Triterpene Saponin Content in the Roots of Red Beet (*Beta vulgaris* L.) Cultivars

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ABSTRACT: Triterpene saponins in the roots of *Beta vulgaris* cultivars Red Sphere, Rocket, and Wodan were profiled and quantitated using reverse-phase liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-ESI/MS/MS). Results obtained indicated that the roots of all three cultivars contained 11 saponins consisting of oleanolic acid or hederagenin aglycone and varying numbers of sugars, with the dominant triglycoside derivative of oleanolic acid. The relative proportions of derivatives of these two aglycones were similar in the three subspecies: cv. Red Sphere contained 99.1 and 0.9%; cv. Rocket, 98.2 and 1.8%; and Wodan 98.8 and 1.2% of oleanolic acid and hederagenin glycosides, respectively. To the best of our knowledge this is the first report on the occurrence, structure, and content of triterpenoid saponins in red beet.

KEYWORDS: reed beet (Beta vulgaris L.), triterpene saponins, oleanolic acid, hederagenin, HPLC-ESI-MS/MS, quantitation

INTRODUCTION

Red beet (*Beta vulgaris* L.) is cultivated throughout the world for its roots, which are used as a food and as a source of natural dye. The roots are usually cooked to be eaten in salads or pickled. Extracts obtained from red beet roots have been shown to have anticancer, detoxifying, and tonic properties.[1]

The chemical composition of red beet roots has been well characterized with regard to the betalains, the water-soluble nitrogenous pigments that give the root its characteristic color, and were determined as dietary cationized antioxidants.[2] However, there have not been any reports concerning the content of saponins in this plant, although oleanolic acid and hederagenin saponins have been isolated from sugar beet, the other cultivated variety of *Beta vulgaris* L., and were found to exhibit hypoglycemic effects.[3−6]

Pentacyclic triterpenoids of the oleanane type are compounds commonly found in higher plants in the form of the free acids or more frequently as aglycones of saponins.[7,8] They participate in mechanisms of plant defense and protection as well as in environmental interactions.[9] The presence of these triterpenes has been documented in many herbal or edible plants; therefore, these compounds constitute an integral part of the human diet.[7] In addition, there have been numerous reports of the anti-inflammatory, immunomodulatory, cytotoxic, antitumor, antimutagenic, antihepatotoxic, antidiabetic, hemolytic, antiviral, antibacterial, trypanocidal, and antiparasitic activities of oleanolic acid and its saponins.[7−12] Because of the wide spectrum of pharmaceutical activities of oleanane triterpenoids, these compounds appear to be promising drugs of natural origin.

Using conventional procedures such as NMR for the identification of saponins, especially those occurring in smaller quantities, is time-consuming and difficult due to the complexity of saponin mixtures present in plants. An alternative approach employs liquid chromatography–mass spectrometry (LC-MS) giving information on the structure of these compounds. Recent studies have demonstrated the advantages of the use of multistage MS combined with LC for the characterization of constituents in crude extracts or in compound mixtures. LC-MS/MS methods have played an important role in phytochemistry and are more often used in research on natural compounds, e.g., triterpenoid saponins.[13,14] Previously, the FAB-MS detection mode was used, but recently ESI-MS detection is more widely employed in saponin analysis.[14−17]

In light of the wide occurrence of oleanolic acid derivatives in plants forming part of the human diet and their potential health benefits, we decided to investigate the triterpenoid composition of three cultivars of *B. vulgaris* var. *esculenta* belonging to cv. Red Sphere, Rocket, and Wodan by a combination of chromatographic and spectroscopic methods. To our knowledge, this is the first report on the occurrence, structure, and content of triterpenoid saponins in this worldwide known vegetable. The presented strategy allows a rapid and complete analysis of saponin composition in *B. vulgaris* and is particularly suitable for the screening of extracts designed for pharmaceuticals in agricultural and industrial applications.

