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Chemical Analysis of Bioactive Substances in Seven Siberian *Saussurea* Species

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Abstract. Main groups of biologically active substances of seven siberian *Saussurea* species (*S. controversa* DC., *S. latifolia* Ledeb., *S. parviflora* (Poir.) DC., *S. frolowii* Ledeb, *S. amara* (L.) DC., *S. salicifolia* (L.) DC. and *S. daurica* Adams) have been studied using paper, thin-layer, performance liquid chromatography, IR spectroscopy, spectrophotometry and mass spectrometry with inductively coupled plasma. Siberian *Saussurea* species have a rich elemental composition and contain a variety of phenolic compounds, amino acids, polysaccharides. The majority of polysaccharides are accumulated by *S. controversa*, *S. salicifolia* and *S. frolowii*. These plants contain a significant amount of calcium that may be a species characteristic. All plants contain quercetin and its glycosides, in some species luteolin, kaempferol, glycosides of apigenin and myricetin were revealed. Phenolic acids with predominant content of caffeic, chlorogenic and cinnamic acids were found in all the species. The maximum amount of phenolic acids and flavonoids was determined in the grass of *S. latifolia, S. controversa* and *S. daurica*. Characteristic absorption bands of lactone carbonyl of sesquiterpenoids in IR spectrum found in *S. latifolia*, *S. controversa, S. daurica, S. amara* and *S. salicifolia.* HPLC / UV analysis showed that peaks with absorption maxima of 242-246 nm due to the presence of α,β-unsaturated ketone group in the structure of ecdysteroids were found in *S. salicifolia*, *S. controversa*, *S. daurica* and S. *latifolia.*

INTRODUCTION

Genus *Saussurea* DC. includes about 400 species growing in India, China, Korea and Kazakhstan. About 100 species inhabit the territory of the Russian Federation [1]. Representatives of this genus have a rich history of application in folk medicine of the Far East, Siberia, Mongolia and Buryatia in the treatment of respiratory, digestive and musculoskeletal diseases [2-4]. A wide range of biological activity was detected in *S. lappa* (Decne.) Clarke. [5], *S. costus* (Falc.) Lipsch. [4], and *S. involucrata* (Kar. et Kir.) Sch. Bip. [3], used in traditional medicine of Tibet and China along with ginseng.

The chemical composition of some *Saussurea* species growing in Japan and China has been well enough studied. Thus, a number of sesquiterpenoids were isolated from the roots of *S. lappa*: costunolide and its derivatives, cinaropicrin [4,6], hermacrene and saussureamines (sesquiterpenes conjugated with aminoacids) [7,8]. Saussureamines were also isolated from *S. pulchella* (Fisch.) Fisch. [9]. New guanyan-type and eudessman-type sesquiterpenes were determined in *S. laniceps* [10], *S. elegans* Ledeb. and *S. amurensis* Turcz. [11-13].

Many *Saussurea* species are rich in triterpene compounds. A number of new triterpenes and lignans are isolated from the methanol extract of *S. japonica* (Thunb.) DC. [14]. Olean-type triterpenes were isolated from the flowers and roots of *S. muliensis* Hand.-Mazz. [15]. Taraxasterol (saussurol), lupeol, α- and ß-amirines were found in the aerial part of *S. neopulchella* Lipsch. [16].

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Saussurea species are characterized by the content of a variety of phenolic compounds which were studied by several methods in *S. medusa* Maxim. [17], S. *elegans* Ledeb. [18] and *S. involucrata* [19]. 13 phenolic components (glycosides of lyteolin, apigenin, quercetin, coumarin and syringing) were obtained by HPLC-DAD-ESI-MS^n from *S. tridactyla* Sch. Bip. ex Hook. f. [20].

As a result of the chemical screening of *Saussurea* species for the presence of ecdysteroids, it was shown that 20-hydroxyecdysone was found in *S. latifolia* [21]. The presence of ecdysteroids was established by biotest in leaves of *S. krylovii* Schischk. et Serg., *S. orgaadayi* V. Khan. et Krasnob., *S. pulchella* (Fisch.) Fisch., *S. salsa* (Pall. ex Bieb.) Spreng. [22]. The analysis of 18 *Saussurea* species growing in the Far East showed the absence of the desired compounds in the all species studied [23].

However, *Saussurea* is represented in Siberia by 54 species and 2 subspecies [24], but the chemical composition of the most of them has been studied little and superficial. The aim of the presented study is to determine the plants among the seven *Saussurea* species that accumulate the main groups of biologically active substances.

