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CHEMICAL COMPOSITION OF *Aloe arborescens* AND ITS CHANGE BY BIOSTIMULATION

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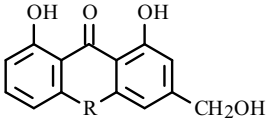
The chemical composition of Aloe arborescens *Mill. (Asphodelaceae) and its change by biostimulation (cold stress) were studied. It was found that aloe-emodin; 2″-*O*-feruloylaloesin; aloins A and B; aloenin; free glucose, saccharose, and fructose; pectinic substances; and malic, malonic, and oxalic acids were present. 4-Methoxy-6-(4′-hydroxy-6′-methylphenyl)-2-pyrone, 7-hydroxy-2,5-dimethylchromone, umbeliferone, esculetin, and vanillic and veratric acids were found for the first time. Changes in the chemical composition of* A. arborescens *due to biostimulation for 5-10 d increased the antiradical and biostimulating activity of the raw material.*

Key words: *Aloe arborescens* Mill., biostimulation, phenolic compounds, free carbohydrates, polysaccharides, organic acids.

Aloe arborescens Mill. (Asphodelaceae) provides raw material for preparing several biostimulants, the production of which includes biostimulation (BS) by the method of Filatov [1]. The change of chemical composition of *A. arborescens* during BS has not been reported. Our goal was to evaluate the stepwise changes of biochemical indicators of *A. arborescens* raw material quality and its biological activity that occur under the influence of cold stress, the principal BS factor.

Considering the literature on the composition and biological activity of compounds from *A. arborescens* [2], we studied the component composition and quantitative content of phenolic compounds, free carbohydrates, polysaccharides, and organic acids in the starting raw material.

Chromatography of *A. arborescens* confirmed the presence of aloe-emodin (**1**), 2″-*O*-feruloylaloesin (**6**), aloins A (**7**) and B (**8**), and aloenin (**9**). We also observed for the first time in this species 4-methoxy-6-(4′-hydroxy-6′-methylphenyl)-2-pyrone (**2**), 7-hydroxy-2,5-dimethylchromone (**3**), umbelliferone (**4**), esculetin (**5**), and vanillic (**10**) and veratric acids (**11**). Compounds **2** and **3** were isolated previously from *A. ferox* Mill. [3, 4].



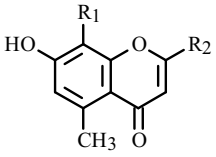
1, 7, 8

R

1: =O

7: Glc, H

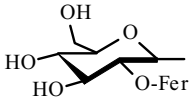
8: H, Glc



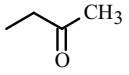
3, 6

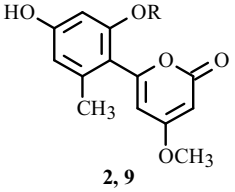
3: R_1 = H, R_2 = CH_3

6: R_1 =



R_2 =





2, 9

2: R = H

9: R = Glc

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TABLE 1. Biochemical Parameters of *A. arborescens* Leaves at Various BS Times

BS time, d	Organic acids, mg/g*				Carbohydrates, mg/g*		Phenolic compound, μg/g*
	free	bound	total	k^f**	FC***	WSPS***	
0	2.16	2.12	4.28	1.02	0.91	6.80	547
5	2.52	2.43	4.95	1.04	1.34	7.56	685
10	2.88	3.29	6.17	0.88	1.35	7.62	603
15	2.25	2.93	5.18	0.77	1.38	8.37	592
20	2.12	1.86	3.78	1.14	1.18	7.35	523

*Of fresh raw material mass; **k^f ratio of free and bound acid contents; ***FC, free carbohydrates; WSPS, water-soluble polysaccharides.

TABLE 2. Content of Phenolic Compounds in *A. arborescens* Leaves at Various BS Times, μg/g of Fresh Raw Material Mass

Compound	BS time, d				
	0	5	10	15	20
1	5.69	7.47	6.45	6.04	5.13
7	71.54	86.53	84.22	18.71	14.75
8	80.96	107.68	89.49	41.32	8.92
9	312.34	345.43	305.91	285.03	247.16

HPLC established the quantitative content of nine components in the starting raw material (μg/g of fresh raw material): **1**, 5.69 ± 0.11; **4**, 10.61 ± 0.21; **5**, 21.22 ± 0.44; **6**, 22.48 ± 0.49; **7**, 71.15 ± 1.42; **8**, 80.96 ± 1.62; **9**, 312.34 ± 6.24; **10**, 13.18 ± 0.26; **11**, 1.48 ± 0.03. We selected **1**, **7**, **8**, and **9** as markers for studying BS of *A. arborescens*.

