

Phenolic compounds of *Bassia prostrata* (Chenopodiaceae)

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Abstract. The composition and content of phenolic compounds in plants of the polymorphic species *Bassia prostrata* (Chenopodiaceae) from geographically distant populations were studied by high-performance liquid chromatography. The material for the study was the aboveground part of the plant. The plants were collected in the flowering phase in Russia (Novosibirsk region, Republic of Tyva, Khakassia, Buryatia, Altai), Kazakhstan, Armenia. The phenolic compounds were extracted by double extraction with 70% ethanol. Component composition of the phenolic complex was investigated by Agilent 1200 chromatograph. Eleven phenolic compounds, including isoquercitrin, kaempferol-3-glycoside, isoramnetin-3-rutinoside, and luteolin were found in the composition. The quantitative content of each compound could vary from 0.1 to 10.8 mg/g in different populations. Chemotypes were determined for the qualitative and quantitative content of phenolic compounds

1 Introduction

Bassia prostrata (L.) A.J. Scott s. l. (\equiv *Kochia prostrata* (L.) Schrad.) is a species of the Chenopodiaceae family. It is a drought- and salt-tolerant species that is a valuable pasture plant. It is used in breeding to improve or create pastures in desert and arid zones. The main range of *B. prostrata* extends from the northern Black Sea region, the Caucasus to Central Asia. In Russia, the eastern border of the range passes through Zabaikalski Krai and eastern Mongolia.

B. prostrata s. l. is characterized by the presence of several cytotypes. Different ploidy levels of this species have been repeatedly reported in the literature. Diploids ($2n = 18$) have been reported from the Republics of Tyva, Altai, Krasnoyarskii Krai, Mongolia, Kazakhstan, Uzbekistan, and Iran; tetraploids ($2n = 36$) from Kazakhstan and China. Hexaploids ($2n = 54$) found in Uzbekistan, Pakistan, and Iran were noted much less frequently.

Mainly the study of the chemical composition of extracts of the aboveground parts of plants of the *Bassia* genus was carried out for pharmacological research, with the detection of different biological activity of the extracts and production of new medicinal preparations. There is information on the anthelmintic, cardiogenic, tonic, and other activities of extracts

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of above-ground parts of plants of genus *Bassia* in folk and traditional medicine. Scientific and clinical studies have been conducted on hypotensive, hypolipidemic, analgesic, anticancer, and other properties [7-8]. For the species *B. prostrata*, *B. scoparia* (L.) A.J. Scott, *B. laniflora* (S.G. Gmel.) A.J. Scott, *B. iranica* (Litv. ex Bornm.) Bornm., *B. muricata* (L.) Asch., data on flavonoids, tannins, saponins, alkaloids are given [9-12]. *B. prostrata* has been the subject of joint pharmacological studies in Kazakhstan and Pakistan [13]. 20 amino acids, 8 fatty acids, and 4 phenolic acids - protocatechuic, vanillic, isovanillic, and *p*-coumaric - were isolated and identified in the studied objects.

This work aimed to study the composition and content of phenolic compounds of *B. prostrata* plants from geographically distant populations of different cytotypes by high-performance liquid chromatography methods. The aim was to investigate the variability of phytochemical traits in natural populations of *B. prostrata* and to analyze the use of biochemical (composition and content of phenolic compounds) traits as additional markers in the identification of diploid and tetraploid cytotypes.

2 Materials and methods

The object of the study is the leaves of the plants of *B. prostrata* in the flowering phase collected in the period 2019-2020 in Novosibirskaja Oblast', the Republics of Khakassia, Buryatia, Altai, Tyva, etc. (Table 1). The raw materials were dried in well-ventilated rooms, crushed to a particle size of 2 mm, mixed and an average sample was taken.

Double extraction with 70% ethanol in a water bath (60–70°C for 1.0–1.5 h.) was carried out to extract the phenolic compounds. An exact weight (0.1 g) of the crushed material was extracted with 30 ml of aqueous ethanol for 30 min followed by 20 ml for 20 min. The residue on the filter was washed with 5 ml of 70% ethanol. The combined extract was concentrated to 10-15 ml (exact volume). To free from impurities, 1 ml of the extract was diluted with bidistilled water to 5 ml and passed through a Diapack C16 cartridge.

