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Original research paper

Isolation and free-radical-scavenging properties of cyanidin 3-*O*-glycosides from the fruits of *Ribes biebersteinii* Berl.

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The reversed-phase preparative high performance liquid chromatographic purification of the methanol extract of the fruits of *Ribes biebersteinii* Berl. (*Grossulariaceae*) afforded five cyanidin glycosides, 3-*O*-sambubiosyl-5-*O*-glucosyl cyanidin (**1**), cyanidin 3-*O*-sambubioside (**2**), cyanidin 3-*O*-glucoside (**3**), cyanidin 3-*O*-(2^G-xylosyl)-rutinoside (**4**) and cyanidin 3-*O*-rutinoside (**5**). They showed considerable free-radical-scavenging properties in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with the RC_{50} values of 9.29×10^{-6}, 9.33×10^{-6}, 8.31×10^{-6}, 8.96×10^{-6} and 9.55×10^{-6} mol L^{-1}, respectively. The structures of these compounds were elucidated by various chemical hydrolyses and spectroscopic means. The total anthocyanin content was 1.9 g per 100 g dried fruits on cyanidin 3-glucoside basis.

Keywords: Ribes biebersteinii Berl. (*Grossulariaceae*), 2,2-diphenyl-1-picrylhydrazyl (DPPH), cyanidin glycoside, free-radical-scavenger

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The red-violet colour in fruits and vegetables is derived mainly from a class of flavonoids called anthocyanins. To date, more than 300 different anthocyanins have been identified in different plants. There has been an increased interest in natural anthocyanins and anthocyanin containing plants because of their potential health promoting properties and, above all, for their protection against free radicals (1). The *Ribes* species are known to contain high amounts of anthocyanins. *Ribes biebersteinii* Berl. (*Grossulariaceae*), commonly known as 'reddish-black berry', is an Iranian medicinal plant (2–4). The fruits of *R. biebersteinii* are used as food and also in the traditional medicine as a hypotensive agent and as a retina-protectant. No phytochemical or bioactivity studies on *R. bieber-*

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steinii have ever been carried out previously. As part of our on-going studies on Iranian medicinal plants, we now report on the isolation and identification of anthocyanin glycosides from the fruits of *R. biebersteinii* and their potential free-radical-scavenging property in the DPPH assay.

EXPERIMENTAL

General

^{1}H NMR spectra were recorded on a Varian FT-400 Unity Plus (400 MHz) spectrometer (USA), HPLC (Shimadzu, LC-8A, Japan) and UV-Visible spectrophotometer (Shimadzu, 2100, Japan) were used. The NMR spectra were recorded in D_2O (10 % trifluoroacetic acid, TFA) at 399.866 MHz for ^{1}H NMR and 100.556 MHz for ^{13}C NMR. Chemical shifts (δ) are given in ppm relative to the internal standard TMS. UV-Visible spectrum of each compound was recorded in MeOH containing 0.01 % HCl and after addition of 3 drops of 5 % $AlCl_3$ in anhydrous MeOH (shift reagent) at 190–700 nm.

TLC plates and chemicals

Silica gel plate (GF_{254}), microcrystalline cellulose plate, $AlCl_3$, methanol, diethyl ether, formic acid, reference sugars, concentrated hydrochloric acid, DPPH, vitamin C and trifluoroacetic acid were purchased from Merck (Germany) and Sigma-Aldrich (Japan), and were of analytical grade. For HPLC analyses, water and MeOH were of HPLC-grade and were purchased from VWR Internatinal Ltd (UK).

