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Content of Biologically Active Substances in the Raw Materials of Some Siberian *Saussurea* Species

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Abstract. This paper focuses on the chemical composition of five Siberian *Saussurea* species: *S. frolowii*, *S. controversa*, *S. latifolia*, *S. parviflora* and *S. salicifolia*. Four groups of substances were found in dry mass of these plants: phenolic compounds (flavonoids, tannins), carotenoids, pectins and saponins. Quantitative spectrophotometric analysis revealed a high content of flavonoids, catechins, tannins, carotenoids and saponins in some samples. Leaves of *S. parviflora* and *S. latifolia*contain high contents of flavonoids (greater than 3.2 %).The greatest amount of catechins accumulates in the leaves of *S. parviflora* and *S. frolowii*. Due to the high content of tannins *S. parviflora* and *S. latifolia*are noteworthy. Leaves of *S. parviflora*and *S. latifolia* accumulate a particularly large number of carotenoids. Leaves of *S. parviflora* and *S. controversa*are particularly rich in saponins. Our results confirm the hypothesis about the prospects of the use of these species for the development of herbal medicines.

INTRODUCTION

Genus *Saussurea* DC. (Asteraceae) includes about 415 species [1] with 53 species and 1 subspecies growing in Siberia [2]. Species of this genus have a long history of applying in traditional medicine [3, 4], and their chemical components show the availability of *Saussurea*'s representatives to develop drugs based on natural raw materials [5-7]. However, until now phytochemical study of Siberian *Saussurea* species was fragmented and concerned only 50 % of the species [8].

Our study focuses on five species of *Saussurea* from Siberia inhabiting the territory of the system of mountain ranges Kuznetsk Alatau: *S. frolowii* Ledeb., *S. controversa* DC., *S. latifolia* Ledeb., *S. parviflora* (Poir.) DC. and *S. salicifolia* (L.) DC. Usual habitats of *S. controversa* are mountain forests, its range covers the Ural, mountains of Southern Siberia and reaches Mongolia [10]. *S. frolowii* and *S. latifolia* are alpine plants. *S. frolowii* grows mainly in the subalpine zone in the meadows and rare forests. *S. latifolia* has a broader ecological niche occurring not only in the high mountains, but also in the forest belt. The ranges of these species mostly confined to the territory of the Altai and Sayan Mountains [9]; their southern border is located in China [1]. *S. frolowii* grows also in Targabatay and Jungar Alatau [10]. The northern boundary of the range of this species lows in the Kuznetsk Alatau. *S. parviflora* has an extensive range covering the northeast European Russia, the Urals, Siberia and Mongolia [10]. This species is quite common for mountain and lowland forests. *S. salicifolia* is characterized by a disjunctive geographical range with the main fragment located in the Republic of Buryatia and the Irkutsk region. Another part of the range is in Altai and Tuva. *S. salicifolia* occurs in the north of Mongolia [11] and China [1]. The Kuznetsk Alatau is the western boundary of the small fragment of the range occupying the south of the Krasnoyarsk Territory and the Republic of Khakassia. *S. frolowii* and *S. salicifolia* are rare in the study area [12] and the prospects of its

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further use as raw material for the manufacture of medicinal products should be associated with cultivation. *S. controversa*, *S. latifolia* and *S. parviflora* are quite common species, so one can consider the possibility to exploit their natural populations in the mode of sustainable use.

Previously, it was shown that the chemical composition of these species is promising. They found a variety of phenolic compounds including phenolic acids, flavonoids (flavones and flavonols), coumarins, phenylpropanoids, tannins and lignans [8,13]. Furthermore, there is an evidence of the content of vitamins [14], alkaloids [15] triterpenoids [16, 17] and steroid compounds [18, 19] in these plants. For this reason, it can be expected that the herbal preparations based on these plants will possess antibacterial, antioxidant, antitumor, hemostatic, wound-healing, anti-inflammatory and antipyretic activities [20-23].

Data on quantitative content of biologically active substances listed in the raw materials of *Saussurea* species are not enough [8], and they don't reflect an intraspecific variability of these plants within the range.

