Secondary Metabolites of *Hypericum* L. Species as Xanthine Oxidase Inhibitors

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SUMMARY

Nine *Hypericum* species (*H. barbatum*, *H. hirsutum*, *H. linarioides*, *H. olympicum*, *H. perforatum*, *H. rochelii*, *H. rumeliacum*, *H. tetrapterum* and *H. umbellatum*) collected in Serbia were assayed for inhibitory potential against xanthine oxidase *in vitro*, on the commercial enzyme, and compared with allopurinol. Seven studied *Hypericum* species (*H. barbatum*, *H. rochelii*, *H. rumeliacum*, *H. umbellatum*, *H. perforatum*, *H. tetrapterum* and *H. olympicum*) inhibit commercial xanthine oxidase with an IC₅₀ below 100 µg/mL. *H. barbatum* exerted the most potent inhibitory effect (IC₅₀ = 31.84 ± 6.64 µg/mL), followed closely by *H. perforatum* (IC₅₀ = 37.12 ± 4.06 µg/mL).

Key words: xanthine oxidase inhibition, *Hypericum*, secondary metabolites

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INTRODUCTION

Xanthine oxidase (XO) is a validated target for therapeutic treatment of gout, hyperuricemia and associated conditions, with a few XO inhibitors present on the market (1). Levels of XO in plasma are enhanced also in ischemia-reperfusion injury, hemorrhagic shock, cholecystis, hypercholesterolemia, adult respiratory distress syndrome, carcinogenesis, which provides additional indications where XO inhibitors may exert their therapeutic potential (2).

Secondary metabolites from plants have a long tradition of being used as therapeutics in medicine (3). Hypericum species are traditionally used as medicinal plants all over the world (4). H. perforatum (St. John’s wort) is one of the best chemically determined species. The investigation of Hypericum species have increased over the years due to evidenced bioactivities of compounds found in H. perforatum. Chemically, naphthodianthrones, primarily hypericin and pseudohypericin, phloroglucinol derivatives, especially hyperforin, and flavonoids quercetin, quercitrin, hyperoside and rutin represent the main constituents in the Hypericum species (5).

Continuing our research on the chemical composition (6-11) and pharmacological activities (11-14) of Hypericum species, in the present study extracts of nine Hypericum species (H. barbatum, H. hirsutum, H. linarioides, H. olympicum, H. perforatum, H. rochelii, H. rumeliacum, H. tetrapterum and H. umbellatum), collected in Serbia, were evaluated for inhibitory potential against XO in vitro, on the commercial enzyme.

MATERIALS AND METHODS

Plant material

Table 1 contains data on the identity of nine assayed Hypericum species, their taxonomic placement within sections of the genus Hypericum, voucher numbers of the deposited herbarium specimens (Herbarium Moesicum Doljevac, Serbia), collection period and locality (15). Collection was done in the blooming stage.

| Table 1. Data on the assayed Hypericum species |
|-----------------------------------------------|
| **Section** | **Plant species** | **Voucher number (HMD)** | **Collection period** | **Locality** |
| Drosocarpium Spach |
| H. barbatum | 7280 | August 2010 | Suva planina, East Serbia |
| H. rochelii | 6578 | July 2010 | Sokobanja, East Serbia |
| H. rumeliacum | 7287 | July 2010 | Suva planina, East Serbia |
| H. umbellatum | 6579 | July 2010 | Surroundings of Pirot, South East Serbia |
| Hypericum |
| H. perforatum | 7291 | July 2010 | Surroundings of Leskovac, South Serbia |
| H. tetrapterum | 8223 | July 2010 | Bosilegrad, South East Serbia |
| Olympia Spach |
| H. olympicum | 7288 | July 2010 | Rujan planina, South Serbia |
| Taeniocarpium Jaub. & Spach |
| H. hirsutum | 7282 | July 2010 | Suva planina, East Serbia |
| H. linarioides | 8224 | July 2010 | Suva planina, East Serbia |

