

The cumulation of Wild pansy (*Viola tricolor L.*) accessions: the possibility of species preservation and usage in medicine

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Summary. Wild pansy (*Viola tricolor L.*) has a history in folk medicine of helping respiratory problems such as bronchitis, asthma, and cold symptoms. The drugs and extracts are prepared from raw material of pansy; it is a component of some prepared antitussives, cholagogues, dermatological medicines, roborants and tonics, alteratives, and anti-phlebitis remedies.

Wild pansy is indigenous to or naturalized in large parts of Europe and the Middle East as far as Central Asia, also found through the United States. In the Lithuanian flora wild pansy habitats areas have been fast reducing; this not only limits the availability of the reserves of medicinal raw materials for pharmacy and therapy needs but also causes a menace to survival of species. The reasons of reduction of natural habitats and areas of wild pansy are not only unfavorable meteorological conditions (including summer droughts) but also the competition of different herbs and irrational human activities. The opportunities of preservation of the species wild pansy need to be cultivated and the most exhaustive adaptation research should be performed.

Introduction

The pansy (*Viola L.*) is the only non-tropical genus of the tropical *Violaceae* Batsch family, possessing a wide growth area (1, 2). Out of the species of pansy found in Lithuania, two are medicinal: wild (*V. tricolor L.*) and field (*V. arvensis* Murray) pansy (3, 4) not much differing in habitus and content of active substances and usage.

Wild pansy contains 0.3% of salicylic acid and its derivatives such as the methyl ester and violutoside (the glucosidoarabinoside of salicylic acid methyl ester); phenol carboxylic acids such as trans-caffeic acid, protocatechuic acid, p-coumaric acid; 10.0% of mucilages (made up of glucose (35%), galactose (33%), arabinose (18%), and rhamnose (8%)); 2.4–4.5% of tannins, flavonoids (rutin, i.e. violaquercitrin, violanthin, scoparin, saponaretin, orientin, vicenin, anthocyanidin glycosides); carotenoids (violaxanthin and four geometrical isomers, zeaxanthin, etc.); coumarins: umbelliferone; small amounts of saponins; ascorbic acid and tocopherol (5–7).

Wild pansy is used externally and internally. The therapeutic activity of pansy has been identified in treating various skin conditions, such as eczema, seborrhea, impetigo, acne, catarrh of the respiratory tract, and whooping cough. It is also helpful in cases of cradlecap in babies. The herb is employed in treating frequent and painful urination in conditions such as

cystitis. The salicylates and rutin contained in the plant are anti-inflammatory. Due to the high concentration of rutin in the herb, it may be employed to prevent bruising and broken capillaries, to check the build up of fluid in the tissues and to help to reduce blood pressure. The drug is mildly laxative. It was formerly in much repute as a remedy for epilepsy and numerous other complaints, and the flowers were considered as cordial and helpful in treating diseases of the heart, from which may have arisen its popular name of Heartsease as much as from belief in it as a love potion. *Viola tricolor L.* was formerly official in the US Pharmacopoeia, and is still employed in America in the form of an ointment and poultice in eczema and other skin troubles, and internally for bronchitis. The overground part of wild pansy has been employed for their mucilaginous, demulcent and expectorant properties. In the older folk medicine the drug of pansy was considered to be a 'blood-cleansing' agent, i.e. it was supposed to deploy a metabolism-promoting action; it was employed as an adjuvant for appropriate indications, as diuretic, diaphoretic, and as well as in rheumatism, arthritis, and arteriosclerosis (3, 5). It can gently alter the functioning of nerves, and the immune system. It is helpful in cases of nightmares, insomnia, and distressed sleep with frequent night awakenings. The herb of wild pansy may be very successfully used after surgery to prevent reoccurring tumors (8).

However, too high dose of the drug may cause nausea or vomiting (1, 3, 8).

Wild pansies are indicated in Lithuanian Flora (9) as annual or wintering plants, though their exact definition is 'polymorphic' (10). It is indicated that their root system is poorly developed, mainly root. Pansy is subdued by gramineous and other plants in natural habitats. Its plants not always mature quality seeds and seeds aren't slung sufficiently widely in habitats containing high different herbs. Small seeds of pansy often don't reach the surface of the soil and don't germinate. It greatly determines reduction of habitats areas of wild pansy.