Plant material

The fruits of *Ribes biebersteinii* Berl. (*Grossulariaceae*) were collected from the Arasbaran area of the eastern Azarbaijan province, Iran, during September-October 2002, and a voucher specimen representing this collection was retained in the herbarium of the School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Extraction

Shed-dried and ground fruits of *R. biebersteinii* Berl. (10 g) were macerated with MeOH containing 0.01 % HCl (100 mL). The extract was refrigerated for 1 h and filtered through a cellulose paper filter. Extraction was repeated twice, each time by 60 mL of 0.01 % HCl in MeOH. Then, the filtered extracts were pooled together and concentrated under vacuum in a rotary evaporator (Büchi, Germany). Diethyl ether was added. Anthocyanins precipitated out due to the decrease in dielectric constant. Subsequently, the precipitate was separated by a centrifuge at 1000 rpm (Beckmann Coulter, USA). This procedure was repeated several times until no precipitation took place in order to get pure anthocyanins. Separated and cleaned precipitate was completely dried under nitrogen gas and stored in the refrigerator.

Isolation

The preparative high performance liquid chromatography (HPLC) (Dr Maisch ODS preparative column 10 μm, 250 mm × 20 mm; mobile phase: 0–260 min, 0–10 % B in A (A = 10 % formic acid in water, B = 10 % formic acid in MeOH, followed by 10 % B in A for 30 min; flow rate 20 mL min^{-1}) of the methanolic extract of *R. bierberstinii* resulted in the isolation of five cyanidin glycosides, 3-*O*-sambubiosyl-5-*O*-glucosyl cyanidin (**1**, 50.6 mg, t_R = 178.0 min), cyanidin 3-*O*-sambubioside (**2**, 5.1 mg, t_R = 215.0 min), cyanidin 3-*O*--glucoside (**3**, 10.8 mg, t_R = 226.3 min), cyanidin 3-*O*-(2G-xylosyl)-rutinoside (**4**, 9.3 mg, t_R = 238.6 min) and cyanidin 3-*O*-rutinoside (**5**, 5.7 mg, t_R = 279.1 min).

Acidic hydrolysis

Purified anthocyanin mixture (3 mg) was dissolved in a minimum amount of acidified MeOH (3 mol L^{-1} HCl). The test tube was placed in a boiling water bath for 1 h. The cooled solution was divided into two parts and the solvent was evaporated with a rotary-evaporator. One of the solutions was treated with acidic MeOH for identification of genins and the other with water for identification of glycones by thin layer chromatography (TLC). TLC analyses of genins and glycones were performed using the solvent system butanol/acetic acid/water (4:1:5) (BAW) and microcrystalline cellulose as a stationary phase. Detection of glycones was carried out using aniline-hydrogen phthalate reagent (5).

Alkaline hydrolysis

Ethanol (2 mL) was added to 2 mg of purified anthocyanins. The test tube was flushed with nitrogen and capped. The solution was saponified with 10 mL, 2 mol L^{-1} NaOH for 2 h in the dark at r. t. After neutralization of the solution with 2 mL of 2 mol L^{-1} HCl, it was decanted using diethyl ether (5 mL). For identification of probable acyls linked to anthocyanin structure, the ether phase was examined. Aqueous phase was used for identification of anthocyanins. The TLC of two phases was carried out using BAW (4:1:5) and F366 microcrystalline cellulose as above (5).

Peroxide hydrolysis

This procedure is specific for sugars on the C-3 position of cyanidins. Approximately 2 mg of anthocyanin pigment was dissolved in MeOH and treated dropwise with 3 % peroxide solution until the pigment was bleached. After addition of conc. ammonia solution (1 mL), the mixture was concentrated, spotted on a microcrystalline cellulose TLC plate together with reference sugar solution and developed for sugars. Chromatography was carried out using BAW (4:1:5) and microcrystalline cellulose. Mono-, di- and tri-glycosides would be removed intact from C-3 and could be identified with appropriate reference sugars (6).

Continuous hydrolysis

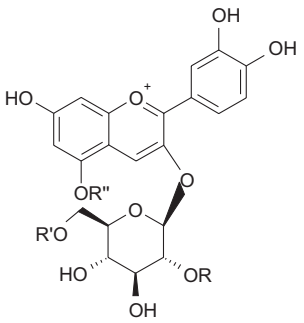
Each anthocyanin pigment (5 mg) was dissolved in 20 mL of 2 mol L^{-1} HCl in a test tube. The tube was placed in boiling water and sampled at 5, 10, 15, 20, 30, 45 and 60 min, and the samples were chromatographed by TLC using the solvent system BAW and microcrystalline cellulose as above (6).

