HIGH RESOLUTION LC-MS/MS SCREENING FOR SECONDARY METABOLITES IN BULGARIAN SPECIES OF GENUS Astragalus L.

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The phytochemical content of some Astragalus species distributed in Bulgarian flora has been previously studied. Among other compounds, flavoalkaloids, acylated flavonoids, flavonoid triglycosides and cycloartane saponins have been isolated so far. The composition of the rest of the representatives of this genus in Bulgaria is not explored yet. The aim of this study was to perform a screening for the presence of selected rare secondary metabolites (flavoalkaloids, acylated flavonoids, flavonoid triglycosides and cycloartane saponins) in selected Astragalus species. Samples were collected in different phenological stages and from different locations in the country. A novel and rapid ultra-high performance liquid chromatography – high resolution electrospray ionisation mass spectrometry (UHPLC-HRESIMS) method was developed and applied. For the first time a flavoalkaloid glycoside was determined from extracts of A. onobrychis var. chlorocarpus and A. glycyphylloides. From A. depressus an acylated derivative of kaempferol was newly identified. The flavonol triglycosides camelliaside A, alcesefoliside and mauritianin were proved in samples of A. glycyphylloides, A. onobrychis var. chlorocarpus and A. cicer for the first time as well.

Keywords: Astragalus species; flavoalkaloids; flavonoids; saponins; qualitative analysis; UHPLC-HRESIMS.

INTRODUCTION

Genus Astragalus L. (Fabaceae) is comprised of more than 3500 species, distributed in every continent except Antarctica.1 In Europe the genus is present with 135 species, 31 of which are distributed in Bulgaria.2,3 The plants have been known to accumulate mainly three groups of pharmacologically active compounds – polysaccharides, flavonoids and saponins.4

Recently, N-(8-methylquercetin-3-O-[α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranosyl]-3-hydroxy-piperidin-2-one and N-(8-methylkaempferol-3-O-[α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranosyl]-3-hydroxy-piperidin-2-one were isolated from A. mnspsssulanus subsp. mnspsssulanus.5 Flavoalkaloids are a rare group of plant secondary metabolites, known previously only as aglycones.6 This leads to a further investigation of possible accumulation of flavoalkaloids in other representatives of the genus.

Long considered a chemotaxonomical marker of genera Rosa and Plathyodon (Rosaceae), flavonoids acylated with 3-hydroxy-3-methylglutaric acid were also isolated from Astragalus species.7,8 Quercetin-3-O-α-L-rhamnopyranosyl-(1→2)-[6-O-(3-hydroxy-3-methylglutaril)-β-D-galactopyranoside and kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-[6-O-(3-hydroxy-3-methylglutaril)-β-D-galactopyranoside were obtained from the overground parts of A. mnspsssulanus subsp. illyricus.9 This presents a new opportunity to establish the chemotaxonomical significance of these acylated flavonoids for the genus.

Flavonoid triglycosides occur quite rarely and their biosynthesis is undoubtedly a result of interconnecting pathways.9 Quercetin-3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (alcesefoliside) and kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (mauritianin) were isolated from the aerial parts of A. mnspsssulanus subsp. mnspsssulanus.5

Kaempferol-3-O-[2-O-β-D-galactopyranosyl-6-O-α-L-rhamnopyranosyl]-β-D-glucopyranoside (camelliaside A) was isolated from the herbs of A. glycyphyllos.10

Studies of Bulgarian Astragalus species showed that they have an intermediate content – some contain pentacyclic triterpenoid saponins, while in other species the accumulation of cycloartane saponins was proved.10,11 Previously, 17(R),20(R)-3β,6α,16β-trihydroxy-cycloartanyl-23-carboxilic acid 16-lactone 3-O-β-D-glucopyranoside was isolated from the herbs of A. glycyphyllos.11 From a taxonomical point of view the saponin content of other species is of particular interest.12

The aim of this study was to conduct an UHPLC-HRESIMS screening for the presence of selected rare secondary metabolites in species of genus Astragalus occurring in Bulgaria

EXPERIMENTAL

Plant material

The overground parts of seven Astragalus species (Table 1) were collected either in flowering or in fructification, from marked plants and/or from different localities in Bulgaria. The species were identified by Dr. D. Pavlova from Faculty of Biology, Sofia University, Bulgaria and two of us (A. S. and I. K.). Voucher specimens were deposited in the Herbarium of the Sofia University (SO) or at the Herbarium of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (SOM).