The component composition of the phenolic complex of the samples was studied by high-performance liquid chromatography on an analytical HPLC system consisting of an Agilent 1200 liquid chromatograph. The chromatographic separation was conducted at 25°C on a Zorbax SB-C18 Column (4.6 × 150 mm, 5 µm internal diameter) with the Agilent Guard Column Hardware Kit. Detection was performed at a wavelength of $\lambda = 360$ nm [11]. Quantification of individual components in plant samples was carried out using the external standard method.

3 Results

Using high-performance liquid chromatography to separate the complex of phenolic compounds, we were able to conduct a comparative study of the qualitative composition and content of phenolic compounds in samples of *B. prostrata* from geographically distant regions of Russia and neighboring countries, 14 samples in total were examined (Table 1).

The quantitative content of each compound in different populations as a whole could vary from 0.1 to 10.8 mg/g. The highest content of isoquercitrin (3.4 mg/g) is characteristic of plants from Armenia (RU-AR2) (hexaploid). The high content of kaempferol-3-glycoside was observed both in diploid cytotype, from Tyva (RU-TU2) - 6.4 mg/g, and in hexaploid from Armenia (RU-AR2) - 4.8 mg/g. The highest content of isoramnetin-3-rutinoside was observed in hexaploid from Armenia (10,3 mg/g), tetraploid from Novosibirsk region (RU-NO1) (7,4 mg/g), and diploid from Tyva (RU-TU2) (6,5 mg/g). The highest luteolin content was observed in hexaploid from Armenia and the lowest in tetraploid from Kazakhstan (KZ-A1). In general, almost all compounds showed instability

in quantitative content, indicating heterogeneity in populations. In almost all plants collected in Kazakhstan from both diploid and tetraploid cytotypes the compounds №№ 1, 2, 7 (kaempferol-3-glycoside), 8 (isoramnetin-3-rutinoside) were the predominant components. In samples from Buryatia (diploid) similarity was observed for the predominance of component № 3 and 6, in samples from Tyva - only for component № 3.

It was found that compounds №3, 4, 6 were either absent in tetraploids and hexaploids (Fig. 1, a), or their content was minimal compared with diploids. This suggests that these compounds can be used as markers in the isolation of positive (Fig. 1, b) and negative chemotypes. The minimum content of these compounds ranged from 0.2 to 0.3 mg/g in tetra- and hexaploid cytotypes, therefore, we refer these cytotypes to a negative chemotype according to the content of compounds № 3,4,6. The content of these compounds in diploid varied from 0,4 to 10,5 mg/g, except for population from Kazakhstan (KZ-A3) - 0.1 mg/g (compound №4) and population from Buryatia (RU-BU3), where compound № 4 was absent. This phenomenon of trait instability, even within selected chemotypes, is consistent with previous studies of polyploid populations of some genera of the family Chenopodiaceae [14].

Table 1. Composition and content (in mg/g) of phenolic compounds in samples of the aboveground part of *B. prostrata*.

№, code, origins material		Phenolic compounds										
		1	2	3	4	5	6	7	8	9	10	11
1. KZ-A4. Kazakhstan, Almaty region, Kopa.	2	1.1	2.4	0.7	1.2	0.9	0.6	0.9	2.3	0.4	0.4	0.2
2. KZ-A3. Kazakhstan, Almaty region.	2	0.9	2.5	0.4	0.1	0.6	0.6	0.4	2.1	0.2	0.1	0.2
3. RU-BU1. Russia. The Republic of Buryatia.	2	0.3	0.4	2.5	1.3	2.3	1.7	0.6	0.6	1.5	1.2	1.0
4. RU-TU1. Russia. The Republic of Tyva. Arjaan. Kyzyl-Erzin.	2	0.2	0.1	1.2	0.5	2.0	1.7	0.1	0.1	0.2	0.2	0.1
5. RU-TU2. Russia. The Republic of Tyva. Sush.	2	1.0	0.9	5.3	0.5	0.6	0.7	6.4	6.5	0.3	0.5	0.3
6. RU-BU3. Russia. The Republic of Buryatia. Udinsk	2	3.1	1.3	7.3	–	0.4	1.7	0.1	0.2	0.6	0.2	0.1
7. RU-NO2. Russia. Novosibirskaja Oblast'. Sharchino.	2	0.3	0.3	1.7	10.5	0.4	0.6	0.03	0.1	0.4	0.5	0.3
8. RU-AL. Russia. The Republic of Altai. Beltir.	2	0.3	0.5	1.1	4.1	0.9	0.4	0.5	0.3	0.3	0.1	0.4
9. KZ-A2. Kazakhstan. Almaty region. Kapchagay.	4	0.3	0.2	–	–	–	–	0.9	0.9	0.5	0.1	0.3
10. KZ-A1. Kazakhstan. Almaty region. Arna.	4	2.2	3.4	–	–	0.1	–	0.9	0.5	0.6	0.04	0.1