FAB-MS analysis

Anthocyanin pigment was dissolved in MeOH for the Fast Atom Bombardment Mass Spectroscopy (FAB-MS) analysis using a Finnigan MAT95 spectrometer (UK). The instrument response was first optimized by infusing a constant flow (1–2 μL min^{-1}) of solution. After evaporation of methanol, 1–2 μL glycerol (matrix liquid) was added to the pigment. Elucidation of the structure of pigments was done by fragmentation of the molecular ions in the cell of the mass spectrometer, using xenon flow.

Total anthocyanin content

Total anthocyanin content of the fruits of *R. biebersteinii* was measured using the pH differential absorbance method (7). The absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid/potassium chloride, 0.2 mol L^{-1}) and 4.5 (acetate acid/sodium acetate, 1 mol L^{-1}). Anthocyanin content was calculated using a molar extinction coefficient of 29,600 (cyanidin 3-glucoside) and absorbance of $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$. Results were expressed as mg cyanidin 3-glucoside equivalents in 100 g of dried fruits.



Compd.	R	R'	R"
1	xylosyl	H	glucosyl
2	xylosyl	H	H
3	H	H	H
4	xylosyl	rhamnosyl	H
5	H	rhamnosyl	H

Fig. 1. Structures of cyanidin glycosides isolated from the fruits of *Ribes biebresteinii* Berl.

Free-radical-scavenging activity (DPPH assay)

The method used by Du *et al*. (8) was adopted with suitable modifications. The sample solutions (0.8 mg mL^{-1}) were prepared by dissolving each sample in water. These sample solutions were diluted to 0.4, 0.2 and 0.1 mg mL^{-1}, stored in a refrigerator at 4 °C before testing for radical scavenging activity. In the test protocol of radical scavenging activity, 2.9 mL of 65 mmol L^{-1} DPPH reagent in methanol/water (80:20, *V*/*V*) was added to 0.1 mL of sample solution to be tested. After 30 min of reaction at 25 °C, the absorbance was measured at 515 nm. The radical scavenging activity R_S was expressed as percentage of DPPH radical elimination calculated from absorbance of the sample at reaction time 0 min and the sample absorbance at 30 min of the reaction. The experiment was performed in duplicate and average absorption was noted for each concentration. The concentration that caused a 50 % reduction in absorbance (RC_{50}) was calculated. The same procedure was followed for the positive control, vitamin C.

RESULTS AND DISCUSSION

Various hydrolyses followed by co-TLC analyses (9, 10) of the glycone and aglycone portions of purified anthocyanin pigments (**1**–**5**) together with reference sugars and cyanidin confirmed the identity of the aglycone as cyanidin in **1**–**5** and respective glycones, *e.g.*, glucose, xylose and rutinose.