MATERIALS AND METHODS

Chemicals and Reagents. Oleanolic acid and hederagenin standards were purchased from Sigma-Aldrich (Steinheim, Germany). Oleanolic acid monoglucuronide was obtained earlier from *Calendula officinalis* flowers in the Department of Plant Biochemistry, University of Warsaw, Poland.[11] Saponin **8** was obtained in the Department of Biochemistry, Institute of Soil Science and Plant Cultivation, State Research Institute, Puławy, Poland. Sugars, namely, glucose, galactose, arabinose, xylose, rhamnose, and glucuronic and galacturonic acids, were purchased from Chempur (Piekary Slaskie, Poland). Diazo-
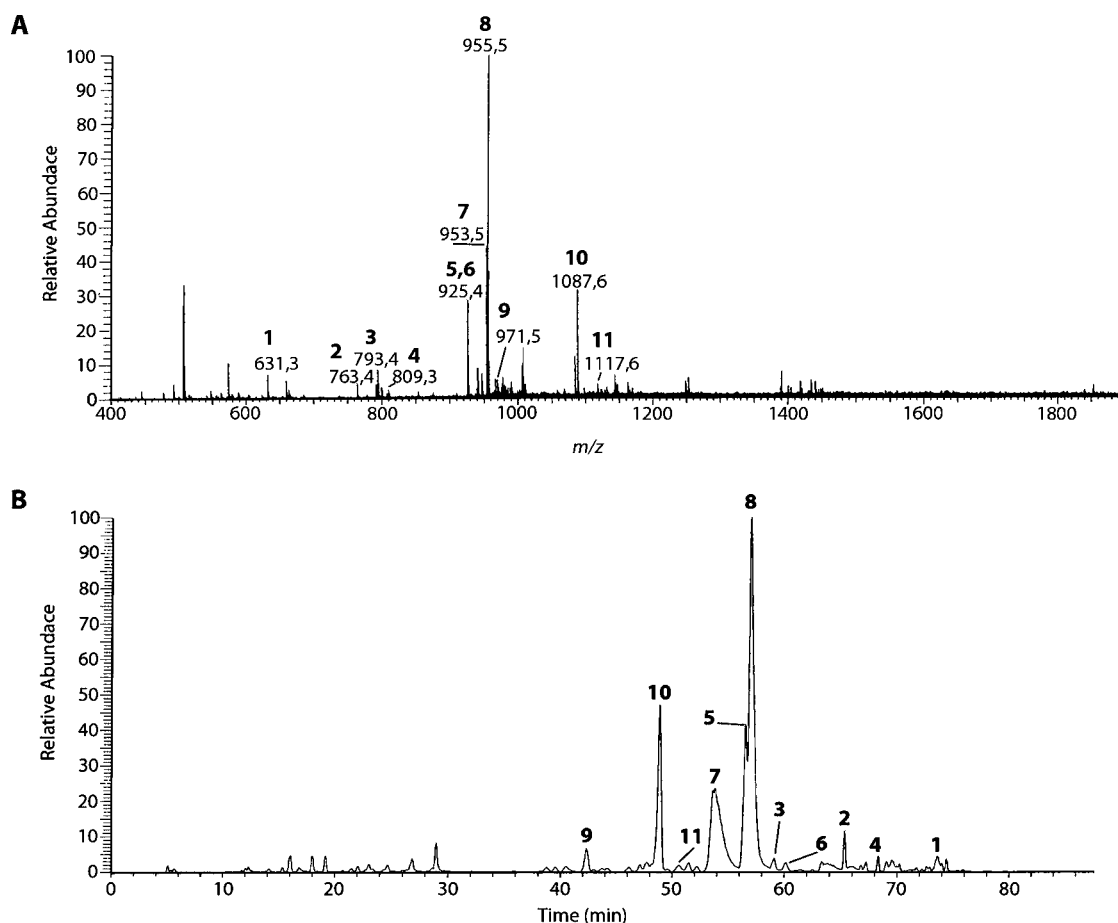
Figure 1. (A) Direct injection ESI/MS of the mixture of *Beta vulgaris* saponins purified by solid-phase extraction; (B) total ion chromatogram obtained by negative-ion HPLC-ESI/MS of SPE purified *Beta vulgaris* saponins.

methane was synthesized according to a method described before.[18] HPLC-grade solvents were produced by J.T. Baker (Phillipsburg, US); all other chemicals were from Linegal Chemicals (Warsaw, Poland).

