Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 75, No 7, 2022

BIOLOGY

Botany

UHPLC-HRMS BASED SAPONIN AND FLAVONOID PROFILING OF *CHENOPODIUM BONUS-HENRICUS* L. ROOTS AND PROLIPASE ACTIVITY OF THE MAIN SAPONINS

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Received on July 17, 2020
Presented by B. Petrunov, Member of BAS, on September 29, 2020

Abstract

A UHPLC-HRMS profiling method was used for a comprehensive study of flavonoid and saponin-rich fractions from the MeOH extract of *C. bonus-henricus* L. roots. Thirty-one compounds, respectively, **16** saponins of five sapogenins (oleanoic acid, 2β-hydroxyoleanoic acid, medicagenic acid, bayogenin, and phytolaccagenin), together with **15** flavonoid glycosides of 6-methoxy-kaempferol, isorhamnetin, spinacetin, and patuletin were detected. Lipase modulatory activities of the main saponins were established by measuring the levels of the released 4-nitrophenol using LC-MS. All saponins exhibited prolipase activity and could find application in the treatment of cachexia.

Key words: *Chenopodium bonus-henricus*, UHPLC-HRMS, saponins, flavonoids, prolipase activity

Introduction. *Chenopodium bonus-henricus* L. (Amaranthaceae) is a perennial herbaceous plant which is spread in the mountainous regions of Bulgaria [1]. It has been recognized by Bulgarian legislation as a medicinal plant. In Bulgarian folk medicine the extracts of the roots have been used for the treatment of bronchitis, laryngitis, rheumatism, gout, constipation, dermatitis, and eczema. A

This work was supported by the Medicinal Science Council at the Medical University of Sofia (grant number D-75/2019).
DOI:10.7546/CRABS.2022.07.06

decoction of the roots of Good King Henry (also known as "chuven") is used in the food industry for the production of "tahin" and "white halva" [2].

Three flavonol glycosides [3] and six saponins [2] have been isolated from the roots of *C. bonus-henricus* L. The MeOH extract together with the isolated flavonoids and saponins of a title plant have exerted hepatoprotective and antioxidant activities comparable to those of silymarin in in vivo and in vitro models of CCl_4-induced liver damage, respectively [3,4]. The tested flavonoids also scavenged $DPPH^+$ and $ABTS^+$ free radicals and significantly inhibited the lipid peroxidation in a linoleic acid system by the ferric thiocyanate method [3]. Besides, the MeOH extract and saponins showed moderate or marginal cytotoxicity on five leukemic cell lines (HL-60, SKW-3, Jurkat E6-1, BV-173, and K-562) and stimulatory effects on interleukin-2 production in PHA/PMA stimulated Jurkat E6-1 cells [2]. Recently a UHPLC-HRMS based profiling of MeOH extract from the roots of *Chenopodium bonus-henricus* L. tentatively identified 15 saponins of six sapogenins [4]. The application of the roots of the title plant in Bulgarian folk medicine and the food industry stimulated us for further fractionation of MeOH extract and LC-MS profiling of saponin and flavonoid rich fractions. Lipase modulatory activities of the main saponins were established by measuring the levels of the released 4-nitrophenol using LC-MS.