The peroxide hydrolysis of compound **1** indicated the presence of sambubiose at C-3 of cyanidin. The UV-visible spectral analysis (MeOH + 0.01 % HCl) of **1** showed absorption maxima (λ_{max}) at 524 and 279 nm, which were in agreement with the absorbance pattern of cyanidin 3-*O*-glycosides (11). The E_{440}/E_{vis} (E_{440} = absorption at 440 nm and E_{Vis} = absorption at band I in the visible wavelength, > 500 nm; for example, in compound **1** the band I was at 524 nm) ratio 25.8 % also supported the above fact (12). Addition of 5 % $AlCl_3$ reagent to methanolic solution showed approximately a 40-nm bathochromic shift, which demonstrated *ortho*-di hydroxyl groups on the B-ring of cyanidin (9–12). The FAB-MS spectrum of 1 displayed the pseudomolecular ions $[M^+ + 1]$ and $[M^+ + 2]$ at *m/z* 744 and 745, respectively (Table I). The fragment ions at *m/z* 611, 581 and 287 corresponded to [M^+-xylose], [M^+-glucose] and [M^+-glucose-sambubiose], respectively. Thus, from the peroxide hydrolysis, and UV-Vis and FAB-MS analyses, it was obvious that the cyanidin aglycone in **1** was substituted at C-3 and C-5, the glycone at C-3 was a disaccharide and at C-5 a glucose (13), while compound **1** was 3-*O*-sambubiosyl--5-*O*-glucosyl cyanidin. The ^{1}H NMR (Table II) and ^{13}C NMR spectra (Table III) of **1** exhibited signals corresponding to cyanidin aglycone (14, 15), sambubiose and glucose. The ^{1}H NMR (Table II) and ^{13}C NMR spectra (Table III) of **1** exhibited signals corresponding to cyanidin aglycone (14, 15) and 2^G-glucosylrutinose glycone. In the ^{1}H NMR spectrum, a 1H singlet at δ 8.98 ppm was typical of H-4 of the cyanidin moiety. Similarly, two 1H doublet at δ 8.09 (J = 2.2 Hz) and 7.95 (J = 8.9 Hz) ppm, a 1H doublets of a doublet at δ 8.32 (J = 8.9, 2.2 Hz) and two 1H singlets at δ 7.02 and 6.91 ppm corresponded, respectively, to H-2′, H-5′, H-6′, H-8 and H-6 of cyanidin. The anomeric proton signals for three sugars, two glucoses and a xylose, appeared at δ 5.57 (1H, d, J = 7.8 Hz), 5.32 (1H, d, J = 7.7 Hz) and 4.69 (1H, d, J = 7.9 Hz) ppm. The ^{1}H NMR signals corresponding to other

*Table I. FAB-MS data of compounds **1**–**5***

Cyanidin glycosides	Pseudomolecular ions (*m/z*)		Major fragment ions (*m/z*)
	$[M^+ + 1]$	$[M^+ + 2]$	
1	744	745	611 [M^+ – xylose], 581 [M^+ – glucose], 287 [M^+ – sambubiose-glucose]
2	582	583	449 [M^+ – xylose], 287 [M^+ – sambubiose]
3	450	451	287 [M^+ – glucose]
4	728	729	595 [M^+ – xylose], 581 [M^+ – rhamnose], 449 [M+ – xylose-rhamnose], 287 [M^+ – xylose – rhamnose-glucose]
5	596	597	449 [M^+ – rhamnose], 287 [M^+ – rhamnose-glucose]

sugar protons appeared as overlapped peaks within the region of δ 3.20–4.30 ppm. The ^{13}C NMR spectrum (Table III) of **1** displayed all 15 signals corresponding to the cyanidin moiety as well as the carbons of all three sugar moieties (14, 15). Thus, compound **1** was identified as 3-*O*-sambubiosyl-5-*O*-glucosyl cyanidin.

Like in case of compound **1** (discussed above), combined hydrolyses and co-TLC of glycosides **2**–**5** revealed the presence of cyanidin as the aglycone in these molecules. This fact was further corroborated by the FAB-MS spectra of the glycosides (Table I) where a major fragment ion at *m/z* 287 corresponding to the cyanidin moiety was present. Addition of 5 % $AlCl_3$ reagent to methanolic solution (containing 0.01 % HCl) of compound **2**–**5** showed an approximately 40-nm bathochromic shift, which demonstrated the presence of *ortho*-di hydroxyl groups on the B-ring of cyanidin (9–12). The UV-Vis spectrum (MeOH + 0.01 % HCl) of **2** showed absorption maxima (λ_{max}) at 280 and 526 nm, supporting the presence of cyanidin 3-*O*-glycoside skeleton in **2**, similar to that of **1**. The glycosylation at C-3 was further confirmed by the E_{440}/E_{Vis} ratio (23 %) (12). The FAB-MS spectrum (Table I) of **2** displayed the pseudomolecular ions $[M^+{+}1]$ and $[M^+{+}2]$ at *m/z* 582 and 583, respectively, which corresponded to the structure of cyanidin 3-*O*--sambubioside (**2**). The ^1H and ^{13}C NMR spectra (Tables II and III) of **2** were similar to those of **1** except for the fact that the signals corresponding to the second glucose moiety (like in **1**) were absent. Thus, glycoside **2** was identified as cyanidin 3-*O*-sambubioside.

