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FLAVONOIDS FROM THE FLOWERS OF *AESCULUS HIPPOCASTANUM*

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Abstract: The flavonoids, kaempferol derivatives: 3-*O*-α-arabinofuranoside, 3-*O*-β-glucopyranoside, 3-*O*-α-rhamnopyranoside, 3-*O*-α-rhamnopyranosyl (1→6)-*O*-β-glucopyranoside and quercetin derivatives: 3-*O*-α-arabinofuranoside, 3-*O*-β-glucopyranoside, 3-*O*-α-rhamnopyranosyl (1→6)-*O*-β-glucopyranoside, were isolated from the flowers of *Aesculus hippocastanum* and identified. The structures of these compounds were confirmed by a chemical analysis and spectrophotometric methods (UV, ^{1}H-, ^{13}C-NMR, ESI-MS). The presence of free aglycones: kaempferol and quercetin was confirmed chromatographically by comparison with standards.

Keywords: *Aesculus hippocastanum*, *Hippocastanaceae*, flavonoids, kaempferol and quercetin glycosides, identification

Aesculus hippocastanum L. (*Hippocastanaceae*) is a large tree, commonly known as horse chestnut. It is native to a small area in the mountains of the Balkans in southeast Europe, but it is widely cultivated throughout the temperate zone, as an ornamental tree.

Horse chestnut seeds, bark and flowers (*Hippocastani semen, cortex, flos*) have been used for healing purposes for several centuries. The extracts from the seeds, containing mainly escin, a mixture of triterpenoid saponins, are constituents of numerous preparations. They have traditionally been used to treat patients with chronic venous insufficiency and to alleviate its associated symptoms, including leg swelling and heaviness. The efficacy of preparations that contain the horse chestnut seed extract is believed to be largely due to an inhibitory effect on the catalytic breakdown of capillary wall proteoglycans (1, 2). The bark of horse chestnut, rich in coumarins (e. g. esculin, fraxetin), is especially used in the treatment of hemorrhoids (3).

In folk medicine, horse chestnut flowers are prescribed both for external and internal use in cases of inflammatory conditions of veins, lower leg varicose veins and hemorrhoids as well as frostbites, burns, epidermis abrasion and skin inflammation (4–6). In Poland, horse chestnut flowers are available as single herbs (produced by: Kawon-Hurt, Flos), in addition to being a component of herb mixtures, e.g., Rektosan (Herbapol, Lublin).

The studies of horse chestnut flowers carried out in the 1950s, by means of the analysis methods available at that time, proved the presence of the following kaempferol derivatives: 3-arabinoside, 3-glucoside, 3-rhamnoglucoside and quercetin derivatives: 3-glucoside i 3-rhamnoglucoside (7). The development of the modern identification methods gives an opportunity to study the structure of the compounds more thoroughly and to determine the configuration of the sugar moiety and the type of the intermolecular bonds.

The aim of this study was to isolate the flavonoid compounds from the flowers of horse chestnut and to determine their structure with the use of the modern identification methods (NMR, MS).

EXPERIMENTAL

Plant material

The flowers of *Aesculus hippocastanum* L. (horse chestnut) were collected from the flowering trees in the Botanical Garden of Adam Mickiewicz University in Poznań in 2007 and dried in normal conditions.

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Extraction and isolation

The air-dried flowers of horse chestnut (600 g) were extracted by maceration with MeOH (room temperature); next, they were extracted twice with MeOH (60°C, 2 h) and with 70% aq. MeOH (boiling temperature, 2h). The obtained extracts had a similar qualitative composition (TLC, mobile phase S_1, S_2), so they were combined and concentrated. The dry extract was purified by precipitation of ballast with hot water. The aqueous filtrate was successively reextracted with $CHCl_3$, and then with Et_2O and EtOAc.

