plant cells to provide protection against plant pathogens and herbivores (Cirak et al., 2014a; Crockett & Boeve, 2011), higher ultraviolet B (UV-B) radiation, and other environmental stressors during propagule reproduction (Falcone Ferreyra et al., 2012).

Table 1. The main plant organ for accumulation of bioactive secondary metabolites in some *Hypericum* species from Turkey (species are listed alphabetically)

Species	Compounds																			References
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
H. aviculariifolium Jaub. and Spach subsp. *aviculariifolium* var. *albiflorum*	F	F	–	–	L	L	L	L	F	F	F	F	–	–	F	–	–	F	F	Cirak et al. (2013, 2016a, 2016b)
H. aviculariifolium Jaub. and Spach subsp. *depilatum* (Freyn and Bornn.) N. Robsonvar. *depilatum*	F	F	F	F	L	L	–	–	L		F	F	–	L	–	–	–	–	–	Cirak et al. (2006b, 2013)
H. bithynicum Boiss.	F	F	F	F	L	L	–	L	F	L	L	L	L	F	F	F	F	F	F	Cirak et al. (2016a, 2016b; 2017b)
H. bupleuroides Gris.	F	F	F	F	F	F	–	–	F	–	F	F	–	F	F	F	–	–	–	Ayan et al. (2009)
H. calycinum L.	–	–	F	F	L	L	–	L	L	L	L	L	L	L	F	F	–	F	F	Cirak et al. (2016a, 2016b; 2017b)
H. capitatum Choisy var. *capitatum*	F	F	F	F	L	L	–	L	F	F	F	F	L	F	F	F	–	F	F	Cirak et al. (2016a, 2016b)
H. capitatum var. *luteum* N. Robson	F	F	F	F	L	L	–	L	F	F	F	F	L	F	F	F	–	F	F	Cirak et al. (2016a, 2016b)
H. cardiophyllum L.	–	–	F	F	L	L	L	L	L	F	F	F	F	L	F	F	L	L	L	Cirak et al. (2016a, 2016b; 2017b)
H. confertum Choisy	F	F	F	F	L	L	L	F	L	L	F	F	F	F	L	F	–	F	L	Cirak et al. (2010b); Camas et al. (2014b)
H. elongatum var. *elongatum* Ledeb. Ex Rchb.	F	F	F	F	L	L	–	L	F	L	F	F	L	F	F	F	F	–	F	Cirak et al. (2016a, 2016b)
H. elongatum subsp. *microcalycinum* (Boiss. and Heldr.) N. Robson	F	F	–	–	L	L	L	L	L	L	L	F	F	F	F	–	–	L	L	Cirak et al. (2016a, 2016b)
H. hircinum L.	F	F	F	F	F	L	L	L	F	F	–	F	–	–	–	F	–	F	F	Odabas et al. (2016)
H. hirsutum L.	F	F	F	F	L	L	L	L	F	F	F	F	L	L	F	–	–	F	F	Cirak et al. (2016a, 2016b)
H. lanuginosum Lam.	F	F	–	–	F	L	–	L	F	F	F	F	F	–	–	F	–	F	F	Odabas et al. (2016)
H. leptophyllum L.	–	–	–	–	L	L	L	L	F	L	L	F	F	–	–	F	–	L	L	Seyis et al. (2016)
H. linarioides Bosse	F	F	–	–	L	L	F	L	L	L	F	F	L	F	L	F	–	F	L	Camas et al. (2014a)
H. lydium Boiss.	F	F	F	F	F	L	–	F	L	L	L	F	F	F	L	F	–	F	L	Cirak (2006); Camas et al. (2014a); Cirak et al. (2015a)
H. montbretii Spach	F	F	F	F	L	L	–	–	–	–	–	–	–	–	F	–	–	–	–	Cirak and Radusiene (2007a, 2007b); Cirak et al. (2008b; 2015b)
H. olivieri (Spach) Boiss.	F	F	–	–	L	L	–	F	L	L	L	F	F	F	L	F	–	F	L	Camas et al. (2014a)
H. olympicum L.	F	F	–	–	L	L	F	F	L	L	L	F	F	L	F	F	–	F	L	Cirak et al. (2016a, 2016b)
H. orientale L.	F	F	F	F	L	L	–	–	L		F	F	–	L	–	–	–	–	–	Cirak et al. (2012, 2013)
H. origanifolium Willd	F	F	F	F	L	L	–	–	–	L	F	F	–	–	–	–	–	–	–	Cirak et al. (2007b, 2008a)
H. pallens Banks et Sol.	F	F	F	F	L	L	L	L	F	F	F	F	F	L	–	F	–	F	F	Odabas et al. (2016)
H. perfoliatum L.	F	F	F	F	L	L	–	–	L		F	L		F	–	–	–	–	–	Cirak et al. (2007a, 2008b)
H. perforatum L.	F	F	F	F	L	L	–	–	L	–	F	F	F	F	L	–	–	–	–	Cirak et al. (2007c, 2007d, 2008b)
H. polyphyllum Boiss. et Balansa	F	F	–	–	L	L	L	F	L	L	L	F	F	L	F	F	–	F	L	Cirak et al. (2016a, 2016b)

