Chapter 16
Roseroot (*Rhodiola rosea* L.): Effect of Internal and External Factors on Accumulation of Biologically Active Compounds

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Abstract Roseroot (*Rhodiola rosea L.*) is a perennial that grows wild in the mountains of Siberia, Central Europe and North America. Its underground organs (rhizomes with roots) are used as a medicinal raw material; the plant is considered to be one of the most active adaptogens. The most important biologically active constituents of the raw material are phenolic compounds, including tyrosol and its glycoside salidroside, and *trans*-cinnamic alcohol derivatives (rosavin, rosarin and rosin). The results of several years of study carried out at Warsaw University of Life Sciences - SGGW in Poland indicate a high intraspecific variability concerning accumulation of these compounds. It was also stated that both the weight of the underground organs of roseroot and the content of active compounds changes during plant development. The mean weight of air-dry rhizomes with roots of plants grown in central Poland increased by up to 120 g per plant in the 5th year of plant vegetation. In the 6th year the symptoms of plant aging were observed – the oldest, central part of rhizome decayed and the rhizome divided into many smaller parts characterised by lower content of salidroside and rosavin. The yield and quality of roseroot raw material was also significantly affected by climatic and soil conditions. Plants grown in central Poland were characterised by higher weight of underground organs but lower content of rosavin and salidroside in comparison with those grown in southern Poland (mountain area). Post-harvest treatment of the raw material (stabilisation and extraction method) distinctly affected the quality of the obtained extracts. Both convection drying at 80ºC and lyophilisation are good methods of stabilisation of the roseroot raw material. Periodical extraction with ultrasound, and continuous exhaustive extraction using both methanol and 75% ethanol as extraction media allow to get extracts of comparable content of determined phenolic compounds.

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16.1 Introduction

Roseroot (*Rhodiola rosea* L.) is an alpine perennial that belongs to the *Crassulaceae* family. Its underground organs (i.e. rhizomes with roots) have been used as natural remedies in Siberia, Tibet and the Far East for centuries [1]. The biological activity of this raw material has been proven in contemporary studies. The results of pharmacological investigations indicate that the extracts from roseroot reveal antioxidant activity via inhibition of lipid peroxidation in liver cells and clearing of free radicals. They stimulate the central nervous system, improve learning abilities and prevent stress-induced cardiac damage [2–16]. They also show anti-fatigue, anti-inflammatory, hepatoprotective and anti-tumour activity [17–21]. In clinical studies they have been effective in the treatment of physical weakness, heart diseases, depression, memory and learning problems. Roseroot is regarded as one of the most active adaptogens and it is specially recommended for sportsmen, hard-working people, convalescents and elderly people.

In the severe alpine climate, the growth of roseroot is very slow, so that it may be harvested as a raw material for herbal industry even after several dozen years. The slow development of the plant and growing demand for the raw material has resulted in a rapid diminution of its natural sites and has necessitated legal protection for this species [1, 22, 23]. It seems that the only reasonable way of both preserving wild-growing roseroot and providing for the needs of the phytopharmaceutical industry is to introduce this plant into cultivation. However, it is not easy to obtain the raw material of uniformly high quality from wild plants directly introduced into cultivation [24–32]. This will only be possible after preliminary multi-directional studies. In the present paper we discuss the research concerning the effects of genetic, developmental, ecological and post-harvest factors on the accumulation of active compounds in roseroot cultivated in Poland.

16.2 Plant Characteristics

Roseroot is a heterozygous plant that exhibits high morphological, developmental and chemical variability. Plant height ranges from 5 to 70 cm, the leaves are sessile, elliptic to lance-shaped, wax coated, crenulated or serrulate, 7–35 mm long and 3–18 mm wide. Yellow to red flowers are located in terminal umbel-like clusters (Fig. 16.1). Flowers are male, female or bisexual [32]. Its fruit is 4–6 mm long and 3–5 mm wide. Seeds are 0.5–1 mm long. The weight

of 1000 seeds ranges from 185 to 250 mg [33, 34]. The underground part of the plant consists of the fleshy cylindrical rhizome, 2–10 cm in diameter, with sparse roots [1, 23, 35]. The outer part of the rhizome is grey-brown with a golden metallic cork [35]. The inner part of the fresh rhizome is white, and during drying of the sliced raw material those surfaces that have contact with air turn pink (Fig. 16.2).

From the pharmacological point of view, the most important active constituents of the raw material are phenolic compounds, including tyrosol and its glycoside salidroside, and *trans*-cinnamic alcohol derivatives: rosavin, rosarin and rosin (Fig. 16.3) [36–47]. The presence of phenolic acids in roseroot has also been reported [1, 48].

Fig. 16.1 Roseroot plant

Fig. 16.2 Air-dried rhizomes with roots (raw material of roseroot)

