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Antioxidant activity and phenolic compounds in *Hypericum perforatum* L. wild-growing plants collected in the Republic of Macedonia

Original Article

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Abstract:

The aim of this study was to evaluate phenolic compounds composition and antioxidant activity in roots (RO), non-flowering shoots (NFS) and flowering shoots (FS) of Hypericum perforatum L. wild-growing plants collected in the Republic of Macedonia. The analyses of total phenolic compounds included quantification of phenolics, flavonoids, catechin derivatives, flavan-3-ols, condensed tannins and hypericin. Antioxidant activity was determined by the cupric ions reducing antioxidant capacity, phosphomolybdenum test, reducing power and DPPH scavenging. The contents of phenolics in FS and NFS were significantly higher than those found in RO extracts. Hypericin content in FS was 2-fold increased in comparison to NFS, while RO showed low capability for the accumulation of naphthodianthrones. Also, FS and NFS exhibited markedly higher antioxidant activities compared to RO. In summary, aerial parts of H. perforatum accumulated significant levels of antioxidant phenolic compounds that were characterized with hydrogen atom donation, radical scavenging and participation in redox reactions.

Key words:
Antioxidant activity, Flowering shoots, Hypericum perforatum L., Non-flowering shoots, Phenolic compounds, Roots

Apstract:

Antioksidativna aktivnost i fenolna jedinjenja u divljerastućim biljkama *Hypericum perforatum* L. sakupljenim u Republici Makedoniji

Cilj ove studije bio je da utvrdi sastav fenolnih jedinjenja i antioksidativnu aktivnost korena (RO), necvetajućih ((NFS) i cvetajućih izdanaka (FS) divljerastućih biljaka Hypericum perforatum L., sakupljenih u Republici Makedoniji. Analize totalnih fenolnih jedinjenja uključivale su kvantifikaciju fenola, flavonoida, derivata katehina, flavan-3-ola, kondenzovanih tanina i hipericina. Antioksidativna aktivnost određivana je kapacitetom redukcije bakarnih jona, fosfomolibdenskim testom, redukcionim potencijalom i DPPH testom. Sadržaj fenola u FS i NFS bili su značajno viši nego onaj nađen u ekstraktima RO. Sadržaj hipericina u FS bio je dvostruko veći u poređenju sa NFS, dok je RO pokazao nisku sposobnost akumulacije naftodiantrona. Takođe, FS i FNS su ispoljili značajno veću antioksidativnu aktivnost u poređenju sa RO. U zaključku, nadzemni delovi H. perforatum akumulirali su značajni nivo antioksidantnih fenolnih jedinjenja okarakterisanih dinacijom vodonikovih atoma, hvatanjem slobodnih radikala i učešćem u redoks reakcijama.

Ključne reči:
Antioksidativno delovanje, cvetajući izdanci, Hipericum perforatum L., necvetajući izdanci, fenolna jedinjenja, korenovi

Introduction

Hypericum perforatum (St John's wort) is an important medicinal plant with long and worldwide usage in traditional medicine. The crude drug (*Hyperici Herba*) consists of dried flowering tops or aerial parts of the wild-growing plants. The phytochemistry and pharmacology of this species have been extensively studied (Greeson et al., 2001; Nahrstedt & Butterweck, 2010). Phenolic compounds (naphthodianthrones, acyl-phloroglucinols and flavonoids) that contributed to the biological activities of *H. perforatum* are usually accumulated in the leaves and flowers. Naphthodianthrones are chemotaxonomic marker compounds of the genus *Hypericum* that is