For Siberian *Saussurea* species, the qualitative composition of flavonoids and the quantitative content of the individual substances of this group are the most studied. In 2003, the presence of penduletin and quercetin were proved in the grass of *S. parviflora* [17]. Kaempferol, quercetin, hyperoside, quercetin-3,5-glucoarabinoside, myricetin-3-glucoside and myricetin-3-rhamnoside were found in *S. controversa* [24]. Apigenin, hyperoside, quercetin, kaempferol, hispidulin and apigenin-7-O-rhamnosidewere isolated from the aerial parts of *S. salicifolia* [13,25,26].The presence of the flavonoid group has also been shown by qualitative chemical reactions in *S. amurensis*, *S. baicalensis*, *S. neoserrata*, *S. subacaulis* and *S. elongata* [8].

Data on the quantitative content of flavonoids in *Saussurea* species are incomplete and fragmented. There is information about the quantitative content of this group of polyphenols in the aerial parts of *S. salicifolia* (0.72 %), *S. contraversa* (1.2 %) and *S. amara* (2.74 %) [24].

The content of the tannins is confirmed by qualitative reactions in *S. parviflora*, *S. pulchella*, *S. foliosa*, *S. latifolia*, *S. amara*, *S. subacaulis*, *S. alpina*, *S. schanginiana* and *S. frolowii*. Quantitative content of tannins was determine only in *S. salicifolia* (2.03 %) [27].

There is a little known about the pigment composition of *Saussurea* species. Thus, for example, their presence were confirmed by physical-chemical methods in *S. alpina* and *S. discolor* [28, 29].

The aim of this study is to determine the qualitative composition and quantitative content of biologically active substances in the raw material of five *Saussurea* species growing in the Kuznetsk Alatau.

METHODS AND MATERIALS

The material for the research were aerial and subsurface parts of 5 *Saussurea* species harvested in the flowering phase in the territory of the Kuznetsk Alatau (Russia, Republic of Khakassia): *S. frolowii* (Ust-Abakan district, the Hazyr-Teren mountain, subalpine rare forests), *S. controversa* (Shira district, neighborhood of the Efremkino village, larch and birch forest), *S. latifolia* (Shira district, the Podoblachniy mountain, subalpine rare forest), *S. parviflora* (Ordzhonekidzevsky district, neighborhood of the lakes Ivanovskie, alpine shrubs) and *S. salicifolia* (Shira district, neighborhood of the Efremkino village, mountain steppe). We dried samples in the mode of temperate convection to air-dry state and pulverized to a particle size of about 1 mm^2. Then we investigated the chemical composition of samples after extraction of raw materials prepared by conventional reactions by phytochemical qualitative analysis.

Flavonoids

Quantitative content of flavonoids were determined by spectrophotometry using a preliminary reaction of complexation with aluminum chloride. For this purpose, about 1.0 g (accurately weighed) of crushed raw materials were extracted with 30 ml of 70 % ethanol in the wide-mouthed flask in a boiling water bath under reflux for 30 min. The resulting extract was filtered into the volumetric flask of 200 ml. In a similar manner the extraction was repeated 2 more times. The combined extract in the flask was adjusted to 70 % ethanol to the mark. Then we placed 2 ml of extract into the volumetric flask of 25 ml, added 2 ml of 2 % alcohol solution of aluminum chloride and adjusted the solution volume to the mark by 95 % ethanol. Measurement of the optical density of the resulting solution was performed with a spectrophotometer at a wavelength of 415 nm in a cuvette with a layer thickness of 10 mm. The solution was used as a comparison the following solution: we placed 2 ml of extract into a volumetric flask of 25 ml, added 1 ml of 3 % acetic acid solution and adjusted the solution to volume mark with ethanol. The concentration of flavonoids found on the calibration plot constructed from routine.

Catechins

We determined quantitative catechin content spectrophotometrically based on the ability of these substances to form colored complexes with a solution of vanillin in concentrated hydrochloric acid giving main absorption maximum at a wavelength of 504 nm. For this purpose, we transferred 0.8 ml of the solution from the flask a two-dimensional tube with a combined ethanolic extract. 1 ml of 4 % vanillin solution in concentrated hydrochloric acid was added in one vial, 4 ml of concentrated hydrochloric acid was added in another test tube (control), and then we adjusted volumes to 5 ml with acid. The optical density of the solution was measured spectrophotometrically at a wavelength of 504 nm in a cuvette with a layer thickness of 10 mm. We calculated the content of catechins in the sample from a calibration curve prepared with (±)-catechin (Sigma).