The UV-Vis spectra (MeOH + 0.01 % HCl) of glycosides **3**–**5** also displayed absorption maxima (λ_{max}) at 281 and 528, 281 and 529, and 282 and 528 nm, respectively, supporting the presence of cyanidin skeleton with glycosylation at C-3. Like compounds **1** and **2**, glycosylation at C-3 in **3**–**5** was supported by the E_{440}/E_{Vis} ratio, respectively, 23.2, 24.4 and 24.4 % (12). The ^1H and ^{13}C NMR spectra (Tables II and III) of **3**–**5** established the presence of C-3 glycosylated cyanidin skeleton like in **1** and **2**. In addition to the signals associated with the cyanidin moiety, the ^1H and ^{13}C NMR spectra of **3** revealed the presence of a glucose unit, suggesting that compound **3** was cyanidin 3-*O*-glucoside. The pseudomolecular ions $[M^+{+}1]$ and $[M^+{+}2]$ at *m/z* 450 and 451 observed in the FAB-MS spectrum of **3** confirmed the identity of the molecule.

Table II. ^{1}H NMR data (400 MHz, coupling constant J in Hz in parentheses) of cyanidin glycosides ***1–5***

Position	Chemical shift (δ, ppm)				
	1	**2**	**3**	**4**	**5**
4	8.98 s	9.07 s	9.08 s	9.02 s	9.08 s
6	6.91 s br	6.74 s br	6.74 s br	6.87 s br	6.88 s br
8	7.02 s br	6.97 s br	6.95 s br	7.03 s br	7.08 s br
2′	8.09 d (2.2)	8.12 d (2.4)	8.11 d (2.0)	8.10 d (2.5)	8.16 d (2.0)
5′	7.05 d (8.9)	7.15 d (8.8)	7.08 d (8.4)	7.10 d (8.6)	7.31 d (8.5)
6′	8.32 dd (8.9, 2.2)	8.31 dd (8.8, 2.4)	8.30 dd (8.4, 2.0)	8.37 dd (8.6, 2.5)	8.53 dd (8.5, 2.0)
	3-*O*-glucose	3-*O*-glucose	3-*O*-glucose	3-*O*-glucose	3-*O*-glucose
1″	5.57 d (7.8)	5.54 d (7.8)	5.42 d (8.0)	5.71 d (7.6)	5.48 d (8.0)
2″	3.96 m	4.12 m	3.79 m	3.98 m	3.78 m
3″	3.75 m	3.85 m	3.59 m	3.74 m	3.98 m
4″	3.53 m	3.64 m	3.49 m	3.41 m	5.53 m
5″	3.87 m	3.78 m	3.71 m	3.69 m	3.89 m
6″	4.24 dd (11.9, 2.4)	4.02 dd (11.8, 2.1)	4.11 dd (12.0, 2.5)	3.96 br d (10.7)	4.28 dd (11.0, 2.0)
	3.93 m	3.86 m	3.92 m	3.47–3.72	4.08 m
	2″-*O*-xylose	2″-*O*-xylose		2″-*O*-xylose	6″-*O*-rhamnose
1‴	4.69 d (7.9)	4.74 d (7.6)		4.70 d (7.8)	4.85 s
2‴	2.98 m	3.24 m		3.08 m	3.97 m
3‴	3.22 m	3.33 m		3.18 m	3.93 m
4‴	3.36 m	3.37 m		3.21–3.94	3.53 m
5‴	3.68 m, 3.04 t (11.1)	3.71 m, 3.07 t (10.9)		3.21–3.94 3.21–3.94	3.75 m
6‴					1.37 d (6.0)
	5-*O*-glucose			6″-*O*-rhamnose	
1⁗	5.32 d (7.7)			4.65 s	
2⁗	3.56 m			3.76 m	
3⁗	3.34 m			3.15–3.50	
4⁗	3.29 m			3.15–3.50	
5⁗	3.51 m			3.56 m	
6⁗	3.62 m, 3.74 d (10.8)			1.08 d (6.4)	