The combined Et_2O and EtOAc fractions, rich in flavonoids, were submitted to column chromatography on cellulose Whatman CF-11 (eluent S_9–S_{14}). The fractions were chromatographically controlled, appropriately combined and, finally, separated by preparative TLC on cellulose microcrystalline Avicel using mobile phases S_1, S_7, S_8. The bands of compounds were scraped off and eluted with MeOH and, next, with 50% aq. MeOH. The purification of all compounds was carried out on Sephadex LH-20, elution being performed with S_{13}. As a result, the flavonoid glycosides **I–VII** were isolated**.** The presence of free aglycones: kaempferol and quercetin in the combined Et_2O and EtOAc fraction was confirmed chromatographically with standards (S_3, S_4, S_5).

Chromatographic analysis

TLC: Alufolien Cellulose (Merck). S_1: HOAc : H_2O (15:85 v/v), S_2: EtOAc : HCO_2H : H_2O (10:2:3 v/v/v) upper phase, S_3: C_6H_6 : HOAc : H_2O (125:72:3 v/v/v), S_4: HOAc : conc. HCl : H_2O (30:3:10 v/v/v), S_5: n-PrOH : HCO_2H : H_2O (2:5:5 v/v/v).

Silica gel 60 G (Merck, Germany). S_6: n-PrOH : EtOAc : H_2O (7:2:1 v/v/v).

Preparative TLC: Microcristalline cellulose Avicel (Merck, Germany). S_1, S_7: HOAc : H_2O (5:95 v/v), S_8: HOAc : H_2O (30:70 v/v).

Column chromatography: Cellulose Whatman CF-11 (Whatman Chemical Separation Ltd., U.K.). S_9: EtOAc, S_{10}: EtOAc : MeOH : H_2O (100:16:10 v/v/v), S_{11}: EtOAc : MeOH : H_2O (100:25:10 v/v/v), S_{12}: EtOAc : MeOH : H_2O (100:50:20 v/v/v), S_{13}: MeOH, S_{14}: H_2O; Sephadex LH-20 (Pharmacia, Sweden). S_1.

Visualization, reagents

Flavonoids: 0.1% solution of Naturstoff reagent A in MeOH (NA-reagent, Roth, Germany), $UV_{365\ nm}$.

Sugars: aniline phthalate and heating at 105°C, VIS.

Identification

The flavonoids were identified by means of an analysis of the products of acid hydrolyses (total and partial) and, also, by spectroscopic methods: UV, ^{1}H-NMR, ^{13}C-NMR, MS (8–14).

Acid hydrolysis

Total: 1 mg of a compound (**I**, **II**, **III**, **V** and **VI**) was heated in 1% HCl (100°C, 1 h); partial: 1 mg of a compound (**IV** and **VII**) was heated in 0.5% HCl (100°C, 10 min.), the hydrolysis was monitored by TLC in S_1. The hydrolyzates of each compound were extracted with EtOAc. The aglycones present in the organic phase were identified by co-TLC in S_3, S_4, S_5, whereas the water layers were checked for sugars by TLC in S_6.

Spectral analysis

UV spectra were recorded on a UV/VIS Perkin Elmer Lambda 35 spectrometer, in methanol before and after addition of the shift reagents, according to the method by Mabry et al. (8).

^{1}H- (300 MHz) and ^{13}C- (75.5 MHz) NMR spectra were recorded in CD_3OD-d_4 on a Varian Unity–300 MHz Spectrometer with TMS as internal standard; chemical shifts are given in δ (ppm).

The ESI-MS spectra were obtained using a Waters/Micromass (Manchester, U.K.) ZQ Mass spectrometer connected with HPLC (Waters typ 2690; Milford, USA) and spectrometer UV Photodiode Array Detector Waters 996 (λ = 200–500 nm). ESI-MS analyses were performed in positive and negative ionization modes (13, 14).

Kaempferol

TLC R_f S_3 = 0.72, S_4 = 0.61, S_5 = 0.51.

Kaempferol-3-*O*-α-arabinofuranoside (**I**)

Yellow needles; m.p. 223–226°C (10 mg). TLC: R_f S_1 = 0.35, S_3 = 0.96. UV (MeOH, λ_{max}): 266.1, 347.9; + NaOAc 273.9, 301.1, 373.2; + NaOAc/H_3BO_3 266.9, 349.2; + NaOMe 273.9, 392.2; + $AlCl_3$ 275.3; 303.5, 348.4, 399.1; $AlCl_3$/HCl 275.9, 301.9, 344.9, 397.5. Total acid hydrolysis: kaempferol and arabinose. ESI-MS (negative): *m*/*z* 417 $[M–H]^-$, *m*/*z* 285 $[A–H]^-$. ESI-MS (positive): *m*/*z* 419 $[M+H]^+$, *m*/*z* 287 $[A+H]^+$. For ^{1}H- and ^{13}C-NMR data see Tables 1 and 2.