Materials and methods. ***General.*** UHPLC-HRMS analysis was performed using a Thermo Scientific Dionex Ultimate 3000 RSLC (Germering, Germany) consisting of 6-channel degasser SRD-3600, high-pressure gradient pump HPG-3400RS, autosampler WPS-3000TRS, and column compartment TCC-3000RS coupled to a Thermo Scientific Q Exactive Plus (Bremen, Germany) mass spectrometer. UHPLC separations were performed on a Kromasil Eternity XT C18 column (AkzoNobel, Sweeden) (2.1×100 mm, 1.8 µm) equipped with precolumn SecurityGuard ULTRA UHPLC EVO C18 (Phenomenex, USA). The main saponins, 3-O-β-glucuronopyranosyl-medicagenic acid-28-β-xylopyranosyl(1→4)-α-rhamnopyranosyl(1→2)-α-arabinopyranosyl ester **S1**, Bonushenricoside A **S2**, 3-O-β-glucuronopyranosyl-bayogenin-28-O-β-glucopyranosyl ester **S3**, 3-O-L-α-arabinopyranosyl-bayogenin-28-O-β-glucopyranosyl ester **S4**, 3-O-β-glucuronopyranosyl-2β-hydroxygypsogenin-28-O-β-glucopyranosyl ester **S5**, Bonushenricoside B **S6** were previously isolated from the roots of *C. bonus-henricus* L. [2]. Lipase from porcine pancreas and *p*-nitrophenyl laurate were obtained from Sigma Aldrich. Orlistat was obtained from Alfa Aesar, while Tris buffer was purchased from Fisher Chemicals. All used reagents were of analytical grade.

Plant material. The roots of *Chenopodium bonus-henricus* L. were collected from Beglica, Western Rhodopes, Bulgaria in September 2017. The plant was identified by P. Nedialkov and a voucher specimen from the plant population (No. SOM-Co-169848) was deposited at the National Herbarium, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Extraction and fractionation. The roots of *C. bonus-henricus* were dried

Table 1

Characterization of flavonoids and saponins from the purified fractions of the roots of *C. bonus-henricus* L. in the negative and positive ion mode

No	t_R (min)	Identification	Ion mode	Found m/z	δ ppm	Composition	Fragment ions (m/z)	Ref.
1*	2.73	Patuletin-3-O-β-Glu(1→6)-β-Glu	$[M+H]^+$	657.1656	−0.84	$C_{28}H_{33}O_{18}$	495.1130 $[M+H\text{-Glu}]^+$ 333.0600 $[M+H\text{-Glu-Glu}]^+$	[7]
2*	3.17	6-Methoxykaempferol-3-O-[β-Api (1→2)]-β-Glu(1→6)-β-Glu	$[M+H]^+$	773.2119	−2.04	$C_{33}H_{41}O_{21}$	641.1721 $[M+H\text{-Api}]^+$; 479.1181 $[M+H\text{-Api-Glu}]^+$ 317.0654 $[M+H\text{-Api-Glu-Glu}]^+$	[3,7]
3	3.17	Isorhamnetin-Hex-Hex-Pent	$[M+H]^+$	773.2119	-2.04	$C_{33}H_{41}O_{21}$	641.1721 $[M+H\text{-Pent}]^+$; 479.1181 $[M+H\text{-Pent-Hex}]^+$ 317.0654 $[M+H\text{-Pent-Hex-Hex}]^+$	–
4*	3.28	Spinacetin-3-O-[β-Api (1→2)]-β-Glu (1→6)-β-Glu	$[M+H]^+$	803.2236	−0.52	$C_{34}H_{43}O_{22}$	671.1815 $[M+H\text{-Api}]^+$; 509.1289 $[M+H\text{-Api-Glu}]^+$ 347.0756 $[M+H\text{-Api-Glu-Glu}]^+$	[3,7]
5	3.52	Patuletin-Hex-Pent	$[M+H]^+$	627.1548	−1.25	$C_{27}H_{31}O_{17}$	495.1128 $[M+H\text{-Pent}]^+$ 333.0598 $[M+H\text{-Pent-Hex}]^+$	–
6*	3.67	6-Methoxykaempferol-3-O-β-Glu(1→6)-β-Glu	$[M+H]^+$	641.1705	−1.09	$C_{28}H_{33}O_{17}$	479.1173 $[M+H\text{-Glu}]^+$ 317.0655 $[M+H\text{-Glu-Glu}]^+$	[7]
7	3.67	Isorhamnetin-Hex-Hex	$[M+H]^+$	641.1705	−1.09	$C_{28}H_{33}O_{17}$	479.1173 $[M+H\text{-Hex}]^+$ 317.0655 $[M+H\text{-Hex-Hex}]^+$	–
8*	3.79	Spinacetin-3-O-β-Glu(1→6)-β-Glu	$[M+H]^+$	671.1820	+0.24	$C_{29}H_{35}O_{18}$	509.1296 $[M+H\text{-Glu}]^+$ 347.0756 $[M+H\text{-Glu-Glu}]^+$	[3,7]
9	3.96	6-Methoxykaempferol-Hex-Pent	$[M+H]^+$	611.1603	−0.53	$C_{27}H_{31}O_{16}$	479.1177 $[M+H\text{-Pent}]^+$ 317.0656 $[M+H\text{-Pent-Hex}]^+$	–
10	4.05	Spinacetin-Hex-Pent	$[M+H]^+$	641.1715	+0.35	$C_{28}H_{33}O_{17}$	509.1290 $[M+H\text{-Pent}]^+$ 347.0760 $[M+H\text{-Pent-Hex}]^+$	–

