Simultaneous determination of polyphenol content *Vitis amurensis* Rupr. by tandem mass spectrometry

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Abstract. *Vitis amurensis* Ruprecht contains a large number of polyphenolic compounds which are biologically active components. For the most efficient and safe extraction supercritical carbon dioxide was used. In this work, for the first time, a comparative metabolomic study of biologically active substances of wild grapes collected from five different places of the Primorsky and Khabarovsk territories is carried out. To identify target analytes in ethanol extracts of grape berries, high performance liquid chromatography (HPLC) was used in combination with an amaZon SL ion trap (manufactured by BRUKER DALTONIKS, Germany) equipped with an ESI electrospray ionization source in negative and positive ion modes. The mass spectrometer was used in the scan range *m / z* 100 - 1.700 for MS and MS / MS. Used fragmentation of the 4th order. Primary mass spectrometric results showed the presence of 94 biologically active compounds corresponding to the species *V. amurensis*, moreover, salvianolic acids F, D and G, oleanoic, ursolic, myristoleic acids, berbericinin, mearnsetin, esculin, nevadensin, stigmasterol, fucosterol, phlorizin, L-tryptophan identified for the first time in *V. amurensis*.

1 Introduction

The appearance of the first representatives of the *Vitaceae* family, belonging to the genus Vitis, must undoubtedly be attributed to the Upper Cretaceous period, when there were already types of plants very similar in leaves to vines. The absence of seeds does not allow, however, in many cases to have complete confidence in their belonging to the genus *Vitis*. [1, 51].

These types include the *Vitis dakotana* Berry vine found in the Upper Cretaceous deposits found in Harding County in South Dakota, which is very similar in appearance to modern

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vines. [2, 3]. The evolution of grape plants approaching the cultivated vine, judging by the fossil finds, took place especially intensively in Central and Southern Europe during the second half of the Tertiary period and then especially in the Quaternary period. On the territory of Russia, quite a few finds of fossils are also known belonging to the genera Cissites, Ampelopsis, Parthenocissus, and especially to the genus *Vitis*: *V. sachalinensis* Krysht. and V. crenata Heer on Sakhalin, *V. teutonica* A. Br. - near Taganrog and on the Irtysh River, as well as *V. praevinifera* Sap. - on the Krynka river. All these data show that the evolution of the vine on the territory of Russia proceeded from ancient times. This can also be seen from the fact that now the wild grape *V. sylvestris* Gmel grows in many regions of Russia [21, 22, 23].

There is very little information about the culture of East Asian grape varieties. *V. lanata* Roxb is cultivated in eastern India. and *V. tomentosa* Heyne, in Japan and Korea – *V. Thunbergii* Sieb. et Zucc. called *V. Seiboldii* hort [15].

More complete information is available regarding *V. amurensis* Rupr., which was first introduced into the culture by I.V. Michurin. In his work "Results of half a century of work" I.V. Michurin describes four forms of *V. amurensis* Rupr., which were isolated in the Far East [29, 30].

Ripe berries, fruit skins, ridges, leaves, seeds, vine bark, red grape wine are used to isolate biologically active substances. Fruits contain 65-85% water, 10-33% sugar (glucose and fructose), phlobaphene, gallic acid, quercetin, enin, glycosides - monodelphinidin and didelphinidin, acids (malic, silicic, salicylic, phosphoric, tartaric, citric, etc.) pectin and tannins, potassium, magnesium, calcium, manganese, cobalt, iron and vitamins: B_1, B_2, B_6, B_{12}, A, C, P, PP, folic acid, and enzymes.

The dominant class of biologically active compounds of grape, and especially grape ridges, are bioflavonoids and, in particular, the so-called complexes of oligomeric proanthocyanidins or condensed tannins, which are polymeric forms of flavonoids from the class of catechins [44].

