Biological Activity of Phenolic Compounds Extracted from *Halenia corniculata* (L.) Cornaz

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Abstract

The composition of extractives from the top part of *Halenia corniculata* were investigated. Two individual compounds were extracted and characterized.

INTRODUCTION

Halenia corniculata (L.) Cornaz is an annual plant 12–50 cm high; it is widespread in the European part of Russia, West and East Siberia, Far East, Mongolia and Manchzhuria [1, 2]. It occurs in meadows, forest borders, along river and brook banks, willows, and osiers, meadow slopes and among sparse trees [2, 3].

The top part of *Halenia corniculata* is applied in Tibetan medicine as a substitute of sar-tig raw material, which prescribe to cure "bile fever and infection" [4–7]. Extracts and tincture of Halenia corniculata are recommended as the means to stimulate the appetite and improve digestion, and also to cure gastritis, stomach and intestinal ache, liver diseases, colitis, enterocolitis [8]. Polyphenolic complex extracted from *Halenia corniculata* exhibits antioxidant and hepatoprotective action [9, 10].

Ethereal oils, alkaloids, tanning agents, iridoids, phenol compounds (xanthones, flavonoids) were discovered in this plant species [11–17].

Investigations of *Halenia corniculata* were mainly connected with the studies of chemical composition [17] and pharmacological activity of individual xanthones [18, 19]. It was confirmed experimentally that individual xanthones of *Halenia corniculata* act as hepatoprotective agents, which is due to their membrane-stabilizing and antioxidant action [18, 19].

The present investigation deals with the problem connected with extraction of predominant chemically pure phenolic compounds from the top parts of *Halenia corniculata* for the purpose of increasing the yield of these compounds.

EXPERIMENTAL

The top part of *Halenia corniculata* was collected in August 2001 during the blooming period

(the Kabansk district, Buryatia). Freshly distilled solvents of pure (ch.) reagent grade were used.

Melting point was measured with Kofler apparatus. In order to determine molecular masses and elemental composition, we used a high-resolution Finnigan MAT 8200 spectrometer. The NMR spectra were recorded with Bruker AC 200 spectrometer (working frequency: 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C) in $CDCl_3$ solution. The multiplicity of signals in ^{13}C NMR spectra was determined with the help of standard recording procedures in the J modulation mode (JMOD).

High-performance liquid chromatography (HPLC) was carried out using the micro-column liquid chromatograph Milikhrom-4 (PO "Nauchpribor", Orel). Chromatrographic conditions: steel column 2 × 64 mm in size, sorbent: Nucleosil C-18 (5 μm), temperature ~ 20 °C, eluting rate: 100 μl/min, UV detection at 360 nm.

Treatment of the plant material

Air-dry ground top parts (2–3 mm) of *Halenia corniculata* in the amount of 1.5 kg were subjected to five-fold extraction with 70 % ethyl alcohol at the ratio of the raw material to the solvent equal to 1 : 12 (18–20 °C, 72 h for each extraction). The united extracts were concentrated in vacuum to the water residue (0.5 l), which was consecutively treated in a separating funnel with chloroform (25 × 200 ml) and ethyl acetate (25 × 300 ml). The organic extracts were evaporated in vacuum; the residue was subjected to separation. The yield of extractives from the top parts of *Halenia corniculata* was 99.79 g (6.65 % of the mass of absolutely dry raw material) for chloroform as a solvent, and 53.11 g (3.54 % of the mass of absolutely dry raw material) for ethyl acetate.

Separation of the chloroform extract

Procedure I. The chloroform extract was concentrated with a rotary evaporator to the dry state. The dry residue was passed through the chromatographic column with silica gel L 100/400 using a mixture of hexane and ethyl acetate (7 : 3) as eluting agent. The fractions were sampled at the amount of 100 ml. Separation was monitored with the help of TLC with Silufol in hexane – ethyl acetate (7 : 3) system. As a result of separation, an individual xanthone 1-hydroxy-2,3,4,5-tetramethoxyxanthone **1** was isolated; its purity was determined by means of HPLC (Table 1).

Procedure II. The chloroform extract (5.4 g) was separated with column chromatography on silica gel L 100/250 (eluting agent: chloroform → ethanol); two fractions were obtained: 2.57 g of fraction 1 (eluted with chloroform) and 2.83 g of fraction 2 (eluted with ethanol).

Recrystallization of fraction 1 from ethanol resulted in obtaining 0.55 g of precipitate I, 0.12 g of precipitate II, 0.05 g of precipitate III, and 0.02 g of precipitate IV; they are mixtures of xanthones: 1-hydroxy-2,3,4,5-tetramethoxyxanthone **1**, 1-hydroxy-2,3,5-trimethyoxyxanthone **2** and 1-hydroxy-2,3,4,7-tetramethoxyxanthone **3**. The compounds **1**–**3** were identified on the basis of the data of TLC and NMR.

