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Content Dynamics of the Main Groups of Biologically Active Substances in *Rheum Altaicum* **Losinsk. in the Introduction to the Novosibirsk Region**

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Abstract

Accumulation dynamics of biologically active substances (flavonols, catechins, tannins, pectin substances, saponins, and carotenoids) in the organs of aboveground parts of the plants Rheum altaicum Losinsk. growing in the experimental plot of the Central Siberian Botanical Garden SB RAS (Novosibirsk) was studied. The buds and flowers of plants may be a source of flavonols (7.40 and 5.89 %), catechins (0.91 and 2.01 %), tannins (32.37 and 27.83 %), and saponins (34.29 and 17.99 %, respectively), as established. Leaves in budding and flowering phases accumulate significant amounts of almost all groups of compounds, especially many carotenoids (160.05 and 191.81 mg/dL). Leaf petioles during these periods are a rich source of pectins (3.47 and 4.66 %), protopectins (7.22 and 9.20 %), and saponins (24.06 and 22.3 %). Considering the findings, cultivated *R. altaicum* may be recommended as raw materials enriched with a complex of biologically active substances.

Keywords: Rheum altaicum, flavonols, catechins, tannins, pectin substances, saponins, carotenoids

INTRODUCTION

Representatives of the rhubarb genus *Rheum* L. (Polygonaceae Juss.) contain various secondary metabolites, such as anthraquinones, flavonoids, stilbenes, phenolic acids, coumarins, tannins, *etc.* and therefore have a wide spectrum of biological activity [1, 2].

Researchers from different countries are actively exploring the chemical composition and biological activity of rhubarb varieties with a view to developing preventive and curative medicines on their basis [3–5]. Particular attention is usually paid to underground organs of rhubarbs. The medicine of the rhizomes of Chinese rhubarb *Rh. palmatum* var. *tanguticum* is recommended in official medicine as an antiinflammatory, antibiotic remedy regulating the action of the bowels [6]. The aboveground part of rhubarbs (leaves and inflorescences) has yet to be fully explored, although it is a valuable source of various biologically active substances (BAS) and contains flavonoids, organic acids, vitamins C and P, provitamin A (carotene), *etc.* Searches for rhubarb varieties with a high content of BAS both under natural growing conditions and in the culture are of certain interest, however, they are carried out insufficiently actively.

The Altai rhubarb (*Rheum altaicum* Losinsk.) growing on rocks, rocky steppes, on gravelly soils along limestone and rocky slopes in the territory of Altai and northwestern Mongolia is one of the promising south Siberian varieties. The Altai rhubarb was described for the first time as an independent type by Lozina-Lozinskaya in the paper [7]. According to V. G. Minaeva [8], *R. altaicum* may successfully replace the Chinese species *R. tanguticum* Maxim.

Data on the composition and content of the Altai rhubarb are minor. The study of overground organs of cultivated plants by HPLC technique carried by us earlier detected the presence of flavonols quercetin, kaempferol, rhamnetin, rutin, astragalin, wherein the content of rutin reaches 1.35 % [9]. The study of the content of BAS in aboveground organs of the closely-related species *Rheum compactum* L. demonstrated that the buds and flowers might be a source of flavonols, catechins, tannins, pectin substances, and saponins, leaves – carotenoids [10–12].

It is necessary to take into account the dynamics of their accumulation and determine the phase with their maximum content when using plants as a source of BAS. Synthesis and degradation of substances proceeds during plant vital activity, therefore, issues of their seasonal and age variation are always relevant and necessary in resource research. Variability of the component composition and content of lowmolecular-weight compounds during the individual development was studied by many authors, however, these data are "diverse, contradictory, and sometimes conflicting, relate to various objects and stages of their development, and therefore non-comparable" [13].

The purpose of the present work is studying content dynamics of the main groups of BAS in the organs of the aboveground part of the plants R. *altaicum* depending on the vegetation phase under introduction conditions.

