ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 8: 843–855 (2022)
doi: 10.5281/zenodo.7749924
http:// journal.asu.ru

RESEARCH ARTICLE

Morphophysiological characterization of *Bupleurum aureum* in natural populations of the Republic of Tatarstan

Olga A. Timofeeva[1, 2], Svetlana A. Dubrovnaya[1, 3], Landysh Z. Khusnetdinova[1, 4]

1 *Institute of Fundamental Medicine and Biology of Kazan Federal University, 18 Kremlevskaya, Kazan, 420001, Russia*
2 *http://orcid.org/0000-0003-4921-458X*
3 *http://orcid.org/0000-0001-5700-4203*
4 *http://orcid.org/0000-0002-7867-2013*

Corresponding author: Svetlana A. Dubrovnaya (sdubrovnaya@inbox.ru)

Academic editor: R. Yakovlev | Received 5 May 2022 | Accepted 12 December 2022 | Published 23 December 2022

http://zoobank.org/68A3746F-1C72-4F5C-A2C2-4D99140F5B89

Citation: Timofeeva OA, Dubrovnaya SA, Khusnetdinova LZ (2022) Morphophysiological characterization of *Bupleurum aureum* in natural populations of the Republic of Tatarstan. Acta Biologica Sibirica 8: 843–855. https://doi.org/10.5281/zenodo.7749924

Abstract

We have carried out comprehensive studies of the natural populations of *Bupleurum aureum* under the conditions of the Republic of Tatarstan. Therefore, we used population research, morphometric, and physiological methods in this research. In addition, we studied the morphophysiological heterogeneity of the natural populations of *Bupleurum aureum*, the dynamics of the population size over three years in the forests of various natural zones of the Republic of Tatarstan. For all cenopopulations, a proportional contribution of aboveground biomass to the achievement of the generative sphere can be observed, even with a significant deterioration in the growth of particular plants. We noted a decrease in plant size in low illuminated and nitrogen-poor soils (1 CP). However, plants had the best indicators of raw material quality, flavonoids content in terms of rutin, as well as soluble phenolic compounds. Powerful plants were formed under optimal conditions in deciduous forests of the forest-steppe zone, but the content of flavonoids in all parts of the plants was insignificant. However, for plants in this natural zone, a higher content of tannins was recorded. Within the republic, the species was sufficiently stable, had high plasticity, and was self-renewable. However, this species needs to be preserved, so collection of its raw materials is not recommended due to the small area of forest within which *Bupleurum* grows.

We suggested creating artificial plantings of this species with the recreation of conditions that ensure the synthesis and accumulation of various groups of phenolic compounds.

Keywords
Bupleurum aureum, critical state of coenopopulation, biologically active substances, flavonoids

Introduction

The study of population heterogeneity of natural populations is an important research direction in the study of medicinal plants. Analysis of the morphophysiological variability of the samples allowed us to study the conditions that determine the synthesis and accumulation of biologically active substances [BAS], identify the most valuable loci of wild plants and their subsequent establishment as plantation crops or the prospect of harvesting medicinal raw materials in natural communities, taking into account the stability and self-renewability of the population. About 250 compounds have been isolated from plants of the genus *Bupleurum* – triterpene glycosides, sesquiterpenes, flavonoids (Ashour et al. 2011; Olennikov et al. 2013). An essential characteristic of the state of herbaceous plant populations is the dynamics of numbers, the ability to preserve plants in the developed space or the new territory formed, as well as the vitality of cenopopulations. (Soule 1987; Fedorov et al. 2010; Menges 2000; Klimenko, Zlobin 2014).

The study aims to investigate the intraspecific variability of *Bupleurum aureum* and to determine the characteristics within the Republic of Tatarstan (Russia). We planned to reveal the location and state of coenopopulations of *Bupleurum aureum* within the republic and study the influence of ecological-cenotic conditions on the process of accumulation of biologically active substances in various parts of *Bupleurum aureum*.

