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Original scientific paper

PHYTOCHEMICAL VARIATIONS IN THE AERIAL PARTS OF *HYPERICUM PERFORATUM* L., AT DIFFERENT HARVEST STAGES

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Abstract

Hypericum perforatum L., (St. John's Wort) is a medicinal plant that has been used for treating different kind of conditions right from the past. The conditions used were: treating skin wounds and burns together with managing psychological conditions as depression. This study aims to find out and do a research on what is the content of total phenols, flavonoids and total hypericin in the flowers and leaves of *H. perforatum* which are gathered at different stages of harvesting the plant itself. What we did is, we tried to analyze what is the concentrations of total phenols, flavonoids and hypericin in the plant material by using spectrophotometric methods: Folin – Ciocalteu reagent for phenols, $NaNO_2$-$AlCl_3$-NaOH method for flavonoids and water/tertrahydrofuran solvent system for hypericin. The observation process was done in the content of compounds depending on the plant part (flower or leaf) and the harvest time of the flower itself. We came to the conclusion that the flowers of *H. perforatum* contain the highest levels of flavonoids and phenols during the flower bud stage, while the highest content of total hypericin was found in the flowers at full bloom. Based on these findings we identified that in order to maximize the accumulation of bioactive compounds it should be done in the time of the floral budding and full blooming stages.

Keywords: Hypericum perforatum, total phenols, hypericin, flavonoids

Introduction

St. John's Wort known by people while in pharmacy is known by the Latin name *Hypericum perforatum* L., wich is part of the *Hypericaceae* family, recommended and used by everyone for its therapeutic and nutritional uses (Caldeira et al., 2022; Kwiecien et al., 2023). The complexity of phytochemical profile of *H. perforatum* is due to the several bioactive compounds such as napthodianthrones, phloroglucinol derivatives, as well as flavonoids and xanthones (Istikoglou et al., 2010; Russo et al., 2014). These mixtures have different biological effects such as: antibacterial, antiviral, anti-inflammatory and antioxidant effects (Marrelli et al., 2020; Karapandzova et al., 2020). *H. perforatum* is widely known as a natural remedy for mild to moderate depression (Ng et al., 2017), it's precise ways of acting remains only partially understood. According to studies, its pharmacological activity is firstly attributed to naphthodianthrone compound hypericin, the phloroglucinol derivatives hyperforin and adhyperforin, as well as the biflavonoid amentoflavone and other flavonoids (Nahrstedt, A &Butterweck, W., 2010; Jacobs at al., 2013; Schmidt, M &Butterweck, W., 2015; Tian et al., 2014; Olivera et al., 2016; Pochwat et al, 2018).

The activity of cyclooxygenase enzymes COX-1 and COX-2 as well as microsomal prostaglandin E2 synthase, key mediators involved in inflammatory processes is suppressed by hyperforin (Koeberle et al., 2011). A methanolic extract from the aerial part of *H. perforatum* and its active compounds hypericin and hyperforin demonstrated spasmolytic effects (Valeri et al., 2012). According to clinical research *H. perforatum* extract at doses from 270 to 330µg over a two month period led to a reduction of hot flashes, menopausal symptoms and mild depression

(Eatemadnia et al., 2019). Additionally, the extract has been seen as very effective in treating of premenstrual syndrome (Ghazanfarpour et al., 2010).
In different *in vivo* studies it has been noted that amentoflavone had antiepileptic effects by reducing oxidative stress and enhance GABA binding affinity at GABA receptors (Zhang et al., 2015; Diniz et al., 2015; Rong et al., 2019). Lately a huge interest has been raised in its extracts and bioactive compounds, as a result of to their benefits to raise growth performance, improve intestinal flora and effect on sensory characteristics in broiler chickens (Davodi et al., 2014; Zengin et al., 2022; Kogut& Fernandez, 2022; Sur et al., 2023). This scientific study aims to determine the total phenols, hypericins and flavonoids in the flowers and leaves of *H. perforatum* collected in Diber region, during the beginning of flowering, full bloom and fruiting stage.