In 1999–2000, during visits to some of natural habitats, which were detected during Botany Institute's expeditions in 1986–1988, it was established that the natural habitats of the wild pansy have become extinct. Though pansy grows in easy soils and even in sandy soil, one of the functions of its leaves and roots epidermic mucous cells is to accumulate water stock (2, 11). It needs a sufficient content of moisture equal to multiyear average (310–320 mm through April–August under Lithuanian conditions). Competition of different herbs and directed human economical activity is especially harmful to pansy.

The aim of the work was to observe growth and development of wild pansy, moved from natural habitats to the collection plant nursery of medicinal plants of Kaunas Botanical Garden or grown at the collection seminary from seeds gathered at the habitats; to explain differences among the accessions by testing chemical composition of pansy plants, while the main aim was to determine the content of the accumulated polyphenolic compounds in them; as well as to determine the species survival tendency while growing accessions of wild pansy originating from different districts of Lithuania in loamy soil.

Materials and methods

Pansy is cultivated in Gleyic Cambisols (RDg) at Laboratory of medicinal and berry plants of Kaunas Botanical Garden of Vytautas Magnus University, also in light loamy soil on the average supplied with nourishing substances. Nine accessions plants were cultivated in collection plant nursery in 1999–2000, and 11 plants – in 2001–2002 (Table 1).

Twenty model plants were selected for phenological observations within each accession (12); the content of the main stems was calculated, their length and the conditional projection area of the overground part of the shrub, the fresh and the dry overground mass was measured. The morphological characteristics of root system of the plants were evaluated using the trenches

(1) and quantitative accountancy (2) methods (13, 14): 1 – the color of the roots, the number of buds, the root system type, its structure, disposition, rooting depth; 2 – distribution of root system in a certain unit of soil volume and root system mass.

The sum of flavonols in the overground part has been determined photometrically (15, 16), phenol carboxylic acids – using Vorovskij, et al. method (17), the content of tannins – using Anisimova (18) method, pigments (the sum of carotenoids and chlorophylls a+b), the content of ascorbic acid – according to Ermakov method (16). The data have been given by dry mass, statistically calculated (19) using STATISTICA program.

Note: The underground part of work has been completed in the presence of habil. dr. Nijolė Lapinskiene and consulted by her.

Results

Natural habitats. Seeking for natural habitats of wild pansy, eight habitats detected in 1986–1988 have been visited (in Kaunas, Jonava, Lazdijai, Vilnius districts). It has become clear that 4 of them have disappeared because of human activities incompatible with nature protection. One area has been spoilt by domestic animals, a road has been built while the roadsides and the forest square, where wild pansy grew were trampled and polluted. Two neighboring plots between Leipalingis and Veisiejai were ploughed, the other – burnt by drought. The same destiny was of two habitats in Vilnius district. In Jonava district two habitats detected by us back in 1997, have moved a bit (0.5–1 km). The plants have been moved to the collection plant nursery (identification numbers are 51, 52 of the accessions in Kaunas Botanical Garden). The abundance of wild pansy is 5–20% through 11 habitats. Insufficient content of precipitations in May through July doesn't allow wild pansy plants with weak roots to compete with fast growing different herbs. The habitats areas often 'move' to one or another side but one can guess that their survival is conditional. The 4th, 5th, 11th habitats were already not found, the rest – have not been visited.

Variety of pansy blossoms and the more often prevailing combination of yellow-white colors has been observed in habitats 9 and 11, Kaunas district. Already in 1926, Hegi (10) has noted the cross of wild and field pansies, the forms of which are determined only by cytogenetical tests (*V. tricolor* 2n=26, *V. arvensis* 2n=34(31)). So only when collecting accessions from different districts of Lithuania in the plant nursery, observing the adaptation to the growing conditions, researching the relation between the overground and

Table 1. List of *Viola tricolor L.* accessions (Laboratory of Medicinal and Berry Plants (LMP) of Kaunas Botanical Garden of Vytautas Magnus University)