Tannins

Measurement of quantitative content of tannins was carried out by spectrophotometry after the reaction of tannins with ammonium molybdate. 2.0 g of raw material (accurately weighed) was placed in a flask, then we added 250 ml of purified water. We carried out extraction with moderate boiling on a hot plate for 30 min. Then extraction was cooled, transferred to a volumetric flask with 250 ml water and adjusted to the mark. We centrifuged extraction portion (20ml) for 5 min at 3000 rev/min. 10 ml of centrifugatewas transferred quantitatively to a volumetric flask of 100 ml, then we added 10 ml of 2 % aqueous solution of ammonium molybdate to it. The contents of the flask was adjusted to the mark with water and left for 15 min. We measured the optical density of the solution with a spectrophotometer SF-46 at a wavelength of 420 nm in a cuvette with a layer thickness of 10 mm. Calculation of the quantitative content of tannins was made by GSO standard sample tannin.

Carotenoids

The gist of the method consists in measuring the absorbance of the acetone-ethanol extracts of pigments in a spectrophotometer at wavelength of maximum absorption of carotenoids (440.5 nm). For this weighed 0.1 g of vegetative mass was triturated in a mortar with 0.1 g of calcium carbonate to neutralize the acid until a homogeneous mass. Then we added 1 ml of dimethylformamide and 2 mL of anhydrous sodium sulfate. Extraction of carotenoids was performed acetone by trituration in a mortar in a first portion of 20 ml, then twice with 5 ml. Thereafter extraction was continued exhaustively with ethanol and then with acetone. The quantitative content of substances were calculated from equations of Vetshtein and Holm.

Pectins and protopectins

The quantitative content of pectins and protopectins was determined by spectrophotometric method without carbazole based on the measurement of the optical density of the reaction product of uronic acid with thymol in sulfuric acid. For this we removed sugars from the sample of finely ground materials (weighed of 5-10.0 g) with hot 80 % ethanol on a water bath to reflux for 20-30 min3 times. The filtered sample was dried at 50 ° C convectively until the disappearance of residual alcohol. First, we extracted pectins by water, then hydrolyzed protopectins. Density of colored solutions after reaction with carbazole was measured at the SF-46 at 480 nm in a cuvette with a working length of 10 mm. The quantitative content of pectin was determined by a calibration curve for galacturonic acid.

Saponins

Quantitative content of saponins was determined gravimetrically. For this purpose, we extracted about 2.0 g of raw material (accurately weighed) with chloroform in a Soxhlet apparatus until complete bleaching to remove lipids and resins interfering saponins. Then we extracted 2 times consecutively with 50, 60 and 96 % ethanol for 30 min at the temperature of solutionequal to 70 °C. The combined extract was concentrated to 5 ml, then we added sevenfoldvolume of acetone to it. After 18 h, the resulting precipitate was filtered, dried in a heat cabinet at 70 °C, weighed, and we calculated the content of the "crude saponin" in the end.

RESULTS AND DISCUSSION

Qualitative screening of biologically active substances of 5 *Saussurea* species showed that the aerial parts of these plants synthesize flavonoids, tannins, catechins, carotenoids, pectins, protopectins and saponins. Quantitative analysis revealed a high content of some groups (Table 1).This confirms the hypothesis about the prospects of their use for the development of herbal medicines. We found a higher content of biologically active substances in the leaves than in stems.

TABLE 1. The content of biologically active substances in the raw material of *Saussurea* species. All indicators (average of three replicates) were calculated on bone dry weight of the feed. Note: l – leaves, s – stems.