Extraction

Overground parts were dried at room temperature and then individually reduced to a powder. A sample of each (200 mg) was refluxed twice with 3 mL 80% MeOH on a boiling water bath (100 °C) for 30 min each. The extracts obtained were filtered, combined in a volumetric flask and the volume adjusted to 10.0 mL with the same solvent. An aliquot of 2 µL was injected to UHPLC.
Ultra high performance liquid chromatography-high resolution electrospray ionization mass spectrometry (UHPLC-HRESIMS)

A Q Exactive™ Plus Orbitrap mass spectrometer with a heated electrospray ionisation (HESI) ion source (ThermoFisher Scientific, Bremen, Germany) coupled with a UHPLC system ( Dionex UltiMate 3000 RSLC, ThermoFisher Scientific, Bremen, Germany) was used. The full scan MS was set at: resolution 70000 (at m/z 200), AGC target 3e 6, max IT 100 ms, scan range 250 to 1700 m/z. The MS2 conditions were: resolution 17500 (at m/z 200), AGC target 1e 5, max IT 50 ms, mass range m/z 200 to 2000, isolation window 2.0 m/z and (N)CE 20. The ionization device (HESI source) was operating at: +3.5 or -2.5 kV spray voltage and 320 °C capillary and probe temperature, 38 arbitrary units (a.u., as set by the Extactive Tune software) of sheath gas and 12 a.u. of auxiliary gas (both Nitrogen); S-Lens RF level 50.0. UHPLC separations were performed using a Kromasil © C18 column (1.9 μm, 2.1 x 50 mm, Akzo Nobel, Sweden) maintained at 40 °C; mobile phase H2O added 0.1% HCOOH (A) and MeCN added 0.1% HCOOH (B) with a flow rate of 0.3 mL/min and gradient elution (10% B for 0.5 min, then increase to 30% B for 7 min, isocratic with 30% B for 1.5 min, increase to 95% B for 3.5 min, isocratic with 95% B for 2 min, then return to 10% B for 0.1 min).

Reference substances

The structures of the reference substances are shown in Figure 1 and the chemical names are given in Table 2. They were isolated from the plant source (purity more than 99 %, HPLC) and their structures were confirmed by extensive spectral analyses and comparison to data reported before.5,11 Rutin (99.8% purity) was purchased from Sigma Aldrich. Standard solutions of each reference substance were prepared in MeOH (100 ng mL). Two μL of each solution were injected in the UHPLC-HRESIMS system three times.

Detection

Detection of the compounds in the samples was based on the full scan chromatograms in both positive and negative mode, considering the retention time and of the fragmentation pattern with that of the standard in both positive and negative modes.

Software

The software Xcalibur®, Version 4.2 (Thermo Scientific) was used to collect raw data and to process the results.

RESULTS AND DISCUSSION

Method development

Liquid chromatography coupled with mass spectrometry is considered to be one of the most accurate methods to identify multiple compounds in complex samples.15 A novel UHPLC-HRESIMS method was developed for determination of selected secondary metabolites in plant extracts. The method was rapid and efficient. As recommended by the The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) the validation was performed on several parameters.16 Specificity against each reference substance was examined on blank solutions. There were no peaks in the chromatogram of the blank solution with retention time (Rt) similar to any of the reference compounds (Table 2). The limit of detection, based on three times the signal-to-noise ratio, was calculated for each reference substance (Table 2) by injecting 2 μL of each standard solution three times. The repeatability (SD %) on six solutions containing rutin was ± 1.1%.

Rutin, as a well-known flavonoid was initially used to develop the method.10 An ion [M-H]- (m/z 609.1470, C15H20O16 - ) and in MS3 ions with m/z 300.0280 (C9H12O7 - , [Que-H]+) and with m/z 301.0349 (C10H17O7 - , [Que-H]+) were observed, while in the positive mode a precursor ion [M+H]+ with m/z 611.1605 (C15H20O16 +) and in the MS2 a corresponding ion with m/z 303.0496 (C9H12O7 +) for [Que+H]+ were registered.10 The flavonoid was identified in all extracts except those of A. depressus and A. glycyphylloides (Samples 3 and 4, Table 3). These findings are with accordance with previous results.15–17 Moreover, the results of rutin content and their coincidence with the literature prove the accuracy of the screening method presented.