Spectra obtained in CD_3OD.

The ^{1}H and ^{13}C NMR spectra (Tables II and III) of **4** and **5** were similar to those of **3**, except that there were additional signals assignable to a xylosyl and a rhamnosyl unit in **4**, and a rhamnosyl unit in **5**. While the pseudomolecular ions $[M^+ +1]$ and $[M^+ +2]$ at *m/z* 728 and 729 observed in the FAB-MS spectrum (Table I) of **4** confirmed the identity of the molecule as cyanidin 3-*O*-(2^G-xylosyl)-rutinoside, those at *m/z* 596 and 597 in the

*Table III. ^{13}C NMR data (100 MHz) of cyanidin glycosides **1–5***

Position	Chemical shift (δ, ppm)				
	1	**2**	**3**	**4**	**5**
2	163.5	164.5	162.8	161.8	163.2
3	144.4	144.8	144.6	143.9	144.5
4	134.4	136.2	135.8	134.1	135.1
5	161.3	159.3	159.0	158.0	158.0
6	104.1	102.9	102.5	102.4	102.5
7	169.0	170.8	169.5	168.6	169.4
8	96.0	95.7	94.2	94.2	94.2
9	157.8	157.3	158.2	157.0	157.8
10	112.5	113.7	112.4	112.1	112.0
1′	120.1	121.6	120.1	119.8	120.0
2′	117.7	118.1	117.4	118.8	117.1
3′	146.1	148.0	146.3	146.3	146.3
4′	155.8	155.3	154.8	156.1	154.9
5′	117.3	117.0	116.5	117.1	116.4
6′	128.5	129.6	127.3	127.3	127.4
	3-*O*-glucosyl	3-*O*-glucosyl	3-*O*-glucosyl	3-*O*-glucosyl	3-*O*-glucosyl
1″	103.0	102.7	102.8	103.1	102.5
2″	81.6	83.0	73.9	82.0	73.7
3″	77.0	77.4	77.1	77.3	76.5
4″	70.1	71.7	70.1	71.2	70.1
5″	76.3	77.9	77.9	77.6	77.0
6″	61.7	62.4	61.7	65.6	66.8
	2″-*O*-xylosyl	2″-*O*-xylosyl		2″-*O*-xylosyl	6″-*O*-rhamnosyl
1‴	104.1	105.4		105.0	101.1
2‴	74.6	75.7		75.6	70.9
3‴	77.0	77.7		77.4	71.5
4‴	70.0	70.7		70.1	72.9
5‴	66.3	67.1		66.8	68.9
6‴					16.9
	5-*O*-glucosyl			6″-*O*-rhamnosyl	
1⁗	102.3			102.2	
2⁗	77.8			70.6	
3⁗	76.2			71.6	
4⁗	74.1			72.8	
5⁗	73.4			68.8	
6⁗	61.2			16.5	

Spectra obtained in CD_3OD.

*Table IV. Free-radical-scavenging activities (RC_{50}) of cyanidin glycosides **1–5** compared to ascorbic acid in the DPPH assay*

Compd.	RC_{50}	
	mg mL^{-1}	mol L^{-1}
1	6.90×10^{-3}	9.29×10^{-6}
2	5.42×10^{-3}	9.33×10^{-6}
3	3.73×10^{-3}	8.31×10^{-6}
4	6.51×10^{-3}	8.96×10^{-6}
5	5.68×10^{-3}	9.55×10^{-6}
Ascorbic acid	3.25×10^{-3}	1.85×10^{-6}

FAB-MS spectrum of **5** established the identity of this compound as cyanidin 3-*O*-rutinoside. All spectroscopic data of compounds **1–5** were in agreement with the respective published data (12, 16–22).

