Studies on the Chemical Composition and Biological Activities of Some *Hypericum* Species Growing in Turkey

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Abstract

In this study, the antidiabetic and anti-inflammatory potentials of *H. perforatum*, *H. scabroides*, *H. lysimachioides*, *H. amblysepalum*, *H. hyssopifolium*, *H. retusum* and *H. linarioides* species growing naturally in Turkey were evaluated by α-glucosidase, α-amylase and 15-lipoxygenase (15-LOX) enzyme inhibition assays; their chemical profiles were compared by HPLC analyses. IC_{50} values were compared with acarbose and quercetin, and phytochemical similarities and differences among the species were evaluated using chemometric methods. The majority of the tested extracts showed stronger inhibition against the α-glucosidase enzyme than acarbose, while the effect on α-amylase remained limited. *H. linarioides*, *H. retusum* and *H. amblysepalum* exhibited significant 15-LOX inhibitory activity. HPLC analyses showed that the highest naphthodianthrone content was in *H. linarioides*, and the highest total quercetin glycoside and hyperforin content was in *H. perforatum*. The absence of literature data on these three enzymes for *H. scabroides*, *H. amblysepalum*, *H. retusum* and *H. hyssopifolium* makes the study original. The findings support that the examined species are natural resources that can be evaluated in studies developing antidiabetic and anti-inflammatory agents.

Keywords: *Hypericum*, α-glucosidase, α-amylase, 15-lipoxygenase, HPLC, chemometrics

1. Introduction

The therapeutic use of plants dates back to prehistoric times, and it is known that medicinal plants have been used for health purposes for thousands of years [1,2]. Phytochemical research gained momentum in the 19th century with the isolation of active compounds, and today interest in natural products has increased again due to the side effects associated with synthetic drugs [3,4].

The genus *Hypericum*, represented by approximately 100 taxa in the flora of Turkey and including 45 endemic taxa, is known among the public as “kantaron” (St. John’s wort), and *H. perforatum* in particular is used in traditional medicine for the treatment of wounds, diabetes, infections and inflammatory disorders [5]. These effects are thought to originate from secondary metabolites such as naphthodianthrones, phloroglucinol derivatives, flavonoids and biflavonoids [6]. The efficacy of *H. perforatum* in mild-to-moderate depression has been supported by clinical studies and officially recognized by the German Commission E, ESCOP, WHO and EMA monographs [7-10].

In this study, the antidiabetic and anti-inflammatory potentials of seven *Hypericum* species were evaluated by α-glucosidase, α-amylase and 15-LOX inhibition assays, and their chemical profiles were compared by HPLC. The absence of any data in the literature on these three enzymes for *H. scabroides*, *H. amblysepalum*, *H. retusum* and *H. hyssopifolium* makes the study original with respect to these species. In addition, the HPLC-determined phytochemical profiles of all species were comparatively evaluated for the first time.

2. Results and Discussion

2.1 α-Glucosidase Inhibitory Activity

α-Glucosidase inhibitors play an important role in the control of postprandial hyperglycemia [11]. The majority of the tested extracts showed stronger activity compared to acarbose (IC_{50}: 665.90 ± 2.19 µg/mL). The highest inhibition was determined in the *H. lysimachioides* extract (121.20 ± 1.68 µg/mL), followed by *H. linarioides* (169.83 ± 13.80), *H. retusum* (176.73 ± 15.54) and *H. perforatum* (196.63 ± 7.56 µg/mL). *H. hyssopifolium* (455.77 ± 4.28) and *H. scabroides* (551.15 ± 25.39 µg/mL) had moderate activity, while *H. amblysepalum* (924.50 ± 32.67 µg/mL) had the lowest activity.

The strong α-glucosidase activity reported in the literature for *H. linarioides* [12] and the significant inhibition reported for *H. lysimachioides* [13] are consistent with the findings of our study. For *H. scabroides*, *H. amblysepalum*, *H. hyssopifolium* and *H. retusum*, the first data in this area are presented. Considering the gastrointestinal side effects of

acarbose, the examined extracts are natural resources that can be evaluated in studies developing alternative antidiabetic agents.