mainly localized in the dark glands dispersed over the margins of leaves and flower petals (Zobayed et al., 2006). The naphthodianthrones presented by hypericin and pseudohypericin, as well as their protoforms (protohypericin and protopseudohypericin) are included in the analytical term "total hypericins" (Williams et al., 2006). The cytotoxic and photodynamic properties of hypericin are responsible for antiviral, antitumor and antibacterial effects of *H. perforatum* extracts (Delaey et al., 2001). Two closely related acyl-phloroglucinol derivatives, hyperforin and its homolog adhyperforin have been detected in the translucent glands of the leaves, flowers and fruits of *H. perforatum* (Soelberg et al., 2007). It has been considered that hyperofrin is the major constituent responsible for the antidepressant activity of *H. perforatum* extracts through the inhibition of synaptic re-uptake of neurotransmitters (Chatterjee et al., 1998). The *H. perforatum* is widely known for its richness in flavonoids especially by the flavonols quercetin and kaempferol, as well by the catechin derivatives represented with monomeric flavan-3-ols and oligomeric proanthocyanidins (Nahrstedt & Butterweck, 2010). Flavonoids are mainly presented in the leaves as water-soluble glycosides in the vacuoles of epidermal cells due to their UV-protective function (Germ et al., 2010). The flavonoid-containing fractions of *H. perforatum* have been shown to exhibit powerful antioxidant activity and radical scavenging capacity (Barnes et al., 2001).

Even that flowers from *H. perforatum* wild-growing plants have been the subject of many research studies concerning the production of total phenolics, flavonoids and hypericins (Germ et al., 2010; Altun et al., 2013; Becker et al., 2016), the biosynthesis of various groups of phenolic compounds in different plant parts are not yet fully understood. Recent phytochemical investigations revealed that roots from *H. perforatum* wild-growing plants represented a promising source of phenolics with potential biological activities (Crockett et al., 2011; Tusevski et al., 2018). However, the roots of *H. perforatum* wild-growing plants have never been evaluated for their capacity for the production of total phenolic compounds and antioxidant activities. Therefore, it would be of great interest to evaluate the distribution of certain groups of phenolic compounds and antioxidant properties in various plant organs of *H. perforatum*.

The main objective of this study was to determine the contents of six groups of phenolic compounds and antioxidant capacity in *H. perforatum* roots (RO), non-flowering shoots (NFS) and flowering shoots (FS). For the realization of this objective, the following topics were included: (1) quantification of total phenolics, flavonoids, catechin derivatives, flavan-3-ols, condensed tannins and hypericin, (2) evaluation of antioxidant activity by cupric ion reducing antioxidant capacity, reducing power, phosphomolybdenum assay and DPPH radical scavenging and (3) determination of relationship between phenolic compounds contents and antioxidant activities.

Material and methods

Phenolic compound contents

Wild-growing plants of *Hypericum perforatum* (voucher number 060231) collected in the National Park Pelister (1394 m a.s.l.) were separated into three plant sections: roots (RO), non-flowering shoots (NFS) and flowering shoots (FS). Phenolic compounds were extracted from powdered plant material (0.2 g) with 80% (v/v) CH_3OH in ultrasonic bath for 30 min at 4 ºC. Thereafter, methanolic extracts were centrifuged at 12.000 rpm for 15 min, and the supernatants were used for the determination of phenolic compound contents. The phenolic compound contents in plant extracts included determination of total phenolics (TP), flavonoids (TF), catechin derivatives (TCD), flavan-3-ols (TFA), condensed tannins (TCT) and hypericin (TH). Spectrophotometric analyses were performed on SpectraMax 190 Microplate Reader (Molecular Devices Corp., Sunnyvale, CA) supported with SoftMax Pro (v. 5.4.1) software.

Total phenolics (TP). The TP contents were determined when plant extracts were mixed with Folin–Ciocalteau reagent and 0.7 M Na_2CO_3 (Singleton & Rossi, 1965). The samples were incubated at 50 ºC for 15 min and then cooled at room temperature. Then, absorbance was measured spectrophotometrically at 765 nm. The results for TP concentration were expressed as milligrams of gallic acid equivalents (GA) per gram of dry weight (mg GA·g^{-1} DW).

Total flavonoids (TF). The TF contents in plant extracts were determined using the assay described by Zhishen et al. (1999). An aliquot of the extract was mixed with 5% $NaNO_2$ and allowed to react for 5 min. Following this, 10% $AlCl_3$ was added and the mixture stood for further 5 min. Then, 1 M NaOH and distilled water were added to the sample and the absorbance was measured spectrophotometrically at 510 nm. The concentration of TF was expressed as milligrams of catechin equivalents (C) per gram of dry weight (mg C·g^{-1} DW).

