

## Article

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# Chemical constituents of *Caragana bungei* shoots

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**Abstract:** The phytochemical study of flowering shoots of *Caragana bungei* Ledeb., Fabaceae, collected in Tuva Republic (Russian Federation) resulted in the isolation of sixteen compounds identified as  $\beta$ -sitosterol,  $\beta$ -sitosterol-3-*O*-glucoside, umbelliferone, kaempferol, quercetin, isorhamnetin-3-*O*-glucoside, isoquercitrin, rutin, narcissin, nicotiflorin, caffeic acid, 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, gallic acid and sucrose. The structures of the compounds were established by spectral analyses. This is the first phytochemical investigation of *C. bungei*. The nicotiflorin, phenylpropanoids and gallic acid were isolated from the *Caragana* genus for the first time.

## Introduction

*Caragana* Fabr. is a genus of Fabaceae family including about eighty species widely spread in Europe and Asia and used as a medical, decorative and technical raw materials. Despite of wide using the targeted chemical researches of plants of this genus were started relatively recently. To date we have scientific information about chemical compositions of fifteen *Caragana* species (Meng et al., 2009). In Russian Federation this genus presented by 34 species mainly distributed in the steppe and mountain landscapes.

*Caragana bungei* Lebeb. is branched spiny shrub growing in the desert steppes and valleys of mountain rivers of Eastern and Western Siberia. The decoction of flowering shoots of this species is used in traditional medicine of Tuva and Buryatia regions as an antiinflammatory and immunostimulant means (Aseeva et al., 2008). There is no any scientific data about chemical composition of *C. bungei*. In this study, we present the results of phytochemical investigation of *C. bungei* flowering shoots. As a result sixteen compounds were isolated including  $\beta$ -sitosterol (1), umbelliferone (2), kaempferol (3), quercetin (4),  $\beta$ -sitosterol-3-*O*-glucoside (5), caffeic acid (6), gallic acid (7), 3,5-di-*O*-caffeoylquinic acid (8), isorhamnetin-3-*O*-glucoside (9), isoquercitrin (quercetin-3-*O*-glucoside, 10), 3-*O*-caffeoylquinic acid

(11), 5-*O*-caffeoylquinic acid (12), rutin (quercetin-3-*O*-rutinoside, 13), narcissin (isorhamnetin-3-*O*-rutinoside, 14), nicotiflorin (kaempferol-3-*O*-rutinoside, 15) and sucrose (16). This is the first phytochemical investigation of *C. bungei*.

## Material and Methods

### Plant material

The shoots of *Caragana bungei* Ledeb., Fabaceae, were collected in flowering period in Despen locality (Tes-Hemskii region, Tuva Republic, Russian Federation, 50°81'78" N, 93°78'39" E). The plant samples were identified by Prof. Aseeva T.A. (Institute of General and Experimental Biology, Ulan-Ude, Russian Federation). Voucher specimen is deposited at the Herbarium of Department of Biologically Active Substances (Institute of General and Experimental Biology, Ulan-Ude, Russian Federation; voucher specimen number Fb/s-18/02-11/0942).

### General experimental procedures

Elemental composition was determined using MAT 8200 spectrometer (Thermo Finnigan). Melting

points were determined with a 910 DS calorimeter (DuPont). UV spectra were recorded using a SF-2000 spectrophotometer (OKB Specter). MS spectra were registered on a LCQ mass spectrometer (Thermo Finnigan).  $^{13}\text{C}$  NMR spectra were recorded on a VXR 500S spectrometer (Varian). Optical rotations were measured on a DIP-1000 digital polarimeter (Jasco). Chromatography column was performed over silica gel 60 (230-400 mesh, Merck), Sephadex LH-20 (25-100  $\mu\text{m}$ , Pharmacia), polyamide (Woelm) and pTLC was performed on Sorbfil-A silica gel TLC plates (layer thickness 1 mm; Imid Ltd.). All chemicals were analytical-grade. The Folin method was used to determine total phenolic content (TPC), as described by (Shetty et al., 1995) using gallic acid as a standard compound. Total flavonoid content (TFC) was determined by spectrophotometric method (Chirikova et al., 2010) using rutin as a standard compound.  $\beta$ -Sitosterol content was determined using HPTLC-densitometric method (Starek et al., 2007).

