

FLAVONOIDS AND PHENOLIC ACIDS OF *NEPETA CATARIA* L. VAR. *CITRIODORA* (BECKER) BALB. (*LAMIACEAE*)

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Abstract: Luteolin 7-O-glucuronide, luteolin 7-O-glucurono-(1→6)-glucoside, apigenin 7-O-glucuronide as well as free aglycones luteolin and apigenin have been isolated from lemon catnip herb (*Nepeta cataria* L. var. *citriodora*). Luteolin 7-O-glucurono-(1→6)-glucoside is probably a new compound, for the first time described. Two minor constituents of flavonoid fraction have been identified as apigenin 7-O-glucoside and luteolin 7-O-glucoside by means of HPLC method. The percentage of total flavonoids determined by use of spectrophotometric method was in the range from 0.30 to 0.46% of dry mass. In phenolic acid fraction, caffeic, rosmarinic and p-coumaric acids have been identified. Total amount of phenolic acids determined by spectrophotometric method was in the range of 0.75% to 1.4 % and the content of rosmarinic acid quantified by HPLC method fluctuated in the wide range from 0.06% to 0.15% depending on the sample. The results of the investigations showed that the composition of flavonoid compounds and phenolic acids in lemon catnip are similar to those in lemon balm (*Melissa officinalis* L.). The amount of flavonoids are similar in both plants, and the percentage of rosmarinic acid is about ten times lower in lemon catnip than in lemon balm. The presence of luteolin, apigenin and their glycosides, caffeic acid as well as the previously described terpenoids (ursolic acid, citral, nerol, geraniol) suggests the possibility of the use of lemon catnip herb as a constituent of phytopharmaceutical preparations with mild sedative, antispasmodic, antioxidative and antiinflammatory action.

Keywords: lemon catnip, *Nepeta cataria* var. *citriodora*, flavonoids, luteolin 7-O-glucuronide, apigenin 7-O-glucuronide, luteolin 7-O-glucurono-(1→6)-glucoside, caffeic acid, rosmarinic acid.

Nepeta cataria L. – catnip is a perennial herb native to Asia Minor and Mediterranean countries and cultivated in various parts of Europe and North America (1). It is known as the aromatic plant containing nepetalactone, an irydooid compound having opioid-like properties (2, 3). Nepetalactone was also found to be the main component of essential oil from other *Nepeta* species such as *N.caesarea* Boiss (3), *N. nuda* L. (2), *N. cadmea* Boiss (4).

In *N. cataria* L. var. *citriodora* (= *N. cataria* L. ssp. *citriodora*) nepetalactone was not detected; the main constituents of the essential oil of this plant were identified as nerol, geraniol, and citral (5-8). The same monoterpenes occur in lemon balm (*Melissa officinalis* L.) and lemon catnip is used in traditional medicine similarly to melissa as antispasmodic, sedative, and tonic remedy (1). In books of pharmacognosy (9, 10) *Nepeta cataria* var. *citriodora* is mentioned as a possible adulteration or substitute for lemon balm (*Melissae folium*). The content and composition of the essential oil from lemon catnip herb are well known (5-8). Also ursolic acid,

beta-sitosterol, campesterol, alpha-amyrin, beta-amyrin and sitosterol β-glucopyranoside have been reported previously (11). Phenolic compounds have not been investigated in lemon catnip so far. Therefore the present study has been performed to determine flavonoids and phenolic acids and compare the results with those obtained previously for melissa (12, 13).

EXPERIMENTAL

Plant material

The herb of *Nepeta cataria* L. var. *citriodora* (Becker) Balb. derived from plants cultivated in the Garden of Medicinal Plants of the Chair of Pharmacognosy, Medical University of Łódź, dried at 25-30°C. It was collected at flowering time (July and August). Identification of the plant material was performed on the basis of morphological features in comparison with those described in the literature (1, 14, 15). Plant specimen is stored at the Chair of Pharmacognosy, Medical University of Łódź.