(Continued)

Table 1. (Continued)

Species	Compounds																			References
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
H. pruinatum Boiss. and Bal.	F	F	F	F	L	L	F	L	L	L	F	F	L	F	–	L	–	F	L	Cirak et al. (2006b, 2014b, 2015c); Camas et al. (2014a)
H. retusum Aucher	F	F	F	F	L	L	–	L	F	L	F	F	L	F	F	F	F	F	F	Cirak et al. (2016a, 2016b)
H. russeggeri (Fenzl) R. Keller	F	F	F	F	F	L	–	L	F	F	F	F	F	–	–	F	–	F	F	Odabas et al. (2016)
H. salsolifolium Hand. Mazz.	F	F	–	–	L	L	–	L	F	L	F	F	L	F	F	F	–	F	F	Cirak et al. (2016a, 2016b)
H. scabrum L.	F	F	–	–	L	F	–	F	L	L	L	F	F	F	–	F	–	F	L	Ayan et al. (2009); Camas et al. (2014a)
H. spectabile Jaub. Spach	F	F	–	–	L	L	–	L	–	L	F	F	L	F	F	F	F	F	F	Cirak et al. (2016a, 2016b)
H. thymifolium Banks and Sol.	F	F	–	–	L	L	F	L	F	F	F	F	L	F	–	F	–	F	L	Camas et al. (2014a)
H. triquetrifolium Turra	F	F	F	F	L	L	–	–	F	L	F	F	–	–	–	–	–	–	–	Ayan and Cirak (2008); Toker (2009); Cirak et al. (2011; 2014c)
H. xylosteifolium (Spach) N. Robson	–	–	F	F	L	L	L	L	L	L	–	L	–	–	–	–	–	L	L	Cirak et al. (2016a, 2016b)
H. venustum Fenzl	–	–	–	–	F	F	–	–	F	F	F	F	–	F	–	–	–	–	–	Spiteller et al. (2008)

Note. Compounds: 1: hypericin; 2: pseudohypericin; 3: hyperforin; 4: adhyperforin; 5: chlorogenic acid; 6: neochlorogenic acid; 7: caffeic acid; 8: 2,4–dihydroxybenzoic acid; 9: hyperoside; 10: isoquercitrin; 11: quercitrin; 12: quercetin; 13: avicularin; 14: rutin; 15: I3, II8 biapigenin; 16: amentoflavone; 17: mangiferin; 18: (+)-catechin; 19: (-)-epicatechin; F: floral parts; L: leaf; -: absent.

BIOTIC STRESSORS

Plants have evolved a broad range of tactics with the aim of protecting themselves against different stressors (Narayani & Srivastava, 2017), mainly based on induction of new toxic compounds and/or excessive production of preexisting defensive chemicals (Bruce & Pickett, 2007). Similarly, the induction of various phytochemicals has been reported for the return of biotic challenges in many plant species and a number of bioactive compounds such as hypericin (Crockett & Boeve, 2011), rutin, and chlorogenic acid (Kroner et al., 2012) have been considered as a chemical defense of plants against plant pathogens and herbivores. Cirak et al. (2005) reported that hypericin level was significantly increased in greenhouse-grown *H. perforatum* and *H. pruinatum* in response to the inoculation of seedlings with the elevating doses of plant pathogens *Phytophthora capsici* and *Diploceras hypericinum.* Analogous patterns were reported for *H. perforatum* in the defensive role of hypericin in response to biotic challenge by plant pathogens (Sirvent & Gibson, 2002) and herbivores (Sirvent et al., 2003). Furthermore, because of the hypericin insecticidal activity, it has been observed that insects feeding on leaves of *H. perforatum* adjust their diet by abstaining from eating the leaf parts containing dark glands in which hypericin is stored (Guillet et al., 2000). Cirak et al. (2006a) observed that hypericin accumulation in *H. avicullariifolium* subsp. *depilatum* var. *depilatum* and *H. pruinatum* significantly increased at night time. The authors attributed the distinct increase of hypericin content in plants to the patterns of nocturnal feeding of destructive insect herbivores.