Seg. No.	Identification No. of Kaunas Botanical Garden	Origin of the accessions	Notes
1	V49	Kaunas Botanical Garden	The plants were moved to LMP from Sargėnai surroundings
2	V50	Domeikava, Kaunas surroundings	Seeds, gathered on September 26, 1999 have been sown at LMP seminary
3	V51	Habitat of near ditch, Jonava surroundings, towards the river Neris	For the first time on June 5, 1997 the plants were moved to LMP collection plant nursery; for the second time – on May 31, 1999
4	V52	Jonava surroundings, wood border	On May 31, 1999 the plants were moved to LMP collection plant nursery
5	V53	Jonava surroundings, meadow, 0,5 km away from V52	On May 28, 1998 the plants were moved to LMP collection plant nursery
6	V54	Vilnius District, Zalieji ezerai surroundings, meadow	On July 25, 1999 the plants were moved to LMP collection plant nursery
7	V55	Vilnius countryside, Vilnius road, 3 km to Juodsiliai, wood border	Seeds gathered on September 27, 1999 have been sown at LMP collection seminary
8	V56	Kaunas countryside, by the right side of the road leading to Šakiai	Seeds gathered on September 30, 1999 have been sown at LMP collection seminary
9	V57	Kaunas countryside, by the left hillside of Ezerelis, meadow	Seeds gathered on September 30, 1999 have been sown at LMP collection seminary
10	V58	Kaunas countryside, near Piliuona, by the right side of a meadow of the river Nemunas	On September 23, 1999 the plants were moved to LMP collection plant nursery
11	V59	Juragiai, by the left side of Marijampolė-Kaunas highway, meadow, about 20 m from the water-field	On September 22, 1999 the plants were moved to LMP collection plant nursery

underground parts an opportunity appears for better knowledge of the interspecies changes and in case of need to identify the raw material of pansies scanning microscopy or other test methods can be used (16).

Cultivation. Alive plants have been displaced from habitats 3, 4, 5, 6, 10, 11 to the collection plant nursery, seeds have been gathered in habitats 2, 7, 8, 9 and sowed in seminary in September. The seeds of pansy are small: the mass of 1000 seeds gathered from the natural habitats is 0.38–0.39 g, seeds from growing plants – 0.47–0.77 g. Their laboratory germination after 6–8 months was 58.0–62.0%, in soil – 25.0–43.3%, and seed germination in soil after 2 years – 9.0–18.6%. At average normal meteorological conditions, when there is enough moisture in soil, the germination of seeds gathered within the vegetation period (from June to autumn) lasts for 10–18 days but the germination doesn't exceed 30.0%. Seeds gathered

within the second half of summer (end of July, beginning of September) germinate faster and the abundance is larger (by 43.3%). The germination process is extended by lack of moisture in soil and high temperature within the period of 24 hours.

In spring (May, the first and the second decades) the shoots of pansy are transplanted to the nursery. In June to the plant nursery displaced plants were watered, weeded, the soil was hoed. After 2 weeks taking of roots was calculated: when planting from seeds grown shoots – 92.0–98.0% have taken roots, when transferring plants from habitats – 42.0–56.0%.

When equaling the 1st generation plants of Kaunas Botanical Garden origin accession to the control in the unfavorable to wild pansy vegetation year 1999 the 2nd generation plants of the same accession V49 grown from seeds have rather exceeded the control ones by height (30.0%), shrub measurements (31.7%), and

average mass of one plant (42.5%). The plants of the 2nd generation V51 were slightly backward, the shrub measurements and average mass of one plant exceeded the control only by 10.0 and 18.0%, but they have grown most of side stems and exceeded the control by 18.0%. The aerial dry mass of one of the distinguished plants (67.1 g) was even 1.8 times larger than the average mass of control plants.

In 2000 the control plants were 1.3–2.0 times more luxuriant than those of other accessions. The adaptation degree of the first was higher (wild pansy has been cultivated at Kaunas Botanical Garden collection of medicinal plants for 25 years), while the speed of growth – stable. The plants of the third generation accession V51 developed equally intensively as the control ones. The plants of accessions V55, V56, V57, V58 grew at 2–2.3 times slower speed; while one fourth of them has got extinct after their root system had languished. The main cause of it was lack of moisture after having planted the plants.

Distinguished individuals have been selected in all accessions, their growing was observed and their seeds were gathered.

Morphometric characteristic of plants. *The overground part.* One on average 50 cm high general stem and 3–7 short side stems are characteristic for wild pansy in natural habitats. The growing wild pansy forms a shrub consisting of 3–9 (up to 12) main stems and 31–43 side ones of the first and second row. The overground mass of the cultivated wild pansy is 32.5–160.0 g as fresh mass (in separate cases – up to 300.0 g), i.e. 20–70 times larger than of plants growing in natural habitats. Even more difference is between their shrub measurements. It has been noticed that when growing pansy of different accessions the differences among them become less significant, though plants of accessions V49, V51, V54 grew more intensively.