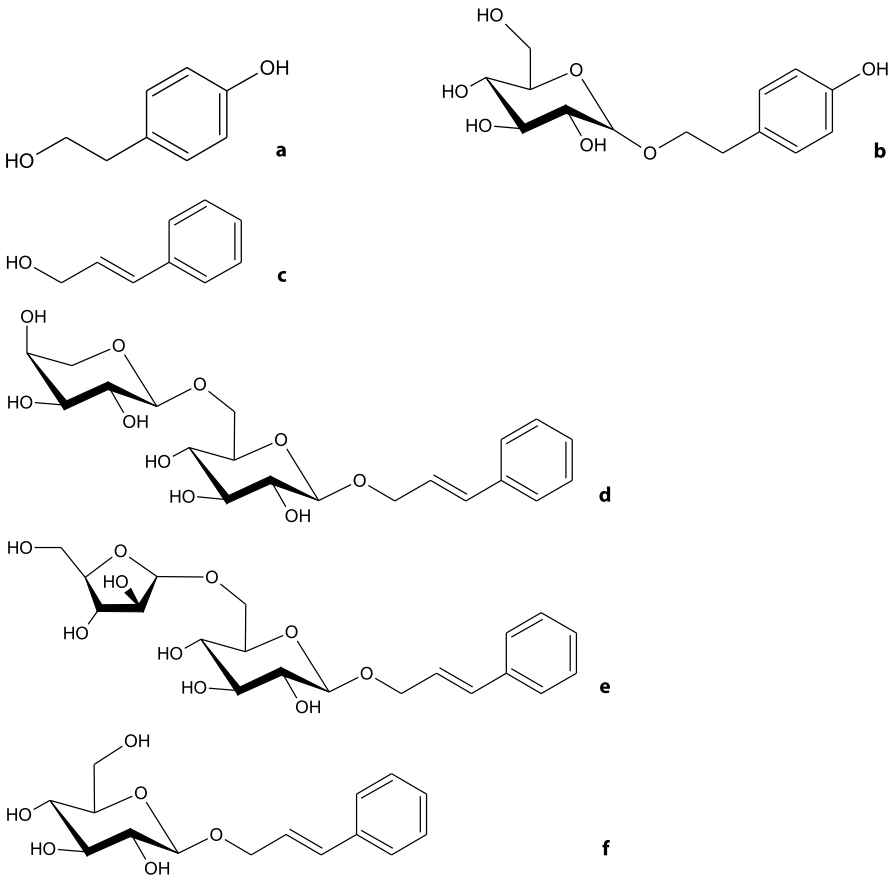


Fig. 16.3 The most important biologically active compounds in the underground organs of *Rhodiola rosea*: **a** tyrosol; **b** salidroside; **c** *trans*-cinnamic alcohol; **d** rosavin; **e** rosarin; **f** rosin

16.3 Intraspecific Variability

16.3.1 Distribution of Phenolic Compounds in Rhizomes and Roots

Phenolic compounds accumulate in the underground organs of *R. rosea* starting from the early stages of plant development. In our investigations they were detected in the roots of 7-week-old seedlings. In the older plants the cells containing phenolics were present mainly in rhizomes. These compounds were located in the parenchymal cells of the secondary conducting tissues of both the rhizomes and roots. In the rhizomes they were also found in the cortical parenchyma cells (Figs. 16.4 and 16.5).

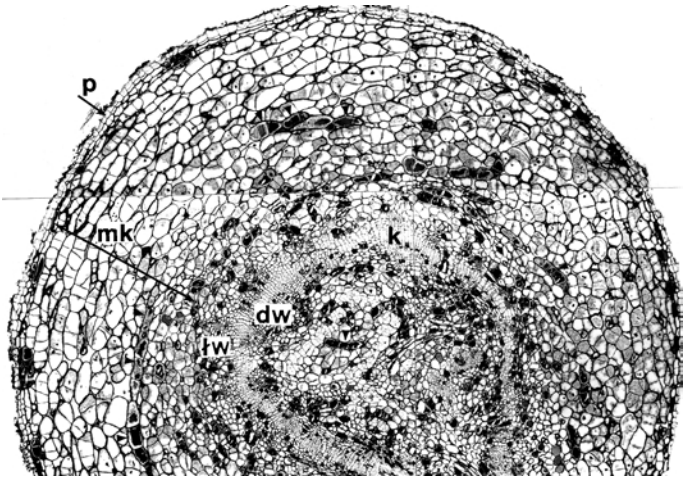


Fig. 16.4 Structure of the rhizome of a 1-year-old plant. Cells containing phenolic compounds are shown with an *arrowhead*. Magnification ×46. *p* Periderm, *mk* cortical parenchyma, *lw* secondary phloem, *dw* secondary xylem, *k* cambium

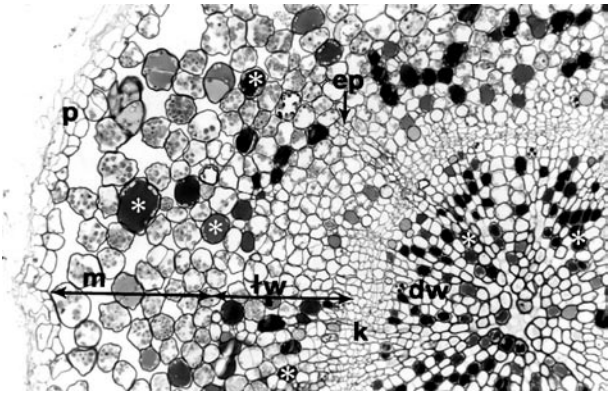


Fig. 16.5 Secondary structure of the root. Cells containing phenolic compounds are shown with an *asterisk*. Magnification ×185. *m* Parenchyma, *ep* conducting elements of secondary phloem

Rhizomes and roots differed significantly with respect to the content of the determined phenolic compounds (Table 16.1). The rhizomes were characterised by higher content of salidroside, rosavin, rosarin and *trans*-cinnamic alcohol, whereas roots by higher content of rosin, tyrosol and phenolic acids.

Table 16.1 Content of phenolic compounds in the rhizomes and roots of 5-year-old plants (mg·100 g^{-1}).

Compound	Rhizomes (n=13)	Roots (n=13)	Mean
Tyrosol derivatives			
Tyrosol	9.7 ± 3.6*	21.3 ± 6.1	15.5 ± 1.8
Salidroside	675.3 ± 565.5	248.9 ± 222.8*	462.1 ± 242.3
***Trans*-cinnamic alcohol derivatives**			
Trans-cinnamic alcohol	44.6 ± 26.6	21.1 ± 16.0*	32.9 ± 7.5
Rosavin	2961.4 ± 633.5	2270.5 ± 594.3*	2616.0 ± 27.7
Rosarin	335.6 ± 60.0	268.6 ± 41.6*	302.1 ± 13.0
Rosin	616.9 ± 169.5*	774.6 ± 205.7	695.8 ± 25.6
Phenolic acids			
Caffeic acid	4.5 ± 2.6*	6.6 ± 2.8	5.6 ± 0.1
Protocatechuic acid	6.1 ± 2.8	4.8 ± 2.2	5.5 ± 0.4
4-Hydroxybenzoic acid	37.0 ± 12.6*	50.1 ± 21.1	43.6 ± 6.0
Syringic acid	48.1 ± 12.6	37.7 ± 14.3*	42.9 ± 1.2