276  Acta facultatis medicae Naiissensis 2017; 34(3): 275-281
Preparation of plant extracts

The extractions were performed using 3 g dry plant material and 30 mL of ethanol, using an indirect sonication method. Sonications lasted 30 min, using a Bandelin Sonorex Digital 10 P apparatus (Bandelin). At the end of extraction, filtration was done in order to separate the extracts from the residual plant material. After washing the residues with 15 mL of ethanol, the filtrates were combined and evaporated using a rotary vacuum evaporator Büchi® Rotavapor® R-210 (Büchi). Dry extracts were then dissolved in dimethyl sulfoxide (DMSO) and stored at -20 °C until measurement.

Evaluation of xanthine oxidase inhibition

Inhibition of XO was evaluated in vitro on the commercial bovine milk enzyme (Sigma-Aldrich), by spectrophotometric measurement of uric acid formation at 293 nm. The assay was performed in a series of test-tubes (total volume 2150 μL) prepared in the following order: i) Test samples: 0.01 units of XO, one of the studied extracts diluted in DMSO (purity ≥ 99.5%; Sigma-Aldrich) (the final concentration of DMSO in the assay was 4.65 % v/v), 232.5 μM of xanthine (purity ≥ 99.5%; Sigma-Aldrich), and 46.5 mM TRIS-HCl buffer (pH 7.8); ii) Solvent control samples: the same amount of XO, appropriate amount of DMSO, xanthine and TRIS-HCl buffer; iii) Control samples: the same amount of XO, xanthine and TRIS-HCl buffer adjusted to the same volume. Blank samples were prepared for each group (i-iii). The tubes were incubated at 37 °C for 15 min, and after that the reaction was stopped by adding 100 μL of perchloric acid. The difference in absorbance, calculated as a percent change of the control with appropriate amount of DMSO, which correlates to uric acid formation, was used for the determination of the percentage of enzyme inhibition. DMSO, at a final concentration of 4.65 % v/v, did not affect the enzyme assay. The evaluation of XO inhibitory potential of all samples was performed at the concentrations of 100 μg/mL. Extracts exerting inhibition greater than 50 % at 100 μg/mL were tested in a broader concentration range in order to allow calculation of IC50 values. Each IC50 curve was generated using four concentrations of inhibitor producing an inhibition. Positive control was allopurinol and experiments were performed in triplicate.

RESULTS AND DISCUSSION

Seven of the nine studied Hypericum species inhibit commercial bovine milk XO with an IC50 below 100 μg/mL (Table 2). H. barbatum showed the most potent inhibitory effect (IC50 = 31.84 ± 6.64 μg/mL). Allopurinol, an approved XO inhibitor, exhibited stronger inhibitory potential (IC50 = 0.20 ± 0.03 μg/mL) than studied Hypericum species. The extracts of Hypericum species belonging to the section Taeniocarpium did not inhibit commercial bovine milk XO with an IC50 below 100 μg/mL.

Table 2. Inhibitory activities of studied Hypericum species against bovine milk xanthine oxidase (IC50, μg/mL)

| Section            | Plant species   | IC50 (μg/mL) XO |
|--------------------|----------------|----------------|
| Drosocarpium Spach | H. barbatum     | 31.84 ± 6.64   |
|                    | H. rochelii     | 42.78 ± 9.32   |
|                    | H. rumeliacum   | 43.70 ± 5.85   |
|                    | H. umbellatum   | 41.40 ± 7.14   |
| Hypericum          | H. perforatum   | 37.12 ± 4.06   |
|                    | H. tetrapertum  | 48.77 ± 7.21   |
| Olympia Spach      | H. olympicum    | 64.76 ± 9.14   |
| Taeniocarpium Jaub. & Spach | H. hirsutum | > 100          |
|                    | H. linarioides  | > 100          |

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Benedí et al. (16) determined IC₅₀ 68.30 μg/mL for the hydroethanolic standardized extract of *H. perforatum* (0.3% total hypericins), while Havlík et al. (17) determined IC₅₀ 46.70 μg/mL for the 80% aqueous ethanolic extract of *H. perforatum* and IC₅₀ 55.40 μg/mL for the methylene chloride - methanolic (50/50 CH₂Cl₂/MeOH) extract of *H. perforatum*.