Kaempferol-3-*O*-β-glucopyranoside (**II**)

Yellow needles; m.p. 173–175°C (50 mg). TLC: R_f S_1 = 0.41, S_3 = 0.75. UV (MeOH, λ_{max}) 266.2, 348.1; + NaOAc 274.5, 305.1, 383.0; + NaOAc/H_3BO_3 267.2, 350.4; + NaOMe 277.3, 394.1; + $AlCl_3$ 275.5; 303.3, 345.9, 397.7; $AlCl_3$/HCl 275.5, 301.6, 344.4, 397.2. ESI-MS (neg-

Table 1. ^{1}H-NMR data of compounds **I–VII** in CD_3OD, δ (ppm), (*J* Hz).

Protons	Compounds						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Aglycone							
2'	7.96 d (8.9)	8.10 d (8.8)	7.76 d (8.8)	8.10 d (8.9)	7.52 d (2.4)	7.70 d (2.1)	7.66 d (2.0)
3'	6.91 d (8.9)	6.88 d (8.8)	6.93 d (8.8)	6.93 d (8.9)			
5'	6.91 d (8.9)	6.88 d (8.8)	6.93 d (8.8)	6.93 d (8.9)	6.90 d (8.3)	6.86 d (8.5)	6.87 d (8.6)
6'	7.96 d (8.9)	8.10 d (8.8)	7.76 d (8.8)	8.10 d (8.9)	7.47 dd (8.30;2.20)	7.58 dd (8.5; 2.1)	7.63 dd (8.6; 2.2)
8	6.40 d (2.1)	6.40 d (2.0)	6.40 d (2.0)	6.45 d (1.8)	6.37 d (2.2)	6.38 d (2.1)	6.40 d (2.2)
6	6.20 d (2.3)	6.20 d (2.0)	6.20 d (2.0)	6.25 d (1.8)	6.19 d (2.2)	6.19 d (2.1)	6.20 d (2.2)
Sugar 1''							
Arabinose	5.48 d (0.4)				5.45 s		
Glucose		5.25 d (7.3)		5.17 d (7.3)		5.25 d (7.5)	5.10 d (7.6)
Rhamnose			5.37 d (1.8)	4.55 d (1.7)			4.51 d (1.7)
CH_3 of rhamnose			0.91 d (5.6)	1.15 d (6.2)			1.11 d (6.1)

ative): *m/z* 447 $[M–H]^-$, *m/z* 285 $[A–H]^-$. ESI-MS (positive): *m/z* 449 $[M+H]^+$, *m/z* 287 $[A+H]^+$. Total acid hydrolysis: kaempferol and glucose. For ^{1}H- and ^{13}C-NMR data see Tables 1 and 2.

Kaempferol-3-*O*-α-rhamnopyranoside (III)

Yellow needles; m.p. 172–174°C (7 mg). TLC: R_f S_1 = 0.45, S_3 = 0.95. UV (MeOH, λ_{max}): 265.1, 341.9; + NaOAc 273.2, 377.7; + $NaOAc/H_3BO_3$ 265.9, 345.8; + NaOMe 277.2, 384.5; + $AlCl_3$ 274.4; 301.7, 343.7, 396.7; $AlCl_3$/HCl 274.6, 300.0, 340.6, 396.7. ESI-MS (negative): *m/z* 431 $[M–H]^-$, *m/z* 285 $[A–H]^-$. ESI-MS (positive): *m/z* 433 $[M+H]^+$, *m/z* 287 $[A+H]^+$. Total acid hydrolysis: kaempferol and rhamnose. For ^{1}H- and ^{13}C-NMR data see Tables 1 and 2.