Total catechin derivatives (TCD). The TCD contents in plant extracts were determined by the vanillin method (Sun et al., 1998). A diluted plant extract was mixed with 1% vanillin-HCl solution. The samples were incubated at room temperature

for 30 min and the absorbance was measured spectrophotometrically at 500 nm. The concentration of TCD was expressed as milligrams of catechin equivalents (C) per gram of dry weight (mg C·g^{-1} DW).

Total flavan-3-ols (TFA). The TFA in plant extracts were estimated by DMACA (4-dimethylaminocinnamaldehyde) assay (Arnous et al., 2002). The 0.1% DMACA reagent in CH_3OH/HCl (91.4/8.6, v/v) was added to the extracts. Samples were incubated for 10 min at room temperature and the absorbance was measured at 640 nm. The TFA concentration was expressed as milligrams of catechin equivalents (C) per gram of dry weight (mg C·g^{-1} DW).

Total condensed tannins (TCT). The TCT contents in plant extracts were determined by the method of Porter et al. (1985) with the following modifications. The samples were prepared by mixing the diluted plant extracts with *n*-butanol/HCl (95:5, v/v) and 2% $NH_4Fe(SO_4)_2$ x $12H_2O$. The mixture was incubated at 95°C for 50 min and the absorbance was measured at 535 nm. The molar extinction coefficient of cyanidin-3-glucoside (ε_{535}=34700 L·mol^{-1}·cm^{-1}) was used for determination of TCT in the extracts. The results were expressed as milligrams of cyanidin-3-glucoside equivalents (CG) per gram of dry weight (mg CG·g^{-1} DW).

Total hypericin (TH). The TH contents in plant samples were determined according to European Pharmacopoeia (2008). The powdered plant sample was extracted with 80% tetrahydrofuran at 65 ºC for 30 min. Samples were centrifuged at 3000 rpm for 10 min and the collected supernatant was lyophilized under vacuum (0.22 mbar). The dry residue was dissolved with CH_3OH in an ultrasonic bath and then centrifuged at 12.000 rpm for 10 min. The absorbance of the supernatant was measured at 590 nm. The concentration of TH was expressed as milligrams of hypericin equivalents (H) per gram of dry weight (mg H·g^{-1} DW).

Antioxidant capacity assays

The antioxidant capacity of plant extracts was determined by the following methods: cupric ion reducing antioxidant capacity (CUPRAC), reducing power (RP), phosphomolybdenum assay (PM) and DPPH radical scavenging activity. Those methods were performed in the same extracts used for the determination of phenolic compound contents.

Cupric ion reducing antioxidant capacity (CUPRAC). The CUPRAC assay of plant extracts was determined by the method of Apak et al. (2004). The reaction mixture consisted of plant extract, 10 mM $CuCl_2$, 7.5 mM neocuproine and 1 M CH_3COONH_4 buffer (pH 7.0). Samples were incubated 30 min at room temperature and the absorbance was recorded at 450 nm. The molar extinction coefficient of trolox (ε_{535}=1.67x10^4 L·mol^{-1}·cm^{-1}) was used for the determination of CUPRAC. The CUPRAC values were expressed as micromoles of Trolox equivalents (T) per gram of dry weight (μmol T·g^{-1} DW).

Reducing power (RP). The RP of plant extracts was determined according to the method of Oyaizu (1986), with the following modifications. The reaction mixture contained plant extract, phosphate buffer (0.2 M KH_2PO_4/K_2HPO_4, pH 6.6) and 1% $K_3[Fe(CN)_6]$. After incubation at 50 ºC for 20 min, an aliquot of 10% TCA was added to the mixture followed by centrifugation at 1000 rpm for 10 min. Finally, the collected supernatant was mixed with CH_3OH and 0.1% $FeCl_3$ and incubated at room temperature for 10 min. The absorbance of the samples was measured at 700 nm. The RP was expressed as micromoles of ascorbic acid equivalents (AA) per gram of dry weight (μM AA·g^{-1} DW).

Phosphomolybdenum (PM) assay. The PM assay was evaluated using the procedure of Prieto et al. (1999). The plant extract was mixed with PM reagent [0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM $(NH_4)_2MoO_4$] and the samples were incubated at 95°C for 90 min. After cooling at room temperature, the absorbance of the green PM complex was measured at 695 nm. The PM capacity was expressed as milligrams of ascorbic acid equivalents (AA) per gram of dry weight (mg AA·g^{-1} DW).

