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Short communication

Contribution of phenolic acids and flavonols to the antioxidant activity of *Hypericum perforatum* L.

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Introduction

Hypericum perforatum (St John's wort) is the most important medicinal plant that is widely used as a source of many biologically active compounds. The medicinal properties of *H. perforatum* have been related to the phenolic composition, particularly flavonoids and phenolic acids that possess ideal chemical structures for antioxidant activity. Chlorogenic acid, quercetin, rutin and catechins have been identified in *H. perforatum* extracts as dominant phenolic compounds with antioxidant properties (Silva et al., 2008). Quantification of total phenolic compounds rather than detection of single compound have a greater importance, since the efficiency of *H. perforatum* is based on the synergism of antioxidant compounds.

Although the detailed phytochemical profile of *H. perforatum* vegetative and reproductive organs using HPLC/DAD/ESI-MS analysis is already known (Tusevski et al., 2018; 2019, 2020), the biosynthetic pathways of phenolic compounds are not yet fully understood. Our recent studies showed that aerial parts of *H. perforatum* wild-growing plants are the main source of phenolics, flavonoids, tannins and hypericins with strong antioxidant activity. On the other hand, *H. perforatum* root extracts enriched with different xanthones exhibited moderate antioxidant properties (Tusevski et al., 2019). Therefore, it is of great importance to analyze the distribution of certain groups of phenolic compounds in various organs of *H. perforatum*.

The main objective of this study was to determine the contents of phenolic acids (TPA) and flavonols (TFL) in roots (RO), non-flowering shoots (NFS) and flowering shoots (FS) of *H. perforatum* wild-growing plants, as well to evaluate their contribution to the antioxidant activity.

Materials and methods

Preparation of plant extracts

Wild-growing plants of *H. perforatum* (Voucher No. 060231) collected in the National Park Pelister, Republic of North Macedonia were separated into three sections (RO, NFS and FS) and phenolic compounds were extracted according to the procedure described by Tusevski et al., (2018).

Determination of phenolic acid and flavonol content

The TPA contents were determined using the Arnow method: plant extract was mixed with 0.5 M HCl, Arnow reagent (5% $NaNO_2$ and Na_2MoO_4) and 2 M NaOH (Fraisse et al., 2007). Absorbance was read at 525 nm and TPA were expressed as pyrocatechol equivalents per dry weight (mg $P \cdot g^{-1}$ DW).

The TFL contents were determined when plant extract was mixed with 2% $AlCl_3$ and 1 M CH_3COONa (Öztürk et al., 2009). The absorbance was read at 440 nm and TFL were expressed as rutin equivalents per dry weight (mg $R \cdot g^{-1}$ DW).

Determination of antioxidant activity

The FRAP assay (Silva et al., 2008) was determined by mixing of plant extracts with FRAP reagent (0.3 M CH_3COONa, 0.01 M TPTZ and 0.02 M $FeCl_3$). The absorbance was read at 593 nm and the results were

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expressed as Fe^{2+} equivalents per dry weight (μM $Fe^{2+}\cdot g^{-1}$ DW).

The LPI was determined according to method of Sun and Ho (2005). The samples were consisted of plant extracts and linoleic acid-β-carotene emulsion. In control sample, the extract was replaced with CH_3OH. The absorbance of samples and controls was read at 470 nm and results were expressed as percentage of LPI inhibition.

Results and Discussion

Present results showed that FS extracts exhibited the highest production of TPA (16.8 mg $P\cdot g^{-1}$), followed by NFS (12.3 mg $P\cdot g^{-1}$), while RO exhibited the lowest value (3.8 mg $P\cdot g^{-1}$). Since *H. perforatum* accumulated various hydroxycinnamic acids (Silva et al., 2008), the Arnow method could be used as a relevant assay for determination of TPA. With regards to TFL, the contents in FS and NFS extracts (52.5 and 46.9 mg $R\cdot g^{-1}$, respectively) were considerably higher than RO extract (5.5 mg $R\cdot g^{-1}$). In accordance with present results, flavonols represented the major flavonoid fraction in *H. peforatum* aerial parts, while trace amounts have been found in roots (Öztürk et al., 2009).

Taking into account the results from FRAP assay, the values in FS and NFS extracts (781.9 and 769.9 μM $Fe^{2+}\cdot g^{-1}$, respectively) were significantly higher than those found in RO (419.9 μM $Fe^{2+}\cdot g^{-1}$), suggesting that aerial plant parts have a capability for accumulation of compounds with elevated reducing properties. The present results for FRAP values are comparable with previous studies indicating that *H. perforatum* extracts have strong reductive potential (Silva et al., 2008). The β-carotene-linoleic acid bleaching inhibition assay is considered as a model system for lipid peroxidation inhibition. With respect to antioxidant activity measured by LPI, the highest value was noticed in FS (85.4%), followed by NFS (78.6%), while the lowest activity was found in root extracts (65%). These findings indicated organ-specific variation in *H. perforatum* plants for the accumulation of antioxidant compounds with hydrogen-donating capacity for lipid peroxidation inhibition.

The correlation analyses performed here showed significant positive correlation between TFL and FRAP assay ($p<0.001$). These results suggested that flavonols in *H. perforatum* could be proposed as preeminent phenolic constituents that exhibit ferric ion reducing capacities. Even that LPI is an indication for the lipophilic antioxidant compounds in plants, present results showed a significant positive correlation between contents of TPA and LPI ($p<0.001$). This relationship could be explained by the presence of caffeoyl-, coumaroyl- and feruloylquinic acids in *H. perforatum* that possess strong antioxidant activity (Tusevski et al., 2020). These hydroxycinnamic acid derivatives with double bond structure conjugated with aromatic ring have been shown to possess strong antioxidant activity measured by β-carotene-linoleic acid assay (Cuvelier et al., 1992).

Conclusion

In this study, phenolic compounds production and antioxidant activity of roots, non-flowering shoots and flowering shoots of *H. perforatum* wild-growing plants were presented as detailed for the first time. The aerial plant samples represented the richest source of phenolic acids and flavonols with strong antioxidant activities. Noteworthy, root extracts showed satisfactory amounts of phenolics with moderate antioxidant activity that make them potential natural source for antioxidant compounds. The initial screening of phytochemical composition and antioxidant activity of *H. perforatum* could be a step forward in the preparation of new phytoproducts in food and pharmaceutical industry.

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