In European medicine, until recently, grapes were widely used as a means of therapy and rehabilitation for a wide range of diseases: chronic recurrent inflammatory processes, tuberculosis, kidney disease, arterial hypertension, etc. The aim of this work was a comparative metabolomic study of biologically active substances of wild grapes collected in five different places in the Far Eastern taiga in the Primorsky and Khabarovsk territories (***Fig. 1***). High-performance liquid chromatography in combination with a BRUKER DALTONIKS ion trap (tandem mass spectrometry) was used to identify target analytes in extracts. This work presents a detailed study of the metabolomic composition of grape juice taken from five habitats of *V. amurensis* in the Far East: Pakhtusov Islands and Rikorda Island (Peter the Great Bay, Japanese Sea), the vicinity of Artem (Primorsky Territory), the vicinity of the river Arsenyevka (Primorsky Territory), environs of Vyazemsky (Khabarovsk Territory).

Fig.1. Wild grape *V. amurensis* harvested in the vicinity of Artem (Primorsky Territory).

2 Materials and Methods

2.1 Materials

The object of the study was the berries of the wild grape V. amurensis, collected in the floodplain of the Arsenyevka River, Primorsky Territory (N. 44°52'18”, E 133°35'12”) in the vicinity of Vyazemsky, Khabarovsk Territory (N 47°32'15", E 134°45'20"), in the vicinity of Artem, Primorsky Territory (N 43°21'34 ”, E 132°11'19”), on Rikord Island, Peter the Great Bay, Sea of Japan (N 42°52'54”, E 131°40'06”), on the Pakhtusov Islands, Peter the Great Bay, Sea of Japan (N 42°53'57”, E 131°38'45 ”).The grapes were harvested at the end of August and September 2020. All samples morphologically corresponded to the pharmacopoeial standards of the State Pharmacopoeia of the Russian Federation [45].

2.2 Methods

2.2.1 Fractional maceration

To obtain highly concentrated extracts, fractional maceration was applied. In this case, the total amount of the extractant (ethyl alcohol of reagent grade) is divided into 3 parts and is consistently infused on grapes with the first part, then with the second and third. The infusion time of each part of the extractant was 7 days.

2.2.2 Liquid chromatography

HPLC was performed using Shimadzu LC-20 Prominence HPLC (Shimadzu, Japan) was used, equipped with an UV-sensor and a Shodex ODP-40 4E reverse phase column to perform the separation of multicomponent mixtures. The gradient elution program was as follows: 0.01-4 min, 100% CH_3CN; 4-60 min, 100-25% CH_3CN; 60-75 min, 25-0% CH_3CN; control washing 75-120 min 0% CH_3CN. The entire HPLC analysis was done with a UV-detector at wavelengths of 230 ηm and 330 ηm; the temperature corresponded to 17 °C. The injection volume was 1 ml.

2.2.3 Tandem mass spectrometry

MS analysis was performed on an ion trap amaZon SL (BRUKER DALTONIKS, Germany) equipped with an ESI source in negative ion mode. The optimized parameters were obtained as follows: ionization source temperature: 70 ° C, gas flow: 4 l / min, nebulizer gas (atomizer): 7.3 psi, capillary voltage: 4500 V, end plate bend voltage: 1500V, fragmentary: 280 V, collision energy: 60 eV. An ion trap was used in the scan range m / z 100 -1.700 for MS and MS/MS. The capture rate was one spectrum/s for MS and two spectrum/s for MS/MS. Data collection was controlled by Windows software for BRUKER DALTONIKS. All experiments were repeated three times. A two-stage ion separation mode (MS/MS mode) was implemented.