DPPH radical scavenging activity. The ability of plant extracts to scavenge stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was determined with the method of Brand-Williams et al. (1995). The reaction mixture consisted of plant extract and 0.25 mM DPPH in CH_3OH. In the control sample, the extract was replaced with CH_3OH. The reaction for DPPH$^{\bullet}$ scavenging was carried out at room temperature in the dark for 10 min, and then decrease in absorbance was recorded at 518 nm. The DPPH radical scavenging activity was expressed as micromoles of Trolox equivalents (T) per gram of dry weight (μM T·g^{-1} DW).

Results

Phenolic compound contents and antioxidant activities in H. perforatum wild-growing plants

The contents of total phenolic compounds in *H. perforatum* extracts (RO, NFS and FS) are summarized in **Tab. 1**. The TP contents in FS and NFS were about 2.9-fold higher than that of RO. The amounts of TF were significantly higher in NFS and FS (2.7- and 2.4-fold, respectively), compared to RO extracts. Also, FS and NFS showed higher TCD contents (2.7- and 2.3-fold, respectively) in comparison to RO sample. Similar amounts of

Table 1. The content of total phenolic compounds in *H. perforatum* wild-growing plants*

Phenolic compounds	RO	NFS	FS
TP (mg GA·g^{-1} DW)	36.51±1.02 [a]	103.88±4.97 [b]	107.38±0.90 [b]
TF (mg C·g^{-1} DW)	28.14±1.56 [a]	76.70±7.66 [b]	68.59±0.26 [b]
TCD (mg C·g^{-1} DW)	15.97±0.46 [a]	36.42±4.58 [b]	42.52±1.10 [b]
TFA (mg C·g^{-1} DW)	9.98±0.78 [a]	22.78±2.09 [b]	24.82±0.45 [b]
TCT (mg CG·g^{-1} DW)	6.52±0.51 [a]	15.58±3.03 [b]	17.60±0.57 [b]
TH (µg H·g^{-1} DW)	7.00±0.25 [a]	581.86±14.45 [b]	1150.28±7.86 [c]

* RO: roots; NFS: non-flowering shoots; FS: flowering shoots; TP: phenolics; TF: flavonoids; TCD: catechin derivatives; TFA: flavan-3-ols; TCT: condensed tannins; TH: hypericin; GA: gallic acid; C: catechin; CG: cyanidin-3-glucoside; H: hypericin; DW: dry weight.

[a] The values in one row marked with lower-cases denoted significant differences between samples at p<0.05 (Duncan's multiple range test).

TFA and TCT were observed between NFS and FS, (about 2.5-fold higher) compared to RO extracts. The TH concentration in FS was about 2-fold higher than that found in NFS extracts, while RO showed significantly lower capability for the accumulation of hypericin derivatives.

The antioxidant activities in *H. perforatum* RO, NFS and FS extracts are presented in **Tab. 2**.

The results for CUPRAC assay showed that FS and NFS extracts had significantly higher antioxidant activities (3-fold) compared to RO. Similar results were observed for RP, where NFS and FS had markedly higher values for antioxidant activity (4.4- and 3.8-fold, respectively) compared to RO extracts. The antioxidant activity measured by PM assay was significantly higher in NFS and FS (2.2- and 1.9-fold, respectively) than RO extracts. Also, FS and NFS showed markedly higher DPPH radical scavenging activity (3.1- and 2.6-fold, respectively) in comparison to RO extracts.

Principal component analysis of phenolic compounds production and antioxidant activities in H. perforatum wild-growing plants

Principal component analysis (PCA) was performed to define the correlation between total phenolic contents and antioxidant activities among different parts of *H. perforatum* wild-growing plants. In order to find out if any ordination of the analyzed RO, NFS and FS extracts exists, a PCA (**Fig. 1**) was applied using the following variables: total contents of phenolics (TP, TF, TCD, TFA, TCT, TH) and antioxidant activities (CUPRAC, RP, PM, DPPH).