Among induced secondary metabolites, plant phenolics have an importance in plant defense, and their role in resistance to fungi is more dynamic than their role against insects or any other attacking organisms. It was reported that the content of phenolics such as luteolin, mangiferin, hyperoside, mangostin, isoquercitrine, quercetine, and their derivatives has increased in *H. perforatum* cultures as a response to *Colletotrichum gloesporioidies* infection, which is one of the most common fungal pathogens inducing bitter rot of many crops (Conceicao et al., 2006). Moreover, Cirak et al. (2014a) described the role of chlorogenic acid, rutin, hyperoside, isoquercetine, quercitrine, and quercetine as part of an inducible plant defense reaction and reported increasing levels of each compound in response to inoculation with the fungal pathogen *D. hypericinum* and plant growth-promoting bacteria *Pseudomonas putida* in *H. perforatum* and *H. triquetrifolium*. The enriched accumulation of phenolic compounds has been reported as a genotype response to pathogen infections by upregulation genes-encoding enzymes involved in the phenylpropanoid pathway leading to the synthesis of these compounds (Foster-Hartnettet al., 2007). In the similar way, Crockett and Boeve (2011) have reported an explicit role of flavonoid glycosides in plant defense mechanisms and an increasing accumulation of phenolic compounds in wild growing *H. perforatum* and *H. hirsutum* L., as a response to the attack of sawfly (*Tenthredo zonula*) larvae.

GROWTH SITE

The growth site along with the wide range of environmental factors, such as soil, radiation, light, temperature, wind velocity, and others, is known to interfere with the synthesis and accumulation of relevant secondary metabolites in plants. In most of *Hypericum* species, investigations were conducted using a plant material from wild populations that were exposed to different environments. Significant differences among *H. perforatum* populations were reported for the content of hyperforin, hypericin, and pseudohypericin from Australia (Southwell & Bourke, 2001), Turkey (Cirak et al., 2006a, 2007d), Lithuania (Bagdonaite et al., 2010), Canada (Jensen et al., 1995), and Armenia (Kirakosyan et al., 2002). Wild populations of other *Hypericum* species were also reported to display significant variations in the content of several bioactive compounds. In terms of variability of essential oil composition, significant differences were detected in compliance with the geographic distribution of wild populations of *H. perfoliatum, H. humifusum* L., *H. linarifolium* Vahl., and *H. pulchrum* L. (Nogueira et al., 2008). Cirak and Bertoli (2013) reported significant compositional variations in essential oil between two populations of *H. aviculariifolium* subsp. *depilatum* var. *depilatum*. Significant variations were detected in the content of hypericin, pseudohypericin, hyperforin, and several flavonoids as rutin, hyperoside, apigenin-7-O-glucoside, kaempferol, quercitrin, quercetin, and amentoflavone among four wild populations of *H. triquetrifolium* from Turkey (Camas et al., 2008; Cirak et al., 2011). Accordingly, 5 wild populations of *H. lydium* (Cirak et al., 2015a), *H. montbretii* (Cirak et al., 2015b), and *H. pruinatum* (Camas et al., 2013) and 11 populations of *H. orientale* (Cirak et al., 2012) were significantly different in the quantity of hypericin, pseudohypericin, hyperforin, adhyperforin, chlorogenic acid, neochlorogenic acid, 2,4-dihydroxybenzoic acid, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, (+)-catechin and (-)-epicatechin, and amentoflavone.

The results have indicated the importance of the growth localities with different environment characteristics on the expression of secondary metabolites along with the chemical intraspecific diversity of wild *Hypericum* populations.

ABIOTIC STRESSORS

Temperature and UV-B radiation

Among the abiotic stressors of the site, temperature and UV-B radiation have a prominent impact on plant secondary metabolism. Decreased temperature and increased UV-B radiation have caused plant cells to produce reactive oxygen species (ROS) resulting in oxidative damage to lipids, DNA, structural proteins, and other cellular structures. As a result, higher UV-B radiation and lower temperature have stimulated the production of secondary metabolites with UV-B-absorbing and ROS-scavenging qualities, such as phenolic acids, proanthocyanidins, anthocyanins, and flavonoids (Turkan & Demiral, 2009). Similarly, leaf flavonoid concentrations increased in response to enhancing UV-B radiation in cultivated *H. perforatum* plants (Germ et al., 2010). Abreu and Mazzafera (2005) observed a significant increase in total soluble phenols, rutin, betulinic acid, and quercetin contents in *H. brasiliense* for reaction to low-temperature treatments. Experimentally increased light intensity enhanced the content of leaf hypericin (Briksin & Gawienowski, 2001; Pardaz et al., 2013) and hyperforin (Odabas et al., 2009a) in *H. perforatum.*

