

Flavonol glycosides from aerial parts of *Astragalus thracicus* Griseb

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ABSTRACT

Five flavonol glycosides, including a new glutaryl-conjugated compound, were isolated from methanolic extract of the endemic Balkan species *Astragalus thracicus* Griseb. Based on NMR and MS spectra, one new compound was identified as kaempferol 3-O-[β-glucopyranosyl-(1→4)-α-rhamnopyranosyl-(1→6)-(2"-3-hydroxy-3-methylgutaroyl)-β-galactopyranoside] (1). The other compounds, all known, were identified as mauritianin (2), kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-β-galactopyranoside (3), kaempferol-3-O-β-apiofuranosyl-(1→2)-β-galactopyranoside (4), and quercetin-3-O-β-apiofuranosyl-(1→2)-β-galactopyranoside (5). All of them, except 2, were found for the first time in a representative of genus *Astragalus*.

1. Introduction

With its more than 2200 taxonomically classified species, genus *Astragalus* (Fabaceae) is considered to be one of the largest genera of dicotyledonous plants (Frodin, 2004). Species of *Astragalus* have been found growing at altitude up to 1700 m in dry and semi-dry regions all over the world (Pistelli, 2002). One hundred and thirty-three species grow in Europe, 29 in Bulgaria (Tutin et al., 1972; Assyov et al., 2006). Of those Bulgarian species of *Astragalus*, 14 are currently protected by the Bulgarian Biodiversity Act and included in the Red List of Bulgaria because of their vulnerable, endangered, or critically endangered status (Petrova and Vladimirov, 2009).

Among the protected species designated “vulnerable” is *Astragalus thracicus* Griseb., a tertiary relict endemic to the Balkans. This plant is a xeromorphic shrub with robust roots, hairy, densely branched stems (16–40 cm in height), and leaves that terminate in the shape of a spine. Nowadays, it is found in Bulgaria only around the cities Sliven, Yambol, and Haskovo, but it also occurs in some limited areas in the Greek and Turkish regions of Thracia (Stoeva, 2020, Red Data Book of the Republic of Bulgaria - Digital edition).

The long history of using *Astragalus* as a medicine or food or for

cosmetic purposes has led to extensive chemical investigation. To date, approximately 100 species of this genus have been studied phytochemically. In general, these species show high contents of saponins and phenolics that contribute a variety of biological activities to their extracts (Ionkova et al., 2014; Bratkov et al., 2016). The phenolics isolated from *Astragalus* comprise a wide range of flavonoids, covering most of the subfamilies flavones, flavonols, flavanones, dihydroflavonols, chalcones, isoflavones, and isoflavans, and also include pterocarpanes (Bratkov et al., 2016; Pistelli, 2002). Of the *Astragalus* flavonoids, flavonol glycosides are the most abundant group with the greatest diversity (Bratkov et al., 2016). Isorhamnetin, kaempferol, and quercetin are the most common flavonol aglycones, while the glycosides are mainly represented by astragalin, isoquercitrin, hyperoside, and rutin (Bratkov et al., 2016; Pistelli, 2002).

As a part of the Bulgarian research program for the phytochemical and chemotaxonomical characterization of Bulgarian species of *Astragalus*, this study reports on the isolation and identification of *Astragalus* flavonoids, including a new compound 1, three known compounds, isolated for the first time in the genus *Astragalus* 3–5, and mauritianin (2), which has been found in *A. thracicus* for the first time.

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Table 1
¹H and ¹³C NMR spectroscopic data for compound **1** in CD₃OD.