Plant Material. Red beet (*Beta vulgaris* L.) cv. Red Sphere, Rocket, and Wodan were collected from fields in central Poland in October, 2010. Ten plant roots of every cultivar were pooled, then sliced, and dried at 40 °C. Dried material was finely powdered using a grinder (Moulinex, France).

Extraction and Purification. One gram samples of finely powdered material were extracted three times with the assistance of a microwave oven (300W) for 10 min using each time 50 mL of 80% methanol. Three independent extractions were performed for each cultivar. Obtained extracts were filtered and concentrated to dryness under reduced pressure (rotary evaporator Heidolph, Schwabach, Germany). Extracts were diluted in 4 mL of water and loaded into the Lichrolut 1000 mg RP-18 cartridges (Merck, Darmstadt, Germany) preconditioned with methanol and water in a vacuum manifold. The cartridges were washed with water and then eluted with 40% and 80% methanol in water, and finally 100% methanol. Twenty-four milliliter volume of each solution was used, and elutes were monitored by TLC on silica gel plates (Merck, Darmstadt, Germany) developed in ethyl acetate/acetic acid/water (7:2:2 v/v/v). Triterpenoids were visualized by spraying the TLC plates with 50% sulfuric acid in water followed by heating at 130 °C. Saponins were eluted with 80% methanol, evaporated under reduced pressure, and redisolved in 2 mL of methanol for direct injection MS/MS analyses. For HPLC, 0.5 mL was transferred to another vial, evaporated, and redisolved in 2 mL of 25% acetonitrile in water. For acid hydrolysis, 0.5 mL was transferred to a test tube and evaporated.

Acid Hydrolysis. A saponin crude mixture was treated with 4 mL of 2 N HCL in 80% aqueous methanol at 100 °C for 2 h in closed test tubes. Subsequently, an equal volume of water was added to each hydrolyzate, methanol was evaporated, and aglycones were extracted with three equal volumes of ethyl ether. Aqueous residue containing sugars was loaded into columns (10 × 20 mm i.d.) filled with Amberlite 120 IR (OH form, Merck, Darmstadt, Germany) and subsequently kationite Amberlite IR 440 (Cl⁻ from, Merck, Darmstadt, Germany). Elutes were evaporated to dryness.

TLC Analysis of Sapogenins. Aglycones were identified by the TLC method in the presence of sapogenin standards. Silica gel coated TLC plates (Merck, Darmstadt, Germany) were developed in chloroform/methanol (98:2 v/v). Triterpenoids were visualized as described above.

GLC Analysis of Sapogenins. Aglycones were derivatized in order to obtain the methyl esters by treating with diazomethane in diethyl ether (1.5 mL).[18] The reaction mixtures were held overnight at room temperature. GLC of methylated samples was performed using a Shimadzu GC-2014 gas chromatograph fitted with a glass column 2 m × 3 mm i.d., 3% SE-30 on 80/100 Chromosorb WHP matrix (Supelco, Munich, Germany), and equipped with a flame-ionization detector (FID). The injector, column, and detector temperatures were 260, 280, and 280 °C, respectively, while the flow rate of the carrier gas (nitrogen) was 30 mL/min.

Sugar Analysis. Sugars were separated on cellulose plates (Merck, Darmstadt, Germany) with butanol/toluene/pyridine/water (5:1:3:3 v/v/v/v), made visible with 10% silver nitrate solution in acetone (1:200), followed by 0.5 N sodium hydroxide in 98% methanol, and 10% sodium thiosulphate in water and identified by comparison with reference compounds.