In a similar way, altitude of plant growing site has stimulated secondary metabolism greatly as lower temperature and higher UV-B radiation predominate at higher altitudes. A significant increase in the content of hypericins in *H. triquetrifolium*, *H. perforatum, H. perfoliatum,* and *H. empetrifolium* Willd. was reported in response to altitudinal ranging by Xenophontos et al. (2008). Furthermore, Umek et al. (1999) reported a positive correlation between the accumulation of hyperoside, amentoflavone, and rutin and the altitude of habitats of *H. hirsutum, H. perforatum, H. maculatum* Crantz, *H. montanum* L., *H. tetrapterum* Fries., and *H. humifusum* L. The content of naphtodianthrones, phloroglucinol derivatives and phenolic compounds such as chlorogenic acid, neochlorogenic acid, caffeic acid, 2,4-dihydroxybenzoic acid, 13,II8-biapigenin, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, and epicatechins was reported to increase significantly in wild *H. androsaemum* L., *H. polyphyllum* Boiss. and Bal., *H. orientale* and *H. pallens* with elevating altitude of plant growing site (Camas et al., 2014a; Cirak et al., 2017b). The authors attributed the observed increase in content of bioactive compounds in *Hypericum* species as adaptation to low temperature and high UV-B radiation, which are prevalent at higher altitudes.

Salinity and drought

Salinity and drought stress are also considered as important abiotic factors influencing growth, productivity, and secondary metabolism of plants, especially in arid and semi-arid regions (Temizel et al., 2014). On top of specific ion effects (salinity stress), nutritional imbalance, and low water potential in a soil solution (drought stress), the detrimental effect of salinity on plant physiology is also related to the production of defensive secondary metabolites (Munns & Tester, 2008). However, the results of recent studies have indicated that the oxidative stress that could also be caused by drought and the imbalance between the production and elimination of ROS is a major cause of salt sensitivity (Turkan & Demiral, 2009).

Plants have evolved various defense mechanisms to eliminate the oxidative damage induced by salt and drought stress, which involves the excessive production of antioxidant chemicals that prevent the expansion of oxidative chain reactions. In the circumstances, phenolic compounds such as anthocyanins, phenolic acids, proanthocyanidins, and flavonoids have a significant role in eliminating the impairing effect of salinity (Hichem et al., 2009). The proven antioxidant activity of phenolics allows them to act as ROS-scavenging agents and is the main reason why their

synthesis is stimulated in return for the exposure to abiotic stresses (Souza & Devaraj, 2010). The accumulation level of several phenolics namely rutin, quercetin, and total soluble phenols has been significantly enhanced under conditions of water and temperature stresses in greenhouse-grown *H. brasiliense* (Abreu & Mazzafera, 2005). Moreover, *H. perforatum* plants subjected to drought and salinity stresses exhibited an evident increase in accumulation of total phenolics, quercetin, and rutin (Gray et al., 2003; Temizel 2015). Similarly, hyperforin content in the same species enhanced notably and was nearly double after 12 days of exposure to drought stress (Zobayed et al., 2007). The content of chlorogenic acid, rutin, hyperoside, isoquercetin, quercitrin, and quercetin in *H. pruinatum* plants was also found to be significantly increased by elevating salt treatment doses (Caliskan et al., 2017).

