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FLAVONOID PROFILE OF *Sorbus intermedia*

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The genus *Sorbus* (Rosaceae) comprises about 100 species of deciduous trees or shrubs commonly distributed and often cultivated through the northern hemisphere [1]. *Sorbus intermedia* (Ehrh.) Pers. (Swedish mountainash) is one of five *Sorbus* species native in the Polish flora [2]. It is also an apomictic, polyploidal, stable hybrid of *S. aucuparia* L., *S. aria* (L.) Crantz. and *S. torminalis* (L.) Crantz [1, 2]. The parental species *S. aucuparia* is known as a valuable natural source of antioxidants [3–6]. The present work is therefore a contribution to the phytochemical study of descendant *S. intermedia*, with the objective of discovering natural products with potential antioxidant activity.

According to the literature, little is known about the chemical composition of *S. intermedia*. Only leaf flavonoids have been studied in co-chromatographic PC screening, and four of the compounds have been identified as quercetin and kaempferol 3-*O*-β-sophorosides, astragalin, and isoquercitrin [7].

In the course of the present study, nine flavonoids **1–9** were isolated from inflorescences of *S. intermedia*. The structures of the isolates were identified by spectroscopic methods (UV, ^1H and ^{13}C NMR), co-chromatography (TLC, HPLC) with standards, and by the products of acid hydrolysis. All these data were in good agreement with the respective literature data [8–14]. The plant material afforded sexangularetin (**1**) [8], quercetin (**2**) [9], sorbaroside (**3**) [8], isorhamnetin 3-*O*-β-D-glucopyranoside (**4**) [10, 11], hyperoside (**5**), isoquercitrin (**6**) [9, 12], avicularin (**7**) [9, 12, 13], rutin (**8**), and quercetin 3-*O*-β-sophoroside (**9**) [9, 12, 14]. Although all compounds **1–9** were already known, among them only **6** and **8** have previously been reported in *S. intermedia*. Moreover, compounds **4** and **7** were isolated from the genus *Sorbus* for the first time.

In order to prove the originality of the isolates the HPLC method was used. The methanolic extracts of inflorescences, leaves, and fruits were analyzed by the fingerprint protocol. As a result, the flavonoid profiles of all the plant materials were found to be similar, and rutin and isoquercitrin were predominant components of the extracts. The exception was the presence of sexangularetin and its glucoside (sorbaroside), which were detected in the inflorescences only. The peaks of two unisolated components were identified with standards as chlorogenic acid and astragalin. Kaempferol 3-*O*-β-sophoroside, described earlier as present in the leaves [7], was, however, not detected.

The antioxidant capacity of nine isolated flavonoids was studied by the DPPH test [5, 15]. The highest IC_{50} values were obtained for compounds **2** (5.16 μM), **8** (5.55 μM), **5** (6.05 μM), **6** (6.70 μM), **7** (6.95 μM), and **9** (7.43 μM). Lower activity was found for compound **1** (11.95 μM), whereas compounds **3** and **4** turned out to be inactive (IC_{50} > 1000 μM). The IC_{50} value determined for standard Trolox was 17.26 μM.

Plant Material was authenticated and collected in the Botanical Garden in Lodz in June (inflorescences and leaves) and in October (fruits) in 2006. Voucher specimens (SIt001-SIt003/06) have been deposited at the Department of Pharmacognosy, Medical University of Lodz, Poland.

Methods. Melting points (uncorrected) were determined on a Boethius apparatus. UV spectra with the usual shift reagents (according to the standard procedure [16]) were obtained on a Unicam 500 apparatus, and ^1H and ^{13}C NMR spectra (125.7 MHz) on a Bruker 500 MHz apparatus (in DMSO-d_6, TMS as internal standard). The absorbance was measured using a Spekol spectrophotometer in 10 mm cuvettes. Preparative column chromatography (CC) was performed on polyamide SC6 (MN) and on Sephadex LH-20 (Sigma Aldrich); analytical TLC on silica gel 60 G (Merck) using two solvent systems: S-1 (EtOAc–HCOOH–H_2O 18:1:1, v/v/v) and S-2 (EtOAc–AcOH–HCOOH–H_2O 100:27:1:5, v/v/v/v, organic phase).

HPLC Analysis. Methanolic extracts were analyzed by the fingerprint protocol on an HP 1100 Series instrument (Perlan Technologies) equipped with a UV/VIS detector (HP 1314 A). A Lichrosphere 100 (5 μm, 250 × 4 mm, i.d., Merck)

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C18 column was used for separation, and the mobile phase consisted of solvents A (0.5% water solution of orthophosphoric acid) and B (ACN). The elution profile was as follows: 0–2 min, 5% B; 2–5 min, 5–10% B; 5–7 min, 10–15% B; 7–13 min, 15–20% B; 13–21 min, 20% B; 21–25 min, 20–25% B; 25–28 min, 25–30% B; 28–30 min, 30–40% B; 30–35 min, 40–50% B; 35–36 min, 50–5% B; 36–40 min, 5% B (equilibration). The flow rate was 1.0 mL/min, and detection was at 350 nm.