Kaempferol-3-*O*-α-rhamnopyranosyl (1→6)-*O*-β-glucopyranoside (IV)

Yellow needles; m.p. 182–185°C (10 mg). TLC: R_f S_1 = 0.58, S_3 = 0.42. UV (MeOH, λ_{max}): 265.9, 347.1; +NaOAc 274.0, 379.0; + $NaOAc/H_3BO_3$ 267.5, 352.1; + NaOMe 276.8, 402.8; + $AlCl_3$ 274.3; 303.4, 349.6, 397.3; $AlCl_3$/HCl 273.6, 303.0, 346.6, 394.0. Total acid hydrolysis: kaempferol, glucose and rhamnose. Partial acid hydrolysis: 3-*O*-glucoside as a secondary heteroside, chromatographically identical with compound **II**. ESI-MS (negative): *m/z* 593.4 $[M–H]^-$. ESI-MS (positive): *m/z* 594.9 $[M+H]^+$, m/z 617.5 $[M+Na]^+$, *m/z* 287 $[A+H]^+$. For ^{1}H- and ^{13}C-NMR data see Table 1 and 2.

Quercetin

TLC: R_f S_3 = 0.23, S_4 = 0.42, S_5 = 0.30.

Quercetin-3-*O*-α-arabinofuranoside (V)

Yellow needles; m.p. 252–253°C (5 mg). TLC: R_f S_1 = 0.29, S_3 = 0.80. UV (MeOH, λ_{max}): 258.2, 357.2; + NaOAc 272.4, 382.9; + $NaOAc/H_3BO_3$ 262.0, 376.0; + NaOMe 274.3, 403.2; + $AlCl_3$ 275.3, 429.6; $AlCl_3$/HCl 270.5, 359.8, 401.2. Total acid hydrolysis: quercetin and arabinose. ESI-MS (negative): *m/z* 433 $[M–H]^-$, *m/z* 301 $[A–H]^-$. ESI-MS (positive): *m/z* 435 $[M+H]^+$, *m/z* 303 $[A+H]^+$. For ^{1}H- and ^{13}C-NMR data see Tables 1 and 2.

Quercetin-3-*O*-β-glucopyranoside (VI)

Yellow needles; m.p. 226–228°C (15 mg). TLC: R_f S_1 = 0.39, S_3 = 0.67. UV (MeOH λ_{max}): 260.3, 353.6; + NaOAc 269.7, 383.7; + $NaOAc/H_3BO_3$ 263.1, 372.9; + NaOMe 274.3, 395.5; + $AlCl_3$ 273.5; 422.1; $AlCl_3$/HCl 269.9, 359.8, 395.7. Total acid hydrolysis: quercetin and glucose. ESI-MS (negative): *m/z* 463 $[M–H]^-$, *m/z* 301 $[A–H]^-$. ESI-MS (positive): *m/z* 465 $[M+H]^+$, *m/z* 303 $[A+H]^+$. For ^{1}H- and ^{13}C-NMR data see Tables 1 and 2.

Quercetin-3-*O*-α-rhamnopyranosyl (1→6)-*O*-β-glucopyranoside (VII)

Yellow needles; m.p. 186–188°C (10 mg). TLC: R_f S_1 = 0.48, S_3 = 0.35. UV (MeOH, λ_{max}): 257.6, 358.1; + NaOAc 271.9, 395.6; +

Table 2. ^{13}C-NMR data of compounds **I–VII** in CD_3OD, δ (ppm).