C	δ _C (ppm)	δ _H (mult., J in Hz)	Rha 1→2''	δ _C (ppm)	δ _H (mult., J in Hz)
2	157.3, C		1'''	101.0, CH	5.18, d (1.60)
3	133.0, C		2'''	70.8, CH	4.01, dd
4	177.9, C		3'''	76.5, CH	3.20 dd
5	161.7, C		4'''	82.8, CH	3.54, pt
6	98.4, CH	6.18, d (2.05)	5'''	67.0, CH	4.11, dq
7	164.3, C		6'''	16.3, CH ₃	1.04 d (6.23)
8	93.3, CH	6.39, d (2.11)	HMG 1→6		
9	157.0, C		1	170.7, C	
10	104.5, C		2	44.7, CH ₂	2.33, d (14.94)
1'	121.5, C				2.42, d (14.94)
2'	130.8, CH	8.04, d (8.76)	3	69.1, C	
3'	114.8, CH	6.87, d (8.76)	4	45.0, CH ₂	2.37, d (15.27)
4'	159.9, C	-OH			2.46, d (15.23)
5'	114.8, CH	6.87, d (8.76)	5	174.0, C	
6'	130.8, CH	8.04, d (8.76)	3'	26.2, CH ₃	1.12, s
3-O-Gal			Glc 1→4'		
1''	99.2, CH	5.55, d (7.76)	1''''	104.4, CH	4.49, d (7.54)
2''	76.0, CH	3.90, dd	2''''	74.6, CH	3.15, dd
3''	74.1, CH	3.68 dd	3''''	76.7, CH	3.29, dd
4''	69.3, CH	3.76 d *	4''''	69.8, CH	3.26, dd
5''	72.9, CH	3.67 dd	5''''	76.5, CH	3.21, dt
6''	62.9, CH ₂	4.09 dd	6''''	61.1, CH ₂	3.62, dd
		4.13 dd			3.74, dd

Assignments were made using HSQC, HMBC, and COSY experiments. Gal = galactopyranosyl; Rha = rhamnopyranosyl; Glc = glucopyranosyl; HMG = 3-hydroxy-3-methylglutaryl.

* signals overlapped.

2. Results and discussion

Compounds **1–5** were isolated from methanol extracts of aerial parts of *A. thracicus* Griseb. and their structures were determined by HRESIMS and NMR (¹H, ¹³C, ¹H-¹H-COSY, HSQC, and HMBC) spectral analysis.

Compound **1** was isolated as an amorphous yellow powder. The negative HRESIMS of **1** displayed a molecular ion peak [M-H]⁻ at *m/z* 899.2471 (calcd. [M-H]⁻ *m/z* 899.2463), suggesting the molecular formula C₃₉H₄₈O₂₄. The ¹H NMR spectrum (Table 1), showed signals of

four aromatic protons within the AA'/BB' system of ring B at δ_H 6.87 ppm (2H, d, *J* = 8.8 Hz) and δ_H 8.04 ppm (2H, d, *J* = 8.8 Hz); the two *meta* aromatic protons of ring A at δ_H 6.18 ppm (1H, d, *J* = 2.1 Hz) and 6.39 ppm (1H, d, *J* = 2.1 Hz) were assigned to a flavonol aglycone; three anomeric proton signals at δ_H 4.49 ppm (1H, d, *J* = 7.5 Hz), 5.18 ppm (1H, d, *J* = 1.6 Hz), and δ_H 5.55 ppm (1H, d, *J* = 7.8 Hz); two methyl groups at δ_H 1.04 ppm (3H, d, *J* = 6.2 Hz) and δ_H 1.12 ppm (3H, s); and eight methylene protons: δ_H 2.33 ppm (1H, d, *J* = 14.9 Hz) and 2.42 ppm (1H, d, *J* = 14.9 Hz); 2.37 ppm (1H, d, *J* = 15.2 Hz), and 2.46 ppm (1H, d, *J* = 15.2 Hz); 4.09 ppm (1H, dd), and 4.13 ppm (1H, dd); 3.62 ppm (1H, dd) and 3.74 ppm (1H, dd). The ¹³C NMR and HSQC spectra of **1** showed the signals of 39 carbons, including three carbonyl carbons at δ_C 170.7, 174.0, and 177.9 ppm; nine non-protonated carbon atoms at δ_C 69.1, 104.5, 121.5, 133.0, 157.0, 157.3, 159.9, 161.7 and 164.3 ppm; twenty-one methines at δ_C 67.0, 69.3, 69.8, 70.8, 72.9, 74.1, 74.6, 76.0, 76.5, 76.7, 82.8, 93.3, 98.4, 99.2, 101.0, 104.4, 114.8, 114.8, 130.8, and 130.8 ppm; four methylenes at δ_C 44.7, 45.0, 61.1, and 62.9 ppm, and two methyl carbons δ_C at 16.3 and 26.2 ppm. The HMBC correlations, observed in aglycone are between: H-2'/6' (δ_H 8.04 ppm) and C-2' (δ_C 130.8 ppm)/C-2 (δ_C 157.3 ppm)/C-4' (δ_C 159.9 ppm); between H-3'/5' (δ_H 6.87 ppm) and C-3' (δ_C 114.8 ppm)/C-1' (δ_C 121.5 ppm)/C-4' (δ_C 159.9 ppm); between H-8 (δ_H 6.39 ppm) and C-6 (δ_C 98.4 ppm)/C-10 (δ_C 104.5 ppm)/C-9 (δ_C 157.0 ppm)/C-7 (δ_C 164.3 ppm); between H-6 (δ_H 6.18 ppm) and C-8 (δ_C 93.3 ppm)/C-10 (δ_C 104.5 ppm)/C-5 (δ_C 161.7 ppm)/C-7 (δ_C 164.3 ppm).