#### Extraction and isolation

Air-dried, ground shoots of *C. bungei* (1462 g) were extracted with 70% EtOH at 80 °C three times and the extracts were concentrated under reduced pressure to yield 409.4 g of crude extract. The crude extract was re-suspended in water (1:4, v/v) and successively partitioned with  $\text{CHCl}_3$ , EtOAc, and *n*-BuOH. The organic layers were brought to dryness in vacuo to yield 87.7, 43.8 and 175.4 g of  $\text{CHCl}_3$  (CB-C), EtOAc (CB-E) and *n*-BuOH fraction (CB-B) residue respectively. The CB-C fraction (42 g) was dissolved in 1000 mL of chloroform and chromatographed over Sephadex LH-20 column (7x120 cm), eluting with  $\text{CHCl}_3$  containing increasing concentrations of MeOH (100:0 $\rightarrow$ 0:100) to obtain fifty fractions (fr.C1-fr.C50). Fr.C3-fr.C6 were combined and chromatographed on a silica column (3x50 cm), eluting with  $\text{CHCl}_3$ -MeOH mixtures (100:0 $\rightarrow$ 70:30) to obtain twelve fractions (fr.C3-6/1-fr.C3-6/12). Fr.C3-6/3 (eluent  $\text{CHCl}_3$ -MeOH 90:10) after recrystallization (MeOH) was given 263 mg of **1**. Fr.C5 was chromatographed on a silica column (2x40 cm), eluting with  $\text{CHCl}_3$ -MeOH mixtures (90:10 $\rightarrow$ 60:40) to obtain fifteen fractions (fr.C5/1-fr.C5/15). Fr.C5/7-fr.C5/8 were combined (eluent  $\text{CHCl}_3$ -MeOH 74:25) and was chromatographed on a pTLC (solvent toluene-EtOAc-HCOOH 6:3:1) to yield **2** (15 mg), **3** (27 mg) and **4** (37 mg). The CB-E fraction (35 g) was dissolved in 500 mL of MeOH and chromatographed over Sephadex LH-20 column (5x100 cm), eluting with MeOH- $\text{H}_2\text{O}$  mixtures (100:0 $\rightarrow$ 50:50) to obtain 60 fractions (fr.E1-fr.E60). Fr.E3 was chromatographed on a silica column (2x50 cm), eluting with  $\text{CHCl}_3$ -MeOH mixtures (100:0 $\rightarrow$ 60:40) to obtain fifteen fractions (fr.E3/1-fr.E3/15). Fr.E3/8 (eluent- $\text{CHCl}_3$ -MeOH 70:30) after recrystallization (MeOH) was given 24 mg of **5**. Fr.E5-fr.E8 were combined and