Mass spectral analysis of reference substances

The mass fragmentation of the standards was investigated and the experimental findings are presented in Table 2. The principal fragmentation patterns of flavoalkaloids and flavonoids are shown on Figure 2, except for rutin, since its fragmentation was previously described.10 The fragmentation of ECAS is presented in Figure 3.

Table 1. Samples of Bulgarian Astragalus species

| Sample No. | Overground parts of | Year | Locality; coordinates | Voucher specimen | Phenological stage |
|------------|---------------------|------|-----------------------|-----------------|-------------------|
| 1          | A. cicer           | 2013 | Sofia; 42°41’52”N, 23°19’19”E | SO 102681       | flowering         |
| 2          | A. cicer           | 2016 | Sofia; 42°41’52”N, 23°19’19”E | SOM 1394        | fructification    |
| 3          | A. depressus       | 2015 | Erma; 42°48’45”N, 22°34’52”E | SOM 1402        | flowering         |
| 4          | A. glycyphylloides | 2018 | Vitosha; 42°33’36”N, 23°16’48”E | SO 093817       | flowering         |
| 5          | A. glycyphyllos    | 2019 | Rila; 42°7’40”N, 23°7’56”E | SO 107612       | flowering         |
| 6          | A. glycyphyllos    | 2012 | Vitosha; 42°33’36”N, 23°16’48”E | SO 107613       | flowering         |
| 7          | A. monspessulanus subsp. monspessulanus | 2016 | Devin; 41°43’41”N, 24°26’59”E | SOM 1391        | flowering         |
| 8          | A. monspessulanus subsp. monspessulanus | 2016 | Slavianka; 41°33’57”N, 24°44’41”E | SOM 1392        | flowering         |
| 9          | A. monspessulanus subsp. illyricus | 2015 | Erma; 42°48’45”N, 22°34’52”E | SOM 107532      | flowering         |
| 10         | A. onobrychis var. chlorocarpus | 2013 | Stara Zagora; 42°23’37”N, 25°38’46”E | SOM 107538      | flowering         |
| 11         | A. onobrychis var. chlorocarpus | 2016 | Golo Bardo; 42°35’17”N, 23°3’2”E | SOM 1393        | flowering         |
| 12         | A. onobrychis var. chlorocarpus | 2016 | Vitosha; 42°33’36”N, 23°16’48”E | SOM 1390        | flowering         |
| 13         | A. ponticus        | 2013 | Pleven; 43°24’32”N, 24°37’4”E | SOM 107539      | flowering         |
In the negative MS² spectrum of all flavonol glycosides described above, a fragment ion with m/z 151 (C₉H₁₄O₄) and in the positive MS² spectrum a fragment ion with m/z 153 (C₁₀H₁₆O₆), both due to retro-Diels-Alder (RDA) fragmentation of their aglycone (α,β-A or β,α-A) were observed.20 This was not registered for the flavoalkaloids investigated.