The analysis of NMR data (¹H, ¹³C, ¹H-¹H COSY, HSQC and HMBC) showed the structure of **1** to be kaempferol, but with OH at C-3 (δ_C 133.0 ppm) replaced by a substituent consisting of two hexoses, one deoxyhexose, and a 3-hydroxy-3-methylglutaryl (HMG) group. Further analysis after acid hydrolysis of **1** confirmed the presence of the sugar components β-galactopyranose, β-glucopyranose, and α-rhamnopyranose. Additionally, according to the observed HMBC correlations, the two remaining methylene groups δ_C 44.7 ppm and δ_C 45.0 ppm, two carboxy carbons at δ_C 170.7 ppm and δ_C 174.0 ppm, one quaternary carbon atom at δ_C 69.1 ppm, and a methyl group at δ_C 26.2 ppm belong to a non-sugar substituent, determined to be a 3-hydroxy-3-methylglutaryl residue. The C-1 carbonyl atom, showed a ⁴*J* correlation with H-6a''/6b'' of the galactose, which clearly showed the (1→6) connection between the HMG residue and the galactose. Finally, the compound was characterized as kaempferol 3-O-[[β-glucopyranosyl-(1→4)-α-rhamnopyranosyl-(1→6)-(2''-3-hydroxy-3-methylglutaryl)-β-galactopyranoside]] (**1**), representing a new natural product (Fig. 1).

The known compounds were identified as mauritianin (**2**) (De Simone et al., 1990; Yasukawa and Takido, 1987), kaempferol-3-O-α-rhamnopyranosyl-(1→2)-β-galactopyranoside (**3**) (Yasukawa and Takido, 1987), kaempferol-3-O-β-D-apiofuranosyl-(1→2)-β-D-galactopyranoside (**4**) (De

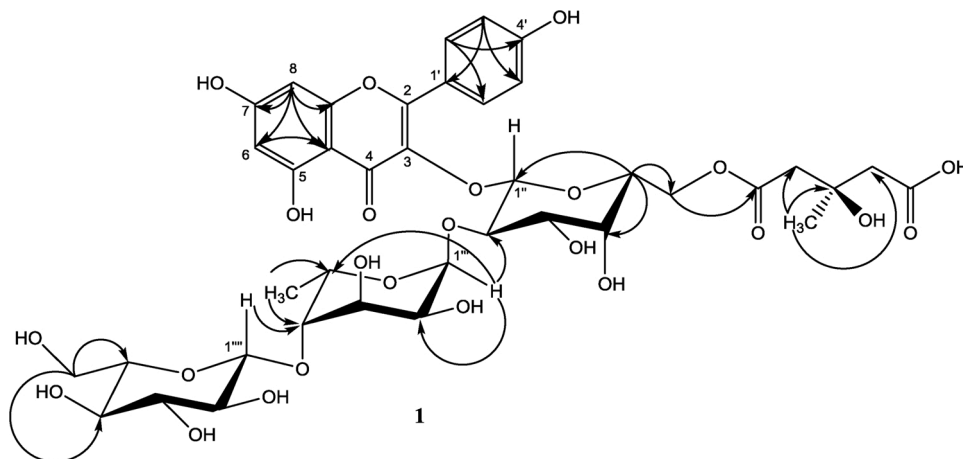


Fig. 1. HMBC correlations of compound **1**.