chromatographed on a polyamide column (6x100 cm), eluting with  $\text{H}_2\text{O}$ -MeOH mixtures (100:0 $\rightarrow$ 0:100) to obtain twenty fractions (fr.E5-8/1-fr.E5-8/20). Fr.E5-8/2-fr.E5-8/3 (eluent  $\text{H}_2\text{O}$ -MeOH 90:10, 85:15) were combined and chromatographed on a pTLC (solvent toluene-EtOAc-HCOOH 5:4:1) to yield **6** (11 mg), **7** (8 mg) and **8** (8 mg). Fr.E5-8/4-fr.E5-8/5 (eluent  $\text{H}_2\text{O}$ -MeOH 80:20, 75:25) were combined and chromatographed on a pTLC (solvent toluene-EtOAc-HCOOH 5:4:1) to yield **8** (18 mg). Fr.E11-fr.E14 were combined and chromatographed on a polyamide column (6x50 cm), eluting with  $\text{H}_2\text{O}$ -MeOH mixtures (100:0 $\rightarrow$ 0:100) to obtain twenty fractions (fr.E11-14/1-fr.E11-14/20). Fr.E11-14/11-fr.E11-14/14 (eluent  $\text{H}_2\text{O}$ -MeOH 60:40, 55:45) were combined and chromatographed on a pTLC (solvent EtOAc-toluene-HCOOH 6:4:1.5) to yield **9** (15 mg) and **10** (27 mg). Fr.E19-fr.E23 were combined and chromatographed on a polyamide column (6x100 cm), eluting with  $\text{H}_2\text{O}$ -MeOH mixtures (100:0 $\rightarrow$ 0:100) to obtain twenty fractions (fr.E19-23/1-fr.E23/20). Fr.E19-23/3-fr.E19-23/5 (eluent  $\text{H}_2\text{O}$ -MeOH 90:10-80:20) were combined and chromatographed on a pTLC (solvent EtOAc-dioxane-AcOH- $\text{H}_2\text{O}$  10:5:1:1) to yield **11** (11 mg) and **12** (9 mg). The CB-B fraction (87 g) was dissolved in 1500 mL of 50% EtOH and chromatographed polyamide column (10x120 cm), eluting with  $\text{H}_2\text{O}$ -EtOH mixtures (100:0 $\rightarrow$ 0:100) to obtain fifty fractions (fr.B1-fr.B50). Fr.B20-fr.B23 (eluent- $\text{H}_2\text{O}$ -EtOH 60:40-54:46) were combined and chromatographed on Sephadex LH-20 column (5x80 cm), eluting with EtOH- $\text{H}_2\text{O}$  mixtures (96:4 $\rightarrow$ 60:40) to obtain twenty fractions (fr.B20-23/1-fr.B20-23/20). Fr.B20-23/5-fr.B20-23/7 (eluent EtOH- $\text{H}_2\text{O}$  80:20) after recrystallization (80% EtOH) was given 86 mg of **13**. Fr.B20-23/7-fr.B20-23/9 were combined and after recrystallization (80% EtOH) was given 43 mg of **14**. Fr.B27-fr.B29 (eluent  $\text{H}_2\text{O}$ -EtOH 46:54-42:58) were combined and chromatographed on a pTLC (solvent EtOAc-dioxane-AcOH- $\text{H}_2\text{O}$  10:5:1:1) to yield **15** (16 mg). Fr.B1 (eluent  $\text{H}_2\text{O}$ ) was concentrated to syrup which crystallized at 2 °C during 5-7 days to yield colorless prisms of **16** (53 mg).

*$\beta$ -Sitosterol* (**1**):  $\text{C}_{29}\text{H}_{50}\text{O}$ , m.p. 141 °C.  $^+\text{FAB-MC } m/z$ : 415  $[\text{M}+\text{H}]^+$ .  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 125 MHz): 11.73 (C-29), 12.14 (C-18), 18.63 (C-26), 19.03 (C-21), 19.39 (C-19), 19.82 (C-27), 21.47 (C-11), 23.08 (C-28), 24.31 (C-15), 26.22 (C-25), 28.18 (C-16), 29.97 (C-23), 31.73 (C-2), 31.95 (C-8), 32.03 (C-7), 34.89 (C-22), 36.11 (C-20), 36.69 (C-10), 37.24 (C-1), 40.27 (C-12), 42.32 (C-4), 42.61 (C-13), 50.33 (C-24), 50.92 (C-9), 56.11 (C-17), 57.02 (C-14), 71.97 (C-3), 121.92 (C-6), 140.87 (C-5).

*Umbelliferone* (**2**):  $\text{C}_9\text{H}_6\text{O}_3$ , m.p. 232 °C. UV  $\lambda_{\text{max}}$  nm MeOH: 214 sh, 248 sh, 299 sh, 327.  $^+\text{FAB-MC } m/z$ : 163  $[\text{M}+\text{H}]^+$ .  $^{13}\text{C-NMR}$  ( $\text{DMSO-d}_6$ , 125 MHz): 103.02 (C-8), 111.60 (C-10), 111.63 (C-3), 113.27 (C-6), 129.97 (C-5),

144.24 (C-4), 156.12 (C-9), 160.71 (C-2), 161.93 (C-7).

**Kaempferol (3):** C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>, m.p. 275 °C. UV λ<sub>max</sub> nm MeOH: 258, 325 sh, 370. +FAB-MC *m/z*: 287 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 125 MHz): 94.38 (C-8), 99.02 (C-6), 103.92 (C-10), 116.18 (C-3',5'), 123.47 (C-1'), 130.38 (C-2',6'), 136.54 (C-3), 147.63 (C-2), 158.03 (C-9), 160.34 (C-4'), 162.14 (C-5), 165.22 (C-7), 176.71 (C-4).