Materials and methods

Characteristics of the areas studied

The object of the study is *Bupleurum longifolium* ssp. *aureum* L. (Fisch. ex Hoffm.) Soo. – Pleistocene relict with a disjunctive European-Siberian range. The species is characterized as a typical mesophyte confined to places with sufficient moisture. It is widely distributed within the territory of Russia, growing in the European part (West and Eastern Siberia) in the Far East (Linchevsky 1950). The species is not widespread under the conditions of the middle Volga region (Chugunov, Khapugin 2020). Within the Republic of Tatarstan [RT], this species can be found in glades and edges of deciduous forests.

This study was carried out in the Republic of Tatarstan, located in the eastern part of the European (Russian) plain. Cenopopulations [CP] in various natural and geological regions of the republic were selected for the study. 1 CP, 2 CP are confined to the forest zone and 3 CP to the forest-steppe.

1 CP. This trial plot was found in the Zelenodolsk district of the Tatarstan Republic. A small area of broad-leave forest was located at the top of the southwestern exposure slope in the Petiyalka River valley. The height above sea level was 160 m. The soils are grey forest. The slope surface was completely overgrown with pasture vegetation. In addition, we noticed grazing on the slope. An arable land adjoins the forest. Furthermore, we found a Bupleurum coenopopulation under the canopy of a broad-leaved forest. The canopy closure was 0.8. The agrochemical characteristics of the soils are presented in Table 1.

2 CP. This trial plot was established in the Baltasinsky district of the Republic of Tatarstan on a flat landscape with 140 m height above sea level in a coniferous deciduous forest. The soils are grey forests and the cenopopulation is confined to the edge of the illuminated forest.

Table 1. Characteristics of the soil in the communities studied

	1 CP	2 CP	3 CP
pH (salt)	6.8	5.3	5.5
Humus, %	3.2	3.1	4.7
Nitrate nitrogen, mg/ kg	3.6	34.7	24.6
Ammonia nitrogen, mg/ kg	15.9	11.3	11.2
Mobile phosphorus, mg/ kg	80	22	80
Exchangeable potassium, mg/ kg	160	110	66
Exchangeable calcium, mmol/ 100 g	28.5	16.0	22.2
Exchangeable magnesium, mmol/ 100 g	2.5	3.0	5.0

3 CP. The trial plot was laid in the forest steppe zone in a broad-leafed forest on the left bank of the Kama River, in the Alekseevsky district of the Republic of Tatarstan. The landscape was smooth with a height of 117 m above sea level. The forest was characterized by significant lighting and the canopy closure was 0.4. On the site, an intensive growth of raspberries can be highlighted. The dark gray soils were slightly acidic (pH 5.5) with enough humus content.

These studies were carried out in 2018–2020. The climatic conditions of the seasons studied are presented in Table 2.

A more significant amount of precipitation was observed in the summer-autumn 2019 period compared to 2018. The indicator 'Precipitation amount' in August 2019 was almost twice the average annual indicator. The 2020 season was also quite wet. At the beginning of the growing season, high precipitation indicators can be observed in May and June, and temperature indicators do not exceed average long-term indicators.

For morphological analysis, we collected 15–20 flowering plants in all cenopopulations, because in the flowering phase it is characterized by the most diverse spectrum of essential oil components (Tykheev et al. 2021). When analyzing plants, we took into account the number of seeds per umbel, the number of inflorescences per shoot, the mass of one inflorescence, the total mass of inflorescences (WG), the mass of leaves (WL), the total mass of aerial shoots (W) and the mass of axial organs (stems). We determined the following allometric parameters based on the data obtained: reproductive effort (RE) = WG / W, photosynthetic effort (LWR) = WL / W, and the weight of the axial organs per total shoot biomass.

To analyze the structure of the cenopopulation, we made temporary transects of 20x1 m. However, we used primary population-ontogenetic approaches in the study (Uranov, Serebryakova 1976). Moreover, we determined the ontogenetic spectrum by the ratio of plants of different ontogenetic states.