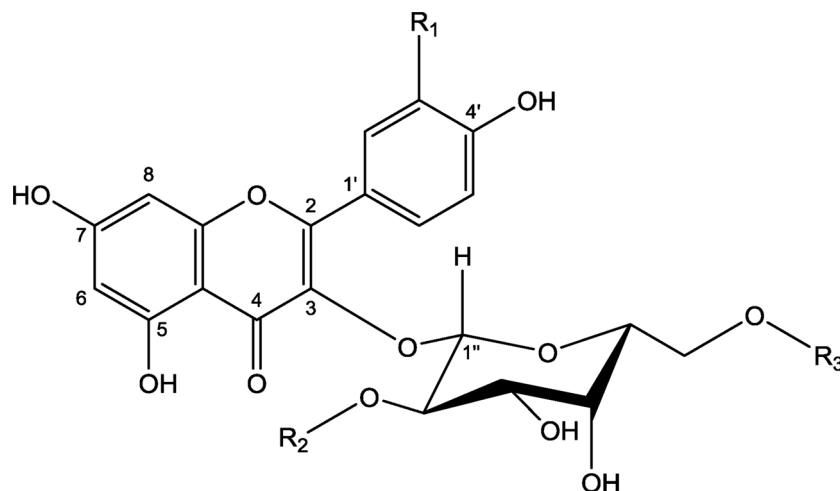


Fig. 2. Chemical structures of compounds 2–5. 2: $R_1 = H$, $R_2 = \alpha$ -L-rhamnose, $R_3 = \alpha$ -L-rhamnose. 3: $R_1 = H$, $R_2 = \alpha$ -L-rhamnose, $R_3 = H$. 4: $R_1 = H$, $R_2 = \beta$ -D-apiose, $R_3 = H$. 5: $R_1 = OH$, $R_2 = \beta$ -D-apiose, $R_3 = H$.

Simone et al., 1990), and quercetin-3-O- β -apiofuranosyl-(1 \rightarrow 2)- β -galactopyranoside (5) (Hamburger et al., 1985). Along with the well-known Mauritanin (2), found previously in *A. sieberi* (Abd El-Mawla and Attia, 2002), *A. armantus* (Khalfallah et al., 2014) and *A. monspessulanus* (Krasteva et al., 2015), this paper for the first time reveals presence of three other natural molecules (3–5) in a plant from genus *Astragalus* (Fig. 2).

Interestingly, compound 1, which is a kaempferol analogue, appears to have been resulted from conjugation with 3-hydroxy-3-methylglutaric acid (HMG), which itself is rarely found in the plant kingdom with fewer than 50 such compounds isolated to date. According to Porter et al. (2012), HMG-substituted flavonoid glycosides were once considered to be chemotaxonomic markers of genus *Rosa* (Rosaceae), but they have also been found in *Astragalus* species such as *A. caprinus* (Semmar et al., 2002), *A. gombiformis* (Montoro et al., 2013), *A. monspessulanus* (Krasteva et al., 2015), and *A. spruneri* (Shkondrov et al., 2018), indicating that HMG-substituted flavonoids may be chemomarkers for the genus *Astragalus* as well. In addition, HMG-substituted flavonoids could be responsible for the bioactivity of *Astragalus* extracts and studying the pharmacological uses of species rich in these flavonoids should be considered.

3. Materials and methods

3.1. General experimental procedures

Optical rotation was measured on a JASCO P-2000 spectropolarimeter (Easton, MD, USA) at 20 °C in MeOH and with Spectramanager software. HPLC was used with a configuration of the Agilent 1100 Series analytical system (Degasser G1322A, Quaternary Pump G1311A, Autosampler ALS G1313A, Column Compartment G1316, DAD G1315B, Loop 20 μ L, UV spectrum 200–900 nm) employing a Kinetex PFP 100 A, 250 \times 4.6 mm i.d., 5 μ m (Phenomenex, CA, USA) column, flow rate: 1 mL/min. Semi-preparative HPLC was carried out using a Dionex UltiMate 3000 system (Pump Dionex UltiMate 3000 UPLC + Focused, Dionex UltiMate 3000 RS Variable Wavelength Detector, fraction collector Dionex UltiMate 3000 with 6 positions, LCO 101 ECOM column oven, constant temperature of 40 °C, autosampler Dionex UltiMate 3000, loop 100 μ L), column: Ascentis RP-AMIDE, 250 mm \times 10 mm, 5 μ m (Supelco, PA, USA), flow rate 5 mL/min. TLC was carried out on precoated silica gel plates (Supelco Kieselgel G, F254, 60, Merck, Darmstadt, Germany) with the solvent systems EtOAc-MeOH-H₂O (100:13.5:10, v/v/v). Spots were visualized under UV light (366 nm) after spraying with NTS/PEG reagent. Column chromatography (CC)

was performed using Diaion HP-20 (Supelco), \varnothing = 80 mm, height 70 cm \sim 700 g and Silica gel (40–63 μ m, Sigma-Aldrich, MO, USA) \varnothing = 35 mm, height 60 cm.