EXPERIMENTAL PART

Method of extracts preparation

The aerial parts of seven *Saussurea* species (*S. controversa* DC., *S. latifolia* Ledeb., *S. parviflora* (Poir.) DC. (section *Saussurea*), *S. frolowii* Ledeb (section *Frolovia*), *S. amara* (L.) DC. (section *Theodorea*), *S. salicifolia* (L.) DC., (section *Laguranthera*), and *S. daurica* Adams. (section *Benedicta*) were harvested in the flowering phase in 2016: *S. controversa* (Krasnoyarsk Territory, near Lake Ingol; Khakassia, surroundings of the village of Mendol; Khakassia, surroundings of the village of Efremkino), *S. latifolia* and *S. frolowii* (Khakassia, the Orlig-Taskhy mountain), *S. parviflora* (Khakassia, the Vershina Turgayula mountain), *S. amara* and *S. daurica* (Khakassia, Lake Bele), *S. salicifolia* (Khakassia surroundings of the village of Efremkino). All species was taken from the territory of the Russian Federation. The plants were dried in the shade at 20-25 °C. The air-dry raw material (humidity 7.0–9.3 %) was extracted three times with 40 % ethanol in a water bath with reverse refrigerator at 80 °C. After removal of the ethanol under vacuum at a temperature not above 45 °C, the extract was consistently extracted with chloroform, ethyl acetate and butanol. Chloroform, ethyl acetate, butanol, and aqueous fractions were dried under vacuum and subjected to acid hydrolysis on heating with 5 % sulfuric acid solution at 100–105 °C (120 min) resulting in hydrolysates of the corresponding fractions. Fractions and their hydrolysates were analyzed by paper chromatography (PC) on paper FN-4, FN-12 (Germany) and thin-layer chromatography (TLC) on "Silufol UV-254" plates (Czech Republic).

Research Methods

Triterpenic saponins of TLC in chloroform-acetone 85:15 and chloroform-methanol-AcOH 94:5.5:0.5 systems were identified (25% phosphoric-tungstic acid detector) with reliable samples: hederagenin, oleanolic and ursolic acid.

Flavonoids PC in systems of 15, 30, 60% AcOH (detectors - UV light, 5% ethanol solution of aluminum chloride) were identified with reliable samples: hyperoside, isoquercitrin, cinaroside, rutin, quercetin, kaempferol, apigenin, myricetin, baicalein, lutelion, dihydroquercetin.

Phenolic acids PC in systems 5, 15% AcOH (detector diazo-sulphanilic acid) were identified with reliable samples: cinnamic, gallic, anise, ferulic, fumaric, caffeic, ellagic, chlorogenic and quinic acids. The content of flavonoids in hydrolysed extracts in terms of quercetin and phenol acids in native extracts in terms of coffee acid was determined by spectrophotometric method at 425 and 330 nm, respectively.

Coumarins TLC in hexane-acetone (1:1), hexane-acetone-AcOH (10:20:0.1) were identified with reliable samples: esculetin and umbeliferon.

Amino acids in aqueous fractions (AF) of PC and TLC in butanol-AcOH-water 4:1:5 and 40:10:5 systems (detector of 0.2 % ninhydrin solution) were identified with reliable samples: histidine, valine, glutamic acid, tryptophan, arginine, phenylalanine, lysine, asparagine, serine, isoleucine, methionine, glycine and threonine.

Lipophilic and hydrophilic sesquiterpene lactones were determined in chloroform fractions (CF), butanol fractions (BF) and AF by IR spectroscopy due to the presence of characteristic absorption bands of lactone carbonyl (1740–1780 cm^{-1}).

The ecdysteroids were analyzed in BF by high performance liquid chromatography (HPLC) using a liquid chromatograph Shimadzu LC-20AD (Japan), a Perfect Sil Target ODS-3 chromatography column, eluted with a mixture of acetonitrile and isopropyl alcohol (5:2 v/v) in a gradient of 0.1 % trifluoroacetic acid from 15 to 35 %. The elution rate was 1 ml/min. Sample volume was 5 µl. Analytical wavelength equaled 254 nm. Detection

of ecdysteroids was carried out by comparing the retention times of peaks on sample chromatograms with those of standards.