Table 1

Continued

No	t_R (min)	Identification	Ion mode	Found m/z	δ ppm	Composition	Fragment ions (m/z)	Ref.
11*	4.46	Spinacetin-3-O-(5‴-O--FA)-β-D-Api(1→2)[β-D-Glu (1→6)]-β-D-Glu	$[M+H]^+$	979.2695	−1.90	$C_{44}H_{51}O_{25}$	817.2170 $[M+H\text{-}Glu]^+$; 671.1804 $[M+H\text{-}FA\text{-}Api]^+$ 509.1120 $[M+H\text{-}FA\text{-}Api\text{-}Glu]^+$ 347.0753 $[M+H\text{-}FA\text{-}Api\text{-}Glu\text{-}Glu]^+$ 177.0544 $Feruloyl^+$	[7]
12	4.53	6-Methoxykaempferol-Hex	$[M+H]^+$	479.1181	−0.63	$C_{22}H_{23}O_{12}$	317.0654 $[M+H\text{-}Hex]^+$	–
13	4.53	Isorhamnetin-Hex	$[M+H]^+$	479.1181	−0.63	$C_{22}H_{23}O_{12}$	317.0654 $[M+H\text{-}Hex]^+$	–
14	4.55	Spinacetin-Hex	$[M+H]^+$	509.1284	−1.07	$C_{23}H_{25}O_{13}$	347.0757 $[M+H\text{-}Hex]^+$	-
15*	4.58	6-Methoxykaempferol-3-O-(5‴-O--FA)-β-D-Api(1→2)[β-D-Glu (1→6)]-β-D-Glu	$[M+H]^+$	949.2596	−1.25	$C_{43}H_{49}O_{24}$	787.2068 $[M+H\text{-}Glu]^+$; 641.1706 $[M+H\text{-}FA\text{-}Api]^+$ 479.1179 $[M+H\text{-}FA\text{-}Api\text{-}Glu]^+$ 317.0652 $[M+H\text{-}FA\text{-}Api\text{-}Glu\text{-}Glu]^+$ 177.0545 $Feruloyl^+$	[7]
16	6.74	Medicagenic acid-Hex-Pent	$[M+HCOO]^-$	841.4237	2.48	$C_{42}H_{65}O_{17}$	795.4210 $[M\text{-}H]^-$; 633.3641 $[M\text{-}H\text{-}Hex]^-$ 501.3244 $[M\text{-}H\text{-}Hex\text{-}Pent]^-$	–
17	7.04	Bayogenin-HexA-Hex-Hex	$[M+HCOO]^-$	1033.4872	2.10	$C_{49}H_{77}O_{23}$	987.4807 $[M\text{-}H]^-$; 825.4277 $[M\text{-}H\text{-}Hex]^-$ 663.3756 $[M\text{-}H\text{-}Hex\text{-}Hex]^-$ 487.3436 $[M\text{-}H\text{-}Hex\text{-}Hex\text{-}HexA]^-$	–
18	7.04	Phytolaccagenin-Hex-Hex-Pent	$[M+HCOO]^-$	1033.4872	2.10	$C_{49}H_{77}O_{23}$	987.4807 $[M\text{-}H]^-$; 825.4277 $[M\text{-}H\text{-}Hex]^-$ 663.3756 $[M\text{-}H\text{-}Hex\text{-}Hex]^-$ 531.3309 $[M\text{-}H\text{-}Hex\text{-}Hex\text{-}Pent]^-$	–
19	7.24	Phytolaccagenin-Hex-HexA	$[M\text{-}H]^-$	869.4181	1.78	$C_{43}H_{65}O_{18}$	707.3636 $[M\text{-}H\text{-}Hex]^-$ 531.3353 $[M\text{-}H\text{-}Hex\text{-}HexA]^-$	–