The underground part. The 1.5–3.5 cm long white or light brown, barky part of the underground stem with 2–3 buds begins below the overground stem of pansy. Several bleached-out thin sprouts with the rudiments of leaves grow from the buds. New overground sprouts may develop from them later. The length of the root neck is 1.5–5.0 cm.

A white-yellow 8.5±1.22 cm long, thin ($d=0.26\pm 0.04$ cm) main root, ending by a small tuft of thread-form roots develops from the underground part of the stem. The main root is surrounded by a thin ($d<1$ mm) 1st–3rd row of roots, also forming tufts. The main root exceeds the other roots by length and thickness very insignificantly, so it is possible to propose that the system of wild pansy root system takes an intermediate position between the main and fibrous ones. Sometimes the main

root becomes numb and its functions are carried on by the first row side roots.

As it has been proved by the tests, the diameter of the wild pansy roots differs from 6.0 (in natural habitats) up to 16.0 cm (of cultivated), rooting depth – from 11.0 to 22.0 cm, but it doesn't exceed 15.0 cm of the plants displaced from natural habitats.

The main roots mass accumulates in the depth of 2.5–15.0 cm.

All such indications change within certain limits every year and have been decreasing since 1999.

The underground mass of wild pansy is much smaller than that of accessions and has changed on average from 15.6 to 18.6 (using the method of quantitative accountancy); 47.7–57.1 – for distinguished individuals. Their overground part is always much more luxuriant than the part of on the average developed plants. The ratio of masses of after cutting again grown plants is 12.0–13.0.

As you can see, wild pansy root system is weak even when cultivating and taking care of plants, however the large content of thin side roots and the mucous cells of their epidermis help the plant to supply itself with biogenic substances and to vegetate quite well under normal meteorological conditions.

Content of active substances in wild pansy. *Flavonols*, the most reactive group of flavonoids, are significant for antioxidant activity and for acting as Vitamin P; their content is an important factor when estimating the raw material of wild pansy. Their content in all accessions with small annual deviations changes from 170.0 to 360.0 mg%. Constantly the greatest content has accumulated in plants of Kaunas Botanical Garden origin (V49) (Table 2). In 1999 flavonols level was the lowest. Under various summer meteorological conditions and a variable cut-time of pansy overground part there was an annual difference of flavonol content.

Higher content of *phenol carboxylic acids* was accumulated in 1995–1996 and in 1998. Results of the investigations of next four years were less exclusive.

Accumulation of *tannins* in wild pansy plants before the cut of overground part was not regular.

The content of *ascorbic acid* in the grass of pansy during a year changes from 318.1 to 195.3 mg%. The largest content of ascorbic acid was found in the beginning of growing of pansy (in 1995).

A generous sum of pigments of wild pansy (*carotenoids* and chlorophylls (a+b)) was determined for only six years. The greatest content of them was obtained in 1996–1997.

In other accessions the active substances were accumulated in the same way but their levels were inferior and distinct.

Table 2. Content of biologically active compounds in overground part of *Viola tricolor* (accession V49) before raw material preparation (Kaunas Botanical Garden, 1995–2002)

Active substances	Year of investigation							
	1995	1996	1997	1998	1999	2000	2001	2002
Flavonols, mg% (for dry mass)	350.4 ±2.84	327.9±7.28	237.2±1.18	279.8±3095	195.8±5.60	267.2±3.46	296.3±6.33	227.9±3.14
Phenol carboxylic acids, % (for aerialdry mass)	0.32±0.006	0.38±0.005	–x)	0.33±0.004	0.29±0.006	0.24±0.001	0.26±0.0033	0.20±0.003
Tannins, % (for aerial-dry mass)	4.50±0.09	3.80±0.005	2.30±0.18	2.60±0.05	3.00±0.022	2.60±0.03	3.60±0.04	1.30±0.031
Ascorbic acid, mg% (for dry mass)	318.1 ±5.11	287.9 ±8.01	244.7±1.90	280.6±4.80	208.1±3.07	237.1±4.82	195.3±3.55	–
Carotenoids, mg% (for dry mass)	29.8±0.73	38.6±0.27	38.5±0.84	35.4±0.67	30.2±0.62	32.2±1.06	–	–
Chlorophylls, mg% (for dry mass)	246.4±8.01	465.0±6.87	447.1±9.37	570.4±4.32	349.1±10.94	333.2±7.79	–	–