To study the polysaccharide complex, the air-dried raw material was successively extracted with deionized water (95 °C, 1 h), water containing hydrochloric acid (50 °C, 3 h, pH 4.0) and 0.7 % ammonium oxalate solution (70 °C, 4 h). The extracts were concentrated under vacuum and precipitated with a three-fold amount of 96 % ethyl alcohol. Thus was obtained watersoluble polysaccharides (WSPS), acid polysaccharides (APS), and pectin substances (PS). The obtained fractions were dialyzed against de-ionized water for 48 hours and hydrolysed with 2 mol/L trifluoroacetic acid solution at 100 °C for 6 hours. The hydrolysates were evaporated to dryness, and a monomer composition with reliable samples of sugar (glucose, galactose, arabinose, rhamnose, xylose, galacturonic and glucuronic acids) PC in a butanol-pyridine-water 6:4:3 (detector of anilinphthalate solution) and TLC in a system of ethyl acetate-methanol-AcOH-water 60:15:15:10 (detector of 0.5 % solution of thymol in concentrated sulfuric acid) was established.

A complete elemental analysis of the raw material samples was carried out with using inductively coupled plasma mass spectrometer Agilent 7900 JP (Japan) after preparation of the raw material by acid opening (HNO_3) in a microwave preparation system.

RESULTS AND DISCUSSION

A number of biologically active substances with a diverse chemical structure in different *Saussurea* species were detected. All samples contain triterpene saponins: oleanolic acid, and *S. amara*, *S. parviflora* and *S. controversa* contain also ursolic acid glycosides (Table 1).

This is consistent with previously published data when high saponin content by gravimetric method in all studied species was established [25].

Chromatographic studies revealed that all species contain aglycons and glycosides of flavonoids, and anthocyanins were also found in *S. frolowii*. In all samples quercetin and its glycosides: isoquercitrin (*S. latifolia, S. frolowii, S. parviflora, S. controversa, S. salicifolia*), rutin (*S. frolowii*, *S. controversa*) and hyperoside (*S. controversa*, *S. salicifolia*) were detected. Apigenin (glycosides) and luteolin in *S. latifolia*, *S. frolowii*, *S. amara* and *S. daurica*, luteolin in *S. parviflora* was identified. Four species (*S. latifolia*, *S. parviflora*, *S. controversa*, *S. salicifolia*) contain kaempferol, and glycosides of myricetin in three species (*S. parviflora*, *S. controversa*, *S. amara*) were revealed. The maximum number of flavonoids in the grass of *S. latifolia*, *S. daurica* and *S. controversa* determined. These results are in agreement with the available data on the content of apigenin, luteolin, quercetin and their glycosides in *S. amara*, *S. salicifolia* and *S. parviflora* [26]. Although in general, published information about the composition of flavonoids of the study species is extremely limited.

Phenolic acids with predominant content of caffeic, chlorogenic and cinnamic acids were found in all the species. Ferulic acid was identified in four species (*S. latifolia*, *S. frolowii*, *S. amara*, *S. daurica*), ellagic acid in *S. frolowii* and *S. parviflora* and gallic acid in *S. controversa* was identified. The maximum amount of phenolic acids was determined in the grass of *S. latifolia* and *S. controversa*.

Coumarins, in particular, esculetin, were determined in *S. daurica* and *S. frolowii*, and umbelliferone was found in *S. parviflora*. Both these substances are also identified in *S. amara*, *S. salicifolia* and *S. controversa* which is consistent with early data about content of coumarins in the three last species [25].

All investigated plants have a rich amino acid composition including non-interchangeable amino acids (valine, lysine, methionine, threonine, phenylalanine, arginine) which are concentrated in aqueous fractions.

It is known that many *Saussurea* species are rich in sesquiterpene compounds characterizing family Asteraceae. Sesquiterpene lactones can be found in the form of conjugates with amino acids [7, 8] and acquire a more hydrophilic character. The presence of sesquiterpene lactones of guaian and eudesman type was established in *S. amara* [27], *S. salicifolia* [28] and *S. parviflora* [29] earlier. The characteristic absorption bands of lactone carbonyl (1740–1780 cm^{-1}) in *S. latifolia*, *S. controversa, S. daurica* chloroform fractions and in *S. amara*, *S. salicifolia* and *S. controversa* water fractions was presented.

Due to the fact that *Asteraceae* is rich of ecdysteroid-containing plants (*Rhaponticum*, *Serratula*, *Centaurea*) it is expedient to search for ecdysteroids in the previously unexplored *Saussurea* species.