Free carbohydrates of *A. arborescens* included glucose, saccharose, and fructose. Water-soluble polysaccharides (WSPS) were a heterogeneous complex containing components in the range of molecular weights 15–90 kDa. Hydrolysis of WSPS produced D-galacturonic acid, D-galactose, L-arabinose, L-rhamnose, D-glucose, and D-xylose in the ratio 77.8:6.9:6.3:4.4:4.1:0.4 (mol%). The presence of positive specific rotation, a high content of D-galacturonic acid, and the nature of the IR spectrum suggested that *A. arborescens* WSPS were pectinic substances.

Malic acid was isolated and identified among the organic acids of *A. arborescens*. The presence of oxalic and malonic acids was established by chromatography (HPTLC).

Next we measured changes in the chemical composition of *A. arborescens* that occurred under the influence of cold stress. Preliminary experiments found that the physicochemical parameters of the raw material changed drastically at +4°C. The content of phenolic compounds reached a maximum on the 5th day of the experiment; of organic acids, on the 10th; of carbohydrate components, on the 15th (Table 1). Lengthening the duration of the process caused all measured parameters to decrease.

We found that BS affected the quantitative composition of *A. arborescens* phenolic compounds. Thus, the contents of all markers increased on the 5th day of the experiment by 31% for **1**; 21, **7**; 33, **8**; and 11, **9** compared with the starting raw material. Then their contents decreased (Table 2).

A study of the composition of *A. arborescens* free carbohydrates showed that the ratio of D-glucose, saccharose, and D-fructose in the intact plant was 14.6:13.8:1.0; on the 15th day, 9.5:8.3:1.0; on the 20th day, 7.8:4.9:1.0 (Table 3). The WSPS content in raw material during BS had increased on the 15th day and decreased on the 20th day. The monosaccharide composition changed insignificantly (Table 3).

Analysis of *A. arborescens* organic acids during BS established that the acids underwent a gradual transition from domination of the salt form to the free acid, i.e., the ratio of free and bound acids (k') changed from 1.02 at the start of the process to 1.14 on the 20th day (Table 1).

TABLE 3. Composition of Free Carbohydrates (FC) and Water-Soluble Polysaccharides (WSPS) in *A. arborescens* at Various BS Times, mol%

BS time, d	Carbohydrate group	Ara	Frc	Gal	Glc	Rha	Sac	Xyl	GalUA
0	FC		3.4		49.6		46.9		
	WSPS	6.3		6.9	4.1	4.4		0.4	77.8
5	FC		3.7		52.1		45.2		
	WSPS	5.5		7.2	4.2	4.6		0.4	78.0
10	FC		4.7		51.8		43.4		
	WSPS	5.6		7.3	4.2	4.3		0.5	78.0
15	FC		5.3		50.4		44.2		
	WSPS	4.3		7.5	4.0	4.5		0.3	79.3
20	FC		7.3		56.7		35.9		
	WSPS	4.2		7.6	3.8	5.3		0.3	78.7

TABLE 4. Antiradical and Biostimulating Activity of *A. arborescens* Extracts

BS time, d	DPPH, IC_{50}, μg/mL	Concentration of yeast cells, % of initial concentration		
		after 24 h	after 48 h	after 72 h
0	396	731	962	1087
5	340	748	980	1153
10	315	814	1022	1201
15	361	741	976	1067
20	377	706	984	1032
Control (water)	–	710	934	1024

Investigations of the biological activity of *A. arborescens* juice obtained at all BS stages indicated that juice obtained on the 10th day of the process had the greatest antiradical and biostimulating activity (Table 4).

Thus, we established that BS is a complicated process that produces a complicated influence on the biochemical parameters of *A. arborescens*. BS over 5-10 days increased in the raw material the contents of the principal biologically active compounds and allowed juice with the greatest biological activity to be obtained.