Two main principal components (PC) were used to characterize phenolic compound composition and antioxidant activities in RO, NFS and FS extracts. The PCA data showed that the PC1 and PC2 explained 100% of the total variation. The PCA plot (**Fig. 1**) indicated that PC1 explained the variance of 96.19% that was positively related to all variables concerning the phenolic compound contents and antioxidant activities. The PC2 showed a variance of 3.81% and it was not connected to the tested parameters.

The PCA plot showed that tested *H. perforatum* samples are well separated on PC1 (**Fig. 1**). In this context, FS and NFS extracts exhibited positive scores on PC1 and they were characterized with high contents of phenolic compounds and antioxidant activities. On the other hand, RO extracts had a negative score on PC1 and possessed a low capacity for phenolic compound production and antioxidant

Table 2. The antioxidant activities in *Hypericum perforatum* wild-growing plants*

Antioxidant activities	RO	NFS	FS
CUPRAC (µM T·g^{-1} DW)	230.11±19.24 [a]	685.60±17.33 [b]	708.30±12.58 [b]
RP (µM AA·g^{-1} DW)	425.05±13.91 [a]	1865.21±78.81 [b]	1584.39±163.80 [b]
PM (mg AA·g^{-1} DW)	57.89±3.55 [a]	108.96±4.99 [b]	128.46±0.51 [c]
DPPH (µM T·g^{-1} DW)	158.81±0.10 [a]	407.86±2.36 [b]	496.67±11.67 [c]

* RO: roots; NFS: non-flowering shoots; FS: flowering shoots; CUPRAC: cupric ions reducing antioxidant capacity; RP: reducing power; PM: phosphomolybdenum test; DPPH: DPPH radical scavenging activity; T: trolox; AA: ascorbic acid; DW: dry weight.

[a] The values in one row marked with lower-cases denoted significant differences between samples at p<0.05 (Duncan's multiple range test).

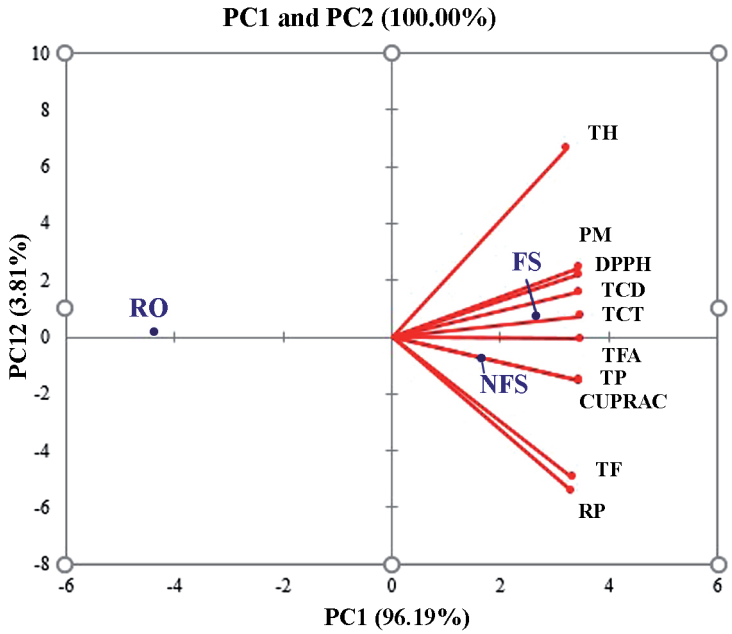

Fig. 7. Canonical discriminant analysis of the six *G. pratensis* populations from Serbia and Montenegro

activities. In addition, FS and NFS extracts were located on the opposite side on PC2 and the major parameters responsible for the separation of these two aerial plant samples were TH content with positive score on PC2, as well TF content and antioxidant activity measured by RP assay with negative scores on PC2. According to these results, FS extracts showed significantly higher TH contents, but lower TF amount and RP value in comparison to NFS extracts.

Pearson's correlation matrix (**Tab. 3**) demonstrated significant positive correlations between phenolic compound contents and antioxidant activities in tested samples. In this view, the TP contents were in significant positive correlation with CUPRAC assay. A significant positive correlation was also found between TF and RP methods. Similarly, TCD amounts were positively related to the antioxidant activity measured by PM and DPPH assays. These results clearly indicated that phenolic compounds greatly contributed to the observed antioxidant activities of plant extracts.