**Quercetin (4):** C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>, m.p. >300 °C. UV λ<sub>max</sub> nm MeOH: 259, 308 sh, 376. +FAB-MC *m/z*: 303 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 125 MHz): 93.74 (C-8), 98.47 (C-6), 104.81 (C-10), 115.21 (C-2'), 115.97 (C-5'), 121.04 (C-6'), 122.49 (C-1'), 134.71 (C-3), 145.33 (C-3'), 146.22 (C-2), 147.27 (C-4'), 158.74 (C-9), 161.52 (C-5), 164.67 (C-7), 178.53 (C-4).

**β-Sitosterol-3-O-glucoside (5):** C<sub>35</sub>H<sub>61</sub>O<sub>6</sub>, m.p. 281 °C. +FAB-MC *m/z*: 577 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125 MHz): 11.62 (C-29), 12.46 (C-18), 18.35 (C-26), 19.37 (C-19), 19.83 (C-21), 20.51 (C-27), 21.82 (C-11), 23.70 (C-28), 25.72 (C-15), 25.94 (C-25), 29.48 (C-23), 29.74 (C-16), 29.93 (C-2), 33.07 (C-8), 33.19 (C-7), 37.15 (C-10), 37.38 (C-20), 38.82 (C-1), 39.73 (C-22), 40.33 (C-12), 42.97 (C-13), 44.01 (C-4), 50.21 (C-9), 50.41 (C-24), 56.26 (C-17), 57.31 (C-14), 61.58 (Glc, C-6'), 70.93 (Glc, C-4'), 74.77 (Glc, C-2'), 76.63 (Glc, C-5'), 77.02 (Glc, C-3'), 81.12 (C-3), 103.57 (Glc, C-1'), 122.15 (C-6), 140.52 (C-5).

**Caffeic acid (6):** C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>, m.p. 224 °C. UV λ<sub>max</sub> nm MeOH: 238, 301 sh, 325. +FAB-MC *m/z*: 181 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 114.92 (C-8), 115.09 (C-2), 116.11 (C-5), 121.24 (C-6), 125.71 (C-1), 144.37 (C-7), 145.37 (C-3), 148.38 (C-4), 168.98 (C-9).

**Gallic acid (7):** C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>, m.p. 250 °C. UV λ<sub>max</sub> nm MeOH: 275. +FAB-MC *m/z*: 171 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>, 125 MHz): 109.08 (C-2, C-6), 120.47 (C-1), 137.84 (C-4), 145.11 (C-3, C-5), 167.74 (C-7).

**3,5-Di-O-caffeoylquinic acid (8):** C<sub>25</sub>H<sub>24</sub>O<sub>12</sub>, m.p. 170 °C. UV λ<sub>max</sub> nm MeOH: 220, 246 sh, 297 sh, 333. +FAB-MC *m/z*: 517 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 74.62 (C-1), 36.08 (C-2), 72.21 (C-3), 70.54 (C-4), 72.04 (C-5), 37.42 (C-6), 114.98 (C-8''), 115.20 (C-2''), 115.27 (C-2'), 115.51 (C-8'), 116.41 (C-5''), 116.41 (C-5'), 123.01 (C-6'), 123.42 (C-6''), 127.71 (C-1''), 127.81 (C-1'), 146.29 (C-3''), 146.74 (C-3'), 147.08 (C-7'), 147.37 (C-7''), 149.20 (C-4'), 149.37 (C-4''), 168.64 (C-9''), 168.91 (C-9'), 176.87 (C-7);

**Isorhamnetin-3-O-glucoside (9):** C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>, m.p. 267 °C. UV λ<sub>max</sub> nm MeOH: 258, 305, 361. +FAB-MC *m/z*: 479 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 55.24 (3'-OCH<sub>3</sub>),

61.37 (Glc, C-6''), 71.48 (Glc, C-4''), 75.63 (Glc, C-2''), 76.40 (Glc, C-5''), 77.11 (Glc, C-3''), 94.37 (C-8), 99.12 (C-6), 104.52 (Glc, C-1''), 105.74 (C-10), 112.87 (C-2'), 114.12 (C-5'), 123.24 (C-6'), 123.97 (C-1'), 135.22 (C-3), 148.61 (C-4'), 150.24 (C-3'), 158.14 (C-2), 158.61 (C-9), 164.27 (C-5), 166.12 (C-7), 179.21 (C-4).