HPLC-ESI-MS/MS. Analyses were carried out in an LC system consisting of a Finnigan Surveyor pump equipped with a gradient controller. Separation was performed using a 250 × 4 mm i.d., 5 μm,

Table 1. Saponins Observed by Negative Ion HPLC-ESI/MS/MS in *B. vulgaris*[a]

saponin	ESI/MS	R_t (min)	ESI/MS/MS	saponin structure
1	631.3[M − H]⁻	73.64	455.3 [M-H-HexUA]⁻	HexUA oleanolic acid
2	763.4[M − H]⁻	65.36	631.3 [M-H-Pen]⁻ 455.3 [M-H-Pen- HexUA] ⁻	Pen + HexUA + oleanolic acid
3	793.4[M − H]⁻	59.10	631.3 [M-H-Hex]⁻ 455.3 [M-H-Hex- HexUA]⁻	Hex + HexUA + oleanolic acid
4	809.3[M − H]⁻	68.10	647.5 [M-H- Hex]⁻ 471.3 [M-H-Hex- HexUA]⁻	Hex + HexUA + hederagenin
5	925.4[M − H]⁻	56.53	(1) 793.3 [M-H-Pen]⁻ 631.3 [M-H-Pen-Hex]⁻ 455.3 [M-H-Pen-Hex-exUA]⁻ (2) 763.4 [M-H-Hex]⁻ 631.3 [M-H-Hex-Pen]⁻ 455.3 [M-H-Hex-Pen-exUA]⁻	Pen + Hex + HexUA oleanolic acid
6	925.4[M − H]⁻	60.08	(11) 793.3 [M-H-Pen]⁻ 631.3 [M-H-Pen-Hex]⁻ 455.3 [M-H-Pen-Hex-exUA]⁻ (2) 763.4 [M-H-Hex]⁻ 631.3 [M-H-Hex-Pen]⁻ 455.3 [M-H-Hex-Pen-exUA]⁻	Pen + Hex + HexUA oleanolic acid
7	953.5[M − H]⁻	53.83		unknown
8	955.5[M − H]⁻	57.03	793.3 [M-H-Hex]⁻ 631.6 [M-H-Hex-Hex]- 455.3 [M-H-Hex-Hex-HexUA]⁻	Hex + Hex + HexUA oleanolic acid
9	971.5[M − H]⁻	42.33	809.3 [M-H-Hex]⁻ 647.5 [M-H-Hex]- 471.3 [M-H-Hex-HexUA]⁻	Hex + Hex + HexUA + hederagenin
10	1087.6[M − H]⁻	48.87	(1) 955.4 [M-H-Pen]⁻ (2) 925.4 [M-H-Hex]⁻	Pen + Hex + Hex + HexUA + oleanolic acid
11	1117.6[M − H]⁻	50.09	955.4 [M-H-Hex]⁻ 793.3[M-H-Hex]⁻ 631.6 [M-H-Hex-Hex]⁻ 455.3 [M-H-Hex-Hex-Hex-HexUA]⁻	Hex + Hex + Hex + HexUA + oleanolic acid

[a]Hex, hexose; Pen, pentose; HexUA, hexuronic acid.

Eurospher 100 C_{18} column (Knauer, Germany). A mobile phase consisting of 0.1% acetic acid in water (B) and 0.1% acetic acid in acetonitrile (A) was used. The flow rate was kept constant at 0.5 mL/min for a total run time of 90 min. The gradient elution was as follows: from 15% to 36% A, 0−55 min; from 36% to 100% A, 55−77 min; from 100% to 15% A, 77−90 min. The sample injection volume was 20 μL.

A Thermo Finnigan LCQ Advantage Max ion trap mass spectrometer with an electrospray ion source was coupled to the HPLC system described above. The following instrumental parameters were used for ESI-MS/MS analysis of saponins: spray voltage, 4.2 kV; capillary offset voltage to −60 V; capillary temperature, 220 °C; nitrogen ion injection time, 200 ms. Calibration of the mass range (400−2000 Da) was performed in negative ion mode. Detection mode was SIM (selected ion monitoring) in negative ion mode.