Discussion

Production of phenolic compounds in H. perforatum wild-growing plants

Present data showed that aerial plant parts of *H. perforatum* were an excellent source of phenolics and flavonoids, while root tissues showed lower capability for accumulation of these phenolic compounds. Outgoing results for total phenolics and flavonoids are in accordance with those previously reported for aerial parts of *H. peforatum* and other *Hypericum* species (Germ et al., 2010; Hernandez et al., 2010). For instance, Gioti et al. (2009) have noticed that shoots, non-flower and flower branches of *H. peforatum* possess significant amounts of phenolic compounds. Taking into account literature data, Kalogeropoulos et al. (2010) reported lower amounts of total phenolics and flavonoids for *H. peforatum* plants collected in Greece in comparison to the results presented here. On the other hand, Altun et al. (2013) observed higher values of these compounds for *Hyperici herba* from Turkey. The variation of phenolic and flavonoid contents in *H.*

Table 3. Correlation analysis between phenolic compounds production and antioxidant activities in *Hypericum perforatum* wild-growing plants (n = 3 samples)*

r	TP	TF	TH	TCD	TFA	TCT	CUPRAC	RP	PM
TF	0.980								
TH	0.889	0.779							
TCD	0.984	0.929	0.956						
TFA	0.996	0.960	0.924	0.996					
TCT	0.992	0.946	0.940	**0.999**	**0.999**				
	0.999	0.980	0.888	0.984	0.996	0.992			
RP	0.974	**0.999**	0.761	0.919	0.952	0.937	0.974		
PM	0.974	0.910	0.969	**0.998**	0.990	0.995	0.974	0.898	
DPPH	0.978	0.916	0.965	**0.999**	0.992	0.996	0.977	0.904	**0.999**

* r: Pearson's coefficient; TP: phenolics; TF: flavonoids; TCD: catechin derivatives; TFA: flavan-3-ols; TCT: condensed tannins; TH: hypericin; CUPRAC: cupric ions reducing antioxidant capacity; RP: reducing power; PM: phosphomolybdenum test; DPPH: DPPH radical scavenging activity. The values in bold indicate significance at $P < 0.05$.

peforatum could be attributed to the genotypic and environmental factors, various plant organs, as well harvesting time (Gioti et al., 2009; Germ et al., 2010). However, the direct comparison of phenolic and flavonoid contents is a complex task due to the differences in solvents and procedures for plant extraction, the usage of reference compounds for quantification and the methods for determination of phenolic compounds. Even that some non-phenolics (ascorbic acid, amino acids, proteins, organic acids and sugars) could react with Folin-Ciocalteu reagent (Prior et al. 2005), the procedure for *H. peforatum* plant extraction used in this study eliminated the possibility for interference of these compounds.

Taking into account the richness of *H. perforatum* extracts with monomeric flavan-3-ols, as well as oligomeric and polymeric tannins (Nahrstedt & Butterweck, 2010), the different assays were applied for determination of catechin derivatives (TCD, TFA and TCT) in plant samples. Present results clearly demonstrated that aerial parts of *H. perforatum* have greater capability for accumulation of those catechin derivatives compared to root samples. The literature data for total contents of flavan-3-ols or tannins in *H. perforatum* are rather scarce. In this view, Germ et al. (2010) have shown higher values for catechin derivatives in *H. perforatum* leaves and flowers than those reported here. The heterogeneity in the results for total catechin derivatives may be due to the high sensitivity of vanillin-HCl assay, as well the solvents, reaction time, temperature and vanillin concentrations used for analysis (Sun et al., 1998). The ratio TCD/TCT could be used as a rough estimation of the polymerization degree of monomeric flavan-3-ols into oligomeric and polymeric tannins (Ribéreau-Gayón & Stonestreet, 1966). In this study, the ratio TCD/TCT in analyzed samples was from 2 to 3 indicating that polymeric tannins in *H. perforatum* are probably represented by proanthocyanidin dimers and trimers. Moreover, the summation of total flavan-3-ol and condensed tannin contents accurately represented the amounts of total catechin derivatives in the analyzed samples. Therefore, vanillin-HCl assay for total catechin derivatives could be proposed as a routine procedure for determination of total catechin derivatives including flavan-3-ol monomers and polymeric condensed tannins in *H. perforatum*.