2.2 α-Amylase Inhibitory Activity

α-Amylase catalyzes the first step of carbohydrate digestion [14]. Acarbose (IC_{50}: 0.03248 ± 8.49×10^{-5} µg/mL) exhibited much stronger inhibitory activity compared to all extracts. The lowest IC_{50} value was found in the *H. lysimachioides* extract (281.15 ± 5.87 µg/mL), followed by *H. retusum* (324.15 ± 3.75) and *H. perforatum* (405.93 ± 4.48 µg/mL). *H. linarioides*, *H. scabroides* and *H. hyssopifolium* showed moderate-to-weak activity, and a measurable IC_{50} value could not be reached for *H. amblysepalum*.

Studies in the literature show that the effect of *Hypericum* species on α-amylase generally remains below that of acarbose [11,15-17]. The extracts exhibiting a more selective inhibition profile on α-glucosidase compared to α-amylase points to an advantageous antidiabetic profile that may limit gastrointestinal side effects.

2.3 15-LOX Inhibitory Activity

Lipoxygenases play a role in arachidonic acid metabolism and are important in the regulation of the inflammatory response [18]. The highest 15-LOX inhibitory activity was determined in the *H. linarioides* extract (IC_{50} = 34.69 ± 3.88 µg/mL), followed by *H. retusum* (43.98 ± 1.12) and *H. amblysepalum* (47.89 ± 6.93 µg/mL); the lowest activity was observed in *H. lysimachioides* (110.30 ± 9.13 µg/mL). All extracts showed lower activity than the positive control quercetin (23.36 ± 3.17 µg/mL).

Although there is no literature on 15-LOX inhibition in *Hypericum* species, flavonoids and hyperforin belonging to the genus have been reported to have inhibitory effects on the 12-LOX and 5-LOX enzymes [19,20]. Similarly, quercetin-3′-O-β-D-glucopyranoside isolated from *H. oblongifolium* [21] and acylphloroglucinol derivatives isolated from *H. empetrifolium* have been reported to show 5-LOX inhibitory activity. These findings indicate that the lipoxygenase inhibitory activity in *Hypericum* species may be related to different secondary metabolite groups such as flavonoids, acylphloroglucinols and hyperforin.

2.4 HPLC Analysis

The highest hypericin (0.0242 ± 0.0010 mg/mL), pseudohypericin (0.0299 ± 0.0015 mg/mL) and total naphthodianthrone (0.0541 mg/mL) content was determined in *H. linarioides*, followed by *H. lysimachioides* and *H. perforatum*. Naphthodianthrone content was found to be low in *H. amblysepalum*, *H. hyssopifolium* and *H. scabroides*. These results are consistent with the literature [22-24].

Total quercetin glycoside was found to be highest in the *H. perforatum* (1.2614 ± 0.0284 mg/mL) and *H. retusum* (1.2282 ± 0.0807 mg/mL) extracts, and the lowest value was determined in *H. hyssopifolium* (0.3582 ± 0.0987 mg/mL). Quercitrin was found at quantifiable levels only in *H. amblysepalum* (0.1342 ± 0.0048 mg/mL) and *H. perforatum* (0.0784 ± 0.0005 mg/mL), and at trace or undetectable levels in the other species. Hyperforin was quantified only in *H. perforatum* (0.3775 ± 0.1275 mg/mL).

The hyperoside-dominant profile reported in the literature for *H. lysimachioides* [25] and the limited phytochemical data for *H. hyssopifolium*, *H. amblysepalum*, *H. retusum* and *H. scabroides* [26-29] were found to be largely consistent with the findings of our study; a comprehensive *Hypericum* characteristic compound profile is presented in the literature for the first time for these four species.

2.5 Chemometric Analysis

In the PCA score plot, *H. perforatum* was clearly separated from the other species due to its high hyperforin and total quercetin glycoside content. The first two principal components explained 80.0% of the total variance (PC1: 47.4%; PC2: 32.6%); the highest positive loadings on PC1 were formed by hypericin, total quercetin glycosides, pseudohypericin and hyperforin, while quercitrin and hyperforin were determinant on PC2.

