DOI 10.1007/s10600-023-03939-3
*Chemistry of Natural Compounds, Vol. 59, No. 1, January, 2023*

# FLAVONOID GLYCOSIDES FROM THE AERIAL PART OF *Lespedeza hedysaroides*

**N. D. Pokhilo,[1] D. V. Tarbeeva,[1] V. P. Grigorchuk,[2] S. S. Starnovskaya,[1] P. G. Gorovoy,[1] and S. A. Fedoreyev[1]***

The genus *Lespedeza* (Fabaceae, Leguminosae) is represented in Primorsky Krai of the Russian Federation by the five species *L. bicolor* Turcz., *L. cyrtobotria* Miq., *L. davurica* (Laxm.) Schindl., *L. hedysaroides* (Pall.) Kitag., and *L. tomentosa* (Thunb.) Maxim. [1], which are sources of biologically active flavonoids [2–6] and their glycosides [2, 7]. According to the International Legume Database and Information Service (ILDIS), the names *L. hedysaroides* (Pall.) King. and *L. juncea* (L. fil.) Pers. refer to one plant species with priority given to *L. hedysaroides* [8].

*L. hedysaroides* is a perennial plant with straight stems and a height of 60–80 cm. It grows on dry rocky slopes in sandy and pebbly deposits along riverbanks and is encountered mainly in the Far East, Mongolia, and northwestern China [1, 9]. Extracts of the plant harvested in China contained orientin, isoorientin, and vitexin [1]. Kaempferol, quercetin, saponaretin, lespedin [10], and bioquercetin [11] were identified in samples of the aerial part of *L. hedysaroides* from eastern Siberia. The homoisoflavanones lesjunceol and lesjuncerol [12] and the phenolic glycosides meosides A and B [13] were isolated from the MeOH extract of roots of this lespedeza species collected in India.

The aim of the present work was to isolate and establish the structures of polyphenolic metabolites from the aerial part of *L. hedysaroides* collected in southern Primorsky Krai of the Russian Federation and to study their antioxidant properties.

Specimens of wild *L. hedysaroides* were collected in Primorsky Krai (near Sinel′nikova-1 village) in September, 2020. Their species was determined by Acad. P. G. Gorovoy. A specimen of the plant (voucher No. 103619) is preserved in the herbarium of G. B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB, RAS.

$^1$H and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ at 30°C using Bruker Avance III DRX-700 and Avance DRX-500 instruments (Karlsruhe, Germany). Analytical HPLC of fractions and high-resolution mass spectra of pure compounds from *L. hedysaroides* used a Shimadzu LCMS-IT-TOF liquid chromatograph–mass spectrometer (Japan) equipped with an LC-20A HPLC, SPD-M20A diode-array detector, and time-of-flight mass spectrometer with an ion trap. Mixture constituents were separated over a Discovery HS C-18 column (150 × 2.1 mm, 3 μm particle size) thermostatted at 36°C by the previously published method [7].

The dry $CHCl_3$–EtOH extract (10 g) obtained from dried stems and leaves of *L. hedysaroides* was chromatographed over a column packed with polyamide 6 DF (Sigma-Aldrich) using $CHCl_3$ with a gradient of increasing content of EtOH up to 100%. The obtained flavonoid fractions were analyzed by HPLC and HPLC-UV-MS-HR and subsequently chromatographed over a column using a gradient of increasing EtOH up to 80%. The flavonoid fractions were additionally purified over YMC-GEL ODS-A sorbent using $H_2O$ with a gradient of increasing EtOH content from 20 to 100% to afford the flavonoid glycosides orientin (**1**) [2, 14]; isoorientin (**2**) [2, 14]; saponaretin (isovitexin) (**3**) [2, 14]; luteolin 6-*C*-(2″-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside) (**4**) [2, 15]; apigenin 6-*C*-(2″-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside) (**5**) [2, 15]; rutin (**6**) [7]; and morin rutinoside (**7**) [7] in yields of 40, 60, 20, 50, 27, 10, and 1 mg, respectively, and gentisic acid (**8**, 5 mg) [16].

1) G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, 159 Prosp. 100 Let Vladivostoku, 690022, Vladivostok, Russia, e-mail: fedoreev-s@mail.ru; 2) Federal Scientific Center for the Biodiversity of Terrestrial Biota of East Asia, Far Eastern Branch, Russian Academy of Sciences, 159 Prosp. 100 Let Vladivostoku, 690022, Vladivostok, Russia. Translated from *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2023, pp. 133–134. Original article submitted July 19, 2022.

  

TABLE 1. Antioxidant Activity of Flavonoid Glycosides **1**–**6**

| Compound | DPPH trapping after 30 min,* % | Fe-reducing activity, mol $Fe^{3+}$/mol compound* |
|---|---|---|
| Quercetin | 8.4 | 4.0 ± 0.6 |
| Ionol | 75.0 | 0.2 ± 0.03 |
| **1** | 10.3 | 2.9 ± 0.3 |
| **2** | 12.6 | 2.3 ± 0.09 |
| **3** | 86.0 | 0.5 ± 0.06 |
| **4** | 10.2 | 2.7 ± 0.6 |
| **5** | 107.0 | 0.4 ± 0.04 |
| **6** | 13.3 | 1.6 ± 0.2 |

*All experiments performed in triplicate, statistical significance determined using Student criterion and SigmaPlot 14.0 (Systat Software Inc., USA).

UV and NMR spectroscopic and mass spectrometric data of isolated 1-8 agreed fully with literature data. Flavonoid diglycosides **4**–**7** and gentisic acid **8** were identified in this plant for the first time.