*P<0.05

For each analysis of phenolic compounds, the underground organs of 20 randomly selected plants (dried at 80 ± 5ºC) were used. One gramme of air-dried, grounded raw material was extracted with 100 ml of methanol in a Büchi B-811 extraction system. After evaporation of the solvent, the residue was dissolved in 10 ml methanol, filtered through a Supelco IsoDisc polytetrafluoroethylene 25 mm×0.45 µm filters, and subjected to high-performance liquid chromatography (HPLC). The analysis was carried out using a Shimadzu chromatograph with SPD-M10A VP DAD detector equipped with a Luna 5-µm C18 (2) 250 mm×4.6 mm column (Phenomenex). A gradient of 0.2% phosphoric acid in HPLC-grade water (A) and acetonitrile (B) was used as follows: 0 min, 4% B; 10 min, 13% B; 20 min, 15% B; 30 min, 20% B; 33 min 25% B; 38 min, 30% B; held constant for 22 min. The following analysis parameters were used: injection volume: 20 µl, flow rate 1.2 ml·min^{-1}, oven temperature 31ºC, time of analysis 60 min, recording wavelength: 190–450 nm, detection wavelength: 275 nm. Peaks were identified by comparison of retention time and spectral data with adequate parameters of standards (*Rhodiola rosea* Standards Kit by ChromaDex). Quantification was based on the peak area. The content of the determined compounds was calculated in mg·100 g^{-1} dry matter. The results were analysed with one-way and multifactor ANOVA Tukey's HSD test at the 0.05 significance level using Statgraphics Plus for Windows v. 4.1

Table 16.2 Content of phenolic compounds in the raw material (rhizomes with roots) of different origins (mg·100 g^{-1})

Compound	Mongolian Altai	Gorkhi Terelj	Russian Altai
Tyrosol derivatives			
Tyrosol	55.1 ± 2.3^{a}	5.3 ± 0.3^{c}	9.2 ± 0.4^{b}
Salidroside	111.4 ± 4.8^{b}	48.2 ± 4.3^{c}	141.6 ± 11.9^{a}
***Trans*-cinnamic alcohol derivatives**			
Trans-cinnamic alcohol	1631.7 ± 40.5^{a}	60.5 ± 3.4^{c}	174.7 ± 14.7^{b}
Rosavin	2250.6 ± 147.9^{b}	813.9 ± 84.1^{c}	3140.9 ± 61.0^{a}
Rosarin	492.7 ± 36.5^{a}	65.9 ± 4.5^{c}	315.7 ± 5.9^{b}
Rosin	275.0 ± 18.2^{b}	95.1 ± 7.9^{c}	596.1 ± 33.8^{a}
Phenolic acids			
Caffeic acid	4.27 ± 1.60^{b}	4.75 ± 0.68^{b}	14.22 ± 1.35^{a}
Protocatechuic acid	5.07 ± 1.54^{a}	1.78 ± 0.58^{b}	7.08 ± 0.73^{a}
4-Hydroxybenzoic acid	4.62 ± 0.75^{b}	21.11 ± 2.51^{a}	8.08 ± 0.17^{b}
Syringic acid	5.03 ± 0.46^{c}	12.11 ± 1.66^{a}	8.28 ± 1.04^{b}

[a–c]Values marked with the same letter do not differ significantly at $\alpha=0.05$

16.3.2 Quality of Raw Material of Different Origin

The results of previous studies [43, 49] indicate that the content of biologically active compounds in the raw material collected from different natural sites of roseroot varies within a wide range. For example, differences in the content of rosavin came up to 60%. One of the most important reasons for such diversity is genetic factors.

In our studies, the raw materials obtained from plants of three different populations originating from distant natural sites – in the area of Russian Altai, Mongolian Altai and Gorkhi Terelj (central Mongolia) – were compared (Table 16.2). The evaluated raw material differed significantly with respect to the content of all determined phenolic compounds. Differences in the content of rosavin were much higher in comparison with those reported by Kir'janov et al. [49] and came up to 400%, and differences in the content of rosin even reached 600%.

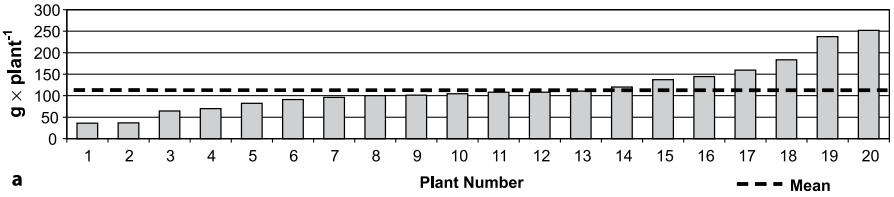


Fig. 16.6 Air-dried weight of the raw material (rhizomes with roots) of individual plants (g·plant^{-1})

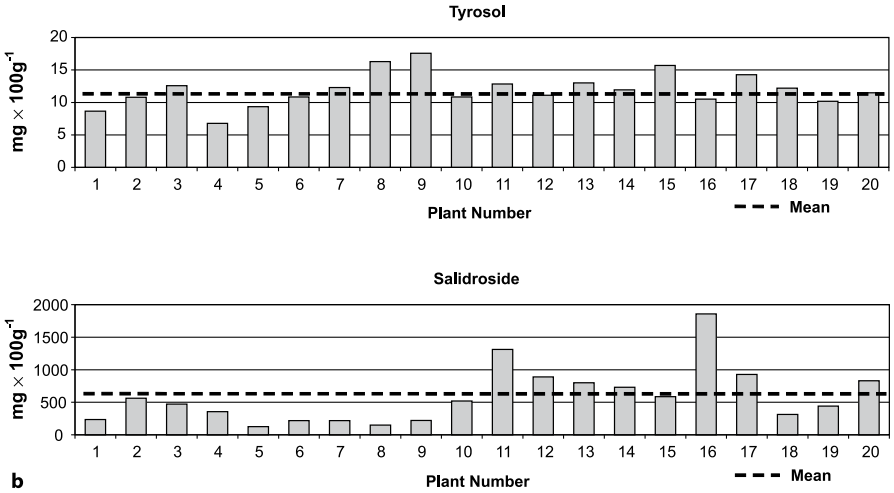


Fig. 16.7 Content of tyrosol derivatives in the raw material (rhizomes with roots) of individual plants (mg·100 g^{-1})

16.3.3 Individual Variation

The chemical variation within the roseroot population originating from the Russian Altai and those cultivated in central Poland was investigated in the 5th year of plant vegetation. High variability concerning both the weight of rhizomes and the content of phenolic compounds was found. The weight of air-dried underground organs ranged from 36 to 250 g (Fig. 16.6). In terms of phenolic compounds, the biggest difference between individual plants concerned the content of salidroside (125–1860 mg·100 g^{-1}; Fig. 16.7) and *trans*-cinnamic alcohol (8.9–79.7 mg·100 g^{-1}; Fig. 16.8). The content of other compounds also varied, but not so remarkably.