Quantitative Analysis. Three independent chromatographic runs were performed for each saponin mixture, and saponins were identified through comparison of their retention times and ion mass. Quantitation of 11 saponins was based on external standardization by employing calibration curve of saponin **8** in the range of concentrations 10−100 μg/mL. Quantitative analyses were based on the peak area calculated from selected ion chromatograms of the corresponding [M − H]⁻ ion. Microsoft Excel 2000 was used for the statistical analysis.

RESULTS AND DISCUSSION

Structural Characterization. In the present study, an HPLC-ESI/MS system was employed to provide a rapid, effective, and convenient method for identification and quantitation of saponins present in the roots of *B. vulgaris* cultivars Red Sphere, Rocket, and Wodan. Initially, solid phase extraction in C-18 cartridges was used as a quick quantitative method for the purification of 80% methanol extract. SPE produced samples which contained saponins with only a few impurities when injected directly in MS negative ion mode (Figure 1A). MS analysis from the direct injection of all samples revealed the presence of 10 peaks ascribed as saponins and which showed the following pseudomolecular [M − H]⁻ ions at m/z 631, 763, 793, 809, 925, 953, 955, 969, 1087, and 1117, which for the $z = 1$ ion correspond to the molecular mass of saponins.

Separation of red beet saponins with good peak resolution was conducted using a modified acetonitrile/water gradient (Figure 1B).[14] This gradient allowed separation of red beet saponins between 40 and 75 min. Saponins with molecular masses 972 (**9**), 1088 (**10**), 1118 (**11**), and 954 (**7**) were separated between 40 and 55 min; saponins with molecular masses 926 (**5**), 956 (**8**), 794 (**3**), 926 (**6**), 764 (**2**), and 810 (**4**) were separated between 55 and 70 min; a saponin with a molecular mass of 632 (**1**) was eluted in 73.7 min. HLPC-MS allowed ascribing two peaks with a molecular mass of 926 to two saponins that differed in retention time −60.8 min (**6**) and 56.5 min (**5**), i.e., in structure and polarity. According to these results, 11 saponins were found in the roots of three red beet cultivars. All cultivars contained the same number of peaks with the same molecular mass.

Subsequent identification of saponins using the MS/MS technique was conducted for all compounds except saponin **7**, which was masked by the strong signal of saponin **8**. Detailed fragmentation patterns are presented in Table 1. Identification of the saponins was obtained not only by analysis of their MS/MS fragmentation but also by comparing the R_t of saponin **1** with the R_t of the known saponin, as well as the sugar composition and default place of bonding the sugar. According to the results, eight saponins gave a fragmentation ion of m/z 455 and have been described as derivatives of oleanolic acid (compounds **1**, **2**, **3**, **5**, **6**, **8**, **10**, and **11**), and two gave an ion of m/z 471 and were described as hydroxyoleanolic acid derivatives (compounds **4** and **9**). Aglycone moieties obtained after hydrolysis of the saponin mixture were identified as oleanolic acid and hederagenin (23-hydroxyoleanolic acid) by TLC by comparing their R_f values with those of reference compounds (data not shown). Also, GLC analysis confirmed the presence of two aglycones, by comparing t_R values of aglycones with those of hederagenin and oleanolic acid standards (data not shown).

The analyzed compounds gave fragmentation with the loss of sugars (uronic acid, pentose, and hexose); however, different monosaccharide epimers cannot be distinguished by the means of MS/MS. As a consequence, TLC of sugars obtained after hydrolysis of saponins, as well as of standards, was performed on cellulose plates, and the sugars were identified as glucuronic acid (GlcA), xylose (Xyl), and glucose (Glc) only (data not shown).

For compound **1**, the loss of glucuronic acid (GlcA) was detected. Fragmentation of this compound occurred at m/z 455, establishing it as oleanolic acid monoglucuronide.

Retention time of **1** was identical to the R_t of 3-O-β-D-glucuronopyranosyl of oleanolic acid isolated from *Calendula officinalis*.[11] Moreover, this structure corresponds to the 3-O-β-D-glucuronopyranosyl of oleanolic acid isolated earlier from fresh sugar beet roots.[3]

Fragmentation of m/z 763 (saponin **2**) resulted in the formation of 631 and 455 ions, which are consistent with the loss of Pen and GlcA in this oleanolic acid derivative. Saponin **2** was determined as the diglycoside of oleanolic acid consisting of Xyl and GlcA.