**Isoquercitrin (10):** C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>, m.p. 226 °C. UV λ<sub>max</sub> nm MeOH: 257, 359. +FAB-MC *m/z*: 465 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 61.42 (Glc, C-6''), 70.95 (Glc, C-4''), 75.31 (Glc, C-2''), 76.37 (Glc, C-5''), 77.25 (Glc, C-3''), 94.52 (C-8), 99.03 (C-6), 102.67 (Glc, C-1''), 103.04 (C-10), 114.93 (C-2'), 115.98 (C-5'), 122.54 (C-6'), 123.24 (C-1'), 135.74 (C-3), 144.35 (C-3'), 149.60 (C-4'), 159.05 (C-9), 160.71 (C-2), 163.58 (C-5), 165.81 (C-7), 179.55 (C-4).

**3-O-Caffeoylquinic acid (11):** C<sub>16</sub>H<sub>18</sub>O<sub>9</sub>, m.p. 206 °C. UV λ<sub>max</sub> nm MeOH: 240, 302 sh, 324. +FAB-MC *m/z*: 354 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 40.84 (C-6), 38.72 (C-2), 71.54 (C-5), 72.67 (C-4), 73.27 (C-3), 77.41 (C-1), 115.14 (C-2'), 115.61 (C-8'), 116.51 (C-5'), 122.71 (C-6'), 127.84 (C-1'), 146.50 (C-3'), 146.90 (C-7'), 149.33 (C-4'), 169.51 (C-9'), 182.38 (C-7).

**5-O-Caffeoylquinic acid (12):** C<sub>16</sub>H<sub>18</sub>O<sub>9</sub>, m.p. 210 °C. UV λ<sub>max</sub> nm MeOH: 241, 305 sh, 326. +FAB-MC *m/z*: 354 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 39.18 (C-2), 40.73 (C-6), 71.87 (C-3), 72.41 (C-4), 73.68 (C-5), 77.75 (C-1), 115.18 (C-2'), 115.52 (C-8'), 116.58 (C-5'), 122.93 (C-6'), 127.78 (C-1'), 146.80 (C-3'), 146.94 (C-7'), 149.75 (C-4'), 169.37 (C-9'), 181.10 (C-7).

**Rutin (13):** C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>, m.p. 243 °C. UV λ<sub>max</sub> nm MeOH: 263, 364. +FAB-MC *m/z*: 611 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 17.81 (Rha, C-6'''), 68.31 (Glc, C-6''), 69.84 (Rha, C-5'''), 71.34 (Rha, C-2'''), 72.23 (Rha, C-3'''), 73.83 (Glc, C-4''), 74.34 (Rha, C-4'''), 75.68 (Glc, C-2''), 77.25 (Glc, C-5''), 78.14 (Glc, C-3''), 94.63 (C-8), 99.82 (C-6), 102.08 (Rha, C-1'''), 104.51 (Glc, C-1''), 105.67 (C-10), 115.47 (C-2'), 116.67 (C-5'), 123.27 (C-6'), 123.75 (C-1'), 135.77 (C-3), 145.61 (C-3'), 149.92 (C-4'), 159.05 (C-9), 159.21 (C-2), 163.41 (C-5), 165.97 (C-7), 179.32 (C-4).

**Narcissin (14):** C<sub>28</sub>H<sub>32</sub>O<sub>16</sub>, m.p. 187 °C. UV λ<sub>max</sub> nm MeOH: 254, 356. +FAB-MC *m/z*: 625 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 17.73 (Rha, C-6'''), 55.01 (3'-OCH<sub>3</sub>), 68.37 (Glc, C-6''), 69.52 (Rha, C-5'''), 71.18 (Rha, C-2'''), 71.48 (Glc, C-4''), 71.82 (Rha, C-3'''), 74.59 (Rha, C-4'''), 75.63 (Glc, C-2''), 76.40 (Glc, C-5''), 77.11 (Glc, C-3''), 94.32 (C-8), 99.30 (C-6), 101.92 (Rha, C-1'''), 104.52 (Glc, C-1''), 105.11 (C-10), 112.96 (C-2'), 114.82 (C-5'), 123.02 (C-6'), 123.74 (C-1'), 135.18 (C-3), 148.71 (C-4'), 150.02 (C-3'), 158.30 (C-9), 158.37 (C-2), 163.37 (C-5), 165.42 (C-7), 178.84 (C-4).