Table 2. Meteorological indicators of the climate of RT

Observation Period	April	May	June	July	August	September	October
				Temperature			
Long-term average	5.5	11.5	18.1	20.2	17.6	11.7	4.6
2018	4.6	14.4	16.9	22.3	19.8	14.1	6.2
2019	6.3	16.5	19.0	18.5	16	10.9	7.9
2020	5.2	13.7	16.8	22.2	17.3	13.3	7.2
				Precipitation			
Long-term average	30	41	63	67.0	60	52	64.0
2018	69	23	35	59	26	25	45
2019	26	64	41	53	104	21	57
2020	60	66	74	69	98	24	42

Furthermore, we determine the total content of soluble phenolic compounds in the extracts by the spectrophotometric method (Burlakova 1992). The number of flavonoids was quantified in terms of rutin (Mirovich et al. 2017), quercetin (Buinov 2016), tannins in terms of tannin (Ministry of Health of the Russian Federation 2015).

In the absence of a normal distribution of the sample, we estimate the median (Me). The hypothesis of equality of the general mean of two independent samples was verified by the nonparametric Wilconson-Mann-Whitney test. The results were processed using MS Excel and Statistica 6.0.

The level of significance of all statistical tests was set to: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Result

One of the criteria for the sustainability of a species in a community is the stability of its sexual reproduction and the ability to carry out its life cycle. The deterioration in the growth of the samples, which was manifested in low indices of the realization of the generative and vegetative spheres due to the studies in the preserved area of the broadleaved forest located on the slope (1 CP) (see Table 3). The medians of the traits 'shoot mass', 'leaves mass', 'stalk mass' were significantly lower than those in other habitats. Minimum values also marked indicators characterizing the realization of the generative sphere. The deterioration of the condition of individual plants was reflected in the structure of the inflorescences (the number of axes is one strand) and the degree of seed productivity. The number of seeds per umbel in 1 CP was more than three times less than in plants growing in the conditions of the coniferous broad-leave forest of the Baltasinsky region (2 CP) and two times less than in the conditions of the deciduous forest of the forest-steppe zone (3 CP). At the same time, the preservation of the reproductive function of plants with deteriorating ecological and cenotic factors is a significant mechanism aimed at conserving species in the community. The plants within 2 CP and 3 CP did not differ in a large number of traits.

The indicators in the cenopopulations were similar (see Table 4), probably reflecting the proportional contribution of aerial biomass to the realization of the generative sphere even with a deterioration of the vital state of samples.

Table 3. Morphological indicators *B. aureum* within different communities

Habitats	Median	Min-Max	Lower Quarti	Upper Quartile	Coefficient of variation *Cv*	P
Mass of leaves						
1 CP	0.645	0.55–0.8	0.63	0.79	14.5	
2 CP	1.7	0.29–2.57	0.81	2.09	50.7	1/2*
3 CP	1.39	0.83–4.92	1.015	1.815	67.57	1/3***
Mass of stems						
1 CP	0.575	0.39–0.75	0.4	0.73	9.51	
2 CP	1.415	0.59–4.85	0.9	2.08	18.5	1/2***
3 CP	1.71	0.55–6.71	1.66	2.24	74.6	1/3***
Mass of aerial plant shoots						
1 CP	1.45	1.27–1.94	1.31	1.56	16.49	
2 CP	3.955	1.57–8.43	3.16	4.88	47.9	1/2***
3 CP	4.285	2.94–4.75	3.655	4.615	63	1/3***
Number of inflorescence on the shoot						
1 CP	1.5	1–3	1	2	45	

Habitats	Median	Min-Max	Lower Quarti	Upper Quartile	Coefficient of variation *Cv*	P
2 CP	3	1–8	2	5	65.8	1/2**
3 CP	3	1–8	3	4	52.4	1/3**
Mass of one inflorescence						
1 CP	0.27	0.1–0.41	0.23	0.28	39.08	
2 CP	0.28	0.1–0.45	0.22	0.34	29.47	1/3*
3 CP	0.31	0.1–0.74	0.28	0.39	37.40	
Number of umbel axes						
1 CP	7	4–9	6	8	23.4	
2 CP	8	3–15	7	9	28.37	1/3**
3 CP	9	4–15	8	11	28.14	
Number of seeds per umbel						
1 CP	8.5	5–12	6	11	36.0	
2 CP	23	8–46	12	33	48.44	1/2**
3 CP	14	10–33	12	22	40.03	1/3**

Table 4. Allometric indicators of *B. aureum*

	1 CP	3 CP	4 CP
Reproductive effort	0.17	0.17	0.25
Photosynthetic effort	0.45	0.42	0.32
Stalk weight per total biomass of shoot	0.4	0.35	0.39

An essential characteristic of herbaceous populations is population dynamics, which reflects its stability over time. Our studies have shown that one can observe an irregular renewal of the population in the deciduous forest on the slope (1 CP). Furthermore, we noted a significant increase in the number of individuals of seed origin only in the 2020 season, which was marked by high precipitation rates at the beginning of the season (see Fig. 1).