Table 1 presents results for the antioxidant activity of 1-6 (DPPH-acceptor and Fe-reducing effects). The antiradical activity of the polyphenolic compounds was assessed from their ability to trap the DPPH radical (2,2-diphenyl-1-picrylhydrazyl radical). The Fe-reducing activity was determined by the FRAP reagent, which was prepared by mixing a solution of 2,4,6-*tris*(2-pyridyl)-*s*-triazine (TPTZ) (2.5 mL, 10 mM) in HCl solution (40 mM) and a solution of $FeCl_3$ (25 mL, 20 mM) in acetate buffer (300 mM, pH 3.6). Solutions of **1**–**6** in EtOH at concentrations of 6–34 μM were added to FRAP reagent (3 mL). The methods for determining antiradical and Fe-reducing activities of polyphenolic compounds were published earlier by us [3, 6]. The reference compounds were the antioxidants quercetin and ionol (2,6-di-*t*-butyl-4-methylphenol).

Quercetin, with five hydroxyls in its structure, possessed the maximum antiradical (DPPH test) and Fe-reducing activities (Table 1). Flavonoids containing four unsubstituted hydroxyls (**1**, **2**, **4**, and **6**) exhibited moderate DPPH-acceptor and Fe-reducing effects comparable to those of quercetin. However, their antioxidant parameters were considerably greater than those of ionol (Table 1). Flavonoid glycosides **3** and **5** possessed low antioxidant activities. Their structures lacked a C-3′ hydroxyl on ring B. Their antiradical and Fe-reducing effects were considerably less than those of ionol and the other antioxidants. Therefore, luteolin *C*-glycosides **1**, **2**, and **4** with C-5 and C-7 OH substituents and *ortho*-dihydroxyls on ring B possessed substantial antioxidant and anti-inflammatory properties. They are known to be capable of inhibiting angiogenesis, inducing apoptosis, preventing carcinogenesis in animal models, and decreasing tumor growth [17].

## REFERENCES


1. *Flora of Siberia. Fabaceae (Leguminosae)*, Vol. 9, A. V. Polozhii and L. I. Malysheva (eds.), Nauka, Novosibirsk, 1994, 280 pp; *Flora of the USSR* [in Russian], Vol. 3, B. K. Shishkina and E. G. Bobrova (eds.), Izd. AS USSR, Moscow, Leningrad, 1948, 380 pp.
2. K. Matsuzaki, Y.-Q. Wang, K. Takahashi, and T. Okuyama, *Shoyakugaku Zasshi*, **44**, 251 (1990).
3. D. V. Tarbeeva, S. A. Fedoreyev, M. V. Veselova, A. S. Blagodatski, A. M. Klimenko, A. I. Kalinovskiy, V. P. Grigorchuk, D. V. Berdyshev, and P. G. Gorovoy, *Fitoterapia*, **135**, 64 (2019).
4. S. Dyshlovoy, D. Tarbeeva, S. Fedoreyev, T. Busenbender, M. Kaune, M. Veselova, A. Kalinovskiy, J. Hauschild, V. Grigorchuk, N. Kim, C. Bokemeyer, M. Graefen, P. Gorovoy, and G. von Amsberg, *Biomolecules*, **10**, 451 (2020).
5. D. V. Tarbeeva, N. V. Krylova, O. V. Iunikhina, G. N. Likhatskaya, A. I. Kalinovskiy, V. P. Grigorchuk, M. Yu. Shchelkanov, and S. A. Fedoreyev, *Fitoterapia*, **157**, 105121 (2022).
6. D. V. Tarbeeva, E. A. Pislyagin, E. S. Menchinskaya, D. V. Berdyshev, A. I. Kalinovskiy, V. P. Grigorchuk, D. L. Aminin, and S. A. Fedoreyev, *Antioxidants*, **11**, 709 (2022).
7. N. D. Pokhilo, S. A. Fedoreyev, D. V. Tarbeeva, M. V. Veselova, V. P. Grigorchuk, and P. G. Gorovoy, *Chem. Nat. Compd.*, **57**, 1023 (2021).
8. Y. R. Roskov et al., *ILDIS World Database of Legumes*, URL: http://www.ildis.org (2005).
9. *Vascular Plants of the Soviet Far East*, Vol. 4, S. S. Kharkevich (ed.), Nauka, Leningrad, 1989, 201 pp.

10. V. I. Glyzin, *Results of Scientific Research on Medicinal Plant Cultivation*, All-Union Scientific Research Institute of Medicinal Plants, Moscow, 1975, p. 148.
11. Ya. Lakhman, V. I. Litvinenko, T. P. Nadezhina, and L. I. Dranik, *Khim. Prir. Soedin.*, 136 (1978).
12. M. A. Tantray, A. S. Shawl, M. A. Khuroo, and N. Ali, *Chem. Nat. Compd.*, **44**, 424 (2008).
13. M. A. Tantray, R. Khan, A. S. Shawl, and M. A. Khuroo, *Chem. Nat. Compd.*, **44**, 591 (2008).
14. O. M. Bello, A. B. Ogbesejana, C. O. Adetunji, and S. Oguntoye, *Turk. J. Pharm. Sci.*, **16**, 437 (2019).
15. S. Rayyan, T. Fossen, and Q. M. Andersen, *J. Agric. Food Chem.*, **53**, 10057 (2005).
16. L. P. D. A. G. Cortez, G. J. Vidotti, M. C. M. Yang, and A. G. Ferreira, *Quim. Nova*, **26**, 157 (2003).
17. M. Lopez-Lazaro, *Mini-Rev. Med. Chem.*, **9**, 31 (2009).