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Methods and equipment

Melting points (m.p.) were determined on Boetius apparatus and are uncorrected. IR spectra were made in KBr tablets using an ATI Mattson Infinity series (FTIR) spectrophotometer and UV spectra were run on Perkin Elmer Lambda 25 apparatus. ¹H-NMR spectra were recorded on Bruker (DRX 500) spectrometer at 500.13 MHz and MS spectra were determined on Finnigan MAT 95 apparatus, using Cs⁺ ions and glycerol as a matrix. The UV analysis of flavonoids was carried out according to the standard procedure (16).

RP-HPLC was performed using Hewlett-Packard 1100 series HPLC system consisting of a quaternary pump (HP model 1311A), vacuum degaser (HP model 1322A), spectrophotometric UV-VIS detector (HP model 1314A) and a 20 µL sample injector (Rheodyne model 7725i, Cotati, CA, USA). The chromatograms were recorded with a 3396B integrator (Hewlett-Packard).

Paper chromatography (PC) was performed on Whatman no. 1 paper; thin-layer chromatography (TLC) on silica gel (Kieselgel 60, Merck) and cellulose (Merck); for column chromatography Diaion HP-20 (Supelco), cellulose (S&S) and silica gel (MN Kieselgel 70-270 mesh) were used. The following solvent systems were employed (all in volumetric ratios): S1: n-BuOH/HOAc/H₂O 4:1:5 (upper layer), S2: HOAc/H₂O 15:85, S3: EtOAc/HCOOH/H₂O 18:1:1, S4: CHCl₃/MeOH/H₂O 16:9:2, S5: HOAc/H₂O/36 % HCl 30:10:3, S6: EtOAc/MeOH/H₂O 200:33:27, S7: n-BuOH/Py/H₂O 6:4:3, S8: EtOH/25 % NH₄OH/H₂O 20:1:4, S9: CHCl₃/cyclohexane/MeOH 3:2:0.5, S10: cyclohexane/HCOOH/butanone/H₂O 6:3:3:1 (upper layer), S11: n-hexane/HOAc/H₂O 6:7:3, S12: HCOOH/HCOONa/H₂O 1:10:200, S13: n-hexane/MeOH/HOAc/ACN 16:2:1:1.

Visualization: in PC and TLC chromatograms were observed under UV light (366 nm) before and after treatment with NH₃ vapor (flavonoids and phenolic acids) and by spraying with reagents: 1 % solution of AlCl₃ in MeOH (flavonoids), diazosulfanilic acid in 10 % sodium carbonate solution (phenolic acids), aniline phthalate in n-BuOH (sugars) and solution of 0,5 g of thymol in 90 mL EtOH 96° and 5 mL of conc. H₂SO₄ (sugars).

RP-HPLC analysis

Separation was carried out on a Lichrospher RP-18 column (250 × 4 mm; I.D., 4 mm, Merck) with mobile phase composed of the mixture of three solvents: 0,5 % aqueous phosphoric acid solution – H₃PO₄ (solvent A), acetonitrile – ACN (solvent B), and tetrahydrofuran – THF (solvent C) using first

gradient followed by isocratic elution. The profile of elution was: 0-17 min: 88 % A/11 % B/1 % C to 69 % A/30 % B/1 % C; 17-25 min: 69 % A/30 % B/1 % C (isocratic); flow rate: 0,7 mL/min; UV detection at λ = 349 nm. Solvents of HPLC grade purity were purchased from Merck (Darmstadt, Germany).

All substances used as the standards were obtained from Roth (Karlsruhe, Germany).