At the same time, the low density (3, 4), low real seed productivity in 1 CP and the dependence of seed germination processes on climatic factors suggest that the cenopopulation needs protection, in as much as its degradation state is close.

For cenopopulations existing under optimal conditions (2 CPs and 3 CPs), one can emphasize a similar type of dynamics-an annual increase in the number of cenopopulations due to newly emerged plants of seed origin (see Fig. 2). The stable state of these cenopopulations is evidenced by a relatively stable number of samples in a middle-aged ontogenetic state, high-density index-15 individuals per $1m^2$.

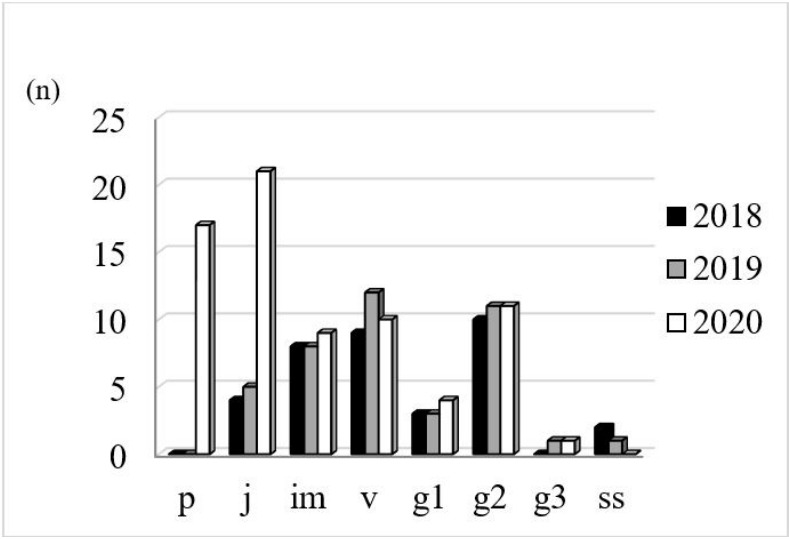

Figure 1. Dynamics of the number of *B. aureum* in various ontogenetic states in 1 CP in 2018–2020. X-axis - ontogenetic stage: p-sprouts, j-juvenile, im-immature, v-virginal, g1-young generative, g2-mature generative, g3 – old generative, ss-subsenile. Y-axis, the number of plants.

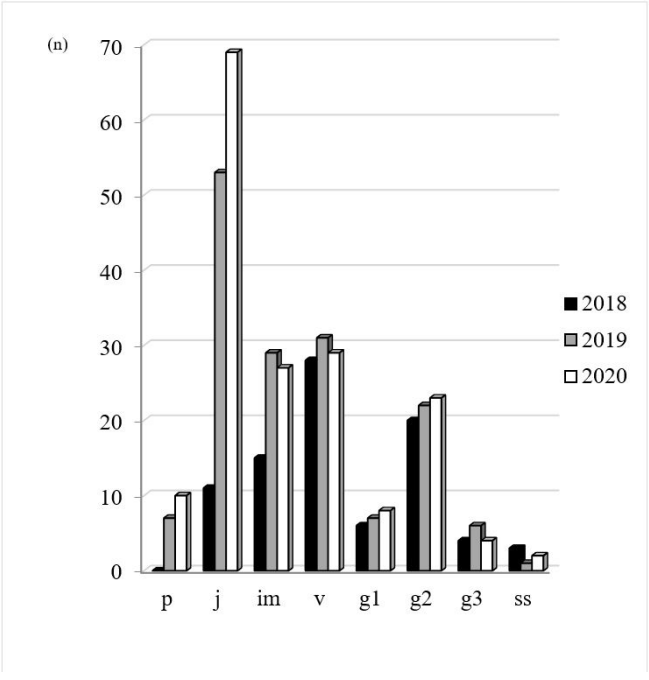

Figure 2. Population dynamics of *B. aureum in various ontogenetic states in 2 CP 2018–2020. X-axis –ontogenetic stage: p-sprouts, j* -juvenile, im -immature, v-virginal, g1-young generative, g2-mature generative, g3-old generative, ss-subsenile. Y-axis – the number of plants.