Table 1

Continued

No	t_R (min)	Identification	Ion mode	Found m/z	δ ppm	Composition	Fragment ions (m/z)	Ref.
20	8.26	2β-Hydroxyoleanoic acid-HexA-Hex-Hex	$[M-H]^-$	971.4860	1.38	$C_{48}H_{75}O_{20}$	809.4346 $[M-H-Hex]^-$ 647.3803 $[M-H-Hex-Hex]^-$ 471.3480 $[M-H-Hex-Hex-HexA]^-$	–
21	8.80	Bayogenin-Hex-Hex	$[M+HCOO]^-$	857.4548	2.22	$C_{43}H_{69}O_{17}$	811.4484 $[M-H]^-$ 649.3955 $[M-H-Hex]^-$ 487.3455 $[M-H-Hex-Hex]^-$	-
22	8.92	Bayogenin-Hex	$[M-H]^-$	649.3597	2.31	$C_{35}H_{53}O_{11}$	487.3056 $[M-H-Hex]^-$	–
23	9.01	Medicagenic acid-Hex-HexA	$[M-H]^-$	839.4075	1.85	$C_{42}H_{63}O_{17}$	677.3614 $[M-H-Hex]^-$ 501.3199 $[M-H-Hex-HexA]^-$	–
24	9.53	Bayogenin-Hex-Hex-Pent	$[M+HCOO]^-$	989.4970	1.84	$C_{48}H_{77}O_{21}$	943.4960 $[M-H]^-$ 781.4379 $[M-H-Hex]^-$ 619.3848 $[M-H-Hex-Hex]^-$ 487.3421 $[M-H-Hex-Hex-Pent]^-$	–
25	9.78	Phytolaccagenin-Hex	$[M+HCOO]^-$	739.3918	2.59	$C_{38}H_{59}O_{14}$	531.3321 $[M-H-Hex]^-$	–
26	10.92	Medicagenic acid-Pent	$[M+HCOO]^-$	679.3704	2.29	$C_{36}H_{55}O_{12}$	633.3646 $[M-H]^-$ 501.3219 $[M-H-Pent]^-$	–
27	11.06	Phytolaccagenin-HexA	$[M-H]^-$	707.3652	2.05	$C_{37}H_{55}O_{13}$	531.3304 $[M-H-HexA]^-$	–
28	13.32	2β-Hydroxyoleanoic acid-Pent-HexA	$[M-H]^-$	779.4230	2.31	$C_{41}H_{63}O_{14}$	647.3810 $[M-H-Pent]^-$ 471.3473 $[M-H-Pent-HexA]^-$	–
29	14.01	2β-Hydroxyoleanoic acid-Pent	$[M+HCOO]^-$	649.3601	2.79	$C_{35}H_{53}O_{11}$	603.3539 $[M-H]^-$ 471.3098 $[M-H-Pent]^-$	–
30	14.76	Medicagenic acid-Hex	$[M-H]^-$	663.3754	2.23	$C_{36}H_{55}O_{11}$	501.3218 $[M-H-Hex]^-$	–
31	20.23	Oleanoic acid-HexA	$[M-H]^-$	631.3856	2.38	C_{36} H_{55} O_9	455.3550 $[M-H-HexA]^-$	–