Extraction and isolation

400 g of pulverized dry herb of *N. cataria* L. var: *citriodora* was extracted with 1 L of 70% methanol by heating on water bath for 3 h. Extraction was repeated four times. The obtained extracts were combined, concentrated to syrup in vacuum, 200 mL of water was added and aqueous solution was extracted first with chloroform and subsequently with n-butanol. After solvent distillation, 20.5 g of butanol extract was obtained. It was separated on the column filled with Diaion HP-20 resin, using methanol as an eluent. Fraction 10-20 contained flavonoid compounds which were further separated on silica gel column using S4 system. Compounds **I** and **II** were obtained from fraction 5-12 after further separation on silica gel with the use of S9 as an eluent. In fractions 13-14 the presence of compounds **III** and **IV** were chromatographically detected, however, they could not be isolated in a homogeneous form because of too small amounts. Compound **V** was obtained from fraction 15-22 after subsequent separation on polyamide using the mixture of methanol with water (gradient from 20% to 30%) as an eluent. Followed the same procedure compound **VI** was obtained from fractions 24-30. Additionally, from aqueous solution which remained after extraction of compounds **I-VI**, compound **VII** was isolated through separation by column chromatography, initially on polyamide (gradient of methanol in water) and subsequently on silica gel (S4 system).

Analysis for the presence of phenolic acids

10 g of lemon catnip was extracted three times with 70% methanol at boiling point. The obtained extract was evaporated to dryness in the vacuum rotary evaporator, the residue was dissolved in water (RW solution) and extracted with diethyl ether (3 × 100 mL). Ether extract was treated with 200 mL of 5% NaHCO₃, alkaline layer was separated, acidified with 10 % HCl to pH 4 and again extracted with diethyl ether. After removing off ether, fraction A was obtained (free phenolic acids). Aqueous solution was concentrated, 4 mL of 10% HCl was added and heated to the boiling point for 1 h. After cooling,

the hydrolysate was extracted with diethyl ether and fraction B was obtained. PC and TLC analyses of fraction A and B were performed for comparison with the authentic substances using following methods: two-dimensional PC with solvent systems S11 (first direction) and S12 (second direction), two-dimensional TLC on cellulose with systems S13 (first direction) and S12 (second direction) as well as TLC on silica gel with systems S6 and S10. Chromatograms were visualized in UV light and by spraying with diazosulfanilic acid in 10 % sodium carbonate solution.

As a result of the performed analysis p-coumaric, caffeic and rosmarinic acids were identified in fraction A and in fraction B caffeic acid was detected what pointed that caffeic acid was present in both free and bound form.

Analysis for the presence of tannins

2 g of powdered plant material and 25 mL of water were heated at boiling point for 15 min. After cooling the mixture was filtered through the paper filter and the filtrate was used for the tests on tannins with specific reagents (17). All reactions gave negative results.

Identification of flavonoids

Total acid hydrolysis

To 2 mg sample 5 mL of 2 M HCl was added and kept at boiling point for 3 h. The hydrolysate was extracted with ethyl acetate. The ethyl acetate extract was rinsed with water, dried with Na_2SO_4 , concentrated and analyzed (PC, TLC) for the presence of aglycones (S1-S5 systems). In the aqueous layer, after evaporation to dryness in rotary evaporator and dissolution in ethanol, the sugars were analyzed using TLC with S7 and S8 solvent systems with the authentic specimens of sugars.

Partial acid hydrolysis of compound VII

To 5 mg sample 5 mL of trifluoroacetic acid was added and kept at boiling point for 15 min. The hydrolysate was extracted with ethyl acetate and the ethyl acetate extract was analyzed chromatographically (TLC, PC) for the presence of luteolin monosides in comparison to authentic substances. Aqueous layer was evaporated, dissolved in small amount of ethanol and analyzed chromatographically for sugars in the same manner as described above.

Compound I (apigenin)

Amorphous powder, m.p. 335-338°C (MeOH), UV fluorescence: brown. PC (R_f): S1 – 0.83, S2 – 0.11; TLC (R_f): S3 – 0.94, S4 – 0.82, S5 – 0.86, S6

– 0.87, the same as for the authentic substance. IR (KBr) ν_{max} [cm^{-1}]: 3297-3095 (OH), 2922-2617 (C-H), 1654 (C=O), 1608-1500 (aromatic rings), 1445, 1354, 1298, 1267. UV λ_{max} nm (CH_3OH): 265, (297), 335. $^1\text{H-NMR}$ (DMSO-d_6): δ [ppm]: 7.93 (2H d, $J = 8.8$ Hz, H-2' H-6'), 6.92 (2H d, $J = 8.7$ Hz, H-3' H-5'), 6.79 s (H-3), 6.48 (d, $J = 2.09$ Hz, H-8), 6.1 (d, $J = 2.02$ Hz, H-6).