**Nicotiflorin (15):** C<sub>27</sub>H<sub>30</sub>O<sub>15</sub>, m.p. 185 °C. UV λ<sub>max</sub> nm MeOH: 268, 351. +FAB-MS *m/z*: 595 [M+H]<sup>+</sup>. <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): 17.68 (Rha, C-6'''), 68.45 (Glc, C-6''), 69.63 (Rha, C-5'''), 71.52 (Glc, C-4''), 71.95 (Rha, C-2'''), 72.27 (Rha, C-3'''), 74.45 (Rha, C-4'''), 75.71 (Glc, C-2''), 76.94 (Glc, C-5''), 78.39 (Glc, C-3''), 94.75 (C-8), 99.97 (C-6), 102.18 (Rha, C-1'''), 104.49 (Glc, C-1''), 105.71 (C-10), 116.40 (C-3',5'), 123.11 (C-1'), 132.37 (C-2',6'), 135.65 (C-3), 158.57 (C-9), 158.63 (C-2), 159.71 (C-4'), 161.82 (C-5), 166.07 (C-7), 179.29 (C-4).

**Sucrose (16):** C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>, m.p. 185 °C. [α]<sub>D</sub> +65.9° (H<sub>2</sub>O; *c* 5.0). <sup>13</sup>C-NMR (D<sub>2</sub>O, 125 MHz): 62.82 (Glc, C-6), 64.02 (Frc, C-1'), 65.05 (Frc, C-6'), 72.01 (Glc, C-4), 73.79 (Glc, C-2), 75.11 (Glc, C-5), 75.37 (Glc, C-3), 76.89 (Frc, C-4'), 79.18 (Frc, C-3'), 84.01 (Frc, C-5'), 95.15 (Glc, C-1), 106.21 (Frc, C-2').

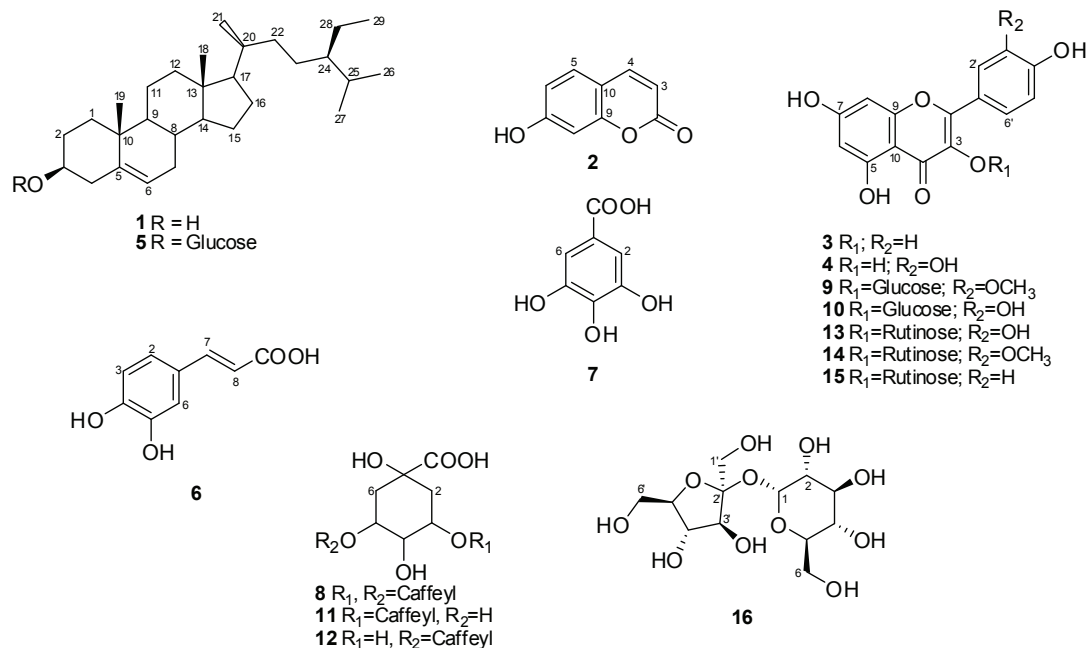
#### HPTLC-analysis conditions

Powdered plant material (500 mg) was extracted for 30 min with 25 mL of MeOH at 80 °C in a water bath two times. The samples were then filtered through a cellulose acetate filter (0.50 μm; Reakhim) and concentrated. The residue was re-dissolved in 5 mL of MeOH. The sample solutions were stored at 4 °C until used. Chromatography was performed on 15 cm x 10 cm Sorbfil-A silica gel HPTLC plates (layer thickness 0.1 mm; Imid Ltd), which used without pretreatment. Standard (1 μL) and sample solutions (2 μL) were applied as 8 mm bands, 5 mm apart, 15 mm from the left of the plate and 15 mm from the bottom edge, by means of a APA-1 automatic sample applicator