The HCA results showed a large degree of agreement with the PCA. The clustering of *H. amblysepalum*, *H. hyssopifolium* and *H. scabroides* in the same cluster reveals that these species share similar characteristics in terms of low hyperforin and relatively low total glycoside content. *H. lysimachioides*, *H. retusum* and *H. linarioides*, on the other hand, formed the second cluster by exhibiting phytochemical profiles close to one another. *H. perforatum* formed the third cluster alone, clearly diverging from the other species due to its high total quercetin glycoside and hyperforin content. This clustering shows that the phytochemical differences determined by HPLC analyses are also statistically supported.

3. Conclusion

This study shows that the examined *Hypericum* species have significant potential in terms of their enzyme inhibitory activities and phytochemical profiles. In the future, it is aimed to isolate active compounds, particularly from species showing high α-glucosidase and 15-LOX inhibitory activity, by bioactivity-guided fractionation, to elucidate their structures, and to investigate their efficacy through molecular docking, cell culture and *in vivo* models.

REFERENCES

1. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev 2012;6(11):1-5.

2. Baytop T. Türkiye'de Bitkiler ile Tedavi Geçmişte ve Bugün. 3rd ed. Ankara: Nobel Tıp Kitabevleri; 2021.

3. Claro AE, Palanza C, Mazza M, Schuenemann GEUM, Rigoni M, Pontecorvi A, et al. Historical use of medicinal plants and future potential from phytotherapy to phytochemicals. Ann Bot (Roma) 2024;14(1).

4. Vaou N, Voidarou C, Rozos G, Saldari C, Stavropoulou E, Vrioni G, et al. Unraveling Nature's Pharmacy: Transforming Medicinal Plants into Modern Therapeutic Agents. Pharmaceutics 2025;17(6).

5. Eroglu Özkan E, Aksu B, Mat A. An overview on Hypericum species of Turkey. Planta Med 2008;74.

6. Otero MC, Ceric F, Miranda-Rojas S, Carreño C, Escares R, Escobar MJ, et al. Documentary Analysis of Hypericum perforatum (St. John's Wort) and Its Effect on Depressive Disorders. Pharmaceuticals (Basel) 2024;17(12).

7. Saddiqe Z, Naeem I, Maimoona A. A review of the antibacterial activity of Hypericum perforatum L. J Ethnopharmacol 2010;131(3):511-521.

8. World Health Organization. WHO Monographs on Selected Medicinal Plants. Geneva: World Health Organization; 2002.

9. European Medicines Agency (EMA), Committee on Herbal Medicinal Products (HMPC). Draft Community herbal monograph on Hypericum perforatum L. and Cimicifuga racemosa (L.) Nutt. 2025.

10. Nathan PJ. The experimental and clinical pharmacology of St John's Wort (Hypericum perforatum L.). Mol Psychiatry 1999;4(4):333-338.

11. Kashtoh H, Baek KH. Recent Updates on Phytoconstituent α-Glucosidase Inhibitors: An Approach towards the Treatment of Type Two Diabetes. Plants (Basel) 2022;11(20):2722.

12. Altay A, Yeniçeri EK, Taslimi P, Taşkın-Tok T, Yılmaz MA, Köksal E. Phytochemical analysis and biological evaluation of Hypericum linarioides bosse: in vitro and in silico studies. ChemistrySelect 2022;7(23):e202200039.

13. Bahsi M, Di Simone SC, Zheleva-Dimitrova D, Zengin G, Cusumano G, Flores GA, et al. Unlocking New Pharma/Nutraceutical Frontiers With Neuroprotective Properties of Three Hypericum Species: A Study Combination With In Vitro and In Silico Methodologies. Food Sci Nutr 2025;13(4):e70069.

14. Dandekar PD, Kotmale AS, Chavan SR, Kadlag PP, Sawant SV, Dhavale DD, et al. Insights into the Inhibition Mechanism of Human Pancreatic α-Amylase, a Type 2 Diabetes Target, by Dehydrodieugenol B Isolated from Ocimum tenuiflorum. ACS Omega 2021;6(3):1780-1786.