Isolation of Flavonoids. Air-dried inflorescences of *S. intermedia* (600 g) were pre-extracted with petroleum ether and $CHCl_3$ in a Soxhlet apparatus and then exhaustively extracted with boiling MeOH and 70% aqueous MeOH. The combined alcohol extracts were partitioned between EtOAc and *n*-BuOH. The EtOAc extract (10.9 g) was first separated by CC on polyamide SC6 (eluent: C_6H_6–MeOH 9:1–1:1, v/v) to yield four fractions A–D. Fractions A and B (eluted with C_6H_6–MeOH 9:1, v/v) were separately purified by gel filtration (Sephadex LH-20, MeOH) to yield compounds **1** (10 mg) and **2** (35 mg), respectively. Fractions C (eluted with C_6H_6–MeOH 8:2, v/v) and D (eluted with C_6H_6–MeOH 7:3, v/v) were re-chromatographed on polyamide (eluent: H_2O–ACN 1:0–3:7, v/v). From fraction C, compounds **3** (40 mg) and **4** (45 mg) were obtained. From fraction D, two further pure compounds, **6** (160 mg) and **7** (90 mg), were isolated, followed by a mixture (30 mg) of compounds **5** and **6**. The *n*-BuOH extract (26.0 g) was separated on polyamide (eluent: H_2O–MeOH 4:1–1:1, v/v) to afford compounds **8** (705 mg) and **9** (18 mg).

Sexangularetin, 8-methoxykaempferol (1), $C_{16}H_{12}O_7$, orange-yellow needles, mp 271–273°C (MeOH); TLC R_f 0.90 (S-1); HPLC RT 16.172 min. UV spectrum (MeOH, λ_{max}, nm): 251, 274 sh, 324 sh, 376; +NaOMe: 287, 336, 428; +$AlCl_3$: 275, 312 sh, 358, 433; +$AlCl_3$+HCl: 274, 311, 357, 433; +NaOAc: 281, 320 sh, 402; +NaOAc+H_3BO_3: 274, 323 sh, 383. ^{1}H NMR (δ, ppm, J/Hz): 12.15 (1H, s, OH-5), 8.05 (2H, d, J = 8.6, H-2′ and H-6′), 6.95 (2H, d, J = 8.6, H-3′ and H-5′), 6.26 (1H, s, H-6), 3.81 (3H, s, MeO-8). ^{13}C NMR (δ, ppm): 176.11 (C-4), 159.29 (C-4′), 156.49 (C-7), 155.42 (C-5), 148.45 (C-9), 146.76 (C-2), 135.71 (C-3), 129.38 (C-2′ and C-6′), 127.46 (C-8), 121.85 (C-1′), 115.60 (C-5′), 115.33 (C-3′), 102.97 (C-10), 98.37 (C-6), 60.97 (8-OMe).

Quercetin (2), $C_{15}H_{10}O_7$, orange-yellow needles, mp 298–303°C (MeOH) [12].

Sexangularetin 3-*O*-*β*-D-glucopyranoside, sorbaroside (3), $C_{22}H_{22}O_{12}$, amorphous yellow powder, mp 241–243°C (MeOH); TLC R_f 0.42 (S-1), 0.72 (S-2); HPLC RT 19.188 min. UV spectrum (MeOH, λ_{max}, nm): 251, 272 sh, 325 sh, 355; +NaOMe: 282, 330, 405; +$AlCl_3$: 282, 312 sh, 354, 410; +$AlCl_3$+HCl: 282, 312, 354, 410; +NaOAc: 279, 310 sh, 380; +NaOAc+H_3BO_3: 272, 327 sh, 353. ^{1}H NMR (δ, ppm, J/Hz): 12.29 (1H, s, HO-5), 8.05 (2H, d, J = 8.7, H-2′ and H-6′), 6.90 (2H, d, J = 8.7, H-3′ and H-5′), 6.28 (1H, s, H-6), 5.46 (1H, d, J = 7.25, H-1″ of Glc), 3.56 (1H, dd, J = 11.4 and 6.0, CH_2-6a″), 3.80 (3H, s, MeO-8), 3.05–3.35 (4H, m, the remaining glucosyl protons). ^{13}C NMR (δ, ppm): 177.64 (C-4), 160.04 (C-4′), 156.09 (C-7), 155.90 (C-2), 155.70 (C-5), 148.61 (C-9), 133.17 (C-3), 130.79 (C-2′ and C-6′), 127.49 (C-8), 121.03 (C-1′), 115.24 (C-3′ and C-5′), 103.90 (C-10), 98.94 (C-6), 100.88 (C-1″), 77.56 (C-5″), 76.41 (C-3″), 74.22 (C-2″), 69.88 (C-4″), 61.01 (C-6″), 60.84 (8-OMe).