Although in CP 2 and CP 3, the capacity for self-recovery was well expressed throughout the years of observation, its vulnerability is associated with intensive agriculture in the Republic of Tatarstan and the reduction of forest phytocenoses.

Studies showed that the amount of biologically active substances in Bupleurum was determined by the ecological-cenotic conditions of the habitats within the botanical-geographical zones. However, within natural zones, the chemical composition of plant raw materials can depend on the type of soil, physical properties, landscape, and ecological cenotic conditions (Dubrovnaya et al. 2020).

In our studies, the maximum content of soluble phenolic compounds (see Fig. 3) can be observed under the conditions of a broad-leafed forest in the forest zone of the Zelenodolsk region (1 CP). Here, plants grown in nitrogen-poor soils exposed to less light can be indicated. The composition of phenolic compounds in the coniferous deciduous forest was significantly lower and did not differ from the relevant indicators for plants growing in the forest-steppe zone (Alekseevsky district). One can observe the minimal content of soluble phenolic compounds in the stems. Moreover, we emphasized a tendency towards a decrease in the content of soluble phenolic compounds in the row of leaves, inflorescences, rhizomes, and stems.

The highest content of flavonoids in terms of quercetin can be observed in the leaves of *B. aureum* that grow in the Baltasinsky region (2 CP)-0.124% (see Fig. 4), where the plants existed under illuminated forest glade conditions. Under the conditions of the Zelenodolsk district (1 CP), the indicator was significantly lower but twice as high as in the conditions of the forest-steppe zone (3 CP).

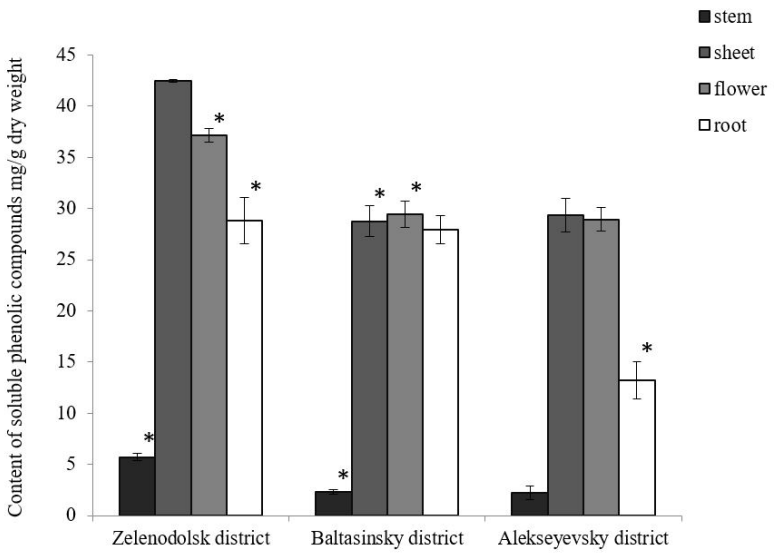

Figure 3. The total content of soluble phenolic compounds mg/g dry weight of the sample.