HPLC / UV analysis showed that peaks with absorption maxima of 242–246 nm due to the presence of α,β-unsaturated ketone group in the structure of ecdysteroids were found in *S. salicifolia*, *S. controversa*, *S. daurica* and S. *latifolia* (Table 2) while no peaks characteristic of ecdysteroids were detected in the extracts of *S. amara*, *S. parviflora* and *S. frolowii*.

TABLE 1. Biologically active substances (BAS) of different *Saussurea* species.

Species	BAS group				
	Saponin	**Flavonoids / content, %**	**Phenolic acids / content, %**	**Coumarins**	**Amino acids**
S. latifolia	oleanolic acid (CF)	kaempferol, luteolin (EF), isoquercitrin (BF), apigenin, quercetin (HBF) / 1.16±0.16	caffeic, ferulic (EF), chlorogenic (BF, AF) / 4.67±0.81	-	phenylalanine, valine, serine, threonine (AF)
S. parviflora	oleanolic acid (CF), ursolic acid (HBF)	luteolin (EF), isoquercitrin (BF), quercetin, kaempferol (HBF), myricetin (HAF) / 0.32±0.04	caffeic, ellagic (EF), cinnamic, chlorogenic (BF, AF) / 1.00±0.13	umbelliferone (CF)	phenylalanine, valine, glutamine, serine, threonine (AF)
S. amara	oleanolic acid, ursolic acid (HBF)	luteolin, apigenin (EF), quercetin (HBF), myricetin (HAF) / 0.47±0.05	caffeic, lilac, ferulic (EF), cinnamic, chlorogenic (BF, AF) / 2.14±0.56	esculletin, umbelliferone (EF)	phenylalanine, valine, glutamine, serine, threonine (AF)
S. daurica	oleanolic acid (CF)	luteolin (HEF), apigenin, quercetin (HBF) / 1.26±0.10	caffeic, ferulic (EF), cinnamic, chlorogenic (BF, AF) / 2.61±0.61	esculletin (EF)	phenylalanine, valine, glutamine, lysine, serine, glycine (AF)
S. frolowii	oleanolic acid (CF)	luteolin (HEF), apigenin, quercetin (HBF), isoquercitrin, rutin (BF) / 0.11±0.04	caffeic, ferulic (EF), ellagic (BF), chlorogenic (BF, WF) / 0.81±0.12	esculletin (EF)	phenylalanine, valine, glutamine, lysine, serine, asparagine, methionine (AF)
S. salicifolia	oleanolic acid (CF)	apigenin, quercetin, kaempferol (EF), hyperoside isoquercitrin (BF) / 0.41±0.02	chlorogenic, cinnamic, ferulic / 2.75±0.28	esculletin, umbelliferone (EF)	phenylalanine, glutamine, methionine, threonine (AF)
S. contro-versa	oleanolic acid (CF), ursolic acid (HWF)	quercetin, kaempferol, myricetin (HBF, HAF), hyperoside isoquercitrin, rutin / 1.20±0.05	caffeic, gallic (EF, BF), chlorogenic, cinnamic (BF, WF) / 4.46±0.76	umbelliferone (CF, HEF), esculletin (EF)	valine, arginine, threonine, lysine, glycine (AF)

Note. CF–chloroform fraction, EF–ethyl acetate fraction, BF–butanol fraction, AF–aqueous fractions, HEF–hydrolysate of ethyl acetate fraction, HBF– hydrolysate of butanol fraction, HAF– hydrolysate of aqueous fractions.

TABLE 2. Characteristics of HPLC/UV of various *Saussurea* species.

Species	Retention time, min	λ max, nm
S. salicifolia	24.679	243
	30.514	243
S. controversa	24.417	243
S. latifolia	22.113	246
S. daurica	24.394	242

Note. λ max – absorption maximum.

The results showed that the *Saussurea* species can be promising sources of polysaccharides. The content of watersoluble polysaccharides (WSPS) reaches 5.87 % and pectic substances (PS) – 13.98 %. Glucose, xylose and galacturonic acid are present in all fractions of polysaccharides. WSPS fractions, in addition, contain arabinose and rhamnose while glucuronic acid was found in PS fractions (Table 3). The majority of polysaccharides are accumulated by *S. controversa*, *S. salicifolia* and *S. frolowii*. It is of interest that these three species contain the greatest amount of calcium which predominates in the elemental composition of *Saussurea* representatives.