The measurement of total hypericin is commonly used for quality control of *H. perforatum* standardized extracts and phytopharmaceuticals (Williams et al., 2006). The results for total hypericin contents showed that flowering shoots accumulated markedly higher contents of hypericin compared to non-flowering shoots, while minor amounts were noticed for roots. In this view, naphthodianthrone accumulation has been connected with the growth and development of *H. perforatum* reproductive parts (Southwell & Bourke, 2001). In addition, the correlation between dark glands formation and hypericin levels in leaves, stems, flower petals, stamens, carpels and sepals of *H. perforatum* has been reported (Zobayed et al., 2006). Therefore, the dark glands could be considered as the limiting factors for hypericin accumulation due to necessity of these glandular structures for deposition of hypericin (Pasqua et al., 2003). Even that aerial parts of *H. perforatum* in full bloom have been proposed as excellent sources of hypericin (Southwell & Bourke, 2001), the roots have never been explored as a hypericin-producing tissues. However, recent histochemical analysis showed the presence of dark-red globules with hypericin in *H. perforatum* leaves, stems and roots (Qian et al., 2012). These findings suggested that dark glands are not the only site of naphthodianthrone accumulation and hypericin is probably synthesized either in mesophyll, root or stem cells and subsequently is transported to the dark glands. All these observations suggested that aerial parts of *H. perforatum* accumulated higher contents of phenolic compounds (flavonoids, catechin derivatives and hypericin) compared to roots.

Antioxidant activity of H. perforatum wild-growing plants

The CUPRAC assay has been widely used to determine the antioxidant capacity of plant extracts as it requires relatively standard equipment and delivers fast and reproducible results (Apak et al., 2004). The CUPRAC assay is useful for determination of antioxidant capacity in a wide variety of antioxidant compounds, such as phenolic acids, flavonoids, carotenoids, anthocyanins, as well for thiols (glutathione), synthetic antioxidants, vitamins C and E. Results from this study demonstrated very strong CUPRAC activity for the aerial parts, while root extracts possessed the lowest CUPRAC value. The relevance of CUPRAC method for evaluation of antioxidant potential of aerial parts from *H. perforatum* and other *Hypericum* species has also been reported (Maltas et al., 2013; Boga et al., 2016). The results obtained from CUPRAC *in vitro* measurements could be efficiently extended to the *in vivo* antioxidant activity because the CUPRAC reaction is carried out at physiological pH (Apak et al., 2004).

The non-flowering and flowering shoots of *H. perforatum* demonstrated high antioxidant activity measured by RP assay, thereby could be proposed as efficient reductones. The reductones terminated free radical chain reactions and thus, RP could be related to the antioxidant activity of plant extracts

(Gordon, 1990). Phenolic compounds as effective reductones have previously been confirmed for *H. perforatum* (Zou et al., 2004; Gioti et al., 2009), as well as other *Hypericum* species (Şerbetçi et al., 2012; Rainha et al., 2013). In this study, aerial parts showed high RP values, which indicated that *H. perforatum* is characterized by abundance of antioxidant compounds that reduce Fe^{3+}/ferricyanide complex into ferrous form.

Present results showed that flowering shoots exhibited the highest value for PM assay, followed by non-flowering shoots, while the lowest antioxidant capacity was found for root extracts. These results for aerial parts were consistent with those previously reported for *H. perforatum* flower, leaf and stem extracts (Radulović et al., 2007). In contrast, Raghu Chandrashekhar et al. (2009) showed that roots have greater antioxidant capacity determined by PM method than those found for aerial parts of *H. hookerianum* wild-growing plants. The discrepancy in the results for PM assay among different plant organs could be related to the interspecies variability concerning the distribution of antioxidant compounds. Despite polyphenols, many other non-phenolic hydrophilic and lipophilic compounds could significantly contribute to the antioxidant activity of plant extracts measured by PM assay (Prieto et al., 1999).