Isorhamnetin 3-*O*-*β*-D-glucopyranoside (4), $C_{22}H_{22}O_{12}$, pale yellow needles, mp 166–168°C (MeOH); TLC R_f 0.37 (S-1), 0.71 (S-2); HPLC RT 21.460 min. UV spectrum (MeOH, λ_{max}, nm): 255, 272 sh, 322 sh, 367; +NaOMe: 272, 328, 418; +$AlCl_3$: 268, 303 sh, 362, 403; +$AlCl_3$+HCl: 270, 300 sh, 358, 403; +NaOAc: 280, 328 sh, 400; +NaOAc+H_3BO_3: 255, 270 sh, 303 sh, 360. ^{1}H NMR (δ, ppm, J/Hz): 12.60 (1H, s, HO-5), 7.93 (1H, d, J = 1.9, H-2′), 7.48 (1H, dd, J = 1.9 and 8.3, H-6′), 6.90 (1H, d, J = 8.3, H-5′), 6.43 (1H, d, J = 1.9, H-8), 6.19 (1H, d, J = 1.9, H-6), 5.56 (1H, d, J = 7.2, H-1″ of Glc), 3.82 (3H, s, MeO-3′), 3.56 (1H, dd, J = 11.2 and 2.9, CH_2-6a″), 3.09–3.25 (4H, m, the remaining glucosyl protons).

Quercetin 3-*O*-*β*-D-galactopyranoside, hyperoside (5), $C_{21}H_{20}O_{12}$, isolated in a mixture with compound **6**, and identified by products of acid hydrolysis and co-chromatographically with standards: TLC R_f 0.27 (S-1), 0.41 (S-2); HPLC RT 17.012 min.

Quercetin 3-*O*-*β*-D-glucopyranoside, isoquercitrin (6), $C_{21}H_{20}O_{12}$, yellow powder, mp 219–222°C (MeOH); TLC R_f 0.35 (S-1), 0.65 (S-2); HPLC RT 17.304 min [12, 15].

Quercetin 3-*O*-*α*-L-arabinofuranoside, avicularin (7), $C_{20}H_{18}O_{11}$, yellow prisms, mp 216–218°C (MeOH) [9, 12, 13].

Quercetin 3-*O*-(6″-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside, rutin (8), $C_{27}H_{30}O_{16}$, yellow powder, mp 189–193°C; TLC R_f 0.07 (S-1), 0.16 (S-2); HPLC RT 16.172 min [12, 15, 17].

Quercetin 3-*O*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside, quercetin 3-*O*-*β*-sophoroside (9), $C_{27}H_{30}O_{16}$, pale yellow needles, mp 198–204°C (MeOH); TLC R_f 0.06 (S-1), 0.13 (S-2); HPLC RT 13.749 min. UV spectrum (MeOH, λ_{max}, nm): 255, 264 sh, 360; +NaOMe: 274, 330, 414; +$AlCl_3$: 270, 300 sh, 364 sh, 423; +$AlCl_3$+HCl: 270, 300, 358, 401; +NaOAc: 270, 393; +NaOAc+H_3BO_3: 261, 303 sh, 379. ^{1}H NMR (δ, ppm, J/Hz): 12.60 (1H, s, HO-5), 7.70 (1H, dd, J = 2.2 and 8.5, H-6′), 7.65 (1H, d, J = 2.2, H-2′), 6.95 (1H, d, J = 8.5, H-5′), 6.50 (1H, d, J = 2.0, H-8), 6.29 (1H, d, J = 2.0, H-6), 5.78

(1H, d, J = 7.5, H-1″ of glucose), 4.70 (1H, d, J = 7.6, H-1‴ of Glc), 3.10–5.65 (12H, 4 × m, the remaining sophorosyl protons). ^{13}C NMR (δ, ppm): 177.58 (C-4), 164.14 (C-7), 161.37 (C-5), 155.74 (C-9), 156.30 (C-2), 148.59 (C-4′), 144.92 (C-3′), 133.12 (C-3), 121.90 (C-6′), 121.24 (C-1′), 116.23 (C-5′), 115.50 (C-2′), 104.30 (C-10), 104.34 (C-1‴), 98.12 (C-1″), 98.73 (C-6), 93.59 (C-8), 82.78 (C-2″), 77.12 (C-5″), 76.92 (C-5‴), 76.82 (C-3‴), 75.50 (C-3″), 74.54 (C-2‴), 69.63 (C-4″), 69.60 (C-4‴), 60.80 (C-6″), 60.80 (C-6‴).

DPPH Test. The scavenging activity for DPPH radicals was determined based on the *in vitro* methods published earlier [5, 18]. Briefly, 2 mL of 90 μM methanolic DPPH solution was mixed with 1 mL of flavonoid methanolic solutions of different concentrations. After 30 min of incubation at room temperature and in the dark, the decrease in the absorbance was measured at 517 nm. The concentration of each compound in the reaction mixture was plotted against the percentage of remaining DPPH radical. Using the curve obtained, the IC_{50} value was calculated. (±)-6-Hydroxy-2,2,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Fluka) was used as positive control. Since the isolated compound **5** was not pure, a commercial standard of hyperoside (Fluka) was used in the test.

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