TABLE 3. Composition of polysaccharide complex of *Saussurea* species.

Species	Amount %	Glucose	Galactose	Xylose	Ramnose	Arabinose	Glucur. acid	Galact. acid
S. latifolia								
WSPS	1.94		+		+	+		+
APS	0.09	+		+			+	+
PS	13.98	+		+			+	+
S. parviflora								
WSPS	0.82	+		+	+	+		+
APS	0.15	+		+			+	+
PS	8.85	+		+			+	+
S. amara								
WSPS	1.25	+		+	+	+		+
APS	0.11	+		+				+
PS	9.38	+		+				+
S. daurica								
WSPS	1.47	+		+		+		+
APS	0.44	+		+				+
PS	12.39	+		+				+
S. frolowii								
WSPS	4.37	+			+	+		+
APS	0.28	+		+				+
PS	11.31			+			+	+
S. salicifolia								
WSPS	5.01	+		+	+	+		+
APS	0.37	+	+	+				+
PS	10.90	+		+			+	+
S. controversa								
WSPS	5.87	+				+		+
APS	0.29	+	+	+				+
PS	10.50	+	+	+			+	+

Note. WSPS –watersoluble polysaccharides, APS–acid polysaccharides, PS–pectin substances.

58 elements were detected in the studied *Saussurea* species. *S. controversa*, *S. salicifolia*, and *S. frolowii* accumulate a significant amount of calcium that may be a species characteristic (Table 4). A lot of magnesium is contained in *S. daurica* and *S. controversa*. Phosphorus and silicon are accumulated in *S. controversa*. A significant amount of sodium is contained in the raw material of *S. daurica*. A high content of zinc was found in *S. frolowii* and *S. salicifolia*.

TABLE 4. Element contents of *Saussurea* species.

Species	Ca	Mg	Si	P	K	Na	Mn	Fe	Li	B	Zn
	mg/g									mcg/g	
S. amara	7.93	1.93	0.62	0.76	17.86	3.92	0.02	0.35	2.21	14.40	23.96
S. controversa	41.38	2.83	1.29	3.78	27.46	0.19	0.09	0.61	0.38	17.40	28.87
S. daurica	9.78	7.18	0.37	1.02	9.36	39.34	0.02	0.34	7.97	32.20	43.54
S. frolowii	23.82	1.25	0.15	1.74	16.58	0.05	0.75	0.15	0.12	20.20	75.06
S. latifolia	12.10	0.89	0.07	0.98	15.32	0.03	0.27	0.08	0.05	12.00	49.88
S. parviflora	4.06	1.31	0.04	1.12	18.23	0.01	0.03	0.04	0.03	11.60	15.62
S. salicifolia	42.36	1.30	0.48	0.79	11.32	0.12	0.04	0.55	0.67	19.90	71.20

Thus, *S. controversa, S. salicifolia, S. daurica* and *S. frolowii* are characterized as the most abundant elemental composition.

CONCLUSIONS

Siberian *Saussurea* species have a rich elemental composition and contain a variety of phenolic compounds, amino acids, and polysaccharides. The majority of polysaccharides are accumulated by *S. controversa*, *S. salicifolia* and *S. frolowii*. These plants contain a significant amount of calcium that may be a species characteristic. These species are promising for further study as a source of immunomodulatory, anti-inflammatory and osteogenic means. Phenolic acids with predominant content of caffeic, chlorogenic and cinnamic acids were found in all the species and may be characteristic of *Saussurea* family. *S. latifolia, S. controversa* and *S. daurica* can be considered as a source of natural flavonoids and phenylpropanoids. A number of species (*S. latifolia*, *S. controversa, S. daurica, S. amara, S. salicifolia*) are interesting for the study of sesquiterpene lactones. The substances of all these groups have numerous biological effects, so their plant sources may be used in the development of new medicines and require further research.