Recrystallization of fraction 2 from ethanol resulted in obtaining 0.37 g of precipitate I (a mixture of 1-hydroxy-2,3,4,5-tetramethoxyxanthone **1** and 1-hydroxy-2,3,5-trimethoxyxanthone **2** at a ratio of 2.5 : 1) and 0.08 g of precipitate II (a mixture of 1-hydroxy-2,3,4,5-tetramethoxyxanthone **1** and 1-hydroxy-2,3,5-trimethoxyxanthone **2** at a

TABLE 1

The yield of individual compounds isolated from the top parts of *Halenia corniculata*

Compound	Purity, %	Yield		
		% of the extracted mass	% of the mass of a.d.r.m.*	
			This work	Literature data
1	95.22	3.76	0.25	0.003 [20], 0.12 [21]
4	96.81	5.93	0.21	0.0096 [17]

*Absolutely dry raw material.

TABLE 2

The characteristics of physicochemical properties of compounds **1** and **4** compared with literature data

Compound	T_m, °C	Chromatographic behaviour R_f	UV spectra, λ_{max} (MeOH), nm	^{1}H NMR spectra (δ_c, ppm, J, Hz)
1	146–147	0.29 (hexane/ethyl acetate 7 : 3)	244, 260, 275 (shoulder), 314, 378	3.92 (3H, s, OCH_3), 4.00 (6H, s, 2×OCH_3), 4.12 (3H, s, OCH_3), 7.22 (2H, m, H-6,7), 7.77 (1H, dd, J = 7.5; 2.0, H-8), 12.55 (1H, s, OH)
1-Hydroxy-2,3,4,5-tetra-methoxyxan-thone [17, 20, 21]	145–147	0.24 (hexane/ethyl acetate 7 : 3)	243,261, 273 (shoulder), 313, 380	3.96 (3H, s, OCH_3), 4.02 (6H, s, OCH_3), 4.16 (3H, s, OCH_3), 7.28 (2H, m, H-6,7), 7.8 (1H, dd, J = 9.0; 2.5, H-8)
4	326–328	0.22 (chloroform/methanol 9 : 1)	253, 265, 292 (shoulder), 347	6.23 (1H, d, J = 2.0, H-8), 6.50 (1H, d, J = 2.0, H-6), 6.56 (1H, s, H-3), 6.99 (1H, d, J = 7.8, H-5′), 7.45 (1H, dd, J = 7.8; 1.8, H-6′), 7.48 (1H, d, J = 1.8, H-2′), 13.0 (1H, s, OH at C-5)
5,7,3′,4′-Tet-rahydroxy-flavon (luteoline) [17, 22, 23]	328–331 (with decomp-osition)	–	242 (shoulder), 253, 267, 291 (shoulder), 349	6.58 (1H, d, J = 2.0, H-6), 6.67 (1H, d, J = 2.0, H-8), 6.79 (1H, s, H-3), 7.08 (1H, d, J = 8.0, H-5′), 7.51 (br. s, H-2′), 7.56 (1H, dd, J = 2.0; 8.0, H-6′)

ratio of 1 : 4) with an admixture of 1-hydroxy-2,3,4,7-tetramethoxyxanthone **3**. Compounds **1**–**3** were identified on the basis of the data obtained with TLC and NMR; the ratio of xanthones in precipitates I and II was determined form the ratio of integral intensities of the signals of protons in the NMR spectra. According to the data of ^{1}H spectra of the chloroform fraction, the concentration of xanthone **3** does not exceed 0.09 %, as calculated for the absolutely dry raw material.

Separation of the ethyl acetate extract

Three fractions were obtained by separating 1 g of the ethyl acetate extract with column chromatography on silica gel L100/250 (eluting agent: chloroform → ethyl acetate → ethanol): fraction 1 (eluted with chloroform) – 0.07 g (the concentration of xanthone **1** according to the data of ^{1}H NMR did not exceed 10 % of the mass of this fraction), fraction 2 (eluted with ethyl acetate) – 0.66 g, fraction 3 (eluted with ethanol) – 0.23 g (accoridng to the data of TLC and NMR, it does not contain the mentioned xanthone compounds).

Recrystallization of fraction 2 from ethanol resulted in obtaining an individual flavor luteoline **4**; its purity was determined by means of HPLC (see Table 1). The content of luteoline in fraction 2, as determined by means of HPLC, was 68.26 %. The content of this compound in the top parts of *Halenia corniculata* was 0.21 %.