EXPERIMENTAL

Leaves of three-year-old *A. arborescens* were collected in 2008 in the greenhouse of the Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch, Russian Academy of Sciences (Irkutsk).

HPTLC was performed on Sorbfil PTSKh-AF-V plates (Sorbpolimer) using solvent systems petroleum ether:$(CH_3)_2CO$ (1, 7:3), EtOAc:EtOH:H_2O (2, 20:3:1), PrOH:EtOH:H_2O (3, 7:1:2, double elution to heights of 3 and 6 cm), and $(CH_3)_2CO$:NH_3 (25%):EtOH:$CHCl_3$:H_2O (4, 60:22:10:6:2, triple elution to a height of 6 cm). The detectors were KOH (5%) in MeOH (1), *p*-hydroxydiphenyl:phthalanilic acid:H_3PO_4 (85%) (2), and methyl red:bromphenol blue (3).

Spectrophotometry was carried out in quartz cuvettes (10-mm) on Cecil CE 2011 and UV-Vis mini (Shimadzu) spectrophotometers. Optical rotation was measured in a cuvette (1-dm) at 20°C on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). IR spectra were recorded in films on KRS-5 plates in the range 4000–450 cm^{-1} on a Spectrum 100 IR-Fourier spectrometer (Perkin–Elmer). ^{13}C NMR spectra were recorded in DMSO-d_6 solutions (1%) on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz.

We used standard reference compounds for comparison: aloe-emodin; aloins A and B; umbelliferone; esculetin; D-galacturonic acid (Fluka); vanillic, veratric, malic, malonic, and oxalic acids; L-arabinose; D-glucose; D-galactose;

D-xylose; L-rhamnose; saccharose; D-fructose (Sigma); 2″-feruloylalosin (ChromaDex); aloenin prepared as before [5]; 4-methoxy-6-(4′-hydroxy-6′-methylphenyl)-2-pyrone; and 7-hydroxy-2,5-dimethylchromone prepared from aloenin by the literature method [6].

Gel chromatography of polysaccharides was carried out over a column of Sephadex G-100 [1.5 × 50 cm, NaCl (0.3%) eluent]. Polysaccharides were hydrolyzed in TFA (2 M, 100°C, 6 h), after which TFA was removed in vacuo at 40°C in the presence of MeOH. The hydrolysate was concentrated to the minimal volume and analyzed by HPTLC (system 3, detector 2). The monosaccharide composition was determined by HPTLC and densitometry.

Extraction and Isolation of Compounds from *A. arborescens* Leaves. Leaves of *A. arborescens* (3 kg) were ground in a homogenizer and extracted with EtOH (3 × 10 L, 95%) on a boiling water bath for 1.5 h. The EtOH extract was concentrated to an aqueous residue and extracted successively with hexane, $CHCl_3$, and EtOAc. The remaining aqueous residue was concentrated and dried in a vacuum drying chamber. This produced four fractions: hexane (0.54 g), $CHCl_3$ (2.57 g), EtOAc (7.83 g), and H_2O (36 g).

The $CHCl_3$ fraction was separated by column chromatography over silica gel (100/250, Chemapol, 5 × 20 cm) using a gradient of hexane:EtOAc (100:0→80:20) with monitoring of the separation by HPTLC (system 1, detector 1) and subsequent preparative HPTLC using system 1. The EtOAc fraction was also separated by column chromatography over silica gel (100/250, Chemapol, 6 × 30 cm) using a gradient of $CHCl_3$:EtOH (100:0→0:100) with monitoring of the separation by HPTLC (system 2, detector 1) and subsequent preparative HPTLC using system 2. Recrystallization from MeOH, physicochemical analysis, and comparison with the literature (mp, $[\alpha]_D$, UV, ^{13}C NMR, HPTLC, HPLC) identified 11 compounds: from the $CHCl_3$ fraction, aloe-emodin (**1**) [7], 4-methoxy-6-(4′-hydroxy-6′-methylphenyl)-2-pyrone (**2**) [6], 7-hydroxy-2,5-dimethylchromone (**3**) [6], umbelliferone (**4**) [8], and esculetin (**5**) [8]; from the EtOAc fraction, 2″-*O*-feruloylaloesin (**6**) [7], aloins A (**7**) and B (**8**) [7], and aloenin (**9**) [6]. The presence of vanillic (**10**) and veratric acids (**11**) was established using HPLC.