3 Results and discussion

Ten of the most consumed extracts of *V. amurensis* have been selected. All of them have a rich polyphenolic composition. There were six extracts from wild *V. amurensis:* Arsenyevka River (Primorsky Territory), Vyazemsky (Khabarovsk Territory), Artem (Primorsky Territory), Rikord Island and Pakhtusov Islands, Peter the Great Bay, Japanese Sea. High accuracy mass spectrometric data were recorded on an ion trap amaZon SL BRUKER DALTONIKS equipped with an ESI source in the mode of negative ions. The four-stage ion separation mode (MS/MS mode) was implemented. All the chemical profiles of the samples were obtained by HPLC – ESI – MS/MS method. A total of 300 peaks were detected in the chromatogram (Fig.2). The combination of both ionization modes (positive and negative) in MS full scan mode gave extra certainly to the molecular mass determination. The negative ion mode provides the highest sensitivity and results in limited fragmentation, making it most suited to infer the molecular mass of the separated polyphenols, especially in cases where concentration is low. By comparing the m/z values, the RT and the fragmentation patterns with the MS^2 spectral data taken from the literature [7; 11; 12; 13; 24; 35; 46; 47; 49; 53; etc] or to search the data bases (MS2T, MassBank, HMDB). A unifying system table was compiled of the molecular masses of the target analytes isolated from the EtOH-extract of *V. amurensis* for ease of identification (Table 1). The 94 polyphenols shown in Table 1 belong to different polyphenolic families: anthocyanidins, flavones, flavonols, flavan-3-ols, flavanones, hydroxycinnamic acids, hydroxybenzoic acids, stilbenes, tannins.

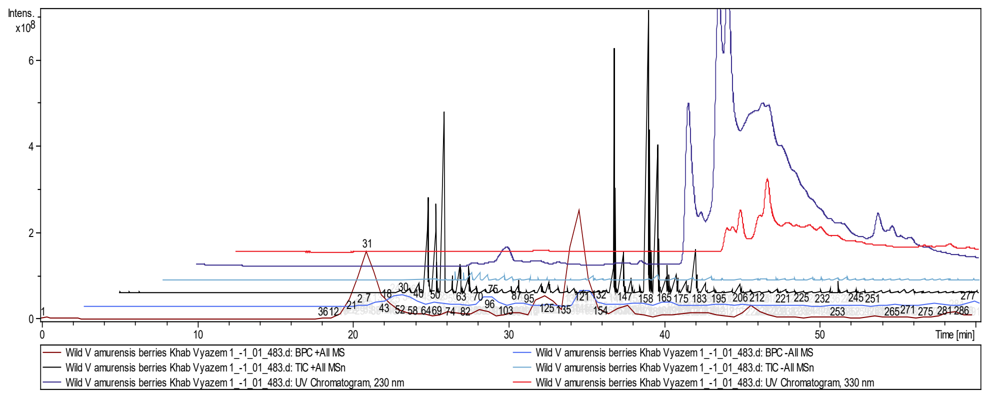

Fig.2. Chemical profiles of the *V. amurensis* (Vyazemsky, Khabarovsk Territory) sample represented total ion chromatogram from EtOH-extract.

A standardized system table of molecular weights and fragmented ions of target analytes isolated in extracts of Vitis amurensis Rupr. was compiled from the results of measurements (Table 1).

Table 1. Metabolomic analysis of biologically active substances from extracts of *Vitis amurensis* Rupr.

№	**Class of compounds**	**Identification**	**Formula**	**Molecular mass**	**Observed mass [M-H]-**	**Observed mass [M+H]+**	**MS/MS fragmentation**	**References**
		Polyphenols						
1	**Anthocyanin**	**Pelargonidin-3-*O*-glucoside (callistephin)**	$C_{21}H_{21}O_{10}$	**433.3854**		433	414; 271; 172; 116	[7; 47; 53]
2	**Anthocyanin**	**Peonidin-3-O-glucoside**	$C_{22}H_{23}O_{11}{}^{+}$	**463.4114**		**463**	301; 286; 258; 230; 202; 174	[12; 24; 53]
3	**Anthocyanin**	**Malvidin 3-*O*-glucoside**	$C_{23}H_{25}O_{12}$	**493.4374**		**493**	331; 315; 179	[12; 24; 53]
4	**Anthocyanin**	**Malvidin 3-(6-*O*-acetyl)glucoside**	$C_{25}H_{27}O_{13}$	**535.478**		**537**	331; 299; 261; 243; 211; 154	[12; 53]