Compound II (luteolin)

Pale yellow amorphous powder, m.p. 325-328°C (70 % MeOH), UV fluorescence: brown. PC (R_f): S1 – 0.8, S2 – 0.06; TLC (R_f): S3 – 0.9, S4 – 0.79, S5 – 0.72, S6 – 0.82, identical as luteolin standard substance. IR (KBr) ν_{max} [cm^{-1}]: 3490-3400 (OH), 2923-2617 (C-H), 1655 (C=O), 1610-1490 (aromatic rings), 1458, 1364, 1267. UV λ_{max} nm (CH_3OH): 253, 268, (290), 348; + NaOMe: 265, (330), 402; + AlCl_3 : 274, (300), (329), 425; + AlCl_3/HCl : 275, 296, 356, 388; + NaOAc: 271, (325), 390; + NaOAc/ H_3BO_3 : 262, (301), 372, (430). $^1\text{H-NMR}$ (DMSO-d_6): δ [ppm]: 7.42 (dd, $J = 8.2$ Hz, $J = 2.1$ Hz, H-6'), 7.39 (d, $J = 2$ Hz, H-2'), 6.88 (d, $J = 8.2$ Hz, H-5'), 6.66 s (H-3), 6.43 (d, $J = 1.8$ Hz, H-8), 6.18 (d, $J = 1.8$ Hz, H-6).

Compound III (apigenin 7-O- β -glucopyranoside)

UV fluorescence: brown. R_f values (PC): S1 = 0.63, S2 = 0.18; t_r (HPLC): 16.131 \pm 0.082 (n = 6) identical as for the authentic substance.

Compound IV (luteolin 7-O- β -glucopyranoside)

UV fluorescence: brown. R_f values (PC): S1 = 0.36, S2 = 0.13; t_r (HPLC): 13.693 \pm 0.045 (n = 6) identical as for the authentic substance.

Compound V (apigenin 7-O- β -glucuronopyranoside)

M.p. 219-225°C (MeOH), UV fluorescence: brown. PC (R_f): S1 – 0.22, S2 – 0.34; TLC (R_f): S3 – 0.22, S4 – 0.43, S6 – 0.11. IR (KBr) ν_{max} [cm^{-1}]: 3424-3323 (OH), 2930-2859 (C-H), 1710 (COOH), 1637 (C=O), 1550 (aromatic rings), 1439, 1379, 1265, 1171, 1071. UV λ_{max} nm (CH_3OH): 268, 332; + NaOMe: 275, 340, 395; + AlCl_3 : 276, 302, 349, 383; + AlCl_3/HCl : 275, 296, 356, 388; + NaOAc: 267, 354, 382; + NaOAc/ H_3BO_3 : 268, 339. $^1\text{H-NMR}$ (DMSO-d_6): δ [ppm]: 7.93 (2H d, $J = 8.6$ Hz, H-2' H-6'), 6.92 (2H d, $J = 8.4$ Hz, H-5' H-3'), 6.82 (2H d, H-3 H-8), 6.43 s (H-6), 5.06 (d, $J = 7.4$ Hz, H-1''), 3.82 (d, $J = 9.7$ Hz, H-5''), 3.3 – 3.6 (m, H-2'', H-3'', H-4'').

Complete acid hydrolysis gave apigenin and glucuronic acid (PC, TLC).

Table 1. The content of the total flavonoids, total phenolic acids and rosmarinic acid in lemon catnip herb.