In the MS spectrum of a saponin **3** (m/z 793), after the initial loss of 162 Da, a loss of 176 Da was observed, corresponding to fragmentation of oleanolic diglycoside consiting of Glc and GlcA. Saponin **4** exhibited a pseudomolecular ion [M − H]$^-$ at m/z 809, i.e., 16 Da greater than that of compound **3**, and its MS/MS spectrum showed an aglycone ion at m/z 471, which is the characteristic ion of hederagenin. Saponin **4**, similar to compound **3**, has a sugar chain consisting of Glc and GlcA, which was stated on the basis of its fragmentation.

Compounds **5** and **6** gave pseudomolecular ions [M − H]$^-$ at m/z 925 and are a pair of isomers with the same number of sugar moieties but linked in a different position. Fragmentation of m/z 925 resulted in the simultaneous loss of pentose and hexose. This indicates that both of the sugar moieties are linked to the GlcA moiety or that one of them is linked to C-28 of aglycone. According to the significant difference in their retention times in HPLC analysis, we can expect that one of them has a branched sugar chain (**6**) and the second one a blocked carboxylic group (**5**). A compound with molecular mass 926 was isolated from a sugar beet root and identified as 3-O-[β-glucopyranosyl(1−2)-(β-D-xylopyranosyl(1−3)-β-D-glucuronopyranosyl] of oleanolic acid.[3]

MS^2 spectra of saponin **8** yielded ions at m/z 793, 631, and 455, which suggested the presence of two Glc and one GlcA moieties connected to oleanolic acid. However, fragmentation of a saponin **9** produced ions at m/z 809, 647, and 471, caused by the loss of two Glc and one GlcA connected to hederagenin.

The mass difference between the pseudomolecular ion [M − H]$^-$ 1087 (saponin **10**) and its product ions 925 [M − H]$^-$ and 955 [M − H]$^-$ was 162 and 132, corresponding to the simultaneous loss of hexose and pentose. Although molecular ions with a lower mass were not observed, we can predict that the fragmentation pattern is similar to that of saponins **5**, **6**, and **7**. Furthermore, 3-O-[β-D-glucopyranosyl(1−2)-(β-D-xylopyranosyl(1−3)-β-D-glucuronopyranosyl] 28-O-β-D-glucopyranosyl ester of oleanolic acid from sugar beet had been identified previously.[3] Fragmentation of a saponin **11** (m/z 1117) resulted in the loss of three 162 fragments, giving a compound with additional glucose in comparison to fragmentation of a saponin with molecular mass 956 (**7**).

In the present study, for the first time, the oleanolic acid and hedragenin derivatives from red beet roots have been described. It can be assumed that all saponins present in red beet roots are 3-O-glucuronides of oleanolic acid or hedragenin with different constituents of glucose or xylose. Some of them had been isolated previously from sugar beet by Ridout at al.[3] They also have a structure pattern similar to that of the saponins isolated from sugar beet in the same laboratory[4] and in the complex and comprehensive study by Yoshikawa et. Al.[5,6] According to these findings, saponins from sugar beet are also derivatives of oleanolic acid and hederagenin, and in opposition to earlier findings,[3] they contain not only sugars but also acetal-type and dioxolane-type substituents, which were presumed to be biosynthesized through the oxidative degradation of a terminal monosaccharide moiety.[6] According to our findings, none of the saponins analyzed by MS/MS contained these types of constituents, yet it cannot be excluded that the undetermined saponin with a molecular mass of 954 contains one of these unique moieties. Nevertheless, betavulgarosides I and II have structures analogous to the structures of saponins **3** and **8** isolated from the roots of red beet, while instead of terminal glucose they have dioxal-type substituents at the 2′ and 3′ positions of glucuronic acid. Betavulgaroside IV has a free carboxylic group and acetal-type substituent derived from glucose substituted in the 3′ position of glucuronic acid, consistent with the structure of diglycoside **3** from red beet. Betavulgaroside V with a number of sugars similar to saponin **11** (molecular mass 1118) has a branched chain and a blocked carboxyl group in the C-17 position. Betavulgaroside IX, a bisdesmoside built from an acetal-type substituent and xylose connected to glucuronic acid in the 2′ and 3′ positions and glucose at the C-28 position of the aglycone, is an analogue of saponin **10**. The hederagenin derivatives, betavulgarosides VI and VII, are derivatives of glucuronide with a unique substituent, and the second additional glucose in C-8 has a structure analogous to that of saponins **4** and **9**.