Material and methods

The aerial parts of *H. perforatum* were collected from the Debar region, North Macedonia during three different harvest stages: the floral budding stage (June), full blooming (July) and the fruit set stage (August).
Total phenolic content was determined with the Folin-Ciocalteu reagent according to the procedure described by Singleton et al. (1965) with slight modifications. Absorbance was measured at 765 nm using a UV-VIS spectrophotometer. The total phenolic content was determined as mg of gallic acid equivalents per gram of plant material (mg GAE/g DW) using an equation obtained from standard Gallic acid calibration graph.
The total flavonoid content was determined using the aluminum chloride assay described by Talari et al. (2012) with slight modification. Absorbance was measured at 510 nm using a UV-VIS spectrophotometer. The total flavonoids were expressed in mg of catechin equivalents per gram of plant material (mg CE/g DW) using an equation obtained from standard (+)-catechin calibration graph.
The total hypericin was determined by official method described in St. John's wort monograph (Ph. Eur. 9.0, 2016). Sample solution was prepared by introducing 0.8g of pulverized drug into a 100 ml round-bottomed flask and 60 ml of a mixture of 20 volumes of water and 80 volumes of tetrahydrophurane. The mixture was put on a magnetic stirrer and then boiled to fall out in a bain-marie at 70°C and centrifuged (2 minutes at 700 g). The supernatant was decanted into a 250 ml flask. The residue was then taken with 60 ml of a mixture of 20 volumes of water and 80 volumes of tetrahydrophurane. The last was repeated once more and the combined extracts were evaporated to dryness. The residue was taken with 15 ml of methanol using ultrasound bath and transfer to a 25 ml volumetric flask. The 250 ml flask was washed with methanol and diluted with 25 ml with the same solvent. Afterwards the solution was centrifuged (2 minutes at 700 g) and 10 ml of the centrifuged sample was filtered through a syringe filter (0.2 µm, Agilent Captiva Premium Syringe Filters). Finally, 5.0 ml of the filtrate was dilute with 25 ml methanol. The absorbance of the sample solution was measured at 590 nm against the blank (methanol) and the percentage of total hypericins, expressed as hypericin, were calculated with the following expression: A x 125/m x 870, where: 870 = specific absorbance of hypericin, A = absorbance at 590 nm, m = weight of drug in grams (Longo and Schulz, 2002).

Results and discussion

The analysis of the content and distribution of total phenols, flavonoids and total hypericin in leaf and flower extracts of *H. perforatum* at three different harvest stages: the floral budding stage, the full-blooming stage and the fruit set stage is presented in the Table 1.

*Table 1.*The content of total phenols, total flavonoids and totalhypericin in flowers and leaves of *H. perforatum* collected in Debar at floral budding stage, the full-blooming stage and the fruit set stage

	Total phenols		**Total flavonoids**		**Total hypericin**	
Harvesting stage	Flos	Folium	Flos	Folium	Flos	Folium
Floral budding stage	121.19±0.78	83.08±0.45	125.35±0.52	102.32±0.90	0.44±0.02	0.16±0.02
Blooming stage	91.05±0.76	68.26±0.71	114.38±0.13	81.58±0.22	0.60±0.03	0.17±0.01
Fruit set stage	80.20±0.30	59.52±0.57	111.81±0.43	69.66±0.47	0.07±0.01	0.03±0.01

(n=3); DW – dry weight; Flos-flower; Folium-leaf

The content of total phenols ranged from 59.52 to 83.08 mg GAE/g in leaves and from 80.20 to 121.19 mg GAE/g in flowers. The highest amount of total phenols (121.19 ± 0.78 mg GAE/g) was found in the samples prepared from flowers collected at the flower bud stage, compared to all other analyzed samples. This was followed by samples collected at the full flowering stage (91.05 ± 0.76 mg GAE/g) and those collected during the fruit formation stage (80.20 ± 0.30 mg GAE/g).

Likewise, the highest amount of total phenols in leaf samples (83.08 ± 0.45 mg GAE/g DW) was found at the flower bud stage. This value gradually decreased in the samples collected at the full flowering stage (68.26 ± 0.71 mg GAE/g) and further declined in those collected during the fruit formation stage (59.52 ± 0.57 mg GAE/g)(Figure 1).