HPLC was performed in a Summit liquid chromatograph (Dionex) using a Prodigy column (5 μm, ODS 3, 250 × 4.6 mm, Phenomenex) with elution using a gradient of aqueous TFA (0.1%, A) and CH_3CN (B) and a UVD 170S UV-detector at λ 220, 254, 280, and 430 nm. Compounds were identified by retention times, experiments with added standards, and UV spectra of the effluent.

The aqueous fraction (20 g) was dissolved in water (200 mL) and passed over a column of cation-exchanger KU-2-8 (H^+-form, Biolar, 6 × 40 cm, H_2O eluent). The effluent was collected and placed on a column of anion-exchanger ASD-4-5p (Cl^--form, Biolar, 6 × 50 cm, H_2O eluent). The aqueous effluent was concentrated and separated by preparative HPTLC (system 3, detector 2) to isolate three compounds that were identified as glucose, saccharose, and fructose. After the H_2O elution, the anion-exchanger column was eluted with HCOOH (5%). The effluent was concentrated in vacuo to dryness. The solid was dissolved in H_2O and separated using HPTLC (system 4, detector 3) to isolate malic acid, which was identified by mp and IR and ^{13}C NMR spectra [9]. The presence of malonic and oxalic acids was established by chromatography.

Raw material remaining after EtOH extraction was extracted with H_2O until the reaction for carbohydrates was negative (phenol–H_2SO_4 reaction). The resulting extract was concentrated and precipitated with EtOH (95%, 1:5). The resulting precipitate was centrifuged, dissolved in water, demineralized over cation-exchanger KU-2-8 (H^+-form, Biolar), deproteinized by *Streptomyces griseus* pronase (KF.3.4.244, Sigma) [10], and precipitated by acetone (1:4) to afford fractions (12.8 g) of *A. arborescens* WSPS. The carbohydrate content was 95.33% (GalUA 77.8% in the hydrolysate; neutral monosaccharide Gal:Ara:Rha:Glc ratio 1.68:1.54:1.07:1.0, and traces of Xyl); proteins and ash, <0.5%. $[\alpha]_D^{20}$ +132° (*c* 1.0; NaOH, 1%). IR spectrum (ν, cm^{-1}): 1731, 1612, 1380, 1107, 1051, 1024, 830.

Biostimulation of *A. arborescens* Leaves. Freshly collected *A. arborescens* leaves were placed in a packet of filter paper that was wrapped in cotton cloth and placed in a thermostat at +4°C. Time intervals were measured starting 4–5 h after loading the raw material. The control times were 0 (starting raw material), 5, 10, 15, and 20 days of storage.

The following classes of compounds were analyzed quantitatively: organic acids [11], free carbohydrates and polysaccharides [12], and phenolic compounds [5].

***A. arborescens* juice** was prepared under laboratory conditions. Thus, raw material was ground in a homogenizer, pressed through a nylon filter, and vacuum filtered through filter paper. The antiradical activity of *A. arborescens* juice was determined using DPPH [13]; biostimulating activity, using a yeast test.

Yeast Test. Rider medium [$(NH_4)_2SO_4$, 3 g; K_2HPO_4, 0.1 g; KH_2PO_4, 1 g; $MgSO_4$, 0.7 g; NaCl, 0.5 g; glucose, 10 g; water to 1 L, pH 4.9] containing Baker's yeast (0.1%) was thermostatted at 28°C for 48–50 h. Cultivation solution was

diluted with Rider medium without yeast until the optical density was 0.15 units at wavelength 453 nm. Working solution of the tested agent (50 mL) was added until the final concentration was 100 mg/L. Solutions were thermostatted at 28°C for 72 h. The optical density of solutions was determined at 453 nm. The concentration of yeast cells (c, cell/mL) was calculated using the formula $c = D13684$, where D is the optical density of the test solution and 13684 is a coefficient accounting for the amount of yeast cells per mL of test solution.

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