In this study, DPPH method was selected as the most effective assay for evaluation of radical scavenging capacity of plant extracts by chain-breaking mechanism (Tusevski et al., 2014). Present results demonstrated that flowering and non-flowering shoots were better DPPH scavengers compared to root extracts. Accordingly, there are many studies that confirm the strong DPPH radical scavenging activity of *H. perforatum* extracts (Silva et al., 2008; Gioti et al., 2009; Kalogeropoulos et al., 2010; Becker et al., 2016).

Taken together, the variation in the results for antioxidant activities of *H. perforatum* extracts could be due to the structural features, antioxidant mechanism, as well as synergistic effects of different classes of phenolic compounds. It is widely accepted that the antioxidant activity of *H. perforatum* extracts is correlated to the content of phenolic compounds. Although present results were focused on this topic, there is still a lack of evidence to determine the contribution of various classes of phenolic compounds to the antioxidant activity of *H. perforatum*. Since the methods used in this study enabled the quantification of various groups of phenolic compounds, it was possible to establish the correlation between those metabolites and antioxidant activity in *H. perforatum*.

Correlation between phenolic compound contents and antioxidant activity in H. perforatum wild-growing plants

The establishment of a relationship between antioxidant activity and phenolic contents is a very difficult approach because plant extracts represented complex mixtures of various metabolites with antioxidant and prooxidant properties that exhibit synergistic actions. In this study, the principal component analysis (PCA) as a statistical tool showed that flowering shoot and non-flowering shoot extracts represented the richest source of phenolics with strong antioxidant activity. On the other side, roots were characterized by low capacity for phenolic compound production and antioxidant activity. The correlation analyses performed in this study demonstrated significant positive correlation between TP and CUPRAC. These results suggested that phenolics in *H. perforatum* wild-growing plants could be proposed as preeminent constituents that exhibit cupric ion reducing capacities. In this view, antioxidant activity of *H. perforatum* and other *Hypericum* species measured with CUPRAC assay has been well correlated with total phenolic and flavonoid contents (Maltas et al., 2013; Moein et al., 2015). Moreover, results presented here showed a significant correlation between TF and RP in *H. perforatum* extracts indicating that high reducing power could be attributed to the total flavonoid contents. Such a correlation between total flavonoids and reducing power of *H. perforatum* extracts has also been reported by Zou et al. (2004). The strong positive correlations of TCD with DPPH and PM assays found in this study were expected since monomeric flavan-3-ols and polymeric proanthocyanidins have already been shown as the most active DPPH scavengers (Froehlicher et al., 2009). In addition, Raghu Chandrashekhar et al. (2009) confirmed that total flavan-3-ol contents in *H. hookerianum* correlated with total antioxidant capacity measured by PM assay. Outgoing results showed a significant positive correlation between PM and DPPH indicating that these assays could be proposed as suitable and reliable methods for evaluation of antioxidant activity of *H. perforatum* extracts. The TH contents did not show any significant correlation with antioxidant methods used in this study. These data could be ascribed to the small percentage distribution of hypericin (up to 1%) in phenolic profile that resulted in their minor contribution to the antioxidant activity of *H. perforatum* extracts.

Conclusion

In this study, the capability for the production of phenolic compounds and antioxidant activities of roots, non-flowering shoots and flowering shoots of *H. perforatum* wild-growing plants was presented as detailed for the first time. The aerial plant samples represented the richest source of phenolics, flavonoids, catechins (flavan-3-ols and condensed tannins) and hypericin with strong antioxidant activities. Noteworthy, root extracts from *H. perforatum* demonstrated satisfactory amounts of various groups of phenolics with moderate antioxidant activities that make them potential candidates as a natural source of antioxidant and radical scavenging compounds. The correlation analysis used in this study indicated that antioxidant activity of *H. perforatum* wild-growing plants is related to the phenolic compounds that are characterized by hydrogen atom donation, radical scavenging and participation in redox reactions. The phytochemical profiling and initial screening of antioxidant activity of *H. perforatum* extracts could be a step forward in the preparation of new phytoproducts in food and pharmaceutical industry.

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