No. of sample and collection data	The mean content* of flavonoids – % of dry mass (spectrophotometric method)	The mean content* of phenolic acids – % of dry mass (spectrophotometric method)	The mean content* of rosmarinic acid – % of dry mass (HPLC method)
1. August 2000	0.46 ± 0,0045	0.75 ± 0,0700	0.14 ± 0,0153
2. July 2001	0.32 ± 0,0006	1.01 ± 0,0608	0.15 ± 0,0424
3. August 2002	0.30 ± 0,0060	1.05 ± 0,0551	0.11 ± 0,0071
4. July 2003	0.31 ± 0,0026	1.40 ± 0,0289	0.06 ± 0,0115
5. August 2003	0.32 ± 0,0021	1.18 ± 0,0265	0.09 ± 0,0058
6. August 2004	0.39 ± 0,0006	1.19 ± 0,0153	

*The mean value from three samples ± SD (standard deviation) is given.

Compound VI (luteolin 7-O- β -glucuronopyranoside)

M.p. 201-206°C (MeOH), UV fluorescence: brown. PC (R_f): S1 – 0.13, S2 – 0.21; TLC (R_f): S3 – 0.26, S4 – 0.31, S6 – 0.06; IR (KBr) ν_{\max} [cm^{-1}]: 3421 (OH), 2956-2854 (C-H), 1736 (COOH), 1650 (C=O), 1603-1460 (aromatic rings), 1379, 1261, 1174, 1122, 1075. UV λ_{\max} nm (CH₃OH): 250, 345; + NaOMe: 261, 323, 399; + AlCl₃: 271, 311, 409; + AlCl₃/HCl: 273, 355, 382; + NaOAc: 266, 353, 403; + NaOAc/H₃BO₃: 261, 371. ¹H-NMR (DMSO-d₆): δ [ppm]: 7.42 (2H dd, J = 6.1 Hz, H-2' H-6'), 6.88 (d, J = 8.6 Hz, H-5'), 6.74 (2H s, H-3 H-8), 6.41 (d, J = 2 Hz, H-6), 5.05 (d, J = 7.4 Hz, H-1''), 3.72 (d, J = 8 Hz, H-5''), 3.1- 3.6 m, 3H (sugar protons). FAB MS, Cs⁺, 13 kV, +VE m/z: 463.1 [M+H]⁺, -VE 461.1 [M-H]⁻, 417.3 [M-CO₂].

Complete acid hydrolysis gave luteolin and glucuronic acid (PC, TLC)

Compound VII (luteolin 7-O- β -glucuronopyranosyl-(1 \rightarrow 6)-O- β -glucopyranoside)

Beige amorphous powder, PC (R_f): S1 – 0.04, S2 – 0.29; TLC (R_f): S3 – 0.38, S4 – 0.06, UV fluorescence: brown. IR (KBr) ν_{\max} [cm^{-1}]: 3392 (OH), 2925-2853 (C-H), 1726 (COOH) 1654 (C=O), 1608 (aromatic rings), 1458, 1421, 1378, 1287, 1177, 1074. UV λ_{\max} nm (CH₃OH): 255, 267, 345; + NaOMe: 261, 399; + AlCl₃: 271, 409; + AlCl₃/HCl: 273, 382, 355, 382; + NaOAc: 266, 353; + NaOAc/H₃BO₃: 261, 371. ¹H-NMR (DMSO-d₆): δ [ppm]: 7.5 (d, J = 1.3 Hz, H-2'), 7.44 (dd, J = 8.5 Hz and 1.5 Hz, H-6'), 7.09 (d, J = 1.6 Hz, H-8), 6.87 (d, J = 8.3 Hz, H-5'), 6.71 s (H-3), 6.56 (d, J = 1.6 Hz, H-6), 5.13 (d, J = 7.5 Hz, H-1''), 4.54 (d, J = 7.2 Hz, H''), 4.2 (dd, 2H, H-6 gluc), 3.1-3.8 (8H, sugar protons). FAB MS, Cs⁺, 13 kV, m/z: 625.5 [M+H]⁺.

Complete acid hydrolysis gave luteolin, glucose and glucuronic acid (PC, TLC). Partial acid

hydrolysis: luteolin 7-O-glucoside and glucuronic acid were identified (PC, TLC).