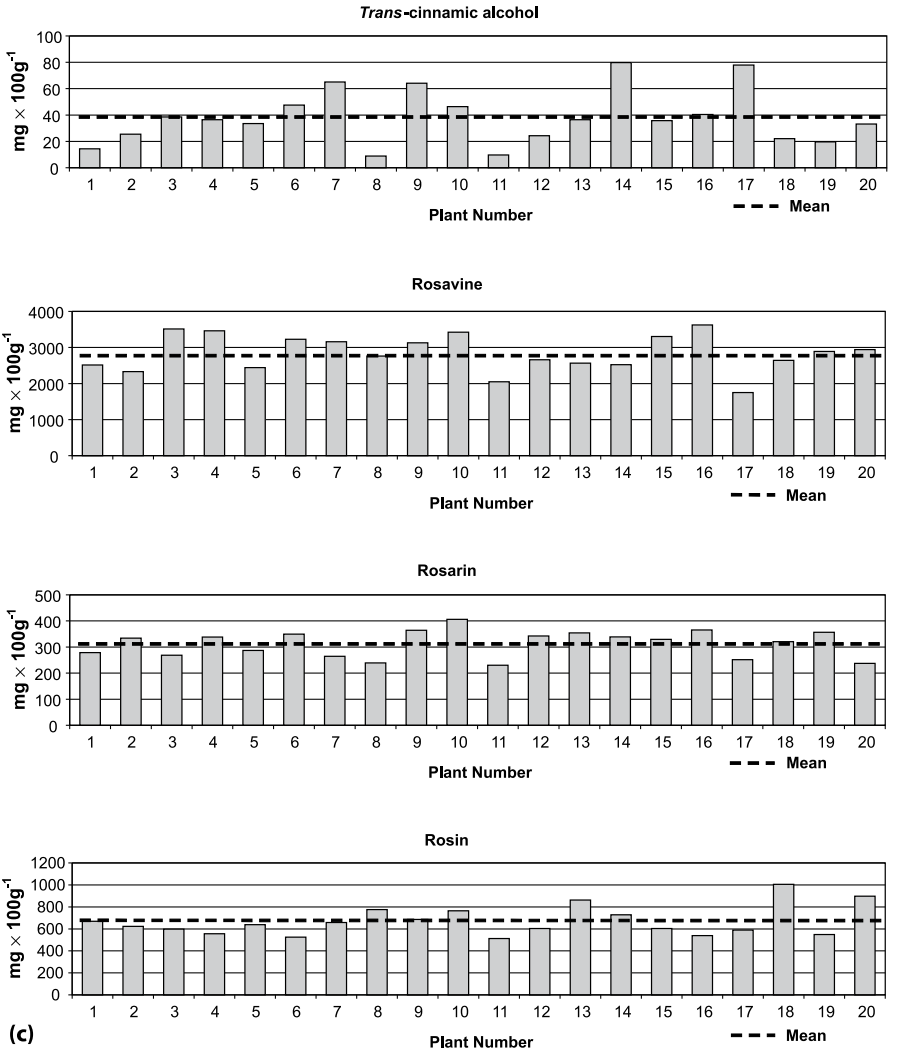


Fig. 16.8 Content of *trans*-cinnamic alcohol derivatives in the raw material (rhizomes with roots) of individual plants (mg·100 g^{-1})

16.4 Accumulation of Biomass and Biologically Active Compounds in the Underground Organs of Roseroot During Plant Development

So far, roseroot is collected mainly from natural sites. The standardisation of such raw material is difficult because it is obtained from the plants of different age. It is easier to control the quality of raw material from cultivation because of the possibility of more precise determination of the dynamics of accumulation of biologically active compounds in such plants.

We studied the growth of the underground organs and the accumulation of phenolic compounds in roseroot grown in central Poland during the period of six vegetation seasons. The mean weight of air-dried rhizomes with roots increased up to 120 g per plant in the 5th year of plant vegetation (Fig. 16.9). Over 60% of 5-year-old plants had underground organs weighing 50–150 g; however, the maximum weight came up to 300 g. Plants collected in the 4th and 5th year of vegetation were characterised by having the highest percentage of rhizome weight in the total weight of the underground part (Table 16.3). In the 6th year of vegetation, symptoms of plant aging were observed. The oldest, central part of rhizome decayed and the rhizome divided into many smaller parts (Fig. 16.10f), so that its mean weight decreased up to 45 g (Fig. 16.9).

Table 16.3 Effect of plant age on the percentage of rhizome weight in the total weight of air-dry raw material (rhizomes with roots; %)

Plant age					
1-year-old	**2-year-old**	**3-year-old**	**4-year-old**	**5-year-old**	**6-year-old**
56[c]	60[bc]	76[a]	81[a]	83[a]	69[b]

[a–c]Values marked with the same letter do not differ significantly at α=0.01

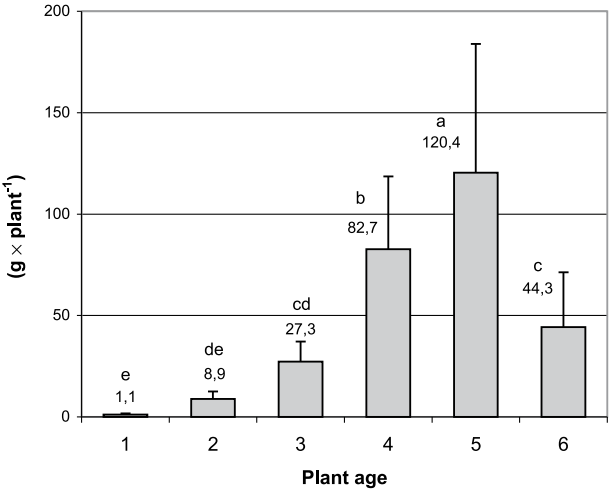
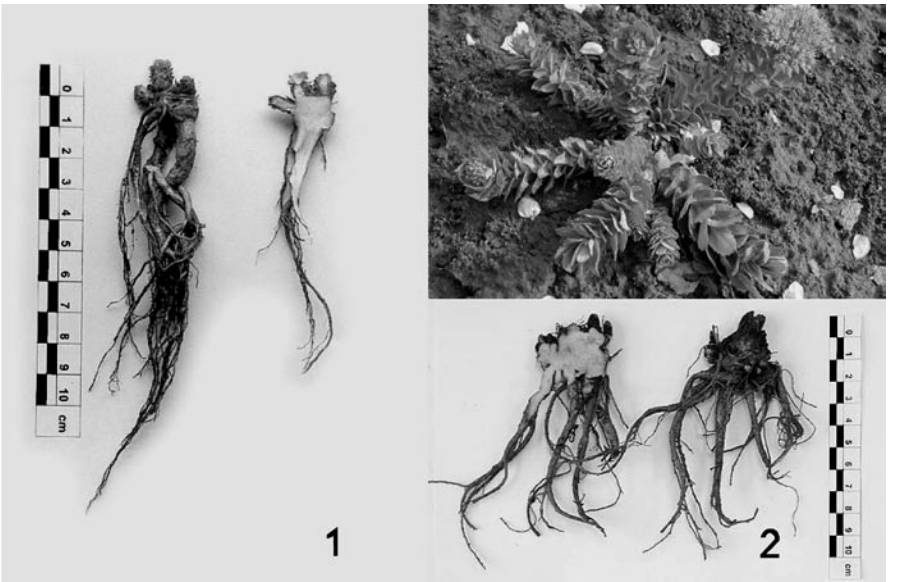