*Flavonoids compared with a reference compound; t_R, retention times of compounds **1**–**31** in the MeOH extract;
Glu, glucose; Api, apiose; FA, ferulic acid; Pent, pentose; Hex, hexose; HexA, hexauronic acid

in the shade and powdered plant material (300 g) was extracted subsequently with MeOH (3 L), 80% aq. MeOH (5 L) and H_2O (1 L) by ultrasonic-assisted extraction. After filtration, the extracts were combined and the solvent was evaporated under reduced pressure to give 150 g white-yellow residue. For further purification, the MeOH extract was subjected to a series of column chromatographic procedures with Diaion HP-20, Silica gel, MCI gel, RP-18 that led to 100 flavonoid and saponin-rich fractions. The resulted fractions were used for UHPLC-HRMS profiling.

Sample preparation and LC-MS conditions. An aliquot (1 mg) of each fraction was dissolved in 5 mL 70% EtOH (solution A). Subsequently, 0.5 mL of solution A was diluted to 10 mL 70% EtOH (solution B). The solution B was used for LC-MS analysis of each fraction. UHPLC-HRMS profiling methods, as described by Kokanova-Nedialkova et al. [4,5] were used for a comprehensive study of flavonoid and saponin-rich fractions.

Lipase activity assay. Lipase modulatory activities of the tested compounds were established by measuring the levels of the released 4-nitrophenol using LC-MS as described by Kokanova-Nedialkova et al. [6].

Results and discussion. UHPLC-HRMS based profiling of flavonoid-rich fractions in a positive ion mode revealed the presence of 15 flavonoids (Table 1). Full MS scans followed by data-dependent (DD) MS^2 experiments detected flavonoid glycosides of four aglycones (6-methoxykaempferol, isorhamnetin, spinacetin, and patuletin) that were identified using pseudo MS^3 experiments [5]. Based on the MS and MS^2 data and by comparison with the reference compounds seven flavonoids were identified as known compounds, respectively, glycosides of 6-methoxykaempferol (compounds, **2**, **6** and **15**), spinacetin (compounds **4**, **8** and **11**) and patuletin (compound **1**) (Table 1). Compound **9** showed a protonated molecule $[M+H]^+$ at m/z 611.1603 supporting the formula for $C_{27}H_{31}O_{16}$. Its MS^2 spectrum showed two fragment ions at m/z 479.1177 and 317.0656 due to the sequential loss of a pentose unit (132 Da) and a hexose unit (162 Da). The former fragment ion corresponds to 6-methoxykaempferol. Thus, compound **9** was established as 6-methoxykaempferol-Hex-Pent. Compound **12** showed a protonated molecule $[M+H]^+$ at m/z 479.1181, which was 132 Da less than that of flavonoid **9**, assuming the elimination of one pentose unit. Thus, compound **12** was tentatively identified as 6-methoxykaempferol-Hex. The protonated molecule at m/z 317.0655 showed two distinctive MS/MS spectra attributed to isorhamnetin and 6-methoxykaempferol. A pseudo MS^3 experiment, published previously, was aided at discriminating 6-methoxykaempferol and isorhamnetin glycosides [5]. Three isorhamnetin glycosides (compounds **3**, **7**, and **13**) were detected in the positive ion mode (Table 1). Compound **3** showed a protonated molecule $[M+H]^+$ at m/z 773.2119. Its MS^2 spectrum gave three fragment ions at m/z 641.1721, 479.1181, and 317.0654 that was due to a sequential loss of one pentose and two hexose units, respectively. Thus, compound **3** was identified as isorhamnetin-Hex-

Hex-Pent. Compounds **7** and **13** showed protonated molecules $[M+H]^+$ at m/z 641.1705 and 479.1181, corresponding to a sequential loss of two hexose units in case of **7** and one hexose unit in case of **13**, respectively. Thus, flavonoids **7** and **13** were established as isorhamnetin-Hex-Hex and isorhamnetin-Hex, respectively. Two spinacetin glycosides (compounds **10** and **14**) and one patuletin glycoside (compound **5**) were detected in the positive ion mode and showed similar sequential sugar unit losses as flavonoids **9** and **12**, respectively, (Table 1). Compounds **5**, **10**, and **14**, were tentatively identified as patuletin-Hex-Pent, spinacetin-Hex-Pent and spinacetin-Hex, respectively.