In the mass spectrum of QueFA, a deprotonated ion [M-H]⁻ m/z 882.2692 (C₃₉H₄₈NO₂₁) with low abundance was observed. The fragmentation led to an ion with m/z 315.0505 (C₁₆H₁₁O₇ fragment ion with m/z hydroxypiperidine-2-one moiety.21–23 A protonated ion [M+H]⁺ [methylKae-H]⁻ with m/z of this adduct the most abundant was the [M-H]⁻ ion (Figure 2 and Table 2).24 A deprotonated molecular ion ([M-H]⁻) was observed (theoretically, m/z 866, C₃₉H₄₈NO₂₁). In the spectrum of QueFA, no deprotonated molecular ion [M]⁻ was observed (theoretically, m/z 866, C₃₉H₄₈NO₂₁), but a stable and abundant ion with m/z 901.2642, corresponding to [M+Cl]⁻ (C₃₉H₄₈NO₂₁Cl⁻) was found (Table 2). In the MS² spectrum of this adduct the most abundant was the [M-H]⁻ ion (m/z 866.2718, C₃₉H₄₈NO₂₁). The aglycone – a deprotonated methylkaempferol [methylKae-H]⁻ with m/z 299.4627 (C₁₅H₁₁O₇, also formed after cleavage of the hydroxypiperidine-2-one moiety) was registered as well.24 A precursor ion [M-H]⁻ (m/z 868.2857, C₃₉H₄₈NO₂₁) was observed in positive ionization mode. Its fragmentation led to the formation of [methylKae-H]⁺ (m/z 301.0711, C₁₅H₁₁O₇) as shown in Figure 2 and Table 2).24 A deprotonated molecular ion (m/z 753.1893, C₃₃H₃₉O₂₀) was observed in the spectrum of QueHMG and after cleavage of the 3-hydroxy-3-methylglutaric residue (HMG), an ion corresponding to deprotonated rutin (m/z 609.1462, C₁₅H₁₄O₇), a fragment ion with m/z 301.0347 (C₉H₁₄O₄) [Que-H⁻]), and an ion of the type [M-(rha-gal-HMG)-H]⁻ (m/z 300.0278, C₈H₁₂O₇), most abundant in MS²) were registered.21–23 A precursor ion [M-H]⁻ (m/z 755.2020, C₃₃H₃₉O₂₀) and an ion with m/z 303.0494 (C₁₃H₁₀O₇) were observed in the positive polarity (see Figure 2 and Table 2).25 In the negative mode in the spectrum of KaeHMG an ion [M-H]⁻ (m/z 739.2072 (C₁₅H₁₀O₇), an ion with m/z 593.1515 (C₁₅H₁₀O₇, after HMG cleavage) were recorded. Characteristic ions of the deprotonated aglycone (m/z 285.0398, C₉H₁₄O₄) and [Kae-H]⁻ with m/z 284.0327 (C₁₅H₁₀O₇) were observed.10,22,23 In the positive mode, a precursor ion [M+H]⁺ with m/z 739.2072 (C₁₅H₁₀O₇), an ion with m/z 593.1515 (C₁₅H₁₀O₇, [Kae-rha-gal+H]⁺, after HMG elimination), and a fragment ion with m/z 287.0545 (C₉H₁₀O₇, [Kae+H]⁺) were observed (see Figure 2 and Table 2).24 In the spectrum of camelliaside A an ion [M-H]⁻ (m/z 755.2035, C₃₃H₃₉O₂₀) was determined and. An ion of [Kae-H], m/z 285.0399 (C₉H₁₄O₄) and [Kae-H]⁻ with m/z 284.0328 (C₁₅H₁₀O₇) were detected.11 A precursor ion [M+H]⁺ with m/z 757.2191 (C₁₅H₁₀O₇) was observed in positive polarity, which gave a fragment ion for [Kae+H]⁺ with m/z 287.0551, C₉H₁₀O₇ (see Figure 2 and Table 2).25 In the spectrum of acesefoliside an ion [M-H]⁻ (m/z 755.2045, C₁₅H₁₀O₇) was identified. The ions [Que-H]⁻ with m/z 300.0279 (C₁₅H₁₀O₇) and [Que-H] with m/z 301.0328 (C₁₅H₁₀O₇) distinguished the compound from camelliaside A, which had similar retention time. A precursor ion [M+H]⁺ (m/z 757.2191, C₁₅H₁₀O₇) was observed, as well in MS² the aglycone with m/z 303.0499 (C₁₃H₁₀O₇), [Que+H]⁺ (see Figure 2 and Table 2).26 In the negative mode in the spectrum of mauritianin an ion [M-H]⁺ (m/z 739.2100, C₁₅H₁₀O₇) and in the MS² ions [Kae-H]⁺ with m/z 284.0329 (C₁₅H₁₀O₇) and [Kae-H] with m/z 285.0393 (C₁₅H₁₀O₇) were recorded.22–24 In the positive mode a precursor ion [M+H]⁺ (m/z 741.2245, C₁₅H₁₀O₇), and in the MS² an ion with m/z 287.0549 (C₁₅H₁₀O₇), corresponding to [Kae+H]⁺ were found (see Figure 2 and Table 2).24

