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Phenolic profile and biological activity of *Hypericum perforatum* L.: Can roots be considered as a new source of natural compounds?

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ABSTRACT

The aim of this study was to evaluate the phytochemical composition, antimicrobial activity and enzyme inhibitory properties of roots (RO), non-flower shoots (NFS) and flower shoots (FS) of *Hypericum perforatum* L. originating from the Republic of Macedonia. The identification and quantification of phenolic compounds in plant material were performed by using HPLC/DAD/ESI–MS^n analysis. The antimicrobial activity on some selected species of bacteria and fungi was tested by microdilution assay. The *in vitro* enzyme inhibitory activity of plant extracts was measured against key enzymes involved in depression (monoamine oxidase A; MAO A), neurodegenerative disorders (acetylcholinesterase; AChE, butyrylcholinesterase; BChE and tyrosinase; TYR) and diabetes (α-amylase; α-AMYL and α-glucosidase; α-GLUC). The aerial plant samples showed biosynthetic potential for the production of phenolic acids, flavan-3-ols, flavonols, naphthodianthrones and acyl-phloroglucinols. The NFS and FS extracts exhibited strong antistaphylococcal and anticandidal activity that was related to the presence of hypericins and hyperforins. Chlorogenic acid, flavonol aglycones and glycosides, as well catechins and procyanidins from aerial parts were considered as potential AChE and TYR inhibitors. This study revealed, for the first time, the co-presence of phenolic acids, flavan-3-ols and xanthones in RO extracts. The antibacterial activity of RO extracts against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* was related to the presence of garcinone C and γ-mangostin. The roots rich in xanthones displayed the strongest MAO A and BChE inhibition highlighting their antidepressant and neuroprotective effects. With respect to antidiabetic properties, RO extracts showed significant α-AMYL and α-GLUC inhibitory activities that were related to the contents of procyanidins and xanthones. Based on phytochemical composition and enzyme inhibitory properties, *H. perforatum* roots could be considered as an alternative source to obtain antidepressant, neuroprotective and antidiabetic active substances.

1. Introduction

Hypericum perforatum L. (St. John's wort) represents one of the best-studied medicinal plants throughout the world with well-characterized secondary metabolites and pharmacological activities (Nahrstedt and Butterweck, 2010; Velingkar et al., 2017). This plant species has received considerable interest in recent years due to the increased market demand for crude material as a source of natural bioactive compounds. The *H. perforatum* extracts contain naphthodianthrones, acyl-phloroglucinols, flavonoids and xanthones with various pharmacological attributes that are associated with anti-inflammatory, antiviral, antimicrobial, antioxidant, antitumoural and wound-healing activities (Nahrstedt and Butterweck, 2010). Considering these medicinal properties, *H. perforatum* preparations represent one of the leading herbal dietary supplements worldwide.

The most significant use of *H. perforatum* preparations comprises symptomatic treatments of mild-to-moderate depression and recently good perspectives emerged in the field of major depression (Linde, 2009). The major biochemical hypothesis of depression is centred on the functional deficiency of cerebral monoamine neurotransmitters (dopamine, norepinephrine and serotonin). The antidepressant activity of hypericin from *H. perforatum* extracts has been represented by the inhibition of monoamine oxidases (MAOs) that catalyze oxidative deamination of neurotransmitters in the brain (Suzuki et al., 1984). Even if hypericins have initially been described as MAO inhibitors, the later studies indicated that this effect was not clinically significant due to their low concentration in the crude drug (Bladt and Wagner, 1994; Thiede and Walper, 1994). The comprehensive investigations on MAO inhibitory properties of *Hypericum* extracts indicated that certain flavonols and xanthones could be proposed as important antidepressants

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; FS, flower shoots; MAO A, monoamine oxidase A; NFS, non-flower shoots; RO, roots; TYR, tyrosinase; α-AMYL, α-amylase; α-GLUC, α-glucosidase.