NMR spectra were recorded using an Agilent DD2 600 MHz NMR with 1D and 2D pulse sequences to produce ¹H, ¹³C, ¹H-¹H COSY, HMBC and HSQC, TOCSY, and NOESY spectra. The spectra were further processed with the software MestReNova 12.0. HRESIMS spectra were recorded using an Exactive Orbitrap GC-MS system (ThermoFisher-Scientific, Inc., Bremen, Germany) operating at 70 eV, ion source temperature 230 °C, interface temperature 280 °C, coupled with a UPLC Dionex Ultimate 3000 RSLC system (Thermo Scientific), equipped with an RP-18 Kinetex column (2.1 \times 100 mm, 2.6 μ m, Phenomenex, Torrance, CA, USA). The software Qual Browser; Thermo Xcalibur™ 3.1.66.10 was used for evaluation (Figs. 1 and 2).

3.2. Plant material

Aerial parts of *A. thracicus* Griseb. (syn. *Astracantha thracica* (Griseb.) Podl.) were collected in June 2015 from the habitat, located on the Bakadzhitsite hills, close to Yambol, Bulgaria (Google Maps coordinates: 42.452025 N, 26.662144 E) and identified by Hristo Vasilev. A voucher specimen has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (SOM) in Sofia with Ref No. SOM001363. The voucher specimen is shown in the supplementary materials (Fig. S1).

3.3. Extraction and isolation

The air-dried and powdered aerial parts (1100 g) of *A. thracicus* were extracted under reflux with 80 % MeOH (5 \times 2 L, 50 min each) at 70 °C. After the combined extracts were concentrated under vacuum and dried, the residue (72.5 g) was re-suspended in H₂O (400 mL) and partitioned by liquid/liquid extraction with dichloromethane (200 mL \times 5) resulting in 9 g of CH₂Cl₂ fraction and 63.5 g of total MeOH extract. The lipid-free methanol extract was put through a Diaion HP-20 column and eluted with the step gradient system H₂O/MeOH (100:0 to 0:100, v/v) to yield 9 major fractions, assigned alphabetically. Fraction C (4 g, eluted with 30 % MeOH) was further fractionated via CC (silica gel, CHCl₃-MeOH-H₂O (v/v/v, 90:10:1 to 60:40:4), resulting in 6 fractions C₁–C₆. Repeated CC of fraction C₂ under the same conditions (silica gel, CHCl₃-MeOH-H₂O 90:10:1 to 60:40:4, v/v/v) gave 5 subfractions C_{2A}–C_{2E}. Subfraction C_{2C} was subjected to purification by semi-preparative HPLC (acetonitrile-H₂O; 21–26 % for 25 min) to yield compound 4 (5.8 mg). After purification by semi-prep HPLC (acetonitrile-H₂O 19–25 % 25

min) fraction C_{2D} yielded compound **3** (8.2 mg). Fraction C₃ was applied directly into semi-preparative HPLC (acetonitrile-H₂O 23–23.5, 25 min) to provide compound **5** (2.6 mg). Fraction C₅ was subjected to purification by semi-preparative HPLC (acetonitrile-H₂O; 19–25 % for 25 min), resulting in compound **1** (5.1 mg). Fraction C₆ was applied directly into semi-preparative HPLC separation (23–24.5 % for 25 min) to obtain fraction C₆₁ (25 mg). Fraction C₆₁ was further purified by a preparative TLC on a silica plate, than was then developed using a CH₃Cl-MeOH-H₂O 80:20:2, v/v/v system. After UV detection at $\lambda = 254$ nm to locate the bands, and the use of MeOH as a recovery solvent, compound **2** (4 mg) was finally obtained. Fig. S2 in the supplementary documents shows a detailed depiction of the isolation process.

3.4. Acid hydrolysis

A small quantity of each of the compounds obtained was treated with 2 M HCl at 95 °C for 1 h. The hydrolysates were separated into aglycones and sugars via SPE on C-18 cartridges (1 g/3 mL, Sigma-Aldrich), aglycones were eluted with 3 mL MeOH. The identities of the sugars were confirmed in the aqueous residue by comparison with reference standards using TLC (EtOH-NH₃-H₂O, 80:4:16, v/v/v) on silica gel plates (Kieselgel 60, F₂₅₄ plates 10 × 20 cm, 0.2 mm, Merck, Germany). The plates were sprayed with aniline phthalate reagent (0.1 M in *n*-butanol/water) and subsequently visualized by heating for 10 min at 110 °C.

