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PHYTOCHEMICAL INVESTIGATION OF BIOLOGICALLY ACTIVE SUBSTANCES IN CERTAIN KAZAKHSTAN *Rumex* SPECIES. 1.

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The qualitative and quantitative compositions of the main groups of biologically active substances from roots of several Kazakhstan Rumex *species were compared. The amino-acid compositions of all species were studied for the first time.*

Key words: *Rumex*, extraction, biologically active substances, chromatography.

The *Flora of Kazakhstan* describes 23 *Rumex* species [1, 2], 13 of which have been studied. Of these, 2 are pharmacoepeic and 7 are used in folk medicine as anti-inflammatory, bactericidal, antitumor, astringent, antidermatitic, and other agents [3-7]. We investigated roots of Kazakhstan *Rumex* species that had not previously been studied: *R. aquaticus* L., *R. pamiricus* Rech. f., *R. pseudonatronatus* Borb., *R. rossicus* Murb., *R. maritimus* L., *R. Marschallianus* R. chb., and *R. thyrsiflorus* F., collected during dormancy.

All species were investigated according to a definite scheme. Raw material was extracted at room temperature for three days with solvents of different polarity: water, aqueous ethanol (50%), ethanol, aqueous isopropanol (50%), isopropanol, aqueous acetone (50%), acetone, ethylacetate, and benzene. The optimal solvents for qualitative and quantitative extraction of biologically active substances (BAS) from all *Rumex* species were aqueous acetone, aqueous ethanol, and aqueous isopropanol (Table 1).

TABLE 1. Extractable Substances (%) in *Rumex* Roots (Averages of Three Determinations)

Extractant	*R. aquaticus*	*R. pseudonatronatus*	*R. Marschallianus*	*R. pamiricus*	*R. maritimus*	*R. rossicus*	*R. thyrsiflorus*
Water	12.72	18.09	13.56	15.29	13.91	11.79	14.01
Ethanol (50%)	13.62	19.54	16.00	20.35	21.91	19.25	17.21
Ethanol	11.10	4.82	11.56	11.87	10.71	8.51	8.62
Isopropanol (50%)	8.24	11.39	15.22	17.26	18.48	18.89	20.97
Isopropanol	2.69	1.61	6.57	6.12	6.41	7.26	9.66
Acetone (50%)	24.05	21.99	24.84	27.07	28.91	25.64	25.25
Acetone	8.39	3.20	6.02	12.54	12.84	11.20	12.34
Ethylacetate	1.07	0.54	1.59	1.08	1.07	1.59	2.15
Benzene	1.26	0.53	1.59	1.09	1.42	1.07	1.62

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TABLE 2. Quantitative Content (%) of Principal Groups of BAS in Roots of Kazakhstan Rumex Species (Averages of Three Determinations)

Rumex species	Amino acids	Tanning agents	Anthraquinones	Polysaccharides	Phenolic acids	Free organic acids	Flavonoids
R. thyrsiflorus F.	1.45	16.77	0.71	1.92	2.73	2.20	2.28
R. pamiricus Rech. f.	4.62	13.36	1.13	1.30	2.70	2.48	4.79
R. pseudonatronatus Borb.	1.34	7.92	1.54	0.42	0.96	2.57	2.18
R. aquaticus L.	1.89	15.37	2.13	0.09	2.66	3.14	6.17
R. Marschallianus R. chb.	3.47	10.42	0.83	3.14	2.42	2.89	9.91
R. rossicus Murb.	4.41	13.39	1.77	0.36	3.44	2.97	6.95
R. maritimus L.	2.93	9.45	1.23	0.41	4.06	2.64	5.23

TABLE 3. Substances Identified in Roots of Six Rumex Species

Rumex species	Substances	Reference
R. thyrsiflorus Fingerh.	Chrysophanol, rhein, emodin, physcion	[12]
Dense-flowered sorrel	Tanning agents	[5, 13-15]
R. pamiricus Rech. fil.	Vitamin K	[16]
Pamir dock	δ-Catechin, 1-epicatechin, 1-epicatechin gallate	[17]
	Tanning agents	[18-21]
	Quercetin, hyperin, rutin, quercitrin	[17, 22-25]
	Rhein, physcion, emodin, chrysophanol, chrysophanein, gluco-frangula-emodin, frangula-emodin methyl ester, chrysophanol and physcion 3-glycosides, frangula-emodin glycosides	[12, 26-28]
R. maritimus L.	Chrysophanol, emodin, physcion	[12, 29]
Golden dock		
R. pseudonatronatus	Tanning agents	[14, 30, 31]
Borb. ex Murb.		
Field dock		
R. rossicus Murb.	Quercetin-3-O-β-D-galactopyranoside, leucocyanidin, emodin, physcion, 8-hydroxy-3-methylanthraquinone-1-O-(4-O-β-D-galactopyranosyl)-α-L-rhamnopyranoside	[32]
Russian dock		
R. aquaticus L.	Vitamin K	[16]
Pond dock	Tanning agents	[31]
	Chrysophanol, multinuclear aromatic compounds: 1,8-dihydroxynaphthalene derivative	[33]