Carbon atoms	Compounds					
	I	**II**	**III**	**IV**	**VI**	**VII**
Aglycone						
2	157.2	158.6	159.3	158.6	158.5	158.6
3	133.6	135.5	136.2	135.5	135.6	135.6
4	178.6	179.5	179.6	179.5	179.5	179.4
5	161.7	163.1	163.2	163.0	163.1	163.0
6	98.5	99.9	99.9	100.0	100.0	100.0
7	164.8	166.2	166.2	166.0	166.3	166.2
8	93.4	94.8	94.8	94.9	94.8	94.9
9	158.0	159.1	158.6	159.4	159.0	159.3
10	104.3	104.1	105.9	105.7	105.6	105.6
1’	121.4	122.8	122.7	122.8	123.2	123.5
2’	130.6	132.3	131.9	132.4	116.0	116.1
3’	115.1	116.5	116.5	116.1	145.9	145.9
4’	160.2	161.6	161.62	161.5	149.9	149.8
5’	115.1	116.5	116.5	116.1	117.5	117.7
6’	130.6	132.3	131.9	132.4	123.1	123.1
Arabinose						
1”	108.2					
2”	82.0					
3”	77.3					
4”	86.7					
5”	61.2					
Glucose						
1”		105.7		104.6	104.3	104.7
2”		75.7		75.8	75.7	75.7
3”		78.4		78.2	78.4	78.2
4”		71.4		71.5	71.2	72.3
5”		78.1		77.2	78.1	77.3
6”		62.7		68.6	62.5	68.6
Rhamnose						
1”’			103.5	102.4		102.4
2”’			71.9	72.1		72.1
3”’			72.1	72.3		71.4
4”’			73.2	73.9		73.9
5”’			72.0	69.7		69.7
6”’			17.7	17.9		17.9

$NaOAc/H_3BO_3$ 262.7, 379.4; + NaOMe 274.7, 409.3; + $AlCl_3$ 274.8; 433.9; $AlCl_3$/HCl 268.6, 399.0. Total acid hydrolysis: quercetin, glucose and rhamnose. Partial acid hydrolysis: 3-*O*-glucoside as a secondary heteroside, chromatographically identical with compound **VI**. ESI-MS (negative): *m*/*z* 609 $[M–H]^-$, *m*/*z* 463 [M-rhamnosyl-H]$^-$, *m*/*z* 301 [A-H]$^-$. ESI-MS (positive): *m*/*z* 611 $[M+H]^+$, *m*/*z* 465 $[M–rhamnosyl+H]^+$, *m*/*z* 303 $[A+H]^+$. For ^{1}H- and ^{13}C-NMR data see Tables 1 and 2.

RESULTS AND DISCUSSION

The extract from the flowers of *Aesculus hippocastanum* L. was partitioned between chloroform, ethyl ether and ethyl acetate. The combined ethyl ether and ethyl acetate fractions, containing a complex of flavonoid compounds, were first separated by column chromatography (cellulose; S_9–S_{14}). After chromatographic analysis, appropriate fractions were separated by PTLC (microcrystalline cellulose Avicel; S_1, S_7, S_8) and, eventually, fractions containing chromatographically homogeneous compounds were purified on Sephadex LH-20 column eluted with S_{13}. As a result, seven flavonoid compounds (**I–VII**) were isolated.

The identification of the flavonoid compounds was carried out by a chromatographic analysis of their hydrolysis products, co-chromatography with standards, and by spectroscopic methods: UV, ^{1}H-, ^{13}C-NMR and ESI-MS (8–14).

Flavonoids **I–IV** and **V–VII** gave brown fluorescence under $UV_{365\ nm}$, typical for the C-3 substituted flavonoid glycosides, changing into yellow (**I–IV**) or orange fluorescence (**V–VII**) after spraying with NA-reagent. The UV spectra indicated the presence of the *ortho*-dihydroxyl group in **V–VII**, the absence of these groups in **I– IV** and, also, free hydroxyl groups at positions C-5, C-7, C-4' in all compounds (8).

Total acid hydrolysis of **I**, **II** and **III** yielded kaempferol and arabinose, glucose and rhamnose, respectively; that of **V** and **VI** gave quercetin and arabinose and glucose, correspondingly. Partial acid hydrolysis of **IV** and **VII** produced kaempferol 3-*O*-glucoside and quercetin 3-*O*-glucoside, respectively, as secondary glycosides.

In the ^{1}H- and ^{13}C-NMR spectra of **I – IV**, all signals were in agreement with the literature data for kaempferol 3-*O*-substituted and those of **V**, **VI** and **VII** were in accordance with the ones reported for quercetin 3-*O*-substituted (8–12).

