

Antioxidant activity of Macedonian St. John's wort (*Hypericum perforatum* L.)

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Introduction

Among all 360 species included in Hypericaceae family, only *Hypericum perforatum* L. is officially accepted for its positive pharmacological activities. This plant has been traditionally used for the treatment of temporary mental exhaustion and mild gastrointestinal complains and stomach ulcer, or externally as an oil extract for skin problems and wound healing agent (Savikin et al., 2017). Chemical composition of *Hypericum perforatum* is very complex, thus several groups of components (naphthodianthrone derivatives hypericin and pseudohypericin, phloroglucinol derivatives hyperforin and adhyperforin, flavonoids and xanthenes) are considered as the active principles. These compounds are connected with variety of biological activities such as antibacterial, antiviral, antiinflammatory and antioxidant. Moreover, flowering aerial parts of this plant have been commonly used in form of tea or extracts as well as for preparation of herbal medicinal products which are mainly recommended in the treatment of mild to moderate depression with less side effects than synthetic drugs (Butterweck and Schmidt, 2007). As neuroprotective agent, it can also prevent neurodegenerative pathologies by regulating neurotransmitter releases as well as by its antioxidant and antiinflammatory activity (Savikin et al., 2017).

A number of phytochemical investigations of Macedonian *Hypericum perforatum* have been done (Shabani et al, 2019). The aim of this work was to investigate antioxidative properties of this species.

Materials and methods

Plant material: Aerial parts of the plant were collected on three different localities (Mavrovo, Debar and Tetovo) in R.N. Macedonia and was dried at room temperature and on draft.

Extraction procedure: 0.5 g of powdered dried plant material were extracted two times with 5 mL methanol, 10 min in ultrasonic bath. The obtained methanol extracts were transferred in 10 mL volumetric flask and filled with methanol.

Total phenolic and flavonoid content: Procedure described by Singleton et al. with Folin-Ciocalteu reagent and aluminum chloride assay reported by Talari et al. was used, respectively (Talari et al., 2012).

Antioxidant activity: Three different methods were performed. Free radical scavenging activity was determined with *DPPH assay*, according to Gyamfi et al. method (Gyamfi et al., 1999). Ferric reducing antioxidant power was determined with *FRAP assay*, described by Shahat et al., (Shahat et al., 2011). *β -Carotene bleaching assay* was performed according to Dapkevicius et al. procedure (Dapkevicius et al., 1998).

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Results and discussion

Methanolic extracts of *H. perforatum* showed radical scavenging activity in DPPH assay with IC₅₀ values ranged from 2.35-6.88 mg/mL, 3.52-6.28 mg/mL and 3.15-6.37 mg/mL, for the samples collected from region of Tetovo, Debar and Mavrovo, respectively. In addition, methanol extracts showed antioxidant activity in FRAP assay with IC₅₀ from 2.08-4.84 mg/mL, 2.48-4.24 mg/mL and 2.12-5.10 mg/mL, for the samples collected in Tetovo, Debar and Mavrovo, as well. IC₅₀ values for methanol extracts of *H. perforatum* tested with β -carotene bleaching assay, were higher, ranging from 16.97-34.91 mg/mL, 15.75-37.46 mg/mL and 12.66-34.70 mg/mL for the plant samples originated from Tetovo, Debar and Mavrovo region, respectively. The percentage of inhibition of the tested methanol extracts with concentration of 10 mg/mL were very high and achieved values of 93.56% for DPPH, 90.52% for FRAP and 92.11% for β -carotene bleaching assay. Compared to the total phenol and total flavonoid content, analyzed plant samples contained high amount of phenols (up to 121.56%) and flavonoids (up to 125.34%).

According to the available literature data, the obtained results were similar to the Muzykiewicz et al. and Ozkan et al. findings, as they referred IC₅₀ values from 2.42-4.37 mg/mL and 19.42-37.82 mg/mL for DPPH and FRAP assay as well as 2.49-4.82 mg/mL, for DPPH assay, respectively (Muzykiewicz et al., 2019, Ozkan et al., 2018).

Conclusion

Methanolic extracts of Macedonian *Hypericum perforatum* possessed promising antioxidant activity due to the high content of secondary metabolites such as phenols and flavonoids.

In the future, subsequent assessment of chemical composition and evaluation of biological activity should be done in order to provide more data for better knowledge of biological activity of this Macedonian species.

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