Fig. 16.9 Effect of plant age on the weight of air-dried raw material (rhizomes with roots) (g·plant^{-1}). Columns marked with the same letter (*a–e*) do not differ significantly at α = 0,05



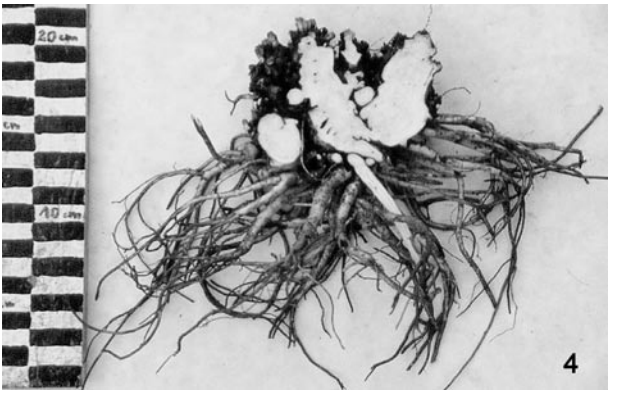


Fig. 16.10 The underground organs:
1 – 1-year-old plant,
2 – 2-year-old plant,
3 – 3-year-old plant,
4 – 4-year-old plant
5–6 see next page

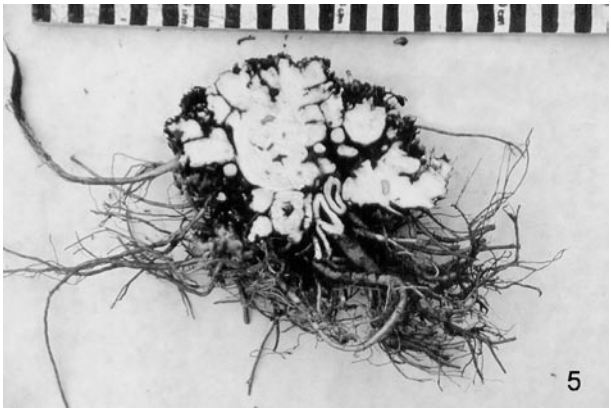


Fig. 16.10 The underground organs: *(continued) 5* – 5-year-old plant, *6* – 6-year-old plant (the rhizome divided into smaller autonomic parts)

There was no simple relationship between plant age and the content of determined phenolic compounds in the underground organs (Table 16.4). The highest content of the most pharmacologically active compounds (salidroside and rosavin) was found in the raw material obtained from 5-year-old plants.

16.5 Effect of Ecological Factors on the Accumulation of Biomass and Biologically Active Compounds in the Underground Organs of Roseroot

The climatic and soil conditions may significantly affect the yield and quality of the obtained plant raw material. Our studies confirmed the effect of these factors on the development of roseroot, morphology and yield of its underground organs, as well as the content of biologically active compounds in the raw material. The mean weight of air-dried rhizomes with roots of plants

Table 16.4 Effect of plant age on the content of biologically active compounds in the raw material (rhizomes with roots; mg·100 g^{-1})

Compound	Plant age					
	1-year-old	2-year-old	3-year-old	4-year-old	5-year-old	6-year-old
Tyrosol derivatives						
Tyrosol	7.5^{c}	10.0^{bc}	14.4^{ab}	14.4^{ab}	13.8^{ab}	15.7^{a}
Salidroside	182.2^{c}	207.9^{bc}	259.1^{bc}	350.9^{abc}	535.7^{a}	441.4^{b}
***Trans*-cinnamic alcohol derivatives**						
Trans-cinnamic alcohol	25.3^{ab}	16.4^{b}	14.5^{b}	23.4^{ab}	40.9^{a}	24.9^{ab}
Rosavin	2415.3^{ab}	2361.9^{ab}	2196.1^{ab}	2186.1^{ab}	2744.4^{a}	2014.6^{b}
Rosarin	354.9^{a}	254.8^{ab}	235.5^{b}	180.9^{b}	322.5^{ab}	243.2^{ab}
Rosin	193.3^{a}	727.3^{a}	601.6^{a}	483.6^{a}	669.2^{a}	462.2^{a}
Phenolic acids						
Caffeic acid	3.2^{c}	7.6^{ab}	7.3^{abc}	4.2^{bc}	5.4^{bc}	11.4^{a}
Protocatechuic acid	6.6^{a}	5.8^{ab}	4.0^{b}	5.2^{ab}	5.9^{ab}	6.1^{ab}
4-Hydroxybenzoic acid	21.8^{c}	38.6^{ab}	31.5^{abc}	27.2^{bc}	41.9^{a}	41.1^{a}
Syringic acid	10.4^{c}	21.0^{bc}	30.6^{ab}	28.9^{ab}	41.7^{a}	22.6^{bc}

$^{a–c}$Values marked with the same letter do not differ significantly at α=0.05

grown in central Poland (typical temperate climate, 99 m above sea level, vegetation period 216 days, alluvial soil) was twice as high as the weight of underground organs of plants grown in north-eastern Poland (transitional area between continental and Atlantic climates, 164 m above sea level, vegetation period 208 days, sandy soil) and in the mountains (alpine climate, 1000 m above sea level, vegetation period 184 days, clayey soil; Table 16.5). Soil type affected the size and shape of the underground part of a plant. Plants grown on sandy soil formed a highly branched rhizome with few roots, whereas on clayey and alluvial soils they formed a compact rhizome with numerous roots of large diameter (Fig. 16.11).