On the basis of HPLC-MS/MS analysis, as well as the interpretation of sugar beet saponin structures, the structure of red beet saponin can be predicted as shown in Figure 2.

Quantitative Analysis. Quantitative analysis of saponins from the roots of three red beet cultivars was achieved by HPLC-ESI/MS. Saponins were separated for quantitation in the conditions described earlier. For the determination of all saponins, it was necessary to prepare a standard calibration curve in the range of concentrations 10−100 μg/mL for saponin **8**. The curve showed good linearity, with R^2 values of 0.98. This curve was used for the quantitation of all triterpenic compounds.

For evaluation of the instrument precision and extraction and purification repeatability, three samples from the same plant powder were independently extracted and purified within the SPE procedure. For each sample, three independent HPLC-ESI/MS runs were performed. It was shown that for repetition of the same sample ($n = 3$) the relative standard deviation did not exceeded 5%. In general, variation of the data caused by the instrument was satisfactory. Relative standard deviation between results obtained for three independent extractions ranged from 3 to 16%. Considering difficulties in obtaining homogeneous plant material and that purification of samples included several steps of extraction/purification, the relative standard variation was satisfactory and was comparative to that obtained in our previous studies[15] and to works performed in different laboratories.[19,20]

The three cultivars varied in total concentration of saponins (Table 2). The cultivar with the highest concentration turned out to be cv. Red Sphere (12.2 mg/g DW). In comparison to Red Sphere, saponin concentration in Rocket and Wodan cultivar roots were, respectively, 15% and 37% lower. In relation to dry mass, the content of saponins equaled 1.21% in Red Sphere, 1.03% in Rocket, and 0.77% in Wodan. Moreover, differences in the relative content of individual saponins were observed.

When we consider oleanolic acid derivatives, the most abundant saponin detected in all cultivars had a molecular mass 956 (**8**). Its content equaled 79% of total saponins in Red
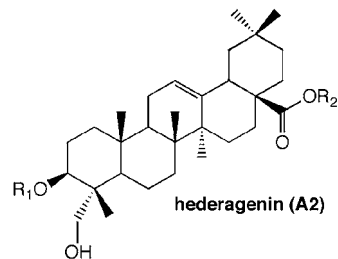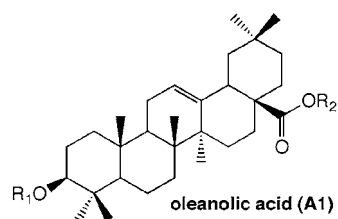
No.	Aglycone	R_1	R_2
1.	A1	GlcA	H
2.	A1	Xyl —GlcA	H
3.	A1	Glc —GlcA	H
4.	A2	Glc —GlcA	H
5.	A1	Glc / Xyl — GlcA	H
6.	A1	Xyl — GlcA	Glc
8.	A1	Glc —GlcA	Glc
9.	A2	Glc —GlcA	Glc
10.	A1	Glc / Xyl — GlcA	Glc
11.	A1	Glc / Glc — GlcA	Glc

Figure 2. Structures of saponins from red beet roots. Glc, glucose; Xyl, xylose; GlcA, glucuronic acid.