In the negative ionization mode, 17(R),20(R)-3β,6α,16β-trihydroxyxycycloartanyl-23-carboxylic acid 16-lactone 3-O-β-D-glucopyranoside (ECAS) forms a formate adduct [M-H+HCOOH]⁻ with
Table 2. Standard substances and analytical parameters used to identify secondary metabolites in the samples

| Standard substance (abbreviation) | Rt ± SD, min | Exact mass; molecular formula | ESI (+) Precursor ion; MS² (relative abundance in %)* | LOD ± SD, ng/mL |
|----------------------------------|-------------|-------------------------------|---------------------------------------------------|----------------|
| N-(8-methylquercetin-3-O-[α-L-rhamnopyranosyl-[(1→2)-[α-L-rhamnopyranosyl-[(1→6)]-β-D-galactopyranosyl]3-hydroxypiperidin-2-one (QuEFA)] | 2.47 ± 0.015 | 883.2695; C₃₂H₅₁O₉ | 882.2692 C₁₃H₂₀NO₆ (100) | 0.18 ± 0.01 |
| N-(8-methylkaempferol-3-O-[α-L-rhamnopyranosyl-[(1→2)-[α-L-rhamnopyranosyl-[(1→6)]-β-D-galactopyranosyl]3-hydroxypiperidin-2-one (KaeFA)] | 3.00 ± 0.010 | 867.2747; C₃₂H₅₁O₉ | 901.2642 C₁₃H₂₀NO₆ (100) | 0.17 ± 0.02 |
| Quercetin-3-O-[α-L-rhamnopyranosyl-[(1→2)-[6-O-(3-hydroxy-3-methylglutaryl)-β-D-galactopyranoside (QueHMG)] | 5.79 ± 0.010 | 754.1956; C₁₅H₂₀O₇ | 753.1893 C₁₃H₂₀O₇ (100) | 0.18 ± 0.02 |
| Kaempferol-3-O-[α-L-rhamnopyranosyl-[(1→2)-[6-O-(3-hydroxy-3-methylglutaryl)-β-D-galactopyranoside (KaeHMG)] | 6.21 ± 0.010 | 738.2007; C₁₅H₂₀O₇ | 737.1942 C₁₃H₂₀O₇ (100) | 0.17 ± 0.02 |
| Kaempferol-3-O-[2,0-β-D-galactopyranosyl-6-O-α-L-rhamnopyranosyl-β-D-glucopyranoside (camelliaside A, CamA)] | 4.99 ± 0.010 | 756.2112; C₁₅H₂₀O₇ | 755.2035 C₁₃H₂₀O₇ (100) | 0.19 ± 0.01 |
| Quercetin-3-O-[α-L-rhamnopyranosyl-[(1→2)-[α-L-rhamnopyranosyl-[(1→6)]-β-D-galactopyranoside (alicafolioside, Ale)] | 4.94 ± 0.010 | 756.2114; C₁₅H₂₀O₇ | 755.2045 C₁₃H₂₀O₇ (100) | 0.16 ± 0.03 |
| Kaempferol-3-O-[α-L-rhamnopyranosyl-[(1→2)-[α-L-rhamnopyranosyl-[(1→6)]-β-D-galactopyranoside (mauritianin, Maur)] | 5.37 ± 0.020 | 740.2164; C₁₅H₂₀O₇ | 739.2100 C₁₃H₂₀O₇ (100) | 0.17 ± 0.03 |
| Quercetin-3-O-[α-L-rhamnopyranosyl-[(1→6)]-β-D-glucopyranoside (Rutin)] | 5.69 ± 0.010 | 610.1533; C₁₃H₂₀O₇ | 609.1470 C₁₁H₁₈O₆ (100) | 0.14 ± 0.01 |
| 17(R),(20(R)-3)f; 6,16β-trihydroxyxycloartan-23-carboxylic 16-lactone-3-O-β-D-glucopyranoside (ECAS)] | 10.27 ± 0.010 | 578.3526; C₁₃H₂₀O₇ | 579.3531 C₁₁H₁₈O₆ (100) | 0.19 ± 0.01 |

*Fragment ions in MS² are in order of their m/zs.