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(Rocha et al., 1994; Chimenti et al., 2006). Depression is strongly associated with Alzheimer's and Parkinson's diseases that are characterized by cognitive abnormality and neurodegeneration (Orhan et al., 2006). According to these authors, Alzheimer's disease is related to the shortage of acetylcholine and butyrylcholine, which are hydrolyzed by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), respectively. In addition, tyrosinase (TYR) plays an important role in neuromelanin formation in the human brain (Asanuma et al., 2003). The excessive activity of TYR enzyme causes dopamine neurotoxicity and contributes to the neurodegeneration associated with Parkinson's disease. Thus, the inhibition of cholinesterases (AChE and BChE) and TYR is an important approach in drug discovery for treatments of neurodegenerative diseases. It has already been shown that chlorogenic acid, rutin, hyperoside, isoquercitrin and quercitrin from *Hypericum* extracts are potential AChE inhibitory compounds (Hernandez et al., 2010; López et al., 2016; Ozkan et al., 2018). Even if BChE and TYR inhibitory properties of *H. perforatum* extracts have been evaluated (Altun et al., 2013), the compounds responsible for those activities remain to be discovered. On the other side, chronic diseases such as diabetes are characterized by hyperglycaemia and disturbance of carbohydrate, fat and protein metabolism due to deficiency in insulin secretion and/or action. An effective therapeutic approach to control hyperglycaemia is to slow down or retard the activity of carbohydrate-hydrolyzing enzymes, α-amylase (α-AMYL) and α-glucosidase (α-GLUC) linked to the absorption of glucose in the digestive tract. The inhibition of α-AMYL and α-GLUC activities prolongs overall carbohydrate digestion causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose elevation (Rhabasa-Lhoret and Chiasson, 2004). Although *H. perforatum* extracts were found to be endowed with anti-hyperglycaemic effect on streptozotocin-induced diabetic rats (Arokiyaraj et al., 2011), there are no available studies on *in vitro* inhibition of α-AMYL and α-GLUC activities. However, *Hypericum maculatum* and *Hypericum ascyron* have been shown to possess promising α-AMYL and α-GLUC inhibitory activities associated with the presence of quercetin and kaempferol derivatives (Kang et al., 2011; Kladar et al., 2015).

The bioactive metabolites (hypericins, hyperforins and flavonoids) from *H. perforatum* that contribute to antimicrobial, antidepressant, neuroprotective and antidiabetic activities are usually accumulated in leaves and flowers (Velingkar et al., 2017). Consequently, *H. perforatum* based products are prepared from Hyperici herba crude material as a source of natural bioactive pharmaceuticals. Even if the medicinal properties of Hyperici herba have been extensively studied (Linde, 2009; Nahrstedt and Butterweck, 2010), the chemical composition and biological activities of root extracts are rather scarce. The phytochemical analysis performed on wild-growing *H. perforatum* plants demonstrated that roots are the main sites for accumulation of xanthones (Tocci et al., 2018). The contribution of xanthones to the pharmacological activities of *H. perforatum* has not been considered due to the apprehension that this class of compounds occurs in traces in Hyperici herba and commercially available products. To the best of our knowledge, the accumulation of xanthones in *H. perforatum* roots was correlated with antifungal activity against plant and human pathogens (Crockett et al., 2011; Tocci et al., 2013). Despite the exclusive capability to accumulate xanthones, *H. perforatum* roots have never been used in the manufacturing process for the preparation of commercially exploitable extracts. All data mentioned above lay down the basis for the central hypothesis that phenolic compounds, particularly xanthones from *H. perforatum* roots could significantly contribute to the biological activities.

2. Materials and methods

2.1. Collection of plant material

Plant material of *H. perforatum* was collected during full flowering time (July 2013) from a natural population in the National Park Pelister at about 1394 m asl. Voucher specimen number (060231) of the plant is deposited in the Herbarium at the Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, Republic of Macedonia (MKNH).