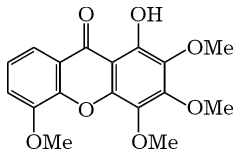
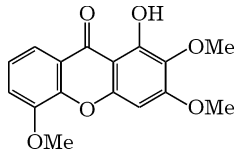
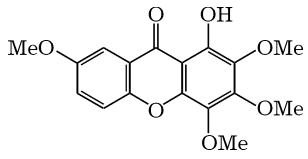
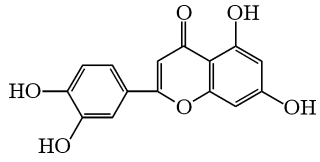
One can see in Table 2 that the quality of phenolic compounds **1** and **4** extracted from the top parts of *Halenia corniculata* corresponds to chemically pure 1-hydroxy-2,3,4,5-tetra-methoxyxanthone and 5,7,3',4'-tetra-hydroxyflavon (luteoline), respectively.

RESULTS AND DISCUSSION

The most suitable method of separating the chloroform extract of *Halenia corniculata* was the first one of two methods tested; it allowed us to separate the individual xanthone **1**

TABLE 3

Structural formulas of the compounds isolated from *Halenia corniculata*

Compound	Structural formula	Title
1		1-hydroxy-2,3,4,5-tetramethoxyxanthone
2		1-hydroxy-2,3,5-trimethoxyxanthone
3		1-hydroxy-2,3,4,7-tetramethoxyxanthone
4		5,7,3',4'-tetrahydroxyflavon (luteoline)

(1-hydroxy-2,3,4,5-tetramethoxyxanthone); separating the ethyl acetate extract we succeeded in isolating the individual flavon **4** (luteoline). The proposed methods of isolating these individual compounds allowed us to increase their yield.

The structures of xanthone compounds **1**–**3** and flavonoid 4 are shown in Table 3.

So, the chloroform extract from the top parts of *Halenia corniculata* can be used as a source to obtain xanthone compounds. The predominant xanthone of this extract is 1-hydroxy-2,3,4,5-tetramethoxyxanthone (0.25 % of the mass of absolutely dry raw material). Luteoline (5,7,3',4'-tetrahydroxyflavon) can be isolated from the ethyl acetate extract with an yield of 0.21 % of the mass of absolutely dry raw material. Compound **1** (1-hydroxy-2,3,4,5-tetramethyxyxanthone) was used to carry out synthetic transformations.

The characteristics of individual compounds are shown below.

1-Hydroxy-2,3,4,5-tetramethoxyxanthone 1. Melting point 146–147 °C (from ethanol). UC spectrum: λ_{max} (MeOH) 244, 260, 275 (shoulder), 314, 378 nm. Mass spectrum, *m/z* (%): 347 (13.55), 332 (83.87), 317 (100), 302 (13.27), 289 (10.83), 259 (11.17), 175 (11.68). $C_{17}H_{16}O_7$. Calc. *m/z*: 332.08959. Experim. *m/z*: 332.08801. ^{13}C NMR spectrum (δ_c, ppm): 56.42 (CH_3), 61.47 (CH_3), 61.72 (2×CH_3), 95.12 (C-4), 106.79 (C-8a), 115.92 (C-6), 116.49 (C-7), 120.83 (C-5a), 123.56 (C-8), 132.69 (C-4a), 135.42 (C-1a), 145.62 (C-5), 148.66 (C-3), 150.49 (C-2), 154.08 (C-1), 181.58 (C-9). The data of ^1H NMR spectrum are similar to those resported in [17, 20, 24].

5,7,3',4'-Tetrahydroxyflavon (luteoline) 4. Melting point 326–328 °C. UV spectrum: λ_{max} (MeOH) 253, 265 (shoulder), 347 nm; NaOAc: 270, 380 nm; NaOAc + H_3BO_3 263, 378 nm; $AlCl_3$ 273, 303 (shoulder) 332, 430 nm; $AlCl_3$ + HCl 265, 272, 300 (shoulder), 64, 385; NaOMe 272, 420 nm. ^1H NMR spectrum (δ_c, ppm, *J*, Hz): 6.23 (d, 1H, H-8, *J* = 2.0); 6.50 (d, 1H, H-6, *J* = 2.0); 6.56 (s, 1H, H-3); 6.99 (d, 1H, H-5′, *J* = 7.8); 7.45 (dd, 1H, H-6′, *J* = 7.8 and *J* = 1.8); 7.48 (d, 1H, H-2′, *J* = 1.8); 13.0 (s,

OH at C-5). The data are similar to those reported in [17, 22, 23].

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