EXPERIMENTAL

Reproductive organs and leaves (leaf blade and petiole) of the plants *Rheum altaicum* of the third year of life growing under culture conditions in the experimental plot of the Central Siberian Botanical Garden (CSBG) SB RAS in Akademgorodok (Novosibirsk) were studied. The collection of raw materials was carried out in 2014 in accordance with plant development phases: the beginning of vegetation (regrowth), budding, mass flowering, fruiting. The average sample consisted of organs of 25 plants. The material dried out in the shade in ventilated areas was used. All biochemical indicators were calculated per the mass of absolutely dry raw materials. The average of data of three parallel determinations by each indicator was taken as the result.

To determine the content of flavonoids, catechins, tannins (hydrolysable tanning agents) the techniques, the detailed description of which was published in [14] were used.

Flavonols were determined spectrophotometrically by the method of V. V. Belikov and M. S. Schreiber [15], which used the complexation reaction of flavonols with aluminium chloride. The concentration of flavonols was found by the graph constructed according to rutin. The content of catechins was also determined by the spectrophotometric technique according to (\pm) -catechin. The method is based upon the ability of catechins to give crimson staining with a solution of vanillin in concentrated hydrochloric acid [16]. State standard tannin sample was calculated when determining tannins [17]. Pectin substances (protopectins and pectins) were determined by the carbazol method based on obtaining specific purple-pink colouration of uronic acids with carbazol in sulphuric acid medium. The graph was constructed according to galacturonic acid [18]. The amount of saponins was established gravimetrically, therefore, the findings should be considered preliminary [19]. Carotenoids were determined in an acetone/ethanol extract [20]. Measurements and calculations of carotenoid content were carried out at wavelengths of 662 and 644 nm (for chlorophyll a and b), and 440.5 nm (for carotenoids) [18].

RESULTS AND DISCUSSION

The Altai rhubarb begins to vegetate in the first decade of May. Solar insolation and spring frost during this period of its formation are damaging to plants. Phenol compounds, such as catechins and tannins that are inhibitors of free-radical reactions and take part in redox processes occurring in plants during this period of its formation carry out protection of plant

Development phase date of collection	, 0	Flavanols Katechins Tannins		Carote- Pectins noids, mg/dL		Proto- Saponins pectins		
Vegetatin,	Leaf	2.16 ± 0.05	0.34 ± 0.006	17.43 ± 0.25	200.85 ± 5.35	2.92 ± 0.03	7.13 ± 0.03	24.06 ± 0.75
14.05.14	Petiole	$0.81 {\pm} 0.01$	0.16 ± 0.005	5.89 ± 0.05	33.25 ± 1.08	2.21 ± 0.09	7.10 ± 0.08	30.71 ± 0.81
Budding,	Leaf	3.97 ± 0.06	0.29 ± 0.006	19.54 ± 0.30	160.05 ± 5.14	2.81 ± 0.03	6.43 ± 0.23	28.33 ± 0.78
02.06.14	Petiole	0.61 ± 0.01	0.21 ± 0.005	4.24 ± 0.08	26.26 ± 1.01	3.47 ± 0.09	7.22 ± 0.09	24.06 ± 0.80
	Bud	7.40 ± 0.08	0.91 ± 0.036	32.37 ± 0.42	115.01 ± 4.30	1.02 ± 0.08	3.90 ± 0.02	34.29 ± 0.91
Flowering,	Leaf	3.46 ± 0.04	0.33 ± 0.05	18.50 ± 0.38	191.81 ± 4.98	3.86 ± 0.05	6.22 ± 0.21	19.67 ± 0.63
21.06.14	Petiole	0.64 ± 0.01	0.24 ± 0.05	$4.94 {\pm} 0.07$	11.65 ± 0.47	4.66 ± 0.06	9.20 ± 0.27	22.37 ± 0.60
	Flower	5.89 ± 0.08	2.01 ± 0.09	27.83 ± 0.32	90.51 ± 3.81	1.01 ± 0.05	6.07 ± 0.16	17.99 ± 0.52
Fruiting,	Leaf	2.78 ± 0.04	0.30 ± 0.005	16.61 ± 0.20	144.73 ± 4.28	2.46 ± 0.02	7.38 ± 0.03	17.71 ± 0.54
18.07.14	Petiole	0.80 ± 0.03	0.23 ± 0.004	4.07 ± 0.09	23.16 ± 0.70	4.01 ± 0.01	6.02 ± 0.05	11.29 ± 0.48
	Leaf	0.83 ± 0.02	0.22 ± 0.005	5.64 ± 0.08	102.62 ± 3.34	0.53 ± 0.02	1.24 ± 0.03	$0.44 {\pm} 0.01$

