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FLAVONOIDS FROM *Calendula officinalis* FLOWERS

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Medicinal calendula (*Calendula officinalis* L., Asteraceae) is widely cultivated in the Russian Federation and is used to produce many medicines [1, 2].

The chemical composition of *C. officinalis* includes carotinoids (a leading group of biologically active compounds), flavonoids, triterpene saponins, and several associated compounds [3, 4]. However, until now standardization of the raw material and preparations of this plant has not been fully implemented.

In our opinion, this situation is to some extent due to contradictory literature data on the flavonoid composition of medicinal calendula flowers.

Thus, one reference [5] lists the dominant component as rutin; another [6], isorhamnetin diglucoside. However, the foreign literature [7] reports that calendula flowers contain isorhamnetin 3-*O*-rutinoside.

Considering the high flavonoid content in medicinal calendula flowers and their definite significance in determining the biological activity of this material, it seemed advisable to study the component composition of calendula in order to justify the standardization of flavonoids in this medicinal plant material.

We investigated flowers of medicinal calendula (Kal′ta variety, 2006) cultivated industrially at specialized facilities in Samara District SGPU Sergievskii (Antonovka), ZAO Samaralektravy, and OOO Kentavr (Berezovka).

Ground air-dried flowers of medicinal calendula (Kal′ta variety) (100.0 g) were extracted exhaustively with ethanol (70%), combining for this maceration (24 h) and subsequent thermal extraction at 85-90°C. The aqueous ethanol extracts were evaporated in vacuo to a thick residue (about 50 mL). The condensed extract was dried over silica gel (SG) L 40/100, eluted with $CHCl_3$, and placed on a SG layer formed in $CHCl_3$. The column was eluted with $CHCl_3$ and $CHCl_3$:C_2H_5OH in various proportions (97:3, 95:5, 93:7, 90:10, 88:12, 85:15, 80:20, 70:30).

Fractions with the dominant flavonoid were combined and placed on polyamide for further purification. Dry powder (extract + polyamide) was transferred to a chromatography column (10.0 g) (4.0 cm of sorbent, 5 cm diameter) with elution by water and aqueous ethanol (20%, 40, 70, 96). The separation was monitored by TLC. The column purification over polyamide produced **1** (the dominant diagnostic flavonoid).

The next set of fractions, which also contained flavonoid-type compounds, was combined, evaporated, and dried over SG L 40/100 (1.0 g). The dry powder (extract + silica gel) was transferred to a chromatography column (4.0 cm of sorbent, 5 cm diameter). The column was eluted with $CHCl_3$ containing various amounts of ethanol (5%, 10, 15, 20, 25, 30, 35) to produce yellow flavonoid **2**, R_f 0.6.

Comparison of the chromatographic behavior of the dominant flavonoid with standard (GSO) rutin (R_f 0.4, $CHCl_3$:CH_3OH:H_2O, 26:14:3) showed that the isolated compound had a different R_f value (about 0.5) and; therefore, was a different flavonoid.

Isolated flavonoids **1** and **2** were identified using UV, NMR, and mass spectrometry and chemical transformations.

Compound 1, yellow crystals (EtOH), $C_{28}H_{32}O_{16}$, mp 173-175°C, $[\alpha]_D^{546}$ +12.0° (*c* 0.83, water). UV spectrum (EtOH, λ_{max}, nm): 257, 268 sh; +$AlCl_3$: 268, 276 sh, 359, 403. PMR spectrum [200 MHz, $(CD_3)_2CO$ + D_2O, δ, ppm, J/Hz]: 1.04 (3H, d, J = 6.0, rhamnose CH_3), 3.2-3.8 (10H, sugar part), 3.95 (3H, s, CH_3O), 4.50 (1H, d, J = 2.0, rhamnopyranose H-1‴), 5.23 (1H, d, J = 7.0, glucopyranose H-1″), 6.24 (1H, d, J = 2.0, H-6), 6.50 (1H, d, J = 2.0, H-8), 6.96 (1H, d, J = 2.0, H-5′), 7.66 (1H, dd, J = 2.0 and 8.5, H-6′), 7.97 (1H, d, J = 2.0, H-2′).

Compound 2, yellow crystals (EtOH), $C_{21}H_{20}O_{12}$, mp 227-229°C. UV spectrum (EtOH, λ_{max}, nm): 257, 267 sh, 360; CH_3ONa: 272, 327, 410; +CH_3COONa: 272, 379; +CH_3COONa + H_3BO_3: 262, 377; +$AlCl_3$: 275, 412; $AlCl_3$ + HCl: 270, 403.

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Acid hydrolysis of **1** (2% HCl, 100°C, 1 h) produced the aglycon, which was identified as isorhamnetin {$C_{16}H_{12}O_7$, $[M]^+$ 316, λ_{max} (EtOH) 257, 268 sh, 367 nm}, and the carbohydrate part, glucose and rhamnose. Compound **1** was hydrolyzed by rhamnodiastase, which led to the conclusion that it contained the biose rutinose. UV spectra with diagnostic reagents indicated that the isorhamnetin 3-OH was glycosylated. Therefore, **1** was isorhamnetin 3-*O*-rutinoside (narcissin).

Acid hydrolysis of **2** gave the aglycon, which was identified as quercetin {$C_{15}H_{10}O_7$, $[M]^+$ 302, λ_{max} (EtOH) 256, 267 sh, 370 nm} and glucose. Furthermore, this glycoside was cleaved by β-glucosidase, which was consistent with a β-bond to the aglycon. A bathochromic shift of the long-wavelength band in the UV spectrum of the aglycon in the presence of $AlCl_3$ (+60 nm), in contrast with the starting compound (+42 nm), indicated that the 3-OH group was glycosylated. Therefore, the spectral data and chemical transformations in addition to direct comparison with an authentic sample identified **2** as quercetin 3-*O*-β-D-glucopyranoside (isoquercitrin).

Compounds **1** and **2** were isolated for the first time in the Russian Federation from cultivated medicinal calendula flowers (Kal′ta variety).

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