– x) – not determined

The results of the investigation of contents of wild pansy active substances showed that the growing of these plants is the possibility of reserves of medicinal raw material and species preservation

Discussion

Wild pansy (*Viola tricolor L.*) is the drug used for treatment from old years. It decreases respiratory inflammations: bronchitis, tracheitis, laryngotracheobronchitis, asthma, is used as expectorant drug, and has anti-inflammatory action by suppressing skin disorders (eczema, seborrhea). The folk medicine recommends using wild pansy in cases of insomnia, nervous strain, as diuretic, cholagogues, diaphoretic, analgetic. The drug may be used after surgery to prevent reoccurring tumors.

Due to unfavorable climatic conditions, competition of different herbs, human economical activities, the areas of wild pansy natural habitats and their number is decreasing; it is necessary to cultivate it not only in order to supply medicinal raw material but mainly in order to preserve the species. Eleven wild pansy accessions have been accumulated at Laboratory of Medicinal and Berry Plants of Kaunas Botanical Garden of Vytautas Magnus University and adaptation of plants to grow in loamy soil is researched for this purpose.

Pansies are plants of local flora but they are sensitive to displacement from one place to another and only plants that have been grown for many years at

the collection and exposition of medicinal plants have taken roots and vegetated after being displaced to the collection plant nursery while their roots were larger comparing with the individuals from natural habitats. However, cultivated for 3–5 years wild pansy plants originating from other districts have gradually adapted themselves a bit lagging behind from the accession of Kaunas Botanical Garden origin by the amount of the overground and underground mass. Plants of wild pansy in natural habitats tiller out 8–10 times less side stems and have 20–70 times smaller overground mass. Their root system is 1.7–2 times less developed than of those grown at the collection nursery.

The vitality of wild pansy is determined by edaphic and meteorological conditions. The speed of the plant development depends upon such factors as the course of the biological processes in them, the content of the overground mass and partly accumulation of active substances.

The accession plants accumulate a different content of active substances (flavonols, phenol carboxylic acids, tannins, etc.). Generalizing the 3–5-year research data, we preliminary conclude, that such accessions can be distinguished by the quantity of the active substances determined in them and only those quantities may be used as a deciding factor when selecting the accessions for preserving and preparation of the quality raw material. Detailed and for years lasting researches are necessary for final conclusions.

Trispalvės našlaitės (*Viola tricolor L.*) kolekcinių pavyzdžių kaupimas – rūšies išsaugojimo bei vartojimo medicinoje galimybė

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Raktažodžiai: trispalvė našlaitė, vartojimas, kolekciniai pavyzdžiai, natūralios augimvietės, auginimas, aktyvios medžiagos.

Santrauka. Trispalvė našlaitė (*Viola tricolor L.*) – jau senovėje gydymui vartotas vaistinis augalas. Jos preparatai slopina kvėpavimo organų uždegimą: bronchitą, tracheitą, laringotracheobronchitą, bronchų astmą, palengvina atsikosėjimą; gydo odos ligas: egzemas, seborėją. Liaudies medicinoje trispalvę našlaitę rekomenduojama vartoti esant nemigai, emocinei įtampai; prakaito, šlapimo, tulžies išsiskyrimui skatinti, skausmui malšinti; onkologijoje – pooperacinių navikų ataugimo profilaktikai.

Lietuvoje mažėjant natūralių jos augimviečių skaičiui ir plotams, Kauno botanikos sodo Vaistinių augalų kolekciniam augyne našlaitės pradėtos auginti nuo 1994 m., o per 1999–2002 m. sukaupta 11 kolekcinių pavyzdžių iš keturių Respublikos rajonų, tirtas augalų prisitaikymas augti priemolio dirvoje. Natūraliose augimvietėse našlaitės nesikrūmija, išaugina 8–10 kartų mažiau šoninių stiebų ir jų antžeminę masę 20–70 kartų mažesnė. Jų šaknų sistema 1,7–2 karto silpnesnė negu auginamų augalų. Jie labiau kenčia nuo sausrų, jas naikina įvairios kitos žolės, augimvietės nuolat niokojamos žmonių bei gyvūnų.