15. Altay A, Yeniçeri EK, Taslimi P, Taşkın-Tok T, Yılmaz MA, Köksal E. Phytochemical analysis and biological evaluation of Hypericum linarioides bosse: in vitro and in silico studies. ChemistrySelect 2022;7(23):e202200039.

16. Gök HN, Bulut S, Orhan DD. Evaluation of the antioxidant and enzyme inhibitory activity of Hypericum scabrum L. extract and its HPLC analysis. J Fac Pharm Ankara 2023;47(2):349-359.

17. Kladar N, Božin B, Bijelić K, Bogavac M, Karaman M, Srđenović Čonić et al. Biological Activity of Genus Hypericum Sect. Hypericum Species-H. tetrapterum, H. maculatum subsp. immaculatum, H. triquetrifolium. Molecules 2023;28:6218.

18. Dobrian AD, Lieb DC, Cole BK, Taylor-Fishwick DA, Chakrabarti SK, Nadler JL. Functional and pathological roles of the 12- and 15-lipoxygenases. Prog Lipid Res 2011;50(1):115-131.

19. Obložinský M, Bezáková L, Holková I, Vanko M, Kartnig T, Pšenák M. Antilipoxygenase activity of compounds from Hypericum perforatum. Biologia (Bratisl) 2006;61(3):331-332.

20. Feißt C, Pergola C, Rakonjac M, Rossi A, Koeberle A, Dodt G, et al. Hyperforin is a novel type of 5-lipoxygenase inhibitor with high efficacy in vivo. Cell Mol Life Sci 2009;66(16):2759-2771.

21. Sajid A, Sajid A, Ahmed E, Sharif A, Manzoor Q, Al-Mijalli SH, et al. In vitro and molecular docking studies of plant secondary metabolites isolated from Hypericum oblongifolium as antibacterial agents and lipoxygenase (5-LOX) inhibitors. J Mol Struct 2024;1312:138549.

22. Jaimand K, Rezaee MB, Mozaffrian V, Azadi R, Naderi Haji Bagher Kandy M, Meshkyzadeh, et al. Determination of Hypericin Content in Flowers and Leaves of Eight Hypericum Species. J Med Plants 2008;7(25):49-55.

23. Jaimand K, Rezaee M, Behrad Z, Mirza M, Mozaffarian V, Azady R et al. Determination of Hypericine content in nine species of Hypericum. Iran J Med Aromat Plants Res 2014;30(1):10-18.

24. Ayan AK, Çirak C. Hypericin and Pseudohypericin Contents in Some Hypericum Species Growing in Turkey. Pharm Biol 2008;46(4):288-291.

25. Akdeniz M, Yilmaz MA, Ertas A, Yener I, Firat M, Aydin F et al. Method validation of 15 phytochemicals in Hypericum lysimachioides var. spathulatum by LC-MS/MS, and fatty acid, essential oil, and aroma profiles with biological activities. J Food Meas Charact 2020;14(6):3194-3205.

26. Maggi F, Ferretti G, Pocceschi N, Menghini L, Ricciutelli M. Morphological, histochemical and phytochemical investigation of the genus Hypericum of the Central Italy. Fitoterapia 2004;75(7):702-711.

27. Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M, Kazaz C. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of Hypericum hyssopifolium L. by activity-guided fractionation. J Ethnopharmacol 2003;87(1):73-83.

28. Keskin C, Aktepe N, Yükselten Y, Sunguroglu A, Boğa M. In-vitro Antioxidant, Cytotoxic, Cholinesterase Inhibitory Activities and Anti-Genotoxic Effects of Hypericum retusum Aucher Flowers, Fruits and Seeds Methanol Extracts in Human Mononuclear Leukocytes. Iran J Pharm Res 2017;16(1):210-220.

29. Keskin C. Antioxidant, anticancer and anticholinesterase activities of flower, fruit and seed extracts of Hypericum amblysepalum HOCHST. Asian Pac J Cancer Prev 2015;16(7):2763-2769.

30. Lam TP, Tran NN, Pham LD, Lai NV, Dang BN, Truong NN, et al. Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: a systematic review of in vitro studies. Nat Prod Bioprospect 2024;14(1):4.