(Imid Ltd). Plates were developed at room temperature (20 °C) with toluene-EtOAc-HCOOH 5:4:1 (v/v, for flavonol aglycones, caffeic and gallic acids) or EtOAc-1,2-dichloroethane-AcOH-HCOOH-H<sub>2</sub>O 10:2.5:1:1:0.8 (v/v, for flavonol glycosides and caffeoylquinic acids) as mobile phases, in a flat-bottomed vertical chamber previously saturated with mobile phase vapor (40 min); the development distance was 6 cm. After development, plates were dried for 10 min in cool air and derivatized with Neu reagent (freshly prepared 0.5% 2-aminoethyl diphenylborinate solution in EtOAc/5% PEG 400 in 1,2-dichloroethane). After derivatization the plates were heated at 100 °C for 5 min with a TLC Plate Heater USP-1M (Imid Ltd) and then scanned 387 nm by use of a Sorbfil Videodensitometer 2.0 TLC Scanner (Imid Ltd) controlled by Sorbfil software; the slit dimensions were 3 mm x 0.40 mm.

#### Results and Discussion

The 70% EtOH-extract of *Caragana bungei* Ledeb., Fabaceae, flowering shoots was partitioned with CHCl<sub>3</sub>, EtOAc, and *n*-BuOH to yield three fractions which were separated by chromatographic column (gel permeation, silica gel and polyamide chromatography) and pTLC, given sixteen compounds. The isolated compounds including seven flavonoids [kaempferol (**3**), quercetin (**4**) (Olenikov et al., 2011b), isorhamnetin-3-*O*-glucoside (**9**) (Manguro et al., 2004), isoquercitrin (quercetin-3-*O*-glucoside, **10**) (Liu et al., 2010), rutin (quercetin-3-*O*-rutinoside, **13**) (Hou et al., 2005), narcissin (isorhamnetin-3-*O*-rutinoside, **14**) (Lu et al.,



**Table 1.** Phenolic compounds distribution, total phenolic content (TPC), total flavonoid content (TFC) and  $\beta$ -sitosterol content in *C. bungei* leaves, flowers and stems.

Compound	Leaves	Flowers	Outer bark of stems	Inner bark of stems
quercetin	tr. <sup>a</sup>	tr.	+	tr.
kaempferol	tr.	-	+	-
isorhamnetin-3- <i>O</i> -glucoside	+ <sup>b</sup>	+	-	-
isoquercitrin	+	+	-	-
rutin	+	+	tr.	-
narcissin	+	+	tr.	-
nicotiflorin	+	-	-	-
caffeic acid	+	-	-	-
3- <i>O</i> -caffeoylquinic acid	+	+	-	-
5- <i>O</i> -caffeoylquinic acid	+	+	-	-
3,5-di- <i>O</i> -caffeoylquinic acid	+	-	-	-
gallic acid	tr.	-	+	-
TPC, mg/g <sup>d</sup>	40.09±0.82	36.24±0.64	12.60±0.38	1.94±0.06
TFC, mg/g <sup>d</sup>	29.32±0.58	18.67±0.37	2.13±0.04	tr.
$\beta$ -sitosterol, mg/g <sup>d</sup>	1.39±0.04	4.52±0.14	1.43±0.04	0.97±0.02

<sup>a</sup>traces; <sup>b</sup>present; <sup>c</sup>not detected; <sup>d</sup>from dry plant weight.

2000), nicotiflorin (kaempferol-3-*O*-rutinoside, **15**) (Hou et al., 2005)], four phenylpropanoids [caffeic acid (**6**) (Olennikov et al., 2011a), 3,5-di-*O*-caffeoylquinic acid (**8**) (Satake et al., 2007), 3-*O*-caffeoylquinic acid (**11**), 5-*O*-caffeoylquinic acid (**12**) (Olennikov et al., 2011a)], two triterpenes [ $\beta$ -sitosterol (**1**),  $\beta$ -sitosterol-3-*O*-glucoside (**5**) (Tomimatsu & Hashimoto, 1968)], umbelliferone (**2**) (Olennikov & Tankhaeva, 2005a), gallic acid (**7**) (Olennikov et al., 2005b) and sucrose (**16**) (Jung et al., 2000) were identified by comparison of their m.p.,  $[\alpha]_D$ , UV, MS and <sup>13</sup>C-NMR data with those reported in the literature.