Quantitative analysis of the content of flavonoids

The total content of flavonoids was determined by means of spectrophotometric method of Christ-Muller and followed the procedure described in Polish Pharmacopoeia VI. The results are given in Table 1.

Quantitative analysis of the content of phenolic acids

Total phenolic acids content was determined by use the spectoscopic method with Arnov's reagent according to the procedure described in Polish Pharmacopoeia VI (monograph of *Taraxaci herba*). The results expressed as caffeic acid content are given in Table 1.

Determination of rosmarinic acid was conducted by means of HPLC and followed the procedure described previously (13). The results are given in Table 1.

RESULTS AND DISCUSSION

Aqueous-methanol extract from the herb of *Nepeta cataria* var. *citriodora* was subjected to the isolation procedure by use of column chromatography. Five flavonoids (compounds I, II, V, VI and VII) were isolated and identified by means of chemical, chromatographic and spectral methods. Two further compounds have been identified chromatographically as components of the complex fraction separated on the column.

Melting point and R_f values of compound I were in accordance with the data for the apigenin authentic sample. The identity of compound I with apigenin was confirmed by IR, UV and ¹H-NMR spectral data. In the same manner compound II was identified as luteolin.

Compounds **III** and **IV** were the minor constituents of the investigated plant material. They have not been isolated as pure substances but could be recognized by chromatographic analysis. The comparison of R_f values (PC) and retention times (HPLC) with these obtained for the authentic samples allowed to identify compound **III** as apigenin 7-O-β-glucopyranoside and compound **IV** as luteolin 7-O-β-glucopyranoside.

Compound **V** liberated apigenin upon total acid hydrolysis and the sugar molecule which had identical R_f values (PC, TLC) as glucuronic acid. The signal of carboxyl group at 1710 in the IR spectrum confirmed the presence of glucuronic acid. The UV spectrum recorded before and after the addition of shift reagents pointed out the sugar moiety attached to OH group at C-7 of apigenin. Also chemical shifts and coupling constants of the proton signals in NMR spectrum of **V** were in accordance with the structure of apigenin 7-O-β-glucuronopyranoside.

Compound **VI** gave luteolin and glucuronic acid after acid hydrolysis; The IR, UV, ¹H-NMR and FAB MS spectral data allowed to establish the structure of compound **VI** as luteolin 7-O-β-glucuronopyranoside.

After total acid hydrolysis of compound **VII**, luteolin, glucose and glucuronic acid were identified. UV analysis before and after addition of the shift reagents showed the lack of the free hydroxyl group at C-7 of luteolin and the presence of free OH at C-5, C-3' and C-4'. Among the products of partial acid hydrolysis luteolin 7-O-glucoside and glucuronic acid were identified chromatographically by comparison with authentic substances. ¹H-NMR spectrum suggested the glucuronic acid moiety attached to luteolin 7-O-glucoside at C6-OH of glucose as the signals of protons at C-6 of glucose were shifted downfield as compared to the respective signals of luteolin 7-O-glucoside. FAB MS spectrum exhibited a quasimolecular ion at 625 (positive ion mode), what pointed out that the molecule of **VII** is enriched by glucose moiety as compared to compound **VI** and confirm the structure of compound **VII** as luteolin 7-O-β-glucuronopyranosyl-(1→6)-O-β-glucopyranoside. This structure was found probably for the first time in plant kingdom, however, a few flavonoid diglycosides having glucuronic acid in sugar moiety have been isolated from other plants of *Lamiaceae* family so far (12, 18, 19).

Total amount of flavonoids determined in six different samples of the lemon catnip herb was in the range from 0,30 to 0,46% of dry mass.

In free phenolic acid fraction isolated from the herb of *Nepeta cataria* L. var *citriodora* three constituents have been found and identified as caffeic acid, rosmarinic acid and p-coumaric acid and in bound phenolic acid fraction only caffeic acid was detected. The total content of phenolic acids was determined using spectrometric method according to Pol. Ph. VI and the content of rosmarinic acid was quantified by use of HPLC method. Depending on the sample, the total phenolic acids amount was in the range from 0,75% to 1,40 %, and the content of rosmarinic acid was from 0,06% to 0,15%. The tannins have not been detected in lemon catnip herb.