The content of salidroside and rosavin in the raw material obtained from the plants grown in the mountains was significantly higher in comparison with that of plants grown in the lowlands (central and north-eastern Poland). In the case of other determined compounds, there was no clear relationship between their accumulation in the raw material and the region of plant cultivation (Table 16.6).

Table 16.5 Effect of climatic and soil conditions on the weight of air-dried raw material (rhizomes with roots) of 3-year-old plants (mg·plant^{-1})

Plant organ	Central Poland	North-eastern Poland	Mountains (south Poland)
Rhizome	20.7 ± 7.3^{c}	12.0 ± 4.8^{b}	4.3 ± 2.6^{a}
Roots	6.6 ± 2.3^{b}	1.6 ± 0.6^{a}	1.8 ± 1.1^{a}
Total	27.3 ± 9.6^{c}	13.6 ± 5.4^{b}	6.1 ± 3.7^{a}

[a–c]Values marked with the same letter do not differ significantly at α=0.05

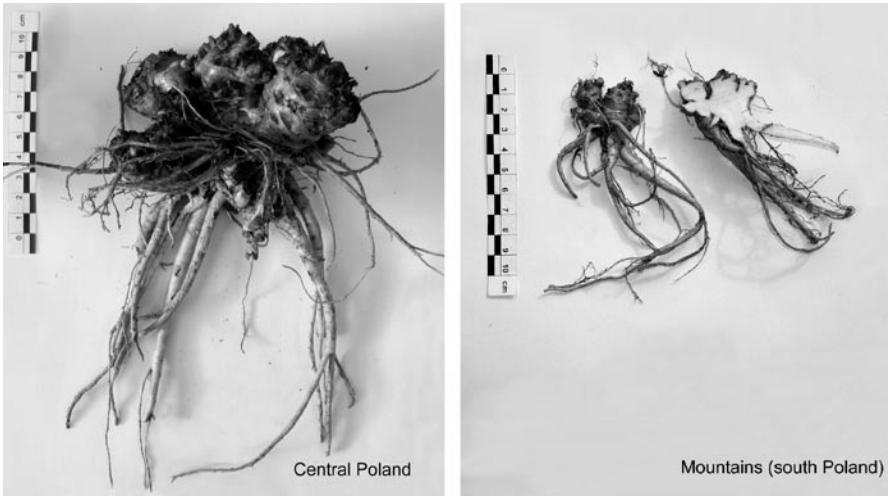


Fig. 16.11 The underground organs of 3-year-old roseroot plants cultivated in different climatic and soil conditions

16.6 Effect of Post-harvest Treatment on the Quality of Raw Material and Extracts

Regarding the high content of water (sometimes over 70%) in fresh rhizomes of roseroot, this plant material is rather difficult to stabilise. Kurkin et al. [42, 45] studied the effect of temperature in the drying chamber on the quality of this raw material. They found that the optimum drying temperature was 80ºC or 20ºC. Drying at 50–60ºC, previously recommended by Syrov [50], resulted in a distinct reduction in salidroside and rosavin content. In our studies, three methods of stabilisation were applied: convection drying at 80ºC, freezing and lyophilisation (Table 16.7). It appeared that the content of determined phenolic compounds in dried and lyophilised raw material was comparable and high,

Table 16.6 Effect of different climatic and soil conditions on the content of biologically active compounds in the raw material (rhizomes with roots) of 3-year-old plants (mg·100 g^{-1})

Compound	Central Poland	North-eastern Poland	Mountains (south Poland)
Tyrosol derivatives			
Tyrosol	14.4[ab]	17.1[a]	10.7[b]
Salidroside	259.1[b]	181.0[c]	378.7[a]
***Trans*-cinnamic alcohol derivatives**			
Trans-cinnamic alcohol	14.5 ns	16.1 ns	15.7 ns
Rosavin	2196.1[ab]	1993.0[b]	2420.3[a]
Rosarin	235.5 ns	237.3 ns	239.4 ns
Rosin	601.6[a]	351.8[b]	441.6[b]
Phenolic acids			
Caffeic acid	7.3[b]	7.2[b]	11.1[a]
Protocatechuic acid	4.0[b]	2.7[c]	7.4[a]
4-Hydroxybenzoic acid	31.5[b]	40.9[a]	33.9[b]
Syringic acid	30.6[a]	13.1[c]	25.0[b]

[a–c]Values marked with the same letter do not differ significantly at α=0.05
ns – differences are not significant at α = 0.05

Table 16.7 Effect of the stabilisation method on the content of biologically active compounds in the raw material (rhizomes with roots; mg·100 g^{-1})

Compound	Freezing	Convection drying	Sublimation drying
Tyrosol derivatives			
Tyrosol	14.0 ± 7.9[b]	26.4 ± 10.4[a]	23.8 ± 9.5[ab]
Salidroside	297.3 ± 151.2 ns	443.9 ± 227.6 ns	491.1 ± 180.8 ns
***Trans*-cinnamic alcohol derivatives**			
Trans-cinnamic alcohol	330.0 ± 115.4[a]	66.2 ± 23.5[b]	13.1 ± 2.9[b]
Rosavin	24.7 ± 8.0[b]	3079.9 ± 329.4[a]	3589.6 ± 739.7[a]
Rosarin	224.5 ± 91.1[b]	302.8 ± 83.4[ab]	388.9 ± 104.1[a]
Rosin	450.8 ± 104.4[b]	1029.1 ± 279.7[a]	849.6 ± 390.2[a]
Phenolic acids			
Caffeic acid	19.6 ± 7.3[b]	17.4 ± 3.7[b]	28.7 ± 5.7[a]
Protocatechuic acid	7.5 ± 2.0[ab]	9.7 ± 3.4[a]	6.6 ± 1.3[b]
4-Hydroxybenzoic acid	27.4 ± 7.2[c]	65.4 ± 18.8[b]	87.9 ± 19.1[a]
Syringic acid	26.0 ± 7.2[c]	45.0 ± 6.5[b]	81.1 ± 15.4[a]

[a–c]Values marked with the same letter do not differ significantly at α=0.05
ns – differences are not significant at α = 0.05

Table 16.8 Effect of solvent and extraction method on the content of biologically active compounds in the raw material (rhizomes with roots; mg·100 g^{-1} dry matter). *S* Ultrasonic extraction, *C* continuous exhaustive extraction