Seniai auginami (KBS kilmės) trispalvės našlaitės augalai perkelti į kolekcinę augyną. Gerai prigijo ir vegetavo, o jų šaknys buvo stambesnės lyginant su našlaitėmis iš natūralių augimviečių. Tačiau 2–3 metus auginamos iš kitur kilusios našlaitės pamažu adaptuojasi, augimo intensyvumu bei antžeminės ir požeminės masės kiekiu kiek atsilikdamos nuo vietinės (KBS) kilmės. Kolekcinių pavyzdžių augalai kaupia skirtingus veikliųjų medžiagų (flavonolių, fenolkarboninių rūgščių, raugų, askorbo rūgšties, pigmentų) kiekius. Nors kiekvienų metų tyrimo duomenys yra skirtingi, tačiau tik tai galima panaudoti kaip lemiamą veiksni atrenkant pavyzdžius saugojimui, taip pat ruošiant kokybišką vaistinę žaliavą, vartojamą daugeliui ligų gydyti.

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References

- Scharfetter R. Biographien von Pflanzensippen. Wien. 1953.
- Spegg H. Planta medica. 1959; 7: 8-23.
- Bakšytė I, Balevičius K, Bandzaitienė Z, et al. Vaistiniai augalai (Medicinal plants). Vilnius: Mintis; 1973. p. 196-9.
- Jankevičienė R., red. Botanikos vardų žodynas (Dictionary of plant names). Vilnius; 1998. p. 312-3.
- Bisset NG, Wichtl M., editors. Herbal drugs and phytopharmaceuticals. CRS Press, L.-N.Y.-W. Medpharm GmbH Scientific publishers Stuttgart; 2001. p. 527-9.
- Serbinova E. Oxidative damage and repair. 1991. p. 77-80.
- Savickienė N, Dagilytė A, Lukošius A, Žitkevičius V. Biologiškai veikliųjų junginių ir augalų reikšmė cukrinio diabeto komplikacijų prevencijai. (Importance of biologically active components and plants for the prevention of diabetes mellitus). Medicina (Kaunas) 2002;38:970-5.
- McGuffin M, Hobbs C, Upton R, Goldberg A. American Herbal Prc Association – Botanical Safety Handbook CRC Press; 1997. p. 518-21.
- Aleksandravičiūtė B, Bagdonaitė A, Butkienė S, et al. Lietuvos TSR flora. (Flora of Lithuanian SSR.) Vilnius; 1971; 4: 725-7.
- Hegi G. Illustrierte Flora von Mitteleuropa. Berlin-Hamburg. 1975.
- Piaseckiene AA, Jakovleva OV, Vaichiuniene JA, Abrutis VA. Rastit. resursy. (Plant resources.) 1991. p. 27-1, 10-21.
- Žalmykova NV. Metodika issledovaniy pri introdukcii lekarstvennykh rasteniy. (Technique of researches over introduction of medicinal plants.) Goskva; 1984.
- Žrasilnikov PŽ. Metodika polevogo izuchenija podzemnykh chastej. (Technique of field research of underground parts.) Leningrad; 1983.
- Lapinskienė NA. Podzemnaja chast' travianistykh rastenij i fitocenzov v Litovskoj SSR. (The underground part of the grassy plants and phytocenosis in the Lithuanian SSR.) Vilnius: Mokslas; 1986.
- Volchonskaja NA. K voprosu kolichestvennogo opredelenija flavonolov v rastenijakh. In: Aktual'nyje voprosy botanicheskogo resursovedenija v Sibiri. (Topic questions of botanic resources in Siberia.) Novosibirsk; 1976. p. 206-9.
- Ermakov AI, editor. Metody biokhimicheskogo issledovaniya rastenij. (Methods of biochemical researches of plants.) Leningrad, LO; 1987.
- Vorovskij VV, Dubinin NS, Dranik LL. Fenolkarbonovyje kisloty Calligonum leucocladum (Schrenk) Bunge. Rastit. resursy. (Plant resources.) 1976.
- Anisimova KT. Opredelenije kolorimetriceskimi metodom dubil'nykh veshchestv v korniakh Polygonum coriarium Grig. Rastit. resursy. (Plant resources.) 1967.
- Shmidt VM. Matematicheskije metody v botanike. (Mathematical methods in botany.) Leningrad; 1984.

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