Accordingly to literature data some of isolated compounds were previously detected in other *Caragana* species: kaempferol in *C. spinosa* (Shpekina, 1990); quercetin in *C. arborescens* (Boinik et al., 1986), *C. alaica* (Butayarov et al., 1999), *C. jubata* (Umarov et al., 1971), *C. pygmaea* (Polovinko & Yakovlev, 1985), *C. sinica* (Tai et al., 2010) and *C. spinosa* (Shpekina, 1990); isorhamnetin-3-*O*-glucoside in *C. alaica* (Butayarov, 1999); isoquercitrin in *C. arborescens* (Boinik, 1986), *C. alaica* (Butayarov, 1999), *C. sinica* (Tai et al., 2010) and *C. spinosa* (Shpekina, 1990); rutin in *C. arborescens* (Boinik, 1986), *C. aurantiaca* (Polovinko & Shostakovskaya, 1987), *C. pygmaea* (Polovinko & Yakovlev, 1985) and *C. spinosa* (Shpekina, 1990); narcissin in *C. aurantiaca* (Polovinko & Shostakovskaya, 1987), *C. pygmaea* (Polovinko & Yakovlev, 1985), *C. sinica* (Tai et al., 2010) and *C. spinosa* (Shpekina, 1990);  $\beta$ -sitosterol in *C. conferta* (Khan et al., 2009) and *C. microphylla* (Huo et al., 2007);  $\beta$ -sitosterol-3-*O*-glucoside in *C. conferta* (Khan

et al., 2009); umbelliferone in *C. frutex* (Boinik et al., 1983); sucrose in *C. microphylla* (Huo et al., 2007). The presence of nicotiflorin, caffeic, 3-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, 3,5-di-*O*-caffeoylquinic and gallic acids was firstly shown for *Caragana* species in this work.

Using HPTLC analysis we were investigated the distribution of phenolic compounds in different *C. bungei* organs (leaves, flowers, stems). It was shown that leaves were concentrated the flavonoid glucosides and phenylpropanoids and traces of flavonoid aglycones and gallic acid (Table 1). The composition of flowers phenols was similar to those of leaves excluding of kaempferol, nicotiflorin, caffeic, 3,5-di-*O*-caffeoylquinic and gallic acids. Characteristic feature of stems was ability of outer bark (epidermis) to concentration of quercetin, kaempferol and gallic acid with absence or traces amounts of flavonoids and phenylpropanoids. Only traces of quercetin were detected in inner bark of stems. The values of total phenolic content in leaves, flowers, outer and inner barks of stems were 40.09, 36.24, 12.60 and 1.94 mg/g from dry plant weight respectively, and values of total flavonoid content in leaves, flowers and outer bark of stems were 29.32, 18.67 and 2.13 mg/g from dry plant weight respectively.

The known data about biological activity of plant sterols as a hypocholesterolemic and antiatherosclerotic substances (Lees et al., 1977) was initiated the quantitative analysis of  $\beta$ -sitosterol in *C. bungei*. It was established that  $\beta$ -sitosterol contents in leaves, flowers, outer and inner barks of stems were 1.39, 4.52, 1.43 and 0.97 mg/g respectively (Table 1). High content of  $\beta$ -sitosterol was allowed to consider *C. bungei* flowers as a potential

raw material for production of hypocholesterolemic preparations.

## Conclusions

The phytochemical study of *Caragana bungei* Ledeb. flowering shoots using in traditional medicine of Tuva and Buryatia Republics (Russian Federation) indicated that this plant species contain flavonols (kaempferol, quercetin, isorhamnetin-3-*O*-glucoside, isoquercitrin, rutin, narcissin, nicotiflorin), phenylpropanoids (caffeic acid, caffeoylquinic acids), triterpenes ( $\beta$ -sitosterol,  $\beta$ -sitosterol-3-*O*-glucoside), coumarins (umbelliferone), simple phenols (gallic acid) and carbohydrates (sucrose) and can be regarded as perspective for further pharmacological investigation.

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