Compound	Extraction method	Water	Ethanol	Methanol	Mean
Tyrosol derivatives					
Tyrosol	S	7.5 ± 1.8	10.3 ± 1.1	10.3 ± 0.5	**9.4 ± 1.1**
	C	2.8 ± 1.3	11.8 ± 2.1	12.0 ± 1.9	**8.9 ± 1.8**
	Mean	**5.2 ± 1.6^{b}**	**11.1 ± 1.6^{a}**	**11.2 ± 1.2^{a}**	
Salidroside	S	335.4 ± 7.2	590.0 ± 50.4	664.8 ± 54.2	**530.1 ± 37.3**
	C	486.8 ± 52.1	604.6 ± 27.6	576.5 ± 22.4	**556.0 ± 35.0**
	Mean	**411.1 ± 29.7^{b}**	**597.3 ± 39.0^{a}**	**620.7 ± 38.3^{a}**	
***Trans*-cinnamic alcohol derivatives**					
Trans-cinnamic alcohol	S	21.7 ± 13.4	17.5 ± 3.9	35.9 ± 24.1	**25.0 ± 13.8**
	C	8.2 ± 2.8	46.5 ± 8.8	46.1 ± 15.2	**33.6 ± 8.9**
	Mean	**15.0 ± 8.1^{b}**	**32.0 ± 6.4^{ab}**	**41.0 ± 19.7^{a}**	
Rosavin	S	1160.1 ± 55.4	3015.7 ± 107.8	3088.5 ± 82.7	**2421.4 ± 81.9**
	C	1702.6 ± 199.4	2731.4 ± 46.7	2801.6 ± 63.8	**2411.9 ± 103.3**
	Mean	**1431.4 ± 127.4^{b}**	**2873.6 ± 77.2^{a}**	**2945.1 ± 73.3^{a}**	
Rosarin	S	124.5 ± 4.0	426.0 ± 22.4	413.1 ± 30.3	**321.2 ± 18.9**
	C	176.2 ± 24.1	341.8 ± 17.4	342.6 ± 22.3	**286.9 ± 21.3***
	Mean	**150.4 ± 14.1^{b}**	**383.9 ± 19.9^{a}**	**377.9 ± 26.3^{a}**	
Rosin	S	114.4 ± 2.2	471.8 ± 136.7	531.7 ± 26.2	**372.6 ± 55.0**
	C	100.0 ± 10.5	566.9 ± 51.3	612.4 ± 34.5	**426.4 ± 32.1**
	Mean	**107.2 ± 6.4^{b}**	**519.4 ± 94.0^{a}**	**572.1 ± 30.4^{a}**	
Phenolic acids					
Caffeic acid	S	3.4 ± 0.6	2.3 ± 0.5	4.0 ± 0.7	**3.2 ± 0.6**
	C	2.4 ± 0.3	3.3 ± 0.6	2.8 ± 0.4	**2.8 ± 0.4**
	Mean	**2.9 ± 0.5 ns**	**2.8 ± 0.6 ns**	**3.4 ± 0.6 ns**	
Protocatechuic acid	S	1.9 ± 0.1	4.4 ± 0.1	5.3 ± 0.4	**3.9 ± 0.2**
	C	3.5 ± 0.4	4.1 ± 0.2	4.4 ± 0.4	**4.0 ± 0.3**
	Mean	**2.7 ± 0.3^{c}**	**4.3 ± 0.2^{b}**	**4.9 ± 0.4^{a}**	
4-Hydroxybenzoic acid	S	11.0 ± 0.7	39.0 ± 1.2	37.4 ± 2.3	**29.1 ± 1.4**
	C	14.9 ± 1.9	36.9 ± 3.3	37.4 ± 2.0	**29.7 ± 2.4**
	Mean	**13.0 ± 1.3^{b}**	**38.0 ± 2.3^{a}**	**37.4 ± 2.2^{a}**	
Syringic acid	S	12.3 ± 5.6	14.1 ± 0.6	23.6 ± 0.3	**16.7 ± 2.2***
	C	4.3 ± 0.9	33.3 ± 1.5	34.2 ± 3.0	**23.9 ± 1.8**
	Mean	**8.3 ± 3.3^{c}**	**23.7 ± 1.1^{b}**	**28.9 ± 1.7^{a}**	

$^{a–c}$Values marked with the same letter do not differ significantly at α=0.05
ns – differences are not significant at α = 0.05

whereas the frozen rhizome was characterised by a lower content of the majority of these compounds. A remarkable decrease in rosavin content and increase of the content of its aglycone (*trans*-cinnamic alcohol) indicates that freezing was not effective in inactivating hydrolytic enzymes, which is essential for plant material stabilisation.

In order to reliably evaluate the quality of a raw material it is necessary to find the best method for extraction of the main biologically active compounds. Data concerning the recommended solvent and extraction method for standardisation of roseroot is contradictory [39, 40, 49]. Our studies indicate that periodical ultrasonic extraction and continuous exhaustive extraction (in a Soxhlet-like Büchi Universal Extraction System) allowed to get extracts characterised by a similar content of phenolic compounds. Both 70% ethanol and 100% methanol appeared to be better extraction media than water (Table 16.8).

The results of several years studies carried out in the Warsaw Agricultural University indicate that the cultivation of roseroot in the lowlands of the temperate zone is possible. In comparison with the natural mountain habitats of roseroot, the region of central Poland is characterised by a longer vegetation period, which results in a faster increment in the weight of its underground organs, which are used as a medicinal raw material. In such conditions it is possible to obtain a high yield and good quality of the raw material as early as in the 5th year of plant vegetation. In the 6th year, the plants divide into smaller autonomic parts that are characterised by a lower content of salidroside and rosavin, the compounds regarded to be the most important for the pharmacological activity of roseroot preparations.

Taking into consideration the high intraspecific variability of roseroot, it is advisable to undertake research on basic breeding problems, as well as on effective methods of vegetative propagation (e.g. in vitro).

Post-harvest treatment of the medicinal raw materials may distinctly affect their quality (i.e. the content and composition of biologically active compounds). Convection drying is the most common method of roseroot raw material stabilisation. Our studies proved that comparable results might be obtained using lyophilisation.

Regarding the necessity for the fast, cheap and reliable evaluation of a raw material, it seems that the best extraction method for determination of phenolic compounds in roseroot is ultrasonic extraction with methanol as a solvent.