UHPLC-HRMS based profiling of saponin-rich fractions in a negative ion mode tentatively identified 16 saponins of five sapogenins (Table 1). Compound **31** displayed a deprotonated molecule $[M–H]^-$ at m/z 631.3856. The MS^2 fragmentation yielded only a product ion at m/z 455.3550 that was a result of a neutral loss of 176 Da, indicating the presence of hexauronic acid. The product ion at m/z 455.3550 corresponds to oleanoic acid. Thus, compound **31** was established as oleanoic acid-HexA. The MS^2 spectra of three saponins **20**, **28,** and **29** showed a product ion at m/z 471.3480 due to aglycone moiety, which was identified as 2β-hydroxyoleanoic acid. Compound **20** showed a deprotonated molecule $[M–H]^-$ at m/z 971.4860 supporting the formula for $C_{48}H_{75}O_{20}$. Its MS^2 spectrum gave three fragment ions at m/z 809.4346, 647.3803, and 471.3480. The former two ions were due to the sequential loss of two hexose units (2 × 162 Da). The later fragment ion at m/z 471.3480 corresponded to the cleavage of hexauronic acid (176 Da) and confirmed the presence of sapogenin 2β-hydroxyoleanoic acid. Thus **20** was tentatively identified as 2β-hydroxyoleanoic acid-HexA-Hex-Hex. Compound **28** showed a deprotonated molecule $[M–H]^-$ at m/z 779.4230. The MS^2 fragmentation yielded product ions at m/z 647.3810 and 471.3473, which were a result of a cleavage of one pentose unit (132 Da) and one hexauronic acid (176 Da). Thus, compound **28** was identified as 2β-hydroxyoleanoic acid-HexA-Pent. Compound **29** exhibited a formyl adduct ion $[M+HCOO]^-$ at m/z 649.3601. Its MS^2 spectrum showed only a product ion at m/z 471.3098 that was a result of a neutral loss of 132 Da corresponding to one pentose unit. Compound **29** was established as 2β-hydroxyoleanoic acid-Pent. The MS^2 spectra of four saponins **16**, **23**, **26**, and **30** showed a distinctive product ion at m/z 501.3244 which was identified as medicagenic acid (Table 1). Compounds **16** and **23** showed a formyl adduct $[M+HCOO]^-$ and a deprotonated molecule $[M–H]^-$ at m/z 841.4237 and 839.4075, respectively. Both MS^2 spectra gave two fragment ions at m/z 633.3641 (677.3614) and m/z 501.3244 corresponding to a sequential loss of hexose and pentose units in case of **16** and one hexose unit and hexauronic acid in case of **23**, respectively. Thus, compounds **16** and **23** were established as diglycosides medicagenic acid-Hex-Pent and medicagenic acid-Hex-HexA, respectively. Compound **26** displayed a formyl adduct ion $[M+HCOO]^-$ at m/z 679.3704 that was 162 Da less than that of saponin **16**, assuming the elimination of one hexose unit.