We found earlier that there is a strong correlation between secondary metabolite contents and the infrageneric classification of Robson (15) among the nine *Hypericum* species (*H. barbatum*, *H. hirsutum*, *H. linarioides*, *H. maculatum*, *H. olympicum*, *H. perforatum*, *H. richeri*, *H. rumeliacum* and *H. tetrapterum*), collected on different locations in Serbia and the F.Y.R. Macedonia (6). It was shown that *H. barbatum* possesses higher (3.9 times) content of hypericin than *H. perforatum* (6), which is in agreement with the study of Kitanov (18), who reported that the content of total hypericins in *H. barbatum* is by 2.4 times higher than in *H. perforatum*. The potent inhibitory effect of *H. barbatum* against XO activity may be related to the high level of hypericin. Also, *H. perforatum* showed a very potent inhibitory effect against XO with an IC₅₀ value of 37.12 ± 4.06 μg/mL. The highest content of hyperforin was found in *H. perforatum* (6).

**CONCLUSION**

Ethanolic extracts of *H. barbatum*, *H. rochelii*, *H. rumeliacum*, *H. umbellatum*, *H. perforatum*, *H. tetrapterum* and *H. olympicum* inhibit commercial bovine milk XO with an IC₅₀ below 100 μg/mL. *H. barbatum* showed the highest inhibitory activity and may be potentially used in the treatment of hyperuricemia and gout.

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References

1. Lü JM, Yao Q, Chen C. 3,4-Dihydroxy-5-nitrobenzaldehyde (DHNB) is a potent inhibitor of xanthine oxidase: a potential therapeutic agent for treatment of hyperuricemia and gout. Biochem Pharmacol 2013; 86(9): 1328-37. https://doi.org/10.1016/j.bcp.2013.08.011

2. Radi R, Rubbo H, Bush K, Freeman B. Xanthine oxidase binding to glycosaminoglycans: kinetics and superoxide dismutase interactions of immobilized xanthine oxidase-heparin complexes. Arch Biochem Biophys 1997; 339: 125-35. https://doi.org/10.1006/abbi.1996.9844

3. Hufsky F, Scheubert K, Böcker S. New kids on the block: novel informatics methods for natural product discovery. Nat Prod Rep 2014; 31: 807-17. https://doi.org/10.1039/c3np70101h

4. Yazaki K, Okuda T. Medicinal and aromatic plants VI. In: Bajaj, Y.P.S. (ed), Biotechnology in agriculture and forestry. Springer-Verlag, Berlin, 1994: 167-78.

5. Stojanović G, Đorđević A, Šmelcerović A. Do other Hypericum species have medical potential as St. John's Wort (Hypericum perforatum)? Curr Med Chem 2013; 20: 807-17. https://doi.org/10.2174/092986731320180001

6. Smelcerovic A, Spiteller M. Phytochemical analysis of nine Hypericum L. species from Serbia and the F.Y.R. Macedonia. Pharmazie 2006; 61: 251-2.