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References

1. Kurkin VA, Zapesochnaya GG (1986) Khim-Farm Zh 20:1231
2. Abidov M, Crendal F, Grachev S, Seifulla R, Ziegenfuss T (2003) Bull Exp Biol Med 136:585
3. Shevtsov VA, Zholus BI, Shervarly VI, Vol'skij VB, Korovin YP, Khristich MP, Roslyakova NA, Wilkman G (2003) Phytomedicine 10:95
4. Spasov AA, Wilkman GK, Mandrikov VB, Mironova IA, Neumoin VV (2000) Phytomedicine 7:85
5. Darbinyan V, Kteyan A, Panossian A, Gabrielian E, Wilkman G, Wagner H (2000) Phytomedicine 7:365
6. Maslov LN, Lishmanov YuB, Maimeskulova LA, Krasnov EA (1998) Bull Exp Biol Med 125:424
7. Afanas'ev SA, Lishmanov YuB, Lasukova TV, Naumova AV (1997) Bull Exp Biol Med 123:447
8. Maslova LV, Kondrat'ev BYu, Maslov LN, Lishmanov YuB (1994) Exp Klin Farmakol 57:61
9. Wagner H, Nörr H, Winterhoff H (1994) Phytomedicine 1:63
10. Afanas'ev SA, Alekseeva ED, Bardamova IB, Maslova LV, Lishmanov YuB (1993) Bull Exp Biol Med 116:480
11. Lishmanov YuB, Maslova LV, Maslov LN, Dan'sina EN (1993) Bull Exp Biol Med 116:175
12. Gossudarstvennaya Farmakopeya SSSR, XI (1991) Meditsina, Moskva
13. Sokolov SYa, Boiko VP, Kurkin VA, Zapesochnaya GG, Rvancova NV (1990) Khim-Farm Zh 24:66
14. Petkov VD, Yonkov D, Mosharoff A, Kamburova T, Alova L, Petkov VV, Todorov I (1986) Acta Physiol Pharm Bulg 12:3
15. Lazarova MB, Petkov VD, Markovska VL, Petkov VV, Mosharoff A (1986) Methods Find Exp Clin Pharmacol 8:547
16. Sokolov SYa, Ivashin VM, Zapesochnaya GG, Kurkin VA, Shchavlinskii AN (1985) Khim-Farm Zh 19:1367
17. Dugan OM, Barilyak IR, Nester TI, Dvornik AS, Kunakh VA (1999) Tsitol Genet 33:19
18. Bocharova OA, Matveev BP, Baryshnikov AYu, Figurin KM, Serebryakova RV, Bodrova NV (1995) Urol Nefrol 2:46
19. Udincev SN, Shakhov VP, Borovskoy IG, Ibragimova SG (1991) Biofizika 36:105
20. Udincev SN, Shakhov VP (1991) Eur J Cancer 27:1182
21. Udincev SN, Shakhov VP (1991) Neoplasma 38:323
22. Polozhii AV, Sakharova NA, Sviridonov GM (1983) Rastit Resur 19:289
23. Nuchimovskii EL (1974) Rastit Resur 10:499
24. Galambosi B (2006) Demand and availability of Rhodiola rosea L. raw material. In: Bogers RJ, Craker LE, Lange D (eds) Medicinal and Aromatic Plants. Springer, the Netherlands, p 223
25. Elsakov GV, Gorelova AP (1999) Agrochimiya 10:58
26. Kirichenko EB, Rudenko SS, Baglay BM, Masikevich YuG (1993) Byulleten' Glavnogo Botanicheskogo Sada 169:50
27. Satsyperova IF, Kurkin VA, Zapesochnaya GG, Pautova IA (1991) Rastit Resur 27:55
28. Kurkin VA, Zapesochnaya GG, Dubichev AG, Voroncov ED (1990) Khim Prir Soedin 26:481
29. Kir'yanov AA, Bondarenko LT, Kurkin VA, Zapesochnaya GG (1989) Khim-Farm Zh 23:449
30. Ishmuratova MM (1998) Rastit Resur 34:13
31. Ishmuratova MM (1998) Rastit Resur 34:72
32. Nukhimovskii EL, Yurtseva NS, Yurtsev BN (1987) Rastit Resur 23:489

33. Ishmuratova MM, Satsyperova IF (1998) Rastit Resur 34:3
34. Tikhonova VL, Kruzhalina TN, Shugaeva EV (1997) Rastit Resur 33:68
35. Khnykina LA, Zotova MI (1966) Aptechnoe Delo 15:34
36. Patov SA, Punegov VV, Kuchin AV (2006) Chem Nat Comp 42:397
37. Kurkin VA (2003) Chem Nat Comp 39:123
38. Tolonen A, Pakonen M, Hohtola A, Jalonen J (2003) Chem Pharm Bull (Tokyo) 51:467
39. Ganzera M, Yayla Y, Khan IA (2001) Chem Pharm Bull (Tokyo) 49:465
40. Linh PT, Kim YH, Hong SP, Jian JJ, Kang JS (2000) Arch Pharm Res 23:349
41. Dubichev AG, Kurkin VA, Zapesochnaya GG, Voroncov ED (1991) Khim Prir Soedin 27:161
42. Kurkin VA, Zapesochnaya GG, Kir'yanov AA, Bondarenko LT, Vandyshev VV, Mainskov AV, Nukhimovskii EL, Klimakhin GI (1989) Khim-Farm Zh 23:1364
43. Kurkin VA, Zapesochnaya GG, Gorbunov JN, Nukhimovskii EL, Sreter AI, Shchavlinskii AN (1986) Rastit Resur 22:310
44. Kurkin VA, Zapesochnaya GG, Shchavlinskii AN (1985) Khim Prir Soedin 21:632
45. Kurkin VA, Zapesochnaya GG, Shchavlinskii AN, Nukhimovskii EL, Vandyshev VV (1985) Khim-Farm Zh 19:185
46. Krasnov EA, Kuvaev VB, Khoruzhaya TG (1978) Rastit Resur 14:153
47. Saratikov AS, Krasnov EA, Khnykina LA, Duvidson LM, Sotova MI, Marina TF, Nechoda MF, Axenova RA, Tscherdinzeff SG (1968) Pharmazie 23:392
48. Kurkin VA, Zapesochnaya GG, Nukhimovskii EL, Klimakhin GI (1988) Khim-Farm Zh 22:324
49. Kir'yanov AA, Bondarenko LT, Kurkin VA, Zapesochnaya GG (1988) Khim-Farm Zh 22:451
50. Syrov YuP (1985) Pravila sbora i suszki lekarstvennych rastenij, Moskwa