5	Anthocyanin	Cyanidin 3,5-O-dihexoside	$C_{27}H_{31}O_{16}$	611.5335		611	287; 449	[7; 35]
6	Anthocyanin	Cyanidin 3,5-O-diglucoside	$C_{27}H_{31}O_{16}$	611.5335		611	287; 449	[24; 53]
7	Anthocyanin	**Peonidin-3,5-diglucoside** [Peonin; Peonidin 3-Glucoside-5-Glucoside]	$C_{28}H_{33}O_{16}$	625.5520		625	301; 463; 286; 258	[24; 53]
8	Anthocyanin	Malvidin 3-(6-*O*-coumaroyl)glucoside	$C_{32}H_{31}O_{14}$	639.5801		639	331; 315; 299; 270; 242; 179	[12; 24; 53]
9	Anthocyanin	**Petunidin 3-*O*-glucoside-5-*O*-glucoside** [Petunidin 3,5-di-*O*-beta-D-glucoside]	$C_{28}H_{33}O_{17}$	641.5514		641	317; 479; 420; 257; 302; 274	[24; 53]
10	Anthocyanin	Malvidin 3-(6'-*p*-caffeoylglucoside)	$C_{32}H_{31}O_{15}$	655.5795		655	493; 331; 315; 313; 179	[11; 12]
11	Anthocyanin	Malvidin 3,5-diglucoside	$C_{32}H_{31}O_{15}$	655.5795		655	331; 493; 299; 179	[24; 53]
12	Anthocyanin	Malvidin 3-acetyl-5-glucoside	$C_{31}H_{37}O_{18+}$	697.6147		697	535; 493; 331; 315; 299; 242; 179	[53]
13	Anthocyanin	Petunidin 3-coumaroylglucoside-5-*O*-glucoside	$C_{34}H_{43}O_{21}$	787.6926		787	625; 479; 317; 302; 301; 274; 246	[24; 53]
14	Anthocyanin	Malvidin 3-coumaroylglucoside-5-*O*-glucoside	$C_{35}H_{45}O_{21}$	801.7192		801	639; 493; 331; 315; 287; 270	[53]
15	Tannin	Procyanidin A-type dimer	$C_{30}H_{24}O_{12}$	576.501		577	425; 397; 373; 287; 245; 181	[12; 37; 49]
16	Flavone	Apigenin	$C_{15}H_{10}O_5$	270.2369		271	253; 181; 137	[46; 54]
17	Flavone	Luteolin	$C_{15}H_{10}O_6$	286.2363		287	271; 225; 175; 158	[18; 28; 48]
18	Flavone	Nevadensin	$C_{18}H_{16}O_7$	344.3154	343		328; 313; 269; 259	[32; 56]
19	Flavone	Apigenin-7-O-glucoside	$C_{21}H_{20}O_{10}$	432.3775		433	414; 287; 241; 186; 158	[12; 18; 27; 46]
20	Flavone	**Isovitexin 6"-*O*-deoxyhexoside** [Apigenin 6-*C*-glucoside 6"-*O*-deoxyhexoside]	$C_{27}H_{30}O_{14}$	578.5187		579	415; 397; 344; 297; 177	[31]
21	Flavone	**Vitexin 2"-*O*-glucoside** [Apigenin 8-*C*-glucoside 2"-*O*-glucoside]	$C_{27}H_{30}O_{15}$	594.5181		595	415; 353; 283; 265; 176	[31]
22	Flavone	Apigenin 6-*C*-[6"-acetyl-2"-*O*-deoxyhexoside]-glucoside	$C_{29}H_{32}O_{15}$	620.5554		621	561; 547; 533; 461; 433	[31]
23	Flavonol	Kaempferol	$C_{15}H_{10}O_6$	286.2363		287	269; 227; 153	[12; 27; 32; 55]
24	Flavonol	Dihydrokaempferol	$C_{15}H_{12}O_6$	288.2522		289	271;243; 199;189; 127	[8; 26; 40]
25	Flavonol	Kaempferide	$C_{16}H_{12}O_6$	300.2629		301	283; 265; 239; 211; 185	[32; 57; 60]
26	Flavonol	Quercetin	$C_{15}H_{10}O_7$	302.2357		303	285; 267; 163; 159	[9; 12; 48; 50]
27	Flavonol	**Dihydroquercetin** (Taxifolin; Taxifoliol)	$C_{15}H_{12}O_7$	304.2516		305	259; 241; 199; 149	[12; 48; 52]