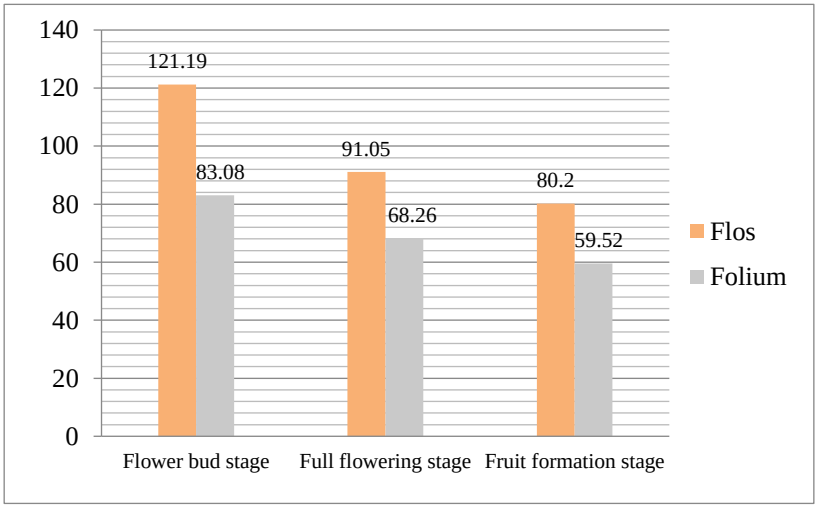

Fig. 1 The content of total phenols in flowers and leaves of *H. perforatum* (mg GAE/g DW) collected in Debar at floral budding stage, the full-blooming stage and the fruit set stage

The highest total flavonoid content was found in the flower samples collected at the flower bud stage (125.35 ± 0.52 mg CE/g), followed by those collected at the full flowering stage (114.38 ± 0.13 mg CE/g) and those collected during the fruit formation stage (111.81 ± 0.45 mg CE/g). On the other hand, the lowest flavonoid content was found in the leaf samples collected during the fruit formation stage (69.66 ± 0.47 mg CE/g) (Figure 2).

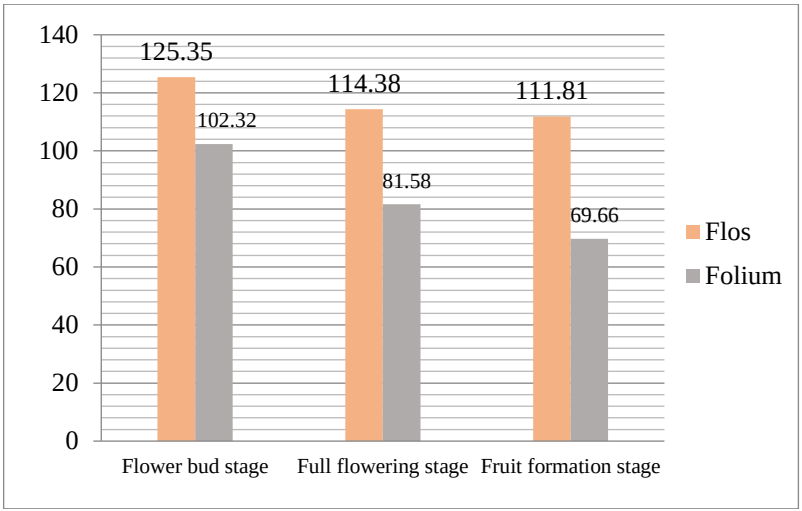

Fig.2 The content of flavonoids in flowers and leaves of *H. perforatum* (mgCE/g DW) collected in Debar at floral budding stage, the full-blooming stage and the fruit set stage

In contrast to the results for total phenols and flavonoid content where the highest concentrations were found in flower samples collected at the flower bud stage, the highest amount of total hypericin were detected in samples collected at the full flowering stage. The content of total hypericin ranged from 0.03±0.01mg/g in leaves and 0.07±0.01mg/g in flowers. The highest amount of total hypericin (0.60 ± 0.03 mg/g) was determined in the flower sample collected at full bloom, followed by the sample collected at the bud formation stage (0.44 ± 0.02 mg/g), while a significant decrease was observed in the sample collected at the fruiting stage (0.07 ± 0.01 mg/g). On the other hand, the lowest amount of total hypericin was determined in the leaf sample collected at the fruiting stage (0.03 ± 0.01 mg/g), while no significant differences were observed between the leaf samples collected at the flower bud stage (0.16 ± 0.02 mg/g) and at full bloom (0.17 ± 0.01 mg/g) (Figure 3).