GENOTYPE

The intraspecific chemical diversity of *Hypericum* species was reported among wild populations, in plants under controlled conditions and even between the plants regenerated from the same clones in the early stages of development by Kosuth et al. (2003) reporting significant genetic differences between phenotypes. Knowledge about genetic diversity of populations is important for effective germplasm selection and breeding improvement for the desired genotypes. The genetic variations occurring within and among populations of *H. perforatum* and other *Hypericum* species have been carried out using several molecular analysis methods. Each molecular technique has limitations in the screening of genetic profiles and therefore more than one method should be used to come to a general conclusion. Haluskova and Cellarova (1997) analyzed the somaclonal variation within *H. perforatum* and R1 progenies at the molecular level via restriction fragment length polymorphism (RFLP) technique, whereas Percifield et al. (2007) practiced DNA fingerprinting using amplified fragment length polymorphism (AFLP) technique to search genetic diversity among 11 *Hypericum* species. Farooq et al. (2014) from India and Morshedloo et al. (2015) from Iran assessed the genetic diversity of *H. perforatum* wild populations from different climatic zones using intersimple sequence repeat markers. Sixteen Tunisian populations of *H. humifusum* L. were evaluated for their genetic variability using isomeric and random-amplified polymorphic DNA (RAPD) markers (Bejaoui et al., 2010, 2012). Furthermore, internal transcribed spacer (Nürk et al., 2013; Pilepic et al., 2011) and RFLP (Pilepic et al., 2010) techniques were used assess the extent of phylogenetic relationships in several sections of *Hypericum* genus. The following molecular studies have revealed high genetic diversity and excess heterozygosity within populations supporting an outbreeding mating system and low gene flow. On the other hand, different populations of the same species exhibited high genetic similarity and had no obvious relationship with climate zones (Bejaoui et al., 2010). The research by Barcaccia et al. (2006) on genetic diversity and reproductive biology of *H. perforatum* has shown that populations are polyclonal, i.e., not dominated by a single genotype, which is in consequence with the prevalent reproductive mode of facultative apomixes. On the other hand, occasional sexual reproduction leads to genetic diversity that is fixed by apomixis. These genetic findings of the aforementioned authors reflect the chemical variations suggesting that the valuable germplasm of *Hypericum* spp. can be detected within populations. The chemodiversity that has been identified among populations of the same *Hypericum* species is likely the result of genetic diversity and adaptive strategies to changing environmental factors.

According to some authors, genetic polymorphism strongly affects the content of bioactive secondary metabolites in *Hypericum* species (Buter et al., 1998). Cytological and compound accumulation analyses have shown the relationship between the ploidy level and total content of hypericins and phloroglucinols with the highest levels found in diploids and the lowest in tetraploids (Kosuth et al., 2003). However, data about the relationship between the genetic structure and bioactive chemical ingredients in *Hypericum* spp. are limited and the results from different sources are often quite discrepant. The significant differences in genetic profile together with the differences in the content of chlorogenic acid, rutin, hyperoside, and quercetin were determined by He and Wang (2013) among 12 wild populations of *H. perforatum* in China using sequence-related amplified polymorphism technique. They observed only a partial correlation between content of bioactive compounds and genetic polymorphism of accessions. Similarly, Verma et al. (2008) reported a partial correlation between hypericins, hyperforin, and flavonoids as rutin, hyperoside, quercitrin, and quercetin contents and data of RAPD and SSR analyses of eight wild *H. perforatum* populations from India. Furthermore, no correlation was found between hypericin content and genetic profile within 27 accessions of *H. triquetrifolium* from Jordan (AI-Rifaee et al., 2010). On the contrary, strong correlations were reported between hypericin content and RAPD data for 19 *H. perforatum* clones cultivated in field conditions during 2 years (Tonk et al., 2011).

The significant correlations between the secondary metabolite contents and RAPD data of the genetic profile were found among different species, namely, *H. barbatum* Jacq., *H. hirsutum*, *H. linarioides* Bosse, *H. maculatum* Crantz, *H. rumeliacum* Boiss., and *H. tetrapterum* Fries, collected from the same site in Serbia (Smelcerovic et al., 2006). In this case, the genotype significantly affects the differences between species, unlike among the accessions of one species. A similar study was conducted with 11 *Hypericum* species and cultivars that exposed correlations between genetic profiles detected using AFLP technique and levels of hyperforin, hypericins, and rutin in plant materials (Aziz et al., 2006).

CONCLUSION FOR FUTURE BIOLOGY

Data from the current literature on *Hypericum* chemistry have revealed that there are major differences in the chemical accumulation levels at the species level. The advantage of this overview is that the plants have been harvested in Turkish territory and evaluated using the same method of extraction and chemical analysis, so the methods did not affect the chemical

composition and thus make the comparison valid. This way we could show how some endogenous and exogenous factors indeed effect the chemical accumulation of pharmacologically important secondary compounds across many species of *Hypericum*. Such kind of data provides a firm basis for assessing the primary quality of wild-harvested plant material as well as avoiding the huge chemical variability that is considered as the main obstacle for standardization and further processing of *Hypericum*-derived products.

Acknowledgments: None.

Funding Statement: The authors declare that there are no sponsors, no funding, and no conflicts of interest.

Data Accessibility: There are no data sets required for supporting this article.

Competing Interests: The authors declare no competing interests.

Authors' Contributions: CC provided the idea and both authors wrote this paper collectively.

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