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METHOD FOR DETERMINING CONTENT OF PHENOLIC COMPOUNDS IN *Aloe arborescens*

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A spectrophotometric method for quantitative determination of total content of phenolic compounds in leaves of Aloe arborescens *Mill. (Liliaceae) calculated as aloenin was developed. Statistical analysis showed that the relative uncertainty of the determination method was less than 2%.*

Key words: *Aloe arborescens* Mill., Liliaceae, phenolic compounds, aloenin, spectrophotometry.

Aloe arborescens Mill. (Liliaceae) is a medicinal plant that is widely used in the practice of scientific medicine. Its chemical composition is variable and consists of various classes of compounds such as polysaccharides [1-3], organic acids [4], phenolic compounds (PC) [5-10], carotinoids [11], triterpenes [5], and essential oils [12]. The phenolic complex of *A. arborescens* includes 11 compounds such as anthraquinones (aloe-emodin) [5], anthrones (aloin A and B, 10-hydroxyaloin A) [6, 11], pyrones (aloenin, aloenin B) [7, 11], chromones (aloesin, 2′-*O*-feruloyl- and 2′-*O*-*p*-coumaroylaloesin) [8, 9], and dimeric anthraquinone-anthrone derivatives (elgonica-dimers A and B) [10]. Standardization of *A. arborescens* and preparations made from it is not planned in Russia.

The goal of our work was to develop a spectrophotometric method for quantitative determination of the total content of PC in *A. arborescens* leaves. We isolated from the ethylacetate fraction of *A. arborescens* a compound that was identified as 4-methoxy-6-(2′-β-glucopyranosyloxy-4′-hydroxy-6′-methylphenyl)-2-pyrone (aloenin). According to HPLC, aloenin is the dominant component of the phenolic complex. Therefore, it could be selected as the standard compound for quantitative analysis of *A. arborescens* (Fig. 1A).

We also developed a simple method for preparing aloenin in 0.011% yield of the raw material mass with 98.5% purity (HPLC, Fig. 1B).

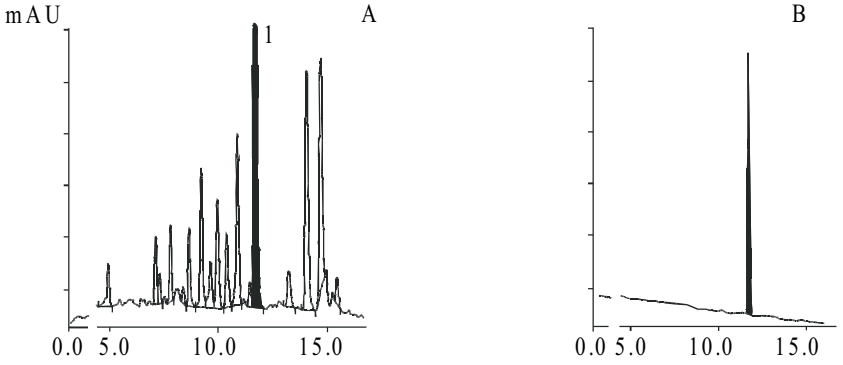

Fig. 1. Chromatogram of alcohol extract of *A. arborescens* (A; 1 = aloenin) and standard aloenin (B).

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TABLE 1. Determination of the Accuracy of the Developed Method by the "Added-Found" Method

Aloenin added, μg	Theoretical content of PC, μg of aloenin	PC found, μg aloenin	Uncertainty	
			abs., μg	rel., %
240	1123	1108	−15	1.34
480	1463	1446	−17	1.17
720	1703	1729	+26	1.53
960	1943	1936	−7	0.36

*Content of PC (983 μg of aloenin) in raw material (1 g).

TABLE 2. Statistical Characteristics of Developed Method [n = 11, P = 95%, $t(p, f)$ = 2.23]

A. arborescens leaves	x_{avg}, %*	S^2	S_x	±Δx, %	E, %
Fresh	0.112	$8.87 \cdot 10^{-6}$	$8.97 \cdot 10^{-4}$	0.002	1.79
Dry	1.51	$2.01 \cdot 10^{-3}$	$1.35 \cdot 10^{-2}$	0.03	1.99

*x_{avg} is the mean value; S^2, the scatter; S_x, the mean-square deviation; ±Δx, the absolute error of the arithmetic mean; E, the standard error.

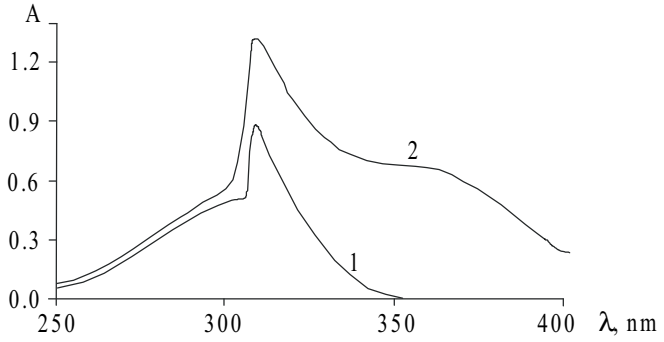

Fig. 2. Absorption spectrum of aloenin (1) and the alcoholic extract of *A. arborescens* (2).

The absorption spectrum of the alcohol extract of *A. arborescens* exhibited a maximum at 307 nm that agreed with that of aloenin and was selected as the analytical wavelength (Fig. 2). The optimum parameters for extracting PC from fresh raw material were chosen during the technical studies. They were ethanol (95%), three-fold extraction (1:10 ratio and 2 × 1:5), 100°C, 30 min each.