7. Smelcerovic A, Verma V, Spiteller M, et al. Phytochemical analysis and genetic characterisation of six Hypericum species from Serbia. Phytochemistry 2006; 67: 171-7. https://doi.org/10.1016/j.phytochem.2005.10.021

8. Smelcerovic A, Spiteller M, Ligon AP, et al. Essential oil composition of Hypericum L. species from Southeastern Serbia and their chemotaxonomy. Biochem Syst Ecol 2007; 35: 99-113. https://doi.org/10.1016/j.bse.2006.09.012

9. Verma V, Smelcerovic A, Zuehlke S, et al. Phenolic constituents and genetic profile of Hypericum perforatum L. from India. Biochem Syst Ecol 2008; 36: 201-6. https://doi.org/10.1016/j.bse.2007.09.003

10. Smelcerovic A, Zuehlke S, Spiteller M, et al. Phenolic constituents of 17 Hypericum species from Turkey. Biochem Syst Ecol 2008; 36: 316-9. https://doi.org/10.1016/j.bse.2007.09.002

11. Đorđević A, Lazarević J, Šmelcerović A, Stojanović G. The case of Hypericum rochelii Griseb. & Schenk and Hypericum umbellatum A. Kern. Kern. essential oils: Chemical composition and antimicrobial activity. J Pharm Biomed Anal 2013; 77: 145-8. https://doi.org/10.1016/j.jpba.2013.01.024

12. Radulović N, Stankov-Jovanović V, Stojanović G, et al. Screening of in vitro antimicrobial and antioxidant activity of nine Hypericum species from the Balkans. Food Chem 2007; 103: 15-21. https://doi.org/10.1016/j.foodchem.2006.05.062

13. Bonkanka CX, Smelcerovic A, Zuehlke S, et al. HPLC-MS analysis and anti-oedematogenic activity of Hypericum grandifolium Choisy (Hypericaceae). Planta Med 2008; 74: 719-25. https://doi.org/10.1055/s-2008-1074526

14. Spiteller M, Özen T, Smelcerovic A, et al. Phenolic constituents and the in vitro antioxidant activity of the flowers of Hypericum venustum. Fitoterapia 2008; 79: 191-3. https://doi.org/10.1016/j.fitote.2007.11.012

15. Robson NKB. Studies in the genus Hypericum L. (Guttiferae) I. Infrageneric classification. Bull Br Mus Nat Hist (Botany) 1977; 5: 293-355.

16. Benedi J, Arroyo R, Romero C, et al. Antioxidant properties and protective effects of a standardized extract of Hypericum perforatum on hydrogen...
peroxide-induced oxidative damage in PC12 cells. Life Sci 2004; 75: 1263-76. https://doi.org/10.1016/j.lfs.2004.05.001

17. Havlik J, Gonzalez de la Huebra R, Hejtmankova K, et al. Xantine oxidase inhibitory properties of Czech medical plants. J Ethnopharmacol 2010; 132: 461-5. https://doi.org/10.1016/j.jep.2010.08.044

18. Kitanov GM Hypericin and pseudohypericin in some Hypericum species. Biochem Syst Ecol 2001; 29: 171-8. https://doi.org/10.1016/S0305-1978(00)00032-6
Inhibicija ksantin-oksidaze sekundarnim metabolitima iz biljnih vrsta roda Hypericum L.

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SAŽETAK

Ispitan je uticaj devet Hypericum vrsta (H. barbatum, H. hirsutum, H. linarioides, H. olympicum, H. perforatum, H. rochelii, H. rumeliacum, H. tetrapterum i H. umbellatum) prikupljenih na području Srbije na aktivnost komercijalne ksantin-oksidaze in vitro i upoređena sa alopurinolom. Sedam ispitivanih Hypericum vrsta (H. barbatum, H. rochelii, H. rumeliacum, H. umbellatum, H. perforatum, H. tetrapterum i H. olympicum) inhibiraju komercijalnu ksantin-oksidazu sa IC₅₀ vrednostima nižim od 100 µg/mL. H. barbatum (IC₅₀ = 31,84 ± 6,64 µg/mL) i H. perforatum (IC₅₀ = 37,12 ± 4,06 µg/mL) su se pokazali kao najefikasniji inhibitori ksantin-oksidaze.

Ključne reči: inhibicija ksantin-oksidaze, Hypericum, sekundarni metaboliti