28	**Flavonol**	**Isorhamnetin** [Isorhamnetol; Quercetin 3'-Methyl ether; 3-Methylquercetin]	$C_{16}H_{12}O_7$	**316.2623**		**317**	299; 270; 230; 207;177; 165; 147; 123	[10; 38; 39; 55]
29	**Flavonol**	**Myricetin**	$C_{15}H_{10}O_8$	**318.2351**	**317**		273; 255; 229; 205; 191	[12; 37; 48]
30	**Flavonol**	**3,7 -Dimethylquercetin**	$C_{17}H_{14}O_7$	**330.2889**		**331**	314; 297; 255; 267; 228; 227; 203; 146	[36]
31	**Flavonol**	**Mearnsetin**	$C_{16}H_{12}O_8$	**332.2617**		**333**	318; 301; 289; 273; 245; 193; 165; 139	[38]
32	**Flavonol**	**Kaempferol-3-*O*-galactoside**	$C_{21}H_{20}O_{11}$	**448.3769**		**449**	287; 269; 217	[9; 12]
33	**Flavonol**	**Dihydrokaempferol glucoside**	$C_{21}H_{22}O_{11}$	**450.3928**	**449**		287; 269; 227; 225	[33]
34	**Flavonol**	**Isorhamnetin 3-O-rhamonoside**	$C_{22}H_{22}O_{11}$	**462.4035**	**461**		315; 152; 219	[38; 39]
35	**Flavonol**	**Hyperoside** (Quercetin 3-*O*- galactoside; Hyperin)	$C_{21}H_{20}O_{12}$	**464.3763**	**463**		301; 179; 257; 255	[9; 12; 48]
36	**Flavonol**	**Quercetin 3-*O*-glucoside** [Isoquercitrin; Hirsutrin; Quercetin-3-O-Glucopyranoside; 3-Glucosylquercetin]	$C_{21}H_{20}O_{12}$	**464.3763**		**465**	303; 285; 257; 229; 201; 150	[9; 12; 18; 33]
37	**Flavonol**	**Taxifolin-3-*O*-glucoside**	$C_{21}H_{22}O_{12}$	**466.3922**		**467**	449; 303; 287; 132; 188	[12]
38	**Flavonol**	**Quercetin-3-*O*-glucuronide**	$C_{21}H_{18}O_{13}$	**478.3598**	**477**		301; 273; 179	[9; 12; 43; 53]
39	**Flavonol**	**Isorhamnetin 3-*O*-glucoside**	$C_{22}H_{22}O_{12}$	**478.4029**		**479**	317; 301; 257; 274; 228	[9; 12; 38]
40	**Flavonol**	**Myricetin-3-O-galactoside**	$C_{21}H_{20}O_{13}$	**480.3757**	**479**		299; 271; 243; 153	[9; 12; 37]
41	**Flavonol**	**Kaempferol-3,7-Di-O-glucoside**	$C_{27}H_{30}O_{16}$	**610.5175**		**611**	449; 287; 229; 213; 165	[25; 34]
42	**Flavan-3-ol**	**Catechin** [D-Catechol]	$C_{15}H_{14}O_6$	**290.2681**	**289**		245; 205; 203; 188	[9; 41; 48]
43	**Flavan-3-ol**	**Epicatechin**	$C_{15}H_{14}O_6$	**290.2681**		**291**	272; 175; 157; 130	[12; 33]
44	**Flavan-3-ol**	**Catechin gallate**	$C_{22}H_{18}O_{10}$	**442.3723**	**441**		289; 245; 205; 169	[12; 17]
45	**Flavanone**	**Naringenin** [Naringetol; Naringenine]	$C_{15}H_{12}O_5$	**272.5228**		**273**	227; 209; 155; 139	[12; 39; 48; 50]
46	**Flavanone**	**Hesperitin** [Hesperetin]	$C_{16}H_{14}O_6$	**302.2788**	**301**		257; 228; 189; 151	[12; 48]
47	**Flavanone**	**Eriodictyol-7-*O*-glucoside** [Pyracanthoside; Miscanthoside]	$C_{21}H_{22}O_{11}$	**450.3928**	**449**		269; 251; 207; 165	[12; 27]
48	**Hydroxycou marin**	**Umbelliferone** [Skimmetin; Hydragin]	$C_9H_6O_3$	**162.1421**	**161**		115	[12; 20]
49	**Hydroxycou marin**	**Esculetin** [Cichorigenin; Aesculetin]	$C_9H_6O_4$	**178.1415**		**179**	133; 115	[12; 58]