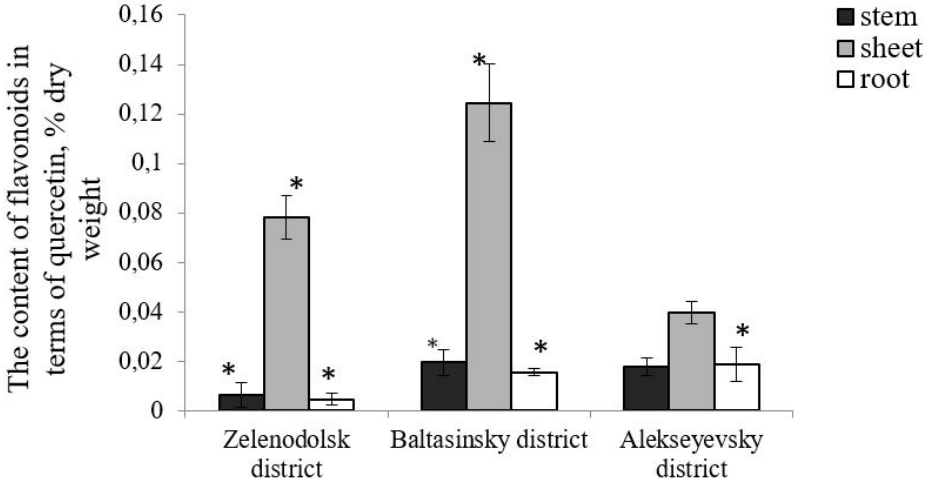

Figure 4. The content of total flavonoids in terms of quercetin and absolute dry raw materials.

Analyzing the distribution of biologically active substances [BAS] within the plant, one can observe that in the flavonoids, calculated for quercetin in all mesoforms, it was higher and statistically different in various cenopopulations. Therefore, we note the minimum concentration in the stems and rhizomes of plants in the Zelenodolsk region. One can observe a similar trend when analyzing the content of flavonoids in terms of rutin (in %) (see Fig. 5). In the leaves of all habitats, this indicator was higher than in the other parts of the plant. The highest indicators of this trait characterized plants in the forest zone of the Zelenodolsk district. Additionally, we determined a tendency for a lower content of flavonoids under terms of rutin in the conditions of the forest-steppe zone.

We identified significant differences during the analysis of tannin content in the above-ground and underground parts of B. aureum plants collected in three districts of the Republic of Tatarstan. In the Baltasinsky district (2 CP), the value of this indicator was lower than in the Zelenodolsky and Alekseevsky districts and was 0.2% in stems, 0.4% in leaves, and 0.63% in rhizomes. Thus, one can emphasize the maximum content of tannins in the conditions of the Alekseevsky district (3 CP), including a high content of humus in the soil. Furthermore, we observed the maximum concentration of tannins in all habitats in the roots and rhizomes (see Fig. 6).

We indicated a differentiated increase in the content of individual groups of phenolic compounds in plants of a particular climatic-geographical zone.

The existence of *B. aureum* populations under extreme conditions was largely ensured by physiological adaptation, which was diagnosed by the high content of flavonoids in the leaves. Under extreme conditions of existence – on poor nitrogen soils, with low illumination and moisture supply (1 CP), one can emphasize the formation of individual plants of smaller size, cenopopulations of low vitality, as-

sociated with significant costs for the protective mechanisms regarding the implementation of physiological processes. However, these plants had high-quality raw materials like 'the total content of soluble phenolic compounds in the dry mass of the sample" and "the content of the sum of flavonoids'.

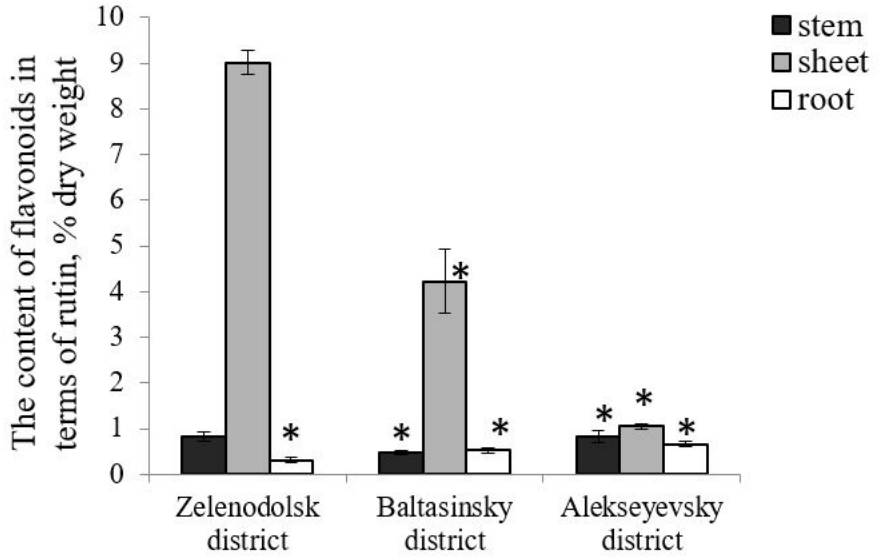

Figure 5. The content of totral rutin in various parts of *B. aureum*.