In the ^{1}H-NMR spectra, the signal of arabinose anomeric proton appeared either in the form of a doublet at 5.48 ppm of a low coupling constant J = 0.4 Hz (compound **I**) or a singlet at 5.45 ppm (compound **V**), which is characteristic for α-arabinofuranose (9). It also confirms the presence of the signal of arabinose anomeric proton at 108.3 ppm in the ^{13}C-NMR spectrum (compound **I**) (10–12). The ^{13}C-NMR analysis was not performed for compound **V** due to an insufficient amount of the isolated compound.

The ^{1}H-NMR and ^{13}C-NMR spectra of compounds **II** and **VI** were consistent with the literature data for kaempferol-3-*O*-β-glucopyranoside and quercetin-3-*O*-β-glucopyranoside, respectively (8–12, 15, 16).

In the ^{1}H-NMR spectra of **III**, a doublet of the anomeric proton of the sugar moiety (5.37 ppm; J = 1.8 Hz) and a doublet of three protons of the methyl group (0.9 ppm; J = 5.6) were observed, which suggested the presence of α-rhamnose in the molecule (9, 16, 17). In ^{13}C-NMR spectra of this compound signals of the anomeric carbon C-1" at 103.5 ppm and carbon of the methyl group C-6"'at 17.7 ppm could be seen, which is consistent with the literature data for α-rhamnose (10–12, 16, 17).

In the ^{1}H-NMR spectra of **IV** and **VII** signals from the anomeric protons of two sugar moieties were present. The doublets at 5.17 ppm with J = 7.3 Hz (**IV**) and at 5.10 ppm with J = 7.6 Hz (**VII**) were assigned to β-glucopyranoses linked to aglycones (kaempferol or quercetin) at C-3, while signals at 4.55 ppm (**IV**) and 4.51 ppm (**VII**) with the coupling constant J = 1.7 Hz corresponded to the anomeric proton of a terminal α-rhamnopyranose (9). The linkage of rhamnose to C-6" of the glucose moiety was determined on the basis of the ^{13}C-NMR spectra. A direct comparison of the ^{13}C-NMR spectrum of **IV** and **VII** with the spectrum of 3-*O*-glucosides (**II** and **VI**) revealed an upfield shift of δ 3.9 ppm (**IV**) and 4.1 ppm (**VII**) for C-6" of glucose and downfield shifts of δ 0.9 ppm (**IV**) and 0.8 ppm (**VII**) for C–5" of glucose, resulting from the presence of the rhamnose moiety. These shifts correspond to those reported for kaempferol and quercetin rhamnopyranosyl (1→6)glucopyranoside (18, 19), thus confirming the sugar linkage in compounds **IV** and **VII**.

In the ESI-MS analysis of **I**, **II**, **II**, **IV**, fragment ions at m/z 285 $[A–H]^-$ and m/z 287 $[A+H]^+$ were observed, which confirmed kaempferol as aglycone in the case of these compounds. The molecular weight 418 of **I** corresponded to kaempferol and pentose, 448 of **II** to kaempferol and hexose, 432 of **III** to kaempferol and methylpentose, 594 of **IV** to kaempferol, hexose and methylpentose. The presence of the fragment ions of **V**, **VI**, **VII** at m/z 301 $[A–H]^-$ and m/z 303 $[A+H]^+$ confirmed quercetin as aglycone in the case of these compounds. The molecular weight 432 of **V** corresponded to quercetin and pentose, 464 of **VI** to quercetin and hexose, 610 of **VII** to quercetin, hexose and methylpentose. In the case of **VII**, fragment ions at m/z 463 [M–rhamnosyl–H]$^-$ and m/z 465 [M–rhamnosyl+H]$^+$ were additionally presented. They appeared after separation of the rhamnose molecule, which confirmed that they were diglycosides (13–17).

As a result of the study, seven flavonoid compounds were isolated and identified. For five of them, previously described in the literature, sugar configuration was additionally determined, in addition to two disaccharides for which intersugar bonds were verified. These compounds were established to be: kaempferol-3-*O*-α-arabinofuranoside (**I**), kaempferol-3-*O*-β-glucopyranoside (**II**), quercetin-3-*O*-β-glucopyranoside (**VI**) and kaempferol-3-*O*-α-rhamnopyranosyl (1→6)-*O*-β-glucopyranoside (**IV**) and quercetin-3-*O*-α-rhamnopyranosyl-(1→6)-*O*-β-glucopyranoside (**VII**). The next two flavonoids, kaempferol-3-*O*-α-rhamnopyranoside (**III**) and quercetin-3-*O*-α-arabinofuranoside (**V**), had not been described as components of horse chestnut flowers before, so they have been indentified in this species for the first time.