TABLE 1

Content of biologically active substances (BAS) in organs of Altai rhubarb plants during the vegetation period in 2014,%

Note. All indicators were calculated per the absolutely dry mass of dry raw materials.

tissues from the damaging effects of external factors [21].

An indirect indication of the protective function of phenol compounds is their high content during this life period. Leaves of vegetating plants contain 2.16 % of flavonols, 0.34 % of catechins, and 17.43 % of tannins. The amount of flavonols increases up to 3.97 % in almost half a month during budding. Their active accumulation (Table 1) in regard to the forthcoming participation in reproductive processes, and then a gradual decrease in phases of mass flowering (3.46 %) and fruiting (2.78 %) occur.

The majority of papers on the content dynamics of flavonoids note an increase in their amount from the beginning of vegetation to a maximum in budding or flowering phases. A decline in the content of total flavonoids and the main components of the flavonoid complex in the flowering phase is observed in some cases, which, in our opinion, is probably due to their consumption in pollination and fertilization processes [22-24].

The amount of catechins in leaves decreases in the budding phase to 0.29 %, but further, there is some rise during mass flowering and fruiting – to 0.33 %. The quantity of tannins remains almost at the same level during the entire season.

Rhubarb buds and flowers are characterized by high contents of flavonols -7.40 and 5.89 %, respectively. Reproductive organs constitute a large proportion in the mass of rhubarbs. Earlier, as found by us in [25], flowers constitute, on average, 8.47 % of the mass of one wavy rhubarb plant. There are little flavonols in fruits -0.83 %. There are also more tannins in buds (32.37 %) than in flowers (27.83 %), there are somewhat more of them in fruits (5.64 %). On the contrary, there are more catechins in flowers more (2.01 %) than in buds (0.91 %, see Table 1).

Flavonols, catechins, and tannins show anti-inflammatory, antibacterial, hemostatic, and astringent properties. Their P-vitamin and antioxidant activity are also known: they strengthen the walls of blood vessels, are active against infections, promote the assimilation of ascorbic acid showing a positive effect on the production of immune bodies in combination with vitamin P, ensuring an antioxidant effect and taking part in the formation of red blood cells and the functioning of white blood cells [26].

Rhubarb leaves are a rich source of carotenoids (provitamin A) that, like vitamin P, are active antioxidants. There are great amounts (to 200.85 mg/dL) of carotenoids in leaves during vegetation. By the beginning of June, their amount becomes much less (160.05) in leaves of budding plants, but in the mass flowering phase, in half a month, there is a significant increase (to 191.81 mg/dL). In the fruiting period, their content in leaves is reduced, still remaining high enough (144.73 mg/dL). There are 1.5-2.0 times less these substances in buds and inflorescences than in leaves. The value of carotenoids for a man is

hard to overvalue, therefore, the search for plants with high contents of these substances is always relevant [27].