The qualitative compositions of the extracts were established by one- and two-dimensional paper and thin-layer chromatography using standards and specific developers [8]. Substances were identified by comparison with standards of carbohydrates, amino acids, phenols, flavonoids, phenolic acids, and anthraquinones using UV (SF-26) and IR (Specord UR-75) spectra, studying acid-hydrolysis products [11], and determining specific rotations (SU-3 polarimeter). We identified in all *Rumex* species: anthraquinones (chrysophanol, emodin, physcion, and glycosides), carbohydrates (glucose, galactose, saccharose; and fructose and rhamnose in *R. thyrsiflorus*), phenolic acids (gallic, caffeic; *p*-hydroxybenzoic in *R. thyrsiflorus*; syringic in *R. Marschallianus*), phenols (pyrogallol, fluoroglucinol; hydroquinone in *R. thyrsiflorus*), flavonoids (quercetin, rutin, myricetin, and glycosides), and amino acids (proline, alanine, tryptophan, glutamine, serine, glutamic acid, threonine, histidine, arginine, phenylalanine, lysine). We detected alkaloids, catechins, coumarins, polysaccharides, and tanning agents (condensed and hydrolyzed). The quantitative contents of the principal groups of BAS were determined by literature methods [9] (Table 2).

Analysis of the characteristic experimental and literature data (Table 3) for the chemical composition of the studied species indicates that it depends on the habitat. Thus, the amino-acid content predominates in *R. pamiricus* (4.62%) and *R. rossicus* (4.41%); flavonoids, in *R. Marschallianus* (9.91%) and *R. rossicus* (6.95%); polysaccharides, in *R. Marschallianus*

(3.14%); tanning agents, in *R. thyrsiflorus* (16.77%), *R. aquaticus* (15.37%), *R. rossicus* (13.39%), and *R. pamiricus* (13.36%); anthraquinones, in *R. aquaticus* (2.13%) and *R. rossicus* (1.77%); phenolic acids, in *R. maritimus* (4.06%), and *R. rossicus* (3.44%).

Six amino acids dominate in *R. thyrsiflorus*, *R. pseudonatronatus*, *R. aquaticus*, and *R. maritimus*; five, in *R. pamiricus* and *R. Marschallianus*; and four, in *R. rossicus*. The same four carbohydrates occur in *R. pamiricus*, *R. pseudonatronatus*, *R. aquaticus*, *R. Marschallianus*, *R. rossicus*, and *R. maritimus*; five, in *R. thyrsiflorus*. Three phenolic acids occur in *R. thyrsiflorus* and *R. Marschallianus*. The remaining four species each have two phenolic acids and three phenols, i.e., all BAS groups are present in the described species, like for other *Rumex* species. However, the component composition and quantitative content of the groups and components are different in them.

In the literature available to us, chemical investigation of *R. Marschallianus* has not been reported. Therefore, we present the properties for six *Rumex* species (Table 3).

The species with a composition and BAS content closest to the pharmacoepeic species are *R. rossicus*, *R. Marschallianus*, and *R. aquaticus*. These were selected for inclusion in the pharmacoepeia of the Republic of Kazakhstan.

EXPERIMENTAL

Chromatographic investigations used paper FN No. 3 and Silufol UV-254 plates. For paper chromatography, the systems were BAW (4:1:5 and 40:12.5:29), acetic acid (2%), benzene:acetone:water (4:1:2), toluene:ethylacetate (8:2), and toluene; for TLC, petroleum ether saturated with methanol, petroleum ether:dioxane:toluene:methanol (8:2:2:2), and petroleum ether. The total extracts were separated on silica gel, polyamide, aluminum oxide, magnesium carbonate, and LH-20.

BAS Isolation Method. An accurately weighed portion of raw material (~10 g) was placed in an Erlenmeyer flask (250 mL) and treated with extractant (150 mL, listed above). The flask was stoppered and left for 3 d at room temperature. The contents of the flask were thoroughly shaken and filtered. An aliquot of the filtrate was transferred into a porcelain dish that was previously weighed and brought to constant weight, evaporated to dryness on a water bath, and dried at 100-105°C to constant mass. The content of extracted substances was determined.

The remaining extracts were concentrated to dryness. The dry solids were re-extracted with solvents of various polarities, concentrated to a small volume, and separated over columns of sorbents of the appropriate nature using eluents of varying polarities. The composition of the eluates was monitored by paper chromatography. Fractions of pure substances were concentrated to dryness. The mixed extracts were rechromatographed to separate the components. Substances were further purified by crystallization or a minilayer of LH-20 sorbent.

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