REFERENCES

1. S. Ju. Lipshic, *Genus Saussurea DC. (Asteraceae)* (Leningrad, 1979), pp. 1–283.
2. *Plant resources of USSR: Flowering plants, their chemical composition and use; Family Asteraceae (Compositae)* (Nauka, St. Petersburg, 1993), pp. 165–169.
3. W-I. Chik, L. Zhu, L-L. Fan, T. Yi, G-Y. Zhu, X-J. Gou, Y-N. Tang, J. Xu, W-P. Yeung, Z-Z. Zhao, Z-L. Yu and H-B. Chenl, J. of Ethnopharmacol. **172**, 44–60 (2015).
4. M. M. Pandey, S. Rastogi and A. K. S. Rawat, J. of Ethnopharmacol. **110**, 379–390 (2007).
5. K. Zahara, S.Tabassum, S. Sabir, M. Arshad, R. Qureshi, M. S. Amjad, and S. K. Chaudhari, Asian Pacific J. of Tropical Med. **7**, 60–69 (2014).
6. A. Robinson, T. V. Kumar, E. Sreedhar, V. G. M. Naidu, S. R. Krishna, K. S. Babu, P. V. Srinivas and J. M. Rao, Bioorgan. and med. chem. Let. **18**, 4015–4017 (2008).
7. H. Matsuda, T. Kageura, Y. Inoue, T. Morikawa and M. Yoshikawa, Tetrahedron **56**, 7763–7777 (2000).
8. J-W. Kraker, M. C. R. Franssen, A. Groot, T. Shibata, H. J. Bouwmeester, Phytochemistry **58**, 481-487 (2001).
9. M. C. Yang, S. U. Choi, W. S. Choi, S. Y. Kim and K. R. Lee, J. Nat. Prod. **71**, 678–683 (2008).
10. H-B. Wang, H-P. Zhang and Y. Zhou, J. Nat. Prod. **68**, 762-765 (2005).
11. I. D. Sham'janov, D. D. Basargin and V. M. Malikov, Chem. Nat. Comp. **1**, 116–117 (1988).
12. I. D. Sham'janov and G. P. Sidyakin, Chem. Nat. Comp. **2**, 258 (1980).
13. I. D. Sham'janov, A. Mullabaev and G. P. Sidyakin, Chem. Nat. Comp. **6**, 788–789 (1983).
14. Y-H. Kuo, S-T. Way and C. Wu, J. Nat. Prod. **59**, 622-624 (1996).
15. C-M. Liu, H.-X. Wang, S-L. Wei and K. Gao, J. Nat. Prod. **71**, 789–792 (2008).
16. T. V. Bukreeva, A. L. Savard, M. A. Morozov and O. V. Matusevich, Rast. Res. **3**(50), 450–452 (2014).
17. C-Q. Fan and J-M. Yue, Bioorgan. and Med. Chem. **11**, 703-708 (2003).
18. I. D. Sham'janov, E. Kh. Batirov, M. P. Yuldashev I. D. Sham'janov A. Mullabaev, Chem. Nat. Comp. **6**, 796–797 (1983).
19. L. Lui, X. Xiao, L. Zhang and R. Zheng, Lanzhou Daxue Xuebao, Ziran Kexueban **21**(4), 80–83 (1985).
20. Z. Dawa, Y. Zhou, Y. Bai, S. Gesang, B. Bai and L. Ding, J. of Pharm. And Biomed. Anal. **48**, 1076–1081 (2008).
21. T.A. Revina, A.S. Revushkin and A.V. Rakitin, Rast. Res. **4**, 565–570 (1988).

22. S. O. Volodina, V. V. Volodin, P. G. Gorovoy, K. G. Tkachenko, E. V. Novozhilova, M. M. Ishmuratova, I. F. Chadin, V. A. Kanev and S. Lei, Turczaninowia **15**, 58–75 (2012).
23. A. N. Vorob`yova, «Taxonomy and phytoecdysteroids of the far Eastern species of the genera *Stemmacantha* Cass., *Serratula* L. and *Saussurea* DC. (Asteraceae)», Ph.D. thesis, Vladivostok, 2004.
24. M.N. Shurupova and A.A. Zverev. Int J Environ Studies. 74(5), 724–731 (2017).
25. M. Shurupova, T. Kukushkina, A. Petruk and P. Shchetinin, «Content of biologically active substances in the raw materials of some Siberian *Saussurea* species», AIP Conference Proceedings 1772, 050008 (2016), doi: 10.1063/1.4964578
26. I. S. Pogodin, E. A. Luksha and N. A. Predeyn, Chem. Nat. Raw Mat. **3**, 43–52 (2014).
27. O. A. Konovalova, K. S. Rybalko and M.T. Pimenov, Chem. Nat. Comp. **6**, 865–866 (1979).
28. V. V. Dudko and K.S. Rybalko, Chem. Nat. Comp. **4,** 424–425 (1982).
29. Z. D. Yang, K. Gao and Z-J. Jia, Phytochemistry **62**(8), 1195–1199 (2003).