Pectin substances in plants exist in two forms, such as pectins and protopectins. These are high molecular-weight saccharides acting as strengthening components and regulating the water metabolism of plants [28]. The content of pectin substances in rhubarb is high, moreover, protopectins that are a reserve in the formation of pectins occupy the major share in total substances. The dynamics of pectins and protopectins in leaves during the vegetation period of Altai rhubarb plants is characterised by the following: the maximum of pectins (3.86 %) falls on the flowering phase, and protopectins (7.38 %) – on the fruiting stage, herewith, there are much more protopectins in all phases than pectins (in 1.6-3.0 times). There are little protopectins in reproductive organs, while protopectins are contained in significant amounts – in 2.3-6.0 times higher (to 6.07 %) in flowers). Leaf petioles contain most of all pectin substances: 4.66 % of pectins and 9.20 % of protopectins in the mass flowering phase that is their maximum accumulation period. Protopectins in petioles are accumulated 1.0-3.0 times higher than pectins during the entire vegetation period. Pectin substances (pectin and protopectin) are very important for human health: they carry out detoxication binding and removing toxins, poisons, and radioactive isotopes [28]. Leaf petioles of budding and flowering Altai rhubarb plants are valuable food product due to a high content of pectin substances.

The maximum content of saponins in leaves and reproductive organs of rhubarb falls on the budding phase (28.33 and 34.29 %, respectively). Leaf petioles of vegetative plants contain 30.71 % of saponins. It is known that saponin-containing plants are used as diuretic, tonic, tonic, antitumor, and sedative remedies. Many of them are efficient at diseases of the cardiovascular system, atherosclerosis, hypertension, and malignant tumours [29, 30].

CONCLUSION

The aboveground part of the plants Rheum altaicum grown in the experimental plot of the

Central Siberian Botanical Garden SB RAS (Novosibirsk, Akademgorodok) is characterised by a high content of flavonols, catechins, tannins, carotenoids, pectin substances, and saponins, which allows considering this rhubarb species as a promising source of valuable biologically active substances. The plants can be used during vegetation periods (budding and flowering), when there are 7.40 and 5.89 %of flavonols, 0.91 and 2.01 % of catechins, 32.37 и 27.83 % of tannins, 34.29 and 17.99 % of saponins, respectively, in buds and flowers. Leaves also accumulate significant amounts of almost all groups during these phases. Young leaves of plants characterised by an extremely high content of carotenoids (200.85 mg/dL) are usually used as food. They become unsuitable for consumption during growth due to high contents of oxalic acid oxalates. Leaf petioles that are traditional food products represent a rich source of pectin substances (3.47-4.66 %)of pectins, 7.22-9.20 % of protopectins, and 24.06-22.37 % of saponins for the budding and flowering of plants.

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REFEENCES

- Rastitel'nye resursy SSSR: Tsvetkovye rasteniya, ikh khimicheskij sostav, ispol'zovanie; Semejstva Magnoliaceae – Limoniaceae. L.: Nauka, 1984. 460 p.
- 2 Rastitel'nye resursy Rossii: Dikorastushchie tsvetkovye rasteniya, ikh komponentnyj sostav i biologicheskaya aktivnost'. Vol. 1. Sem. Magnoliaceae – Juglandaceae, Ulmaceae, Moraceae, Cannabaceae, Urticaceae. M.: Tovarishchestvo nauchnykh izdanij KMK, 2008. 421 p.
- 3 Agarwal S. K., Singh S. S., Lakshmi V., Verma S., Kumar S., J. Sci. Ind. Res. 2001. Vol. 60, No. 1. P. 1–9.
- 4 Bilal S., Mir M. R., Parrah J. D., Tiwari B. K., Tripathi V., Singh P. M., Abidi A. B., Int. J. Pharm. and Biol. Sci. 2013. Vol. 3, No. 3. P. 228–233.
- 5 Huang Q., Lu G., Shen H.-M., Chung M. C. M., Ong Ch. N., Med. Res. Rey. 2007. Vol. 27, No. 5. P. 609-630.
- 6 Kurkin V. A. Farmakognoziya: Ucheb.dlya vuzov. 2-e izd. pererab. i dop. Samara: Ofort, 2007. 1239 p.
- 7 Lozina-Lozinskaya A. S., Flora SSSR. M.; L.: Izd-vo AN SSSR, 1936. Vol. 5. P. 483-501.
- 8 Minaeva V. G. Lekarstvennye rasteniya Sibiri. Novosibirsk: Nauka, 1970. 271 p.
- 9 Kostikova V. A., Vysochina G. I., Petruk A. A., Vestn. VGU. Ser.: khimiya, biologiya, farmatsiya. 2016. No. 2.