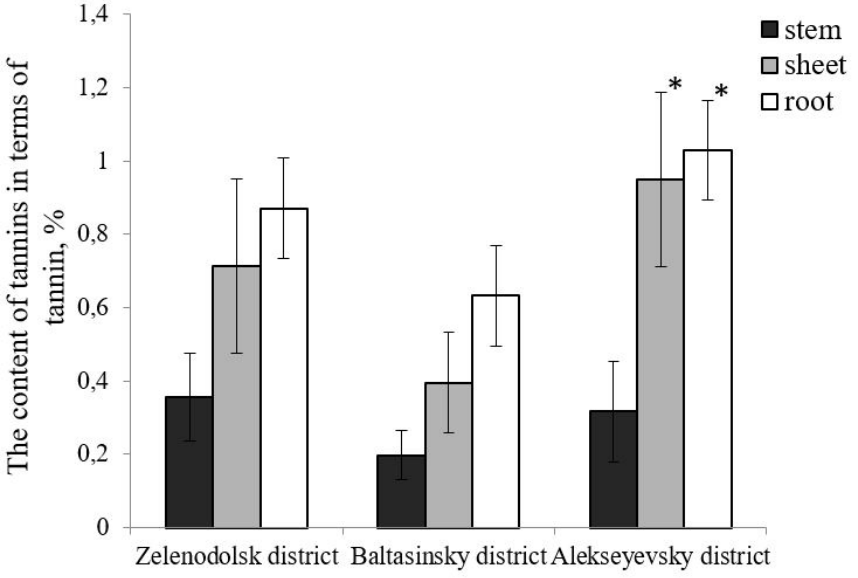

Figure 6. The content of total tannin in terms of tannin.

Discussion

Interest in the study of golden Bupleurum is associated with the possibility of using raw materials to obtain drugs, preventing and treating medicinal liver damage (Naboka et al. 2014; Strelkova et al. 2019). The structure of the co-enopopulation of *B. aureum*, as well as the resource characteristics of the species within the Republic of Tatarstan, have not been previously studied (Soboleva, Krylova 1990). Our studies have shown that the habitats are confined mainly to Predkamya (CP1, CP 2) and Zakamya (CP 3). In the deciduous forests of Predvolzhie, *B. aureum* was not discovered.

Currently, within the republic, this species is experiencing significant anthropogenic pressure associated with a reduction in habitats as a result of deforestation, plowing of meadow communities, overgrowth of forest meadows and glades, which can lead to deterioration of the implementation of generative organs, a decrease in seed yield and a hindered generation turnover process. These aspects do not allow for the procurement of raw materials under natural conditions. At the same time, it is promising to create artificial plantings based on the surviving individuals of the cenopopulations.

Conclusion

In Republic of Tatarstan, the *Bupleurum* populations are limited to forest phytocenoses. However, intensive farming has led to their significant reduction. In this regard, the co-enopopulations of *B. aureum* in the republic should be protected as sources of unique genetic variability as a basis for breeding and creating new varieties. For *B. aureum* within the republic, a tendency to a differential increase in the content of individual groups of phenolic compounds in plants of a particular climatic-geographical zone can be observed. Therefore, the concentration of flavonoids was higher in the forest zone, tannins in the forest-steppe zone.

Acknowledgments

This study was financially supported by the Russian Foundation for Basic Research and the Government of the Republic of Tatarstan (Project No. 18-44-160015) and the Kazan Federal University Strategic Academic Leadership Program.

