

Phenolic compound contents and antioxidant activity in different varieties of *Hypericum perforatum* L. collected from the Republic of North Macedonia

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Introduction

Hypericum perforatum L. is the best studied species of the genus *Hypericum* with a wide variety of phenolic compounds that belong to the groups of naphthodianthrones, phloroglucinols, flavonoids, and xanthenes. The *H. perforatum* extracts showed antidepressant, antitumoral, antiviral, antimicrobial and antioxidant activity (Velingkar et al., 2017). The *Hypericum* species collected around the world have shown to possess different phytochemical composition and biological activities (Patočka, 2003; Çirak et al., 2011; Božin et al., 2013).

In the Republic of North Macedonia, Micevski, (1995) described three varieties of *H. perforatum*: the var. *perforatum* L. with egg-shaped to oval leaves, the var. *angustifolium* DC. with narrow elliptic to linear and usually flat leaves and the var. *microphyllum* DC. with linear to elliptical linear leaves. Since this morphological variability is not sufficiently reliable for proper identification, the phytochemical comparison of these varieties could be of particular interest for chemotaxonomic determination of *H. perforatum* taxa. The phytochemical profile of various plant parts of *H. perforatum* var. *perforatum* have been the subject of extensive studies (Tusevski et al., 2018; 2020). However, the phenolic compounds composition in other varieties of this species is still unknown. Therefore, the comparison of phenolic compounds distribution in different varieties of *H. perforatum* will be a promising approach to find out a novel source of bioactive secondary metabolites.

The main objective of this study was to determine the contents of total phenolics (TP), flavonoids (TF), flavan-3-ols (TFA) and phenolic acids (TPA), as well to analyze

antioxidant activity in aerial parts (AP), stems (ST), leaves (LE) and flowers (FL) of three varieties of *H. perforatum* wild-growing plants.

Materials and methods

Plant material and extraction

The plants were collected at flowering stage from different localities in the North Macedonia. The voucher specimens were deposited in the Herbarium at the Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje (MKNH). The plant material was separated in four sections (AP, ST, LE and FL) and phenolic compounds extraction was performed according to the procedure described by Tusevski et al., (2018).

Determination of phenolic compound contents and antioxidant activity

The TP contents were determined by the method of Folin-Ciocalteu (Singleton and Rossi, 1965) and the results were expressed as gallic acid equivalents per dry weight (mg GA·g⁻¹ DW).

Determination of TF contents was performed according to aluminum chloride method (Zhishen et al., 1999) and the results were expressed as catechin equivalents per dry weight (mg C·g⁻¹ DW).

The TFA amounts were determined by using of 4-dimethylaminocinnamaldehyde method (Arnous et al., 2002) and the results were expressed as catechin equivalents per dry weight (mg C·g⁻¹ DW).

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The TPA contents were analyzed by the Arnou method modified by Tusevski et al., (2020) and the results were expressed as pyrocatechol equivalents per dry weight ($\text{mg P}\cdot\text{g}^{-1}\text{ DW}$).

Antioxidant activity was measured using DPPH radical scavenging method (Tusevski et al., 2020) and the results were expressed as trolox equivalents per dry weight ($\text{mg T}\cdot\text{g}^{-1}\text{ DW}$).

Results and discussion

Present results showed that FL of *angustifolium* and *perforatum* varieties have the greatest capacity for TP accumulation (133.52 and $124.23\text{ mg}\cdot\text{g}^{-1}$, respectively). In contrast, the most productive parts of *microphyllum* variety were AP that accumulated $78.97\text{ mg}\cdot\text{g}^{-1}$ of TP. With respect to TF, the highest content was observed in FL extracts of *angustifolium* and *perforatum* varieties (101.77 and $96.18\text{ mg}\cdot\text{g}^{-1}$). Even that *microphyllum* variety did not show superior TF production, the highest values were found in LE and FL extracts (about $42\text{ mg}\cdot\text{g}^{-1}$). The TPA contents in FL and AP extracts of *perforatum* and *angustifolium* varieties ($19\text{-}23\text{ mg}\cdot\text{g}^{-1}$) were significantly higher than those found in LE and ST ($12\text{-}14\text{ mg}\cdot\text{g}^{-1}$). The TFA contents in *angustifolium* and *perforatum* varieties were comparable in FL (35.86 and $33.69\text{ mg}\cdot\text{g}^{-1}$, respectively) and AP (25.75 and $24.92\text{ mg}\cdot\text{g}^{-1}$, respectively). Noteworthy, LE extracts of *microphyllum* variety were found as the best source of TFA ($15.30\text{ mg}\cdot\text{g}^{-1}$) compared to *angustifolium* and *perforatum* varieties (13.86 and $9.96\text{ mg}\cdot\text{g}^{-1}$, respectively).

The results for DPPH assay showed that FL extracts of *angustifolium* and *perforatum* varieties possess significantly higher antioxidant activities (338.28 and $308.34\text{ }\mu\text{M T}\cdot\text{g}^{-1}$, respectively) than other tested organs. Almost all tested organs of *microphyllum* variety showed moderate antioxidant activities (about $200\text{ }\mu\text{M T}\cdot\text{g}^{-1}$) that were 2-fold higher than that found in ST extract ($92.69\text{ T}\cdot\text{g}^{-1}$).

The statistical analyses performed here showed significant positive correlations between all tested groups of phenolic compounds and DPPH assay ($p < 0.001$), suggesting that flavonoids, flavan-3-ols and phenolic acids in *H. perforatum* taxa represent the most prominent antioxidant compounds.

Conclusion

This study presented for the first time phenolic compounds production and antioxidant activity in

different parts of three varieties of *H. perforatum* wild-growing plants from the North Macedonia. Flowers and aerial parts of *perforatum* and *angustifolium* varieties represented the richest source of phenolic compounds, flavonoids and phenolic acids with strong antioxidant activities. Noteworthy, leaves of *microphyllum* variety were selected as superior source for the accumulation of flavan-3-ols. Present results could be implemented for the authentication of *H. perforatum* taxa and selecting the varieties with similar phytochemical profiles that may have potential application in pharmaceutical, cosmetic and food industry.

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