According to the *Hyperici herba* monograph in the European Pharmacopoeia, the hypericin content must not be below 0.08%. This indicates that the leaf and flower samples collected at the fruiting stage do not meet the quality standards required by the European Pharmacopoeia.

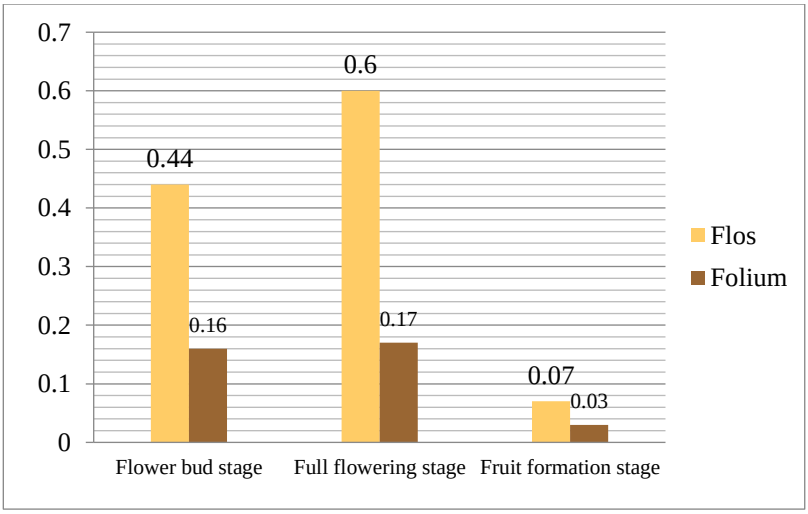

Fig.3 The content of hypericin in flowers and leaves of *H. perforatum* (mg/g DW) collected in Diber atfloral budding stage, the full-blooming stage and the fruit set stage

According to data from the literature, the content of total phenols in aerial parts of *H. perforatum* collected in Bulgaria was reported to be 10,85± 0.04 mg GAE/g (Solak, 2019). Dimitrova et al., 2010 raported that the total flavonoid content in various *Hypericum* species from Bulgaria ranged from 0.20 to 1.22 g/100 g DW. In a study by Maskovič et al., 2011, the content of total

phenols in acetone extracts was found to be 17.6 mg/g, while the total flavonoid content was 16.85 mg/g, also in acetone extract. The distribution of total phenols and flavonoids has been investigated previously, with the highest phenolic content found in leaf extracts (182,93 mg GAE/g) and the highest flavonoid content detected in ethanolic extracts of flowers (20.50 mg QE/g), as reported by Şekeroğlu et al. (2017).

According to Mireon et al., 2002 and Gray, 2003, the content of total hypericins, flavonoids and phenols indicate considerable variation in the aerial parts (flower and leaf) depending the time of harvesting. During the flower bud stage we noticed the highest hypericin content in *H. perforatum* while monoflavonoids and biflavonoids reach their highest levels from the flower bud stage to full blooming (Couceiro et al., 2006; Azizi, 2007). According to Sun et al., 2018, the antioxidant capacity and the content of bioactive compounds in the aerial parts of *H. perforatum* depend hugely on the technological maturity stage at which the plant material is harvested. Our conclusions that were noted and analyzed in Diber region are the same as the study which also noted a decreasing trend in the content of total phenols, flavonoids and hypericin from the flower bud stage to the fruit formation stage.

Our findings revealed that the highest amount of total phenols and flavonoids in the flowers and leaves of *H. perforatum* were determined at the flower bud stage with a gradual decrease toward fruit formation stage. In contrast the higher amount of total hypericin was determined at the full flowering stage.

Conclusion

During this pharmacological research we concluded that the concentration of major bioactive compounds in the aerial parts of *H. perforatum* differs hugely depending on the stage at which they are harvested.The floral budding and full blooming stages have been identified as the optimal harvest stages to achieve the maximum during accumulation of bioactive compounds.

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