The optical density was linear in the range 0.2-0.7 rel. units for aloenin solutions with concentrations 1.75-6.00 μg/mL. The calibration curve obeyed the equation $A = 0.121 \cdot c - 0.029$, where A was the optical density (rel. units) and c, the aloenin concentration (μg/mL) ($r^2 = 0.9994$, $s^2 = 2.95 \cdot 10^{-2}$). The "added—found" method showed that the measurement error was less than 1.6% (Table 1). Table 2 gives the statistical characteristics of the developed method.

The developed method was used to analyze dry leaves of *A. arborescens* used to prepared aloe tablets, having established by this that the total contents of PC calculated as aloenin in fresh and dry raw material were 0.09-0.15% and 1.24-1.65%, respectively.

EXPERIMENTAL

Leaves of three-year-old *A. arborescens* were collected in 2008 in the greenhouse of the Siberian Institute of the Physiology and Biochemistry of Plants of the Siberian Branch, Russian Academy of Sciences (Irkutsk). Dry leaves of *A. arborescens* satisfying requirements of FS 42-2800-91 were prepared at ZAO Vifitekh (Russia).

Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optical Mechanical Plant) in a 1-dm cuvette at 20°C. Spectrophotometry was studied on CE 2011 (Cecil) and UV-Vis-mini (Shimadzu) spectrophotometers with an absorbing layer 10-mm thick. ^{13}C NMR spectra were recorded on a VXR 500S (Varian) NMR spectrometer at operating frequency 125.7 MHz. Spectra of solutions in DMSO-d_6 were recorded. HPLC was carried out on a Summit (Dionex) liquid chromatograph with a Prodigy column (5 μm, ODS 3, 250 × 4.6 mm, Phenomenex) with gradient elution using solvents A (0.1 aqueous TFA) and CH_3CN (B) and a UVD 170S UV-detector at λ 220, 254, 280, and 430 nm.

Isolation of Aloenin. Ground fresh leaves of *A. arborescens* (1 kg) were pressed three times to collect the juice. The remaining raw material was extracted with ethanol (80%, 1:30, 3×). The resulting juice and alcohol extracts were combined, concentrated to 100 mL, and diluted with ethanol (95%, 1:10). The resulting precipitate of polysaccharides was separated by centrifugation. The supernatant was concentrated in vacuo to an aqueous residue that was extracted successively with hexane, $CHCl_3$, and ethylacetate. The ethylacetate extract was concentrated to dryness, dissolved in the minimum volume of ethylacetate:methanol (9:1), and left in the cold for crystallization. The resulting precipitate of aloenin was recrystallized twice from methanol. Yield of aloenin, 107 mg (0.011% of fresh raw material mass).

Aloenin. Fine colorless needles, $C_{19}H_{22}O_{10}$, MW 410 g/mol, mp 145-147°C, $[\alpha]_D$ −28° (*c* 2.0, MeOH). UV (MeOH, λ, nm): 230, 246, 307; +KOH: 227, 254, 353. ^{13}C NMR spectrum (125.7 MHz, DMSO-d_6, ppm): 20.22 (C-6′ CH_3O), 56.04 (C-4, CH_3O), 62.33 (Glc C-6), 71.00 (Glc C-4), 74.52 (Glc C-2), 78.11 (Glc C-3, C-5), 88.4 (C-3), 101.98 (C-3′), 102.66 (Glc C-1), 105.03 (C-5), 112.35 (C-5′), 114.74 (C-1′), 140.02 (C-6′), 157.86 (C-2′), 158.27 (C-6), 161.03 (C-4′), 165.50 (C-2), 171.77 (C-4). HPLC: t_R 11.75 min.

Method for Quantitative Determination of Total Content of Pyrone Compounds in *A. arborescens* Leaves. An analytical sample of raw material was ground in a homogenizer. An accurately weighed portion of the homogenate (about 10 g) was transferred to a one-necked 250-mL flask, treated with ethanol (95%, 100 mL), refluxed for 30 min, cooled, filtered into a 200-mL volumetric flask, and extracted twice under the same conditions with extractant (50 mL). The volume of the combined filtrate was adjusted to the mark with ethanol (95%, solution A). A portion of solution A (2 mL) was transferred to a 25-mL volumetric flask and adjusted to the mark with ethanol (95%, solution B). The optical density of solution B was determined at 307 nm. The reference solution was ethanol (95%).

The total content of pyrone compounds calculated as aloenin (% in fresh raw material) (*X*) was calculated using the formula

$$X = \frac{DK^V}{m} \times \frac{m_0}{D_0 K^V{}_0} \times \frac{100}{100 - W} \times 100,$$

where *D* is the optical density of the sample; K^V, the dilution coefficient of the sample (2500); *m*, the raw material mass (g); D_0, the optical density of a solution of standard aloenin; $K^V{}_0$, the dilution coefficient of the solution of standard aloenin (1250); m_0, the mass of the standard aloenin (g); and *W*, the mass loss on drying raw material (%).

Preparation of Standard Aloenin Solution. An accurately weighed portion of aloenin (15 mg) was transferred to a 100-mL volumetric flask, dissolved in ethanol (95%), and adjusted to the mark with the same solvent (solution A). Solution A (1 mL) was transferred to a 50-mL volumetric flask and adjusted to the mark with ethanol (95%) (solution B).

Dry leaves of *A. arborescens* were analyzed by an analogous method using a weighed portion of raw material (1 g).

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