CRedit authorship contribution statement

Hristo Vasilev: Data curation, Funding acquisition, Investigation, Writing - original draft. **Karel Šmejkal:** Data curation, Funding acquisition, Writing - original draft. **Christian Schulze Gronover:** Funding acquisition, Methodology. **Young Hae Choi:** Formal analysis, Writing - original draft. **Dirk Prüfer:** Formal analysis, Funding acquisition, Methodology. **Dagmar Jankovská:** Formal analysis, Investigation. **Iliana Ionkova:** Conceptualization, Investigation.

Declaration of competing interest

The authors declare that they have no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.phytol.2020.11.012>.

References

- Abd El-Mawla, A.M., Attia, A.A., 2002. Production of flavonoids in cell cultures of *Astragalus sieberi* DC. Bull. Pharm. Sci. Assiut. Univ. 25, 79–83.
- Assyov, B., Petrova, A., Dimitrov, D., Vassilev, R., 2006. In: Assyov, B., Petrova, A. (Eds.), Conspectus of the Bulgarian Vascular Flora, 3rd ed. Sofia.
- Bratkov, V.M., Shkondrov, A.M., Zdraveva, P.K., Krasteva, I.N., 2016. Flavonoids from the genus *Astragalus*: phytochemistry and biological activity. Pharmacogn. Rev. 10 (19), 11–32.
- De Simone, F., Dini, A., Pizza, C., Satunino, P., Schettino, O., 1990. Two flavonol glycosides from *Chenopodium quinoa*. Phytochemistry 29 (11), 3690–3692.
- Prodin, D.G., 2004. History and concepts of big plant genera. Taxon 53 (3), 753–776.
- Hamburger, M., Gupta, M., Hostettmann, K., 1985. Flavonol glycosides from *Securidaca diversifolia*. Phytochemistry 24 (11), 2689–2692.
- Ionkova, I., Shkondrov, A., Krasteva, I., Ionkov, T., 2014. Recent progress in phytochemistry, pharmacology and biotechnology of *Astragalus* saponins. Phytochem. Rev. 13 (2), 343–374.
- Khalfallah, A., Karioti, A., Berrehal, D., et al., 2014. A new flavonol triglycoside and other flavonol glycosides from *Astragalus armatus* willd. (Fabaceae). Rec. Nat. Prod. 8 (1), 12–18.
- Krasteva, I., Bratkov, V., Bucar, F., Kunert, O., Kollroser, M., Kondeva-Burdina, M., Ionkova, I., 2015. Flavonoids and flavonoids from *Astragalus monspessulanus*. J. Nat. Prod. 78 (11), 2565–2571.
- Montoro, P., Teyeb, H., Masullo, M., Mari, A., Douki, W., Piacente, S., 2013. LC-ESI-MS qualitative-quantitative determination of phenolic constituents in different parts of wild and cultivated *Astragalus gombiformis*. J. Pharm. Biomed. Anal. 72, 89–98.
- Petrova, A., Vladimirov, V., 2009. Red list of Bulgarian vascular plants. Phytol. Balcanica 1 (15), 63–94.
- Pistelli, L.F., 2002. Secondary metabolites of genus *Astragalus*: structure and biological activity. Stud. Nat. Prod. Chem. 27 (PART H), 443–545.
- Porter, E.A., Van Den Bos, A.A., Kite, G.C., Veitch, N.C., Simmonds, M.S.J., 2012. Flavonol glycosides acylated with 3-hydroxy-3-methylglutaric acid as systematic characters in *Rosa*. Phytochemistry 81, 90–96.
- Semmar, N., Fenet, B., Gluchoff-Fiasson, K., Hasan, A., Jay, M., 2002. Four new flavonol glycosides from the leaves of *Astragalus caprinus*. J. Nat. Prod. 65 (4), 576–579.
- Shkondrov, A., Krasteva, I., Bucar, F., Kunert, O., Kondeva-Burdina, M., Ionkova, I., 2018. Flavonoids and saponins from two Bulgarian *Astragalus* species and their neuroprotective activity. Phytochem. Lett. 26, 44–49.
- Stoeva, M., 2020. "Red Data Book of the Republic of Bulgaria - Digital Edition." *Astracantha Thracica*. n.d..
- Tutin, T.G., Heywood, V.H., Burges, N.A., Mooze, D.M., Valeutine, D.H., Walters, S.M., Webb, D.A., 1972. Flora Europaea (2). Cambridge University Press, Cambridge.
- Yasukawa, K., Takido, M., 1987. A flavonol glycoside from *Lysimachia mauritiana*. Phytochemistry 26 (4), 1224–1226.