Sample	Organ	Flavonoids, %	Tannins, %	Catechins, mg%	Carotenoids, mg%	Pectins, %	Proto-pectins, %	Saponins, %
S. frolowii, 11.07.2014	l	2.91	16.47	188.0	85.79	1.35	8.28	17.17
S. parviflora,	l	6.21	29.84	153.6	159.23	1.65	10.24	20.48
17.07.2014	s	1.47	5.66	88.6	11.04	0.71	4.27	7.99
S. latifolia,	l	4.36	26.11	121.7	155.22	0.44	9.45	12.45
25.07.2014	s	1.24	5.37	72.8	6.83	0.66	2.61	11.14
S. controversa,	l	3.05	19.24	84.7	79.82	1.08	8.31	19.40
23.07.2014	s	1.35	7.02	83.4	8.46	1.30	3.58	9.82
S. salicifolia,	l	2.02	8.79	95.6	37.86	0.94	8.38	9.46
4.09.2014	s	0.47	4.11	47.9	6.84	0.50	3.13	8.10

Flavonoids and catechins were found in all parts of the studied plants. High contents (greater than 3.2 %) of flavonoids is noted in the leaves of *S. parviflora* (6.21 %) and *S. latifolia* (4.36 %). In comparison with previous data [24,27], in our samples of *S. controversa* and *S. salicifolia* contained significantly more amount of flavonoids. The greatest amount of catechin accumulates in the leaves of *S. parviflora* (153.6 mg%) and *S. frolowii* (188.0 mg%). High polyphenol substances concentrations in *Saussurea*species indicates that they can be considered as a raw material source for drugs that enhance blood vessel wall and having a vitamin P activity, antibacterial and antioxidant properties.

Plants synthesizing tannins are used in official medicine and national health systems as anti-inflammatory, hemostatic, and microbicides. From this point of view, the most noteworthy among studied species are *S. parviflora* and *S. latifolia*: their leaves revealed a high content of tannins (26.11 % and 29.84 % respectively). Less tannins were found in the leaves of *S. controversa* (19.24 %) and *S. frolowii* (16.47 %). *S. salicifolia* contains the least amount of tannins (8.79 %), but this indicator is in 4 times higher than the result of earlier estimates [24].

Carotenoidsprotect cells from oxidative damage, have cardiac andneuroprotective effects, prevent the development of tumors [30-32]. In medicine they are mainly used for the prevention and treatment of avitaminosis A as well as improvements to regenerate tissue under the skin damage including burns. The studied species are of great interest from this point of view because they contain these compounds in large quantities. Leaves of *S. parviflora* and *S. latifolia* accumulate a particularly large amount of carotenoids (159.23 mg% and 155.22 mg% respectively). Probably carotenoids act as additional UV absorbers and allow the protection against the production of free radicals in conditionsof the highlands where the raw material of these species was collected [33].

Pectic substances occurring in plants in the soluble (pectin) and insoluble (protopectin) forms are found in all species examined but their content is low compared to typical sources of vegetable origin.

Saponins containing in the studied raw materials are toxic in high concentrations, however in small doses have a number of useful properties: antisclerotic, expectorant, sedative, adaptogenic and other types of activity [34]. Theleavesof*S. parviflora*and*S. controversa*areparticularlyrichinsaponins (20.48 % and 19.40 % respectively).

CONCLUSION

Thus, diversity and a relatively high content of valuable in practical terms biologically active substances are revealed as a result of phytochemical studies of five Siberian *Saussurea* species. Particular attention should be paid to *S. latifolia* and *S. parviflora* accumulating a significant amount of flavonoids, carotenoids and tannins, while these

species can be attributed to the number of resource on the preliminary expert evaluation. It should be noted that the high content of carotenoids in *Saussurea* representatives is revealed for the first time. The highest content of saponins characterizes *S. parviflora*, much catechins are found in *S. frolowii*. *S. controversa* accumulates also relatively large amounts of saponins. The high content of saponins requires a detailed study of the qualitative composition of this group of substances, since they can have different effects on the body in a dose dependent manner, and their use in medical practice requires special care. The leaves are parts of the sprout system of *Saussurea* species which accumulate secondary metabolites. All species studied are recommended for further pharmacognosic and pharmacologic researches due with a lot of potential as a prospective source of flavonoids, catechins, tannins, saponins and carotenoids.

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