50	**Hydroxycoumarin**	**Esculin** [Aesculin; Esculoside; Polichrome]	$C_{15}H_{16}O_9$	**340.2821**	**339**		177; 293; 131	[12; 13; 18; 58]
51	**Coumarin**	**Fraxin** (Fraxetin-8-O-glucoside)	$C_{16}H_{18}O_{10}$	**370.3081**		**371**	208; 352; 135	[12]
52	**Coumarin**	**Fraxetin-7-O-beta-glucuronide**	$C_{16}H_{16}O_{11}$	**384.2916**		**385**	367; 272; 209; 175; 158; 143	[59]
53	**Stilbene**	**Resveratrol** [trans-Resveratrol; 3,4',5-Trihydroxystilbene; Stilbentriol]	$C_{14}H_{12}O_3$	**228.2433**		**229**	142; 184; 114	[13; 48]
54	**Stilbene**	**Polydatin** [Piceid; trans-Piceid]	$C_{20}H_{22}O_8$	**390.3839**	**389**		227; 343; 184	[33; 48]
55	**Hydroxycinnamic acid**	**p-Coumaric acid**	$C_9H_8O_3$	**164.16**		**165**	146; 119	[12; 33]
56	**Hydroxycinnamic acid**	**Caffeic acid** [(2E)-3-(3,4-Dihydroxyphenyl)acrylic acid]	$C_9H_8O_4$	**180.1574**	**179**		133	[12; 41]
57	**Hydroxycinnamic acid**	**Sinapic acid** [trans-Sinapic acid]	$C_{11}H_{12}O_5$	**224.21**		**225**	179; 153; 133; 115	[4; 12; 42]
58	**Hydroxycinnamic acid**	**Caffeoylmalic acid**	$C_{13}H_{12}O_8$	**296.2296**	**295**		133; 179; 148; 119; 115	[43]
59	**Hydroxycinnamic acid**	**Coutaric acid** [Trans-P-Coumaroyltartaric acid]	$C_{13}H_{12}O_8$	**296.2296**	**295**		163; 119	[12]
60	**Hydroxycinnamic acid**	**Caftaric acid** [Cis-Caftaric acid; 2-Caffeoyl-L-Tartaric acid; Caffeoyl Tartaric acid}	$