P. 135-139.

- 10 Kostikova V. A., Vysochina G. I., Kukushkina T. A., Petruk A. A., Mater. XIV Mezhdunar. nauch.-praktich. konf. "Problemy botaniki Yuzhnoj Sibiri i Mongolii". Barnaul, 25-29 May 2015. P. 194-197.
- 11 Kostikova V. A., Petruk A. A., Mater. III (V) Vseros. molod. konf. s uchastiem inostrannykh uchenykh "Perspektivy razvitiya i problemy sovremennoj botaniki", Novosibirsk, 10–14 November 2014. P. 150–152.
- 12 Vysochina G. I., Kukushkina T. A., Kostikova V. A., Sib. med.zhurn. 2015. No. 1. P. 91–93.
- 13 Minaeva V. G. Flavonoidy v ontogeneze rastenij i ikh prakticheskoe ispol'zovanie. Novosibirsk: Nauka, 1978. 256 p.
- 14 Vysochina G. I., Kukushkina T. A., Vasfilova E. S., Chem. Sust. Dev. 2013. Vol. 21, No. 4. P. 387–393.
- 15 Belikov V. V., Shrajber M. S., Farmatsiya. 1970. No. 1. P. 66-72.
- 16 Kukushkina T. A., Zykov A. A., Obukhova L. A., Mater. VII Mezhdunar. s"ezda "Aktual'nye problemy sozdaniya novykh lekarstvennykh preparatov prirodnogo proiskhozhdeniya". S.-Peterburg, 2–5 July 2003. P. 64–69.
- 17 Gosudarstvennaya farmakopeya SSSR. 11-e izd. No. 1. M.: Meditsina, 1987. 336 p.
- 18 Metody biokhimicheskogo issledovaniya rastenij / Pod red. A. I. Ermakova. L.: Agropromizdat, 1987. 430 p.
- 19 Kiseleva P. V., Volkhonskaya T. P., Kiselev V. E.

Biologicheski aktivnye veshchestva lekarstvennykh rastenij Yuzhnoj Sibiri. Novosibirsk: Nauka, 1991. 135 p.

- 20 Kriventsov V. I. Metodicheskie rekomendatsii po analizu plodov na biokhimicheskij sostav. Yalta: Izd. Nikit. botan. sada, 1982. 21 p.
- 21 Zaprometov M. N. Fenol/nye soedineniya: Rasprostranenie, metabolizm i funktsii v rasteniyakh. M.: Nauka, 1993. 272 p.
- 22 Paris R., Duret S., Bull. Soc. Bot. Fr. 1972. Vol. 119, No. 9. P. 531-542.
- 23 Valutskaya A. G., Past. resursy. 1984. Vol. 20, No. 1. P. 119–123.
- 24 Khramova E. P. Osobennosti nakopleniya flavonolovu Pentaphylloidesfruticosa (L.) O. Schwarz pri introduktsii: Avtoref. dis. ... kand. biol. nauk. Novosibirsk, 1997. 17 p.
- 25 Vysochina G. I., Tez. Vsesoyuz. konf. "Novye lekarstvennye preparaty iz rastenij Sibiri i Dabnego Vostoka", Tomsk, 1989. Vol. 2. P. 38-39.
- 26 Bakhtenko E. Yu., Kurapov P. B. Mnogoobrazie vtorichnykh metabolitov vysshikh rastenij: ucheb. posobie. Vologda: MakrosPrint, 2008. 265 p.
- 27 Nikityuk V. G., Provizor. 1999. No. 6. P. 39–41.
- 28 Ovodov Yu. S., Bioorgan. khimiya. 2009. Vol. 35, No. 3. P. 293-310.
- 29 Rastitel'nye lekarstvennye sredstva / Pod red. N. P. Maksyutinoj. Kiev: Zdorov'e, 1985. 280 p.
- 30 Anisimov M. M., Chirva V. Ya., Usp. sovr. biologii. 1980. Vol. 6, No. 3. P. 351-364.