Flavonoid compounds demonstrate a wide spectrum of biological activity. Derivatives of kaempferol and quercetin may act in a number of different ways on the vascular system. Their activity is related to an inhibition of hyaluronidase *via* chelation of copper ions in enzyme molecules (which decreases the permeability of endothelium) (20, 21). Flavonoids protect the capillary wall and normalize vascular permeability, also by an increase in the endothelium barrier-effect through stabilization of the membrane phospholipids (22). Moreover, flavonoids probably inhibit the activity of lysine oxidase (connected with the production of cross fibres of collagen and elastin) and lysosomal hydrolases (which degrade glucoamino glucans), they also influence on platelet adhesion, aggregation, secretion and an effect on smooth muscles (23).

The flavonoids, together with the other constituents of *Hippocastani flos* can participate in the pharmacological activity.

REFERENCES

1. Suter A., Bommer S., Rechner J.: Adv. Ther. 23, 179 (2006).
2. Blaschek W.: Z. Phytother. 25, 21 (2004).
3. Matysik G., Głowniak K., Soczewiński E., Garbacka M.: Chromatographia 38, 766 (1994).
4. Bombardelli E., Morazzoni B., Griffini A.: Fitoterapia 67, 483 (1996).
5. Buchwald W.: Wiadomości Zielarskie 5, 6 (1993) (Polish).
6. Hagers H., Hänsel R., Keller K., Rimler H., Schneider G.: Drogen A-D. Springer-Verlag, Berlin 1992.
7. Hörrhammer L., Gehermann H.J., Enders L.: Arch. Pharm. 292, 114, (1959).
8. Mabry T.J., Markham K.R., Thomas M.B.: The Systematic Identification of Flavonoids. Springer-Verlag, Berlin, Heidelberg, New York 1970.
9. Harborne J.B.: The Flavonoids: Advances in Research since 1986. Chapman and Hall, London 1994.
10. Harborne J.B., Mabry T.J.: The Flavonoids: Advances in Research. Chapman and Hall, London 1982.
11. Agrawal P.K.: Carbon-13 NMR of flavonoids. Elsevier Science Publishers, Amsterdam, Oxford, New York, Tokyo 1989.
12. Agrawal P.K.: Phytochemistry 31, 3307 (1992)
13. Płaziak A.S.: Mass Spectroscopy of Organic Compounds (Polish). Scientific Publisher (Wydawnictwo Naukowe) UAM, Poznań 1997.
14. Stobiecki M.: Identification of Plant Phenolic Compounds by Mass Spectrometry (Polish). Scientific Publisher (Wydawnictwo Naukowe) UAM, Poznań 1995.
15. Wang K., Yang Ch., Zhang Y.: Food Chem. 101, 365 (2007).
16. Ossipov V., Nurmi K., Loponen J., Prokopiev N., Haukioja E., Pihlaja K.: Biochem. Syst. Ecol. 23, 213 (1995).
17. Bilia A.R., Ciampi L., Mendez J., Morelli I.: Pharm. Acta Helv. 71, 199 (1994).
18. Markham K.R., Ternai B., Staley R., Geiger H., Mabry T.J.: Tetrahedron 34, 1389 (1978).
19. Matławska I., Sikorska M.: Acta Pol. Pharm. Drug Res. 59, 227 (2002).
20. Kuppusamy U.R., Das N.P.: Experientia 47, 1196 (1991).
21. Kuppusamy U.R., Khoo H.E., Das N.P.: Biochem. Pharmacol. 40, 397 (1990).
22. Di Carlo G., Mascolo N., Izzo A.A., Capasso F.: Life Sci. 65, 337 (1999).
23. Harborne J.B., Williams C.A.: Phytochemistry 555, 481 (2000).

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