References

Ashour ML, Wink M (2011) Genus *Bupleurum*: A review of its phytochemistry, pharmacology and modes of action. Journal of Pharmacy and Pharmacology 63 (3): 305–21. https://doi.org/10.1111/j.2042-7158.2010.01170.x

Buinov MV (2016) Pharmacognostic study *Solidago dahurica* Kitag. and drug development based on it. Dissertation, Irkutsk State Medical University. [In Russian]

Burlakova EB (Ed.) (1992) Investigation of Synthetic and Natural Antioxidants in Vitro and in Vivo. Nauka, Moscow, 111 pp. [In Russian]

Chugunov GCh, Khapugin AKh (2020) Population status of *Bupleurum aureum* (Apiaceae), a Critically Endangered plant species in a region of European Russia. Ecological Questions 31: 1–17. https://doi.org/10.12775/EQ.2020.006

Dubrovnaya SA, Khusnetdinova LZ, Attobrah NK (2020) Morpho-Physiological and Ecological Characteristics of *Melilotus officinalis* (Fabaceae, Magnoliopsida) in the Conditions of the Republic of Tatarstan. Povolzhskiy Journal of Ecology 2:151–164. https://doi.org/10.35885/1684-7318-2020-2-151-164 [In Russian]

Fedorov NI, Zhigunova SN, Mikhaylenko OI, Samoilova LYu (2010) The technique of an estimation of productivity of medicinal species in plant communities described in the system of ecological-floristic classification of Braun-Blanquet. Izvestiya Samarskogo Nauchnogo Tsentra Rossiyskoy Akademii Nauk 1 (3): 846–849. [In Russian]

Klimenko AA, Zlobin YuA (2014) Stability and dynamics of rare plant species populations in protected natural areas. Biology Bulletin Reviews 134 (2): 181–191. [In Russian]

Linchevsky IA (1950) Genus *Bupleurum*. In: Shishkin BK (Ed.) Flora of the USSR. Vol. 16. Publishing house of the Academy of Sciences of the USSR, Moscow, 295–296 p.

Menges ES (2000) Applications of population viability analyses in plant conservation. The Use of Population Viability Analyses in Conservation Planning, Ecological Bulletins 48: 73–84.

Ministry of Health of the Russian Federation (2015) State Pharmacopoeia of the Russian Federation XIII. Moscow. [In Russian]

Mirovich VM, Petukhova SA, Dudareva LV (2017) Studying the content of phenolic compounds of *Bupleurum scorzonerifolium* Willd., growing in the Baikal region with HPLC. Acta Biomedica Scientifica 2 (3): 75–77. https://doi.org/10.12737/article_59f035fe65fc57.52277900

Naboka OI, Samer K, Glushchenko AV, Georgiyants V (2014) Antioxidant properties of extracts of aerial part of *Bupleurum aureum*, hillgrowing saltwort herb, *Fumaria schleicheri* and *Cynara scolymus* in vitro and in vivo. Journal of Chemical and Pharmaceutical Research 6: 172–177.

Olennikov DN, Partilkhaev VV (2013) Flavonoids and phenylpropanoids from several species of *Bupleurum* growing in buryatia. Chemistry of Natural Compounds 48 (6): 1078–1082. http://dx.doi.org/10.1007/s10600-013-0471-x

Soboleva LS, Krylova IL (1990) Green pharmacy of Tatarstan. Tatar book, Kazan, 154 pp. [In Russian]

Soule ME (Ed.) (1987) Viable Populations for Conservation. Cambridge University Press Cambridge, UK, 189 pp.

Strelkova LB, Kurmanova EN, Ferubko EV, Panina MI (2019) Hepatoprotective effect of thoroughwax extract in experimental tetracycline-induced hepatitis. Pathological Physiology and Experimental Therapy 63 (1): 78–82. [In Russian]

Tykheev ZhA, Taraskin VV, Zhigzhitzhapova SV, Chimitov DG, Radnaeva, LD (2021) Variation of the Content of Biologically Active Compounds in *Bupleurum scorzonerifolium* Willd. Aerial Parts at Different Phenological Phases. Russian Journal of Bioorganic Chemistry 47: 1432–1438.

Uranov AA, Serebryakova TI (Eds) (1976) Cenopopulation of Plants (Basic Concepts and Structure), Nauka, Moscow, 217 pp. [In Russian]