11. KZ-K1. Kazakhstan. Kyzylorda Region. Aiteke.	4	5.5	5.1	0.2	0.3	0.2	0.3	2.5	2.2	1.7	0.2	0.4
12. RU-NO1. Russia. Novosibirskaja Oblast'. Antonovo.	4	10.8	7.5	–	0.2	0.3	–	0.6	7.4	0.1	2.1	0.6
13. RU-KH. Russia. The Republic of Khakassia. Abakan.	4	1.1	3.1	–	–	1.8	0.3	2.0	1.9	0.2	0.1	0.2
14. RU-AR2. Armenia. Kotayk region. Areni- Jermuk.	6	1.3	1.3	0.3	0.3	3.4	–	4.8	10.3	1.3	3.0	0.1

Compounds №1 - 4 min, №2 - 5 min, №3 - 12 min, №4 - 15 min, №5 - 19 min, №6 - 22 min, №7 - 32 min, №8 - 35 min, №9 - 38 min, №10 - 44 min, №11 - 49 min.

The identified compounds are №5 - isoquercitrin, №7 - kaempferol-3-glycoside, №8 - isoramnetin-3-rutinoside, №10 - luteolin.

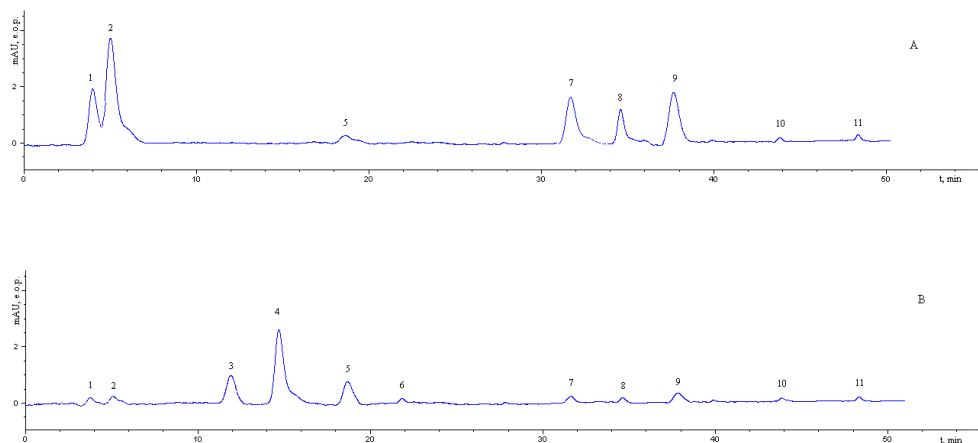


Fig. 1. Chromatograms of 70% water-ethanol extracts from the aerial part of plants: A) tetraploid population of *Bassia prostrata* (Kazakhstan, Almaty region, KZ-A1). Negative chemotype for phenolic compounds №. 3,4,6, which are absent in these plants. B) diploid population of *Bassia prostrata* (Russia, Altai Republic, RU-AL). Positive chemotype for phenolic compounds №. 3,4,6 present in these plants.

The identified compounds - № 5 - isoquercitrin, № 7 - kaempferol-3-glycoside, № 8 - isoramnetin-3-rutinoside, № 10 - luteolin. The abscissa shows the retention time, t, min; ordinate - optical density, e.o.p., mAU. The numbers in the chromatogram indicate the numbers of the compounds.

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