Table 2. Concentration of Individual and Total Saponins in Red Beet Cultivars

saponin	cv. Red Sphere (μg/g dw ± SD)	cv. Rocket (μg/g dw ± SD)	cv. Wodan (μg/g dw ± SD)
1	219.2 ± 5.4	3.2 ± 12	1.4 ± 9.4
2	291.4 ± 16.2	8.0 ± 14.6	103.6 ± 5.9
3	403.0 ± 4.3	225.2 ± 7.8	166.2 ± 5.3
4	48.1 ± 7.9	167.8 ± 3.4	54.7 ± 2.8
5	157.8 ± 7.1	112.6 ± 9.7	53.3 ± 9.6
6	349.0 ± 6.2	18.1 ± 8.2	476.1 ± 7.3
7	142.4 ± 3.8	647.5 ± 6.5	907.6 ± 14.7
8	9543.1 ± 2.5	7935.5 ± 9.8	5239.6 ± 3.7
9	53.2 ± 7.8	13.1 ± 3.9	33.9 ± 6.4
10	736.8 ± 4.2	703.7 ± 9.9	342.3 ± 9.3
11	172.3 ± 9.1	487.9 ± 7.1	290.3 ± 3.8
total	12216.3	10322.6	7669

Sphere, 77% in Rocket, and 68% in Wodan roots. Interestingly, only differences in saponin **8** affected the total amount of saponins in all cultivars and consequently differences in the total saponin mass between cultivars. The content of other saponins was always similar and equaled 2.57, 2.39, and 2.43 mg/g dw. In Red Sphere and Rocket, it was followed by saponin **10** (6.1 and 6.8% of total saponins, respectively) and in Wodan by saponin **7** (11.8% of total saponins). The relative abundance of the rest of the saponins varied between these three cultivars, while in most cases, it did not exceed 6.5% of total saponin.

In the roots of these three cultivars, oleanolic acid derivatives were detected along with two derivatives of hederagenin (saponins **4** and **9**) at 48.1 and 53.2 μg/g of dw, 167.8 and 13.1 μg/g of dw, and 54.7 and 33.9 μg/g of dw in Red Sphere, Rocket, and Wodan, respectively. The sum of both compounds constituted only 0.85%, 1.78%, and 1.16% of total saponin content. This indicates that the concentration of hederagenin derivatives is marginal in all cultivars of *Beta vulgaris* roots. Oleanolic acid derivatives dominated over hederagenin derivatives with ratios of 118:1 in cv. Red Sphere, 56:1 in cv. Wodan, and 85:1 in cv. Rocket.

The results obtained revealed differences in saponin content and indicated accumulation of the triglycoside of oleanolic acid (**8**), and that the difference of total saponin content is connected with the accumulation of this saponin. Roots were collected at the end of the vegetation season, and the accumulation of triglycoside in all tested cultivars may be due to biotic or abiotic factors. It has been shown in different studies that the content of saponins depends on the plant cultivar[15] and also on the plant age, general condition, ambient temperature, and access to water and light.[21,22] However, the relatively high concentration of saponins, e.g., saponin **8**, in the roots of the tested three red beet cultivars can be connected to the agricultural selection of cultivars with good root durability during storage due to their antibacterial and antifungal saponin properties.

Investigation of the saponin content in the roots of red beets has demonstrated that HPLC-ESI/MS/MS is a useful method for preliminary identification as well as for quantitation of total and individual saponins. The method presented in the study demonstrates for the first time the structure of saponins from red beet roots and gives the possibility of assessment of plant material in terms of food storage and potential impact on human health, where correlation between the observed effects and the concentration of active saponins can be monitored.

The occurrence of saponins in red beet whose structure seems to be analogous to the structure of saponins present in sugar beet is not surprising due to the close genetical link between these two plants. They belong to the same species *Beta vulgaris* L. and with other cultivated taxa were brought together in subspecies *vulgaris*.[23] They originate from wild beets that were domesticated about 2500 years ago and then subjected to strict agricultural selection, which led to the occurrence of chemotaxonomic differences between these two plants. Their biochemical profiles differ significantly in regard to betalains, which are not synthesized by sugar beet, but the saponin profile also differs, and the mechanism of the oxidative degradation of sugars probably is not present in red beet.

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