The MS^2 fragmentation yielded only a product ion at m/z 501.3219 due to its aglycone. Compound **26** was identified as medicagenic acid-Pent. Compound **30** exhibited a deprotonated molecule $[M–H]^-$ at m/z 663.3754. Its MS^2 spectrum showed only a product ion at m/z 501.3218 that was a result of a neutral loss of 162 Da corresponding to one hexose unit. Compound **30** was established as medicagenic acid-Hex. The MS^2 spectra of four saponins **17**, **21**, **22**, and **24**, showed a distinctive product ion at m/z 487.3436 which was identified as bayogenin (Table 1). Compounds **17** and **22** showed a similar sequential sugar unit loss as saponins **20** and **30**, respectively. Compounds **17** and **22** were tentatively identified as bayogenin-HexA-Hex-Hex and bayogenin-Hex. Compound **24** exhibited a formate adduct $[M+HCOO]^-$ at m/z 989.4970. Its MS^2 spectrum gave three fragment ions at m/z 781.4379, 619.3848, and 487.3421. The former two ions were due to the sequential loss of two hexose units (2×162 Da). The latter fragment ion corresponded to the cleavage of one pentose unit (132 Da) and confirmed the presence of sapogenin bayogenin. Thus **24** was tentatively identified as bayogenin-Hex-Hex-Pent. Compound **21** displayed a formate adduct $[M+HCOO]^-$ at m/z 857.4548 that was 132 Da less than that of saponin **24**, assuming the elimination of one pentose unit. Its MS^2 spectrum gave two fragment ions at m/z 649.3955 and 487.3455 that was due to a sequential loss of two hexose units, respectively. Thus **21** was identified as bayogenin-Hex-Hex. The MS^2 spectra of four saponins **18**, **19**, **25**, and **27** showed a distinctive product ion at m/z 531.3309 which was identified as phytolaccagenin (Table 1). Compound **25** exhibited a formate adduct $[M+HCOO]^-$ at m/z 739.3918. The MS^2 spectrum showed only a product ion at m/z531.3321 that was a result of a neutral loss of 162 Da corresponding to one hexose unit. Compound **25** was established as phytolaccagenin-Hex. Additionally, compounds **18**, **19**, and **27** showed similar sequential sugar unit losses as saponins **24, 23**, and **31**, respectively. Compounds **18**, **19**, and **27** were tentatively identified as phytolaccagenin-Hex-Hex-Pent, phytolaccagenin-Hex-HexA, and phytolaccagenin-HexA.

Furthermore, six saponins previously isolated from the roots of *C. bonus-henricus* L. [2] were tested for modulation of lipase activity in five concentrations (200, 100, 50, 25, and 12.5 µM). A lipase inhibitor Orlistat (IC_{50} = 291.6 µM) was used as a negative control. All of the tested saponins showed prolipase activity (Table 2). The saponins of medicagenic acid (**S1**), 2β-hydroxygypsogenin (**S5**), and Bonushenricoside B (**S6**) were the most active and stimulated lipase activity with 36.56%, 62.10%, and 46.08%, respectively, at a concentration of 200 µM. All of the tested saponins possessed prolipase activity and could be used in the treatment of cachexia [8].

Conclusion. A UHPLC-HRMS profiling method was used for a comprehensive study of flavonoid and saponin-rich fractions from the MeOH extract of *C. bonus-henricus* L. roots. Thirty-one compounds, respectively, **16** saponins together with **15** flavonoid glycosides were detected. Six saponins were tested for

Table 2

Prolipase activity of tested saponins

Compounds	Anti-lipase activity, IC_{50})	Pro-lipase activity (%)				
		200 µM	100 µM	50 µM	25 µM	12.5 µM
S1	–	36.56±2.50	31.11±1.20	25.15±0.93	14.67±0.61	7.34±0.25
S2	–	14.84±1.81	12.52±1.40	9.85±0.81	4.02±0.46	–
S3	–	13.81±1.60	6.58±1.02	1.38±0.45	–	–
S4	–	28.46±2.07	26.37±1.67	24.79±1.20	20.32±1.03	15.12±0.86
S5	–	62.10±4.10	52.64±3.27	47.10±2.60	37.17±2.10	25.19±1.73
S6	–	46.08±3.52	34.10±2.43	33.86±2.15	31.86±1.68	23.41±1.25
Orlistat	291.6 µM	–	–	–	–	–

lipase modulatory activity by measuring the levels of the released 4-nitrophenol using LC-MS. All saponins exhibited prolipase activity and could find application in the treatment of cachexia.

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