2.2. Preparation of plant extracts

The plant material (100 g) was separated into three sections: roots (RO), non-flower shoots (NFS) and flower shoots (FS). Plant samples were air dried in darkness, ground to powder by laboratory mill, and stored in airtight containers for further analysis.

The plant extracts for identification and quantification of phenolic compounds were prepared when powdered material (0.2 g) was homogenized with 80% (v/v) CH_3OH in an ultrasonic bath for 30 min at 4 °C (Gadzovska et al., 2013). Thereafter, methanolic extracts were centrifuged at 12,000 rpm for 15 min and the supernatants were used for HPLC/DAD/ESI–MS^n analysis.

For determination of *in vitro* biological activities, plant methanolic extracts were evaporated to dryness under reduced pressure by a rotary evaporator (Heidolph Instruments, Germany). Then, dried residues (100–200 mg) were dissolved in 50–100 mL 50% dimethyl sulfoxide (DMSO) to achieve a stock concentration of 10 $mg \cdot mL^{-1}$. The DMSO stock solution was serially diluted (5–0.0025 $mg \cdot mL^{-1}$) for determination of antibacterial and antifungal activity.

The *in vitro* antidepressant, neuroprotective and antidiabetic properties of plant extracts were performed by using three different working concentrations (250, 150 and 50 $\mu g \cdot mL^{-1}$) of DMSO solution (Altun et al., 2013).

2.3. HPLC/DAD/ESI–MS^n analysis of phenolic compounds

The HPLC system for analyses of phenolic compounds in methanolic extracts was described in our previous study (Tusevski et al., 2016). Chromatographic separations were carried out on 150 × 4.6 mm, 5 μm XDB-C18 Eclipse column (Agilent, USA). The mobile phase consisted of two solvents: water–formic acid (A; 99:1, v/v) and methanol (B) in the following gradient programme: 10% B (0–20 min), 20% B (20–30 min), 35% B (30–50 min), 50% B (50–70 min), 80% B (70–80 min) and continued with 100% B for a further 10 min. The flow rate was 0.4 $mL \cdot min^{-1}$ and the injection volume 20 μL. All separations were performed at 38 °C. Commercial standards of chlorogenic acid, rutin, quercetin, kaempferol, catechin, (epi)catechin, hypericin, pseudohypericin, hyperforin and xanthone (Sigma-Aldrich, Germany) were dissolved in 80% CH_3OH and were used as reference compounds. Spectral data from all peaks were accumulated in range 190–600 nm, and chromatograms were recorded at 260 nm for xanthones and hyperforins, at 280 nm for flavan-3-ols, at 330 nm for phenolic acids, at 350 nm for flavonols, at 520 nm for anthocyanins and at 590 nm for hypericins. Peak areas were used for quantification at wavelengths, where each group of phenolic compounds exhibited an absorption maximum.

The HPLC system was connected to the Agilent G2445A ion-trap mass spectrometer equipped with electrospray ionization (ESI) system and controlled by LCMSD software (Agilent, v.6.1). Nitrogen was used as nebulizing gas at a pressure-level of 65 psi and the flow was adjusted to 12 $L \cdot min^{-1}$. Both the heated capillary and the voltage were maintained at 350 °C and 4 kV, respectively. The full scan mass covered the mass range from m/z 100 to 1200. Collision-induced fragmentation experiments were performed in the ion trap using helium as a collision gas, with voltage ramping cycle from 0.3 up to 2 V. Maximum accumulation time of the ion trap and the number of MS repetitions to obtain the MS average spectra were set at 300 and 3 ms, respectively. Identification of the component peaks (Table 1) was based on the UV/Vis spectral data and LC/MS in the negative $[M - H]^-$ or positive $[M + H]^+$ (for anthocyanins) ionization mode with subsequent MS^2, MS^3 and MS^4 analysis for further identification with reference to similar data previously reported (Tusevski et al., 2016).