Identification of compounds in the samples

The difference in retention times of the standards (Table 2) and the compounds in the samples was less than 0.05 min (mean value of three injections) and considered acceptable. The TIC-FS chromatograms of each sample are in Supplementary. QuEFA was identified in extracts from A. monspessulanus subsp. monspessulanus, collected both from Devin (Sample 7) and from Slavianka (Sample 8). This finding corroborates the presence of this flavonoid in the species (Table 3). No other of the species examined accumulated the...
flavoalkaloid. KaeFA was discovered in extracts from A. onobrychis var. chlorocarpus (Samples 10, 11 and 12) as well as in extract from the herbs of A. glycyphylloides (Sample 4). This is the first report of the kaempferol flavoalkaloid presence in species other than A. monspessulanus subsp. monspessulanus. Analysis of extracts from the latter (Samples 7 and 8, Table 3) confirmed its accumulation and coincides with previous reports.5

The presence of QueHMG and KaeHMG was confirmed in the sample of A. monspessulanus subsp. illyricus (Sample 9, Table 3) and consistent with previous results.5 No other of the examined species accumulated QueHMG (Table 3), while for the first time KaeHMG was identified in the extract of A. depressus (Sample 3, Table 3).

Camelliaside A was discovered in samples of A. glycyphylloides (Sample 4) and A. depressus (Sample 3). Alcesefoliside was discovered in samples of A. cicer (Samples 1 and 2), A. glycyphylloides (Sample 4) and A. onobrychis var. chlorocarpus (Samples 10, 11 and
Table 3. Secondary metabolites* identified in the samples

| Sample No. | Species                  | QueFA | KaeFA | QueHMG | KaeHMG | CamA | Alce | Maur | Rutin | ECAS |
|------------|--------------------------|-------|-------|--------|--------|------|------|------|-------|------|
| 1          | A. cicer                 | -     | -     | -      | -      | +    | -    | -    | -     | -    |
| 2          | A. cicer                 | -     | -     | -      | -      | +    | +    | +    | -     | -    |
| 3          | A. depressus             | -     | -     | -      | -      | -    | +    | -    | -     | -    |
| 4          | A. glycyphiloides        | -     | +     | -      | +      | +    | +    | -    | -     | -    |
| 5          | A. glycyphyllos          | -     | -     | -      | -      | -    | +    | +    | +     | -    |
| 6          | A. glycyphyllos          | -     | -     | -      | -      | +    | -    | +    | +     | -    |
| 7          | A. monspessulanus subsp. | +     | +     | -      | +      | +    | +    | +    | +     | +    |
| 8          | A. monspessulanus subsp. | +     | +     | -      | +      | +    | +    | +    | +     | +    |
| 9          | A. monspessulanus subsp. | -     | -     | +      | -      | +    | +    | +    | +     | -    |
| 10         | A. onobrychis var.       | -     | -     | +      | +      | +    | +    | +    | +     | +    |
| 11         | A. onobrychis var.       | -     | -     | +      | +      | +    | +    | +    | +     | +    |
| 12         | A. onobrychis var.       | -     | +     | +      | +      | +    | +    | +    | +     | +    |
| 13         | A. ponticus             | -     | -     | -      | -      | -    | +    | -    | -     | -    |

ECAS [M+H]+ m/z 579.35 (C20H26O8); [M+H]+ m/z 577.35 (C20H24O7)

![Figure 3. Fragmentation of ECAS; abbreviation is as in Table 2](image)

12, Table 3). Analysis of the samples of A. monspessulanus (both subpecies, Samples 7, 8, 9, Table 3) confirmed the previously reported data.1 Mauritianin was identified in the aerial parts of A. glycyphiloideis (Sample 4) and A. onobrychis var. chlorocarpus (Samples 10, 11, 12 of Table 3), in A. cicer (Samples 1 and 2 Table 3) and in A. ponticus (Sample 13, Table 3). The results of the analysis of both subspecies of A. monspessulanus (monspessulanus, Samples 7 and 8, and illyricus, Sample 9, Table 3) coincide with previous reports.13

The presence of the epoxycycloartane saponin in the two samples of A. glycyphyllos confirmed a previous report.14 The ECAS was identified for the first time in all extracts of A. onobrychis var. chlorocarpus (Table 3).

CONCLUSIONS

A novel rapid UHPLC-HRESIMS method was applied to detect rare flavoalkaloids, acylated and highly glycosylated flavonoids as well as a cycloartane saponin in samples of Bulgarian Astragalus species. These results will serve as the basis for thorough phytochemical screening of all Bulgarian representatives of this genus.

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