The results of our previous (6, 11-13, 20) and currently described investigations pointed out that the spectrum of the volatile compounds (essential oil), flavonoids and phenolic acids in lemon catnip are similar to those occurring in lemon balm. In both plants the constituents of the flavonoid fraction are the glycosides of apigenin and luteolin having glucuronic acid and glucose as sugar components and phenolic acids are caffeic, rosmarinic and very small amount of other phenolic acids. Quantitative determination of total flavonoids in lemon catnip gave similar results to that obtained for melissa leaves (13). However, the amount of rosmarinic acid is ca. 10 times lower than in melissa and the tests for tannins gave negative result in lemon catnip herb.

Relatively high percentage of essential oil with nerol, geraniol and citral as well as ursolic acid (11) and polyphenols (flavonoids, phenolic acids) suggest that lemon catnip herb could be used as a raw material for preparation of phytopharmaceutical products having mild sedative (essential oil), antispasmodic (flavonoids), antiinflammatory (ursolic acid) and antioxidative (flavonoids, caffeic acid) activities.

REFERENCES

1. Hegi G.: *Illustrierte Flora von Mittel Europa*, Band 5, Teil 4, p. 2367, Lehmanns, München 1926.
2. Handjieva N. V., Popov S. S.: *J. Essent. Oil Res.* 8, 639 (1996).
3. Aydin S., Beis R., Öztürk Y., Hüsni K., Baser C.: *J. Pharm. Pharmacol.* 50, 813 (1998).
4. Baser K. H. C., Demircakmak B., Altintas A.: *J. Essent. Oil Res.* 10, 327 (1998).
5. Svoboda K., Galambosi B., Hampson J., Hashimoto T.: *Beitr. Züchtungsforsch.* 2, 377 (1996).
6. Chalchat J. C., Lamy J.: *J. Essent. Oil Res.* 9, 527 (1997).

7. Tropnikova I. V., Zenkevich I. G., Budantsev A. L.: *Rastit. Resur.* 35, 64 (1999).
8. Klimek B., Majda T., Góra J., Patora J.: *Herba Polon.* 46, 226 (2000).
9. Kohlmünzer S.: *Pharmacognosy (in Polish)*, p. 577, PZWL, Warszawa 1998.
10. Schier W.: in *Pharmakognosie – Phytopharmazie*, Hänsel R., Sticher O., Steinegger E. Eds., p. 1319, Springer Verlag, Berlin, Heidelberg 1999.
11. Klimek B., Modnicki D.: *Acta Pol. Pharm. – Drug Res.* 62, 231 (2005).
12. Patora J., Klimek B.: *Acta Pol. Pharm. – Drug Res.* 59, 139 (2002).
13. Modnicki D., Patora J., Klimek B.: *Herba Polon.* 51, 23 (2004).
14. Boratyńska W., Leszczakówna W.: *Herba Polon.* 1, 70 (1955).
15. Raszejowa W.: *Herba Polon.* 1, 83 (1955).
16. Mabry T. J., Markham K. R., Thomas M. B.: *The systematic identification of flavonoids*, p. 35, Springer Verlag, Berlin, Heidelberg, New York 1970.
17. Strzelecka H., Kaminska J., Kowalski J., Walewska E.: *Chemical methods of analysis of pharmaceutical plant material (in Polish)* p. 145, PZWL, Warszawa 1982.
18. Makino T., Ito M., Kiuchiu F., Ono T., Muso E., Honda G.: *Planta Med.* 67, 24 (2001)
19. Olechnowicz-Stepień W., Lamer-Zarawska E.: *Herba Polon.* 21, 347 (1975).
20. Patora J., Majda T., Góra J., Klimek B.: *Acta Pol. Pharm. – Drug Res.* 60, 395 (2003).

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