To the best of our knowledge, this is the first report on the occurrence of cyanidin glycosides **1–5** in the fruits of *Ribes biebersteinii* Berl. With the exception of compound **1**, in all other compounds the glycosylation was limited to position C-3. Anthocyanins occur almost in all *Ribes* species. However, significant structural variations have been noted among *Ribes* anthocyanins. Anthocyanins have previously been applied successfully as chemotaxonomic markers for various plant genera (23, 24). The anthocyanins **1–5** isolated and identified in the present study might have some chemotaxonomic implications within the genus *Ribes*.

All cyanidin glycosides **1–5** displayed s high level of free-radical-scavenging activity, comparable to that of the positive control ascorbic acid (vitamin C), in the DPPH assay. The RC_{50} values of these compounds are presented in Table IV. Among the glycosides, cyanidin 3-*O*-glucoside (**3**) exhibited the most potent free-radical-scavenging property with a RC_{50} value of 8.31×10^{-6} mol L^{-1} (RC_{50} value of the positive control, vitamin C was 1.85×10^{-6} mol L^{-1}), while the least active was compound **5** $RC_{50} = 9.55 \times 10^{-6}$ mol L^{-1}). In general, the free-radical-scavenging activity of compounds **1–5**, like other natural phenolic compounds, is a consequence of the phenolic hydroxyl functionalities present in the molecules. The free-radical-scavenging property of anthocyanins is predominantly due to their ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers and, to some extent, could also be due to their metal chelation potential (25).

CONCLUSIONS

The present study demonstrated that fruits of *Ribes biebersteinii* Berl. possess high levels of cyanidin glycosides **1–5**. The presence of these free-radical-scavengers in the fruits of this plant might explain, at least to some extent, the retina-protecting activity of this plant, which is probably mediated through its capacity for protecting retina from any oxidative damage.

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S A Ž E T A K

Izolacija i sposobnost hvatanja slobodnih radikala cijanidin 3-*O*-glikozida iz plodova *Ribes biebersteinii* Berl.

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Pet cijanidin glikozida, 3-*O*-sambubiozil-5-*O*-glukozil cijanidin (**1**), cijanidin 3-*O*-sambubiozid (**2**), cijanidin 3-*O*-glukozid (**3**), cijanidin 3-*O*-(2^{G}-ksilozil)-rutinozid (**4**) i cijanidin 3-*O*-rutinosid (**5**) izolirani su iz metanolnog ekstrakta plodova *Ribes biebersteinii* Berl. (*Grossulariaceae*) koristeći reverzno-faznu preparativnu tekućinsku kromatografiju visoke učinkovitosti. Cijanidin glikozidi pokazali su sposobnost hvatanja slobodnih radikala u pokusu s 2,2-difenil-1-pikrilhidrazilom (DPPH). Dobivene su sljedeće RC_{50} vrijednosti: $9{,}29 \times 10^{-6}$, $9{,}33 \times 10^{-6}$, $8{,}31 \times 10^{-6}$, $8{,}96 \times 10^{-6}$, odnosno $9{,}55 \times 10^{-6}$ mol L^{-1}. Strukture glikozida određene su kemijskom hidrolizom i spektroskopijom masa. Ukupni sadržaj antocijanina bio je 1,9 g na 100 g suhih plodova preračunato na cijanidin 3-glukozid.

Ključne riječi: *Ribes biebersteinii* Berl. (*Grossulariaceae*), 2,2-difenil-1-pikrilhidrazil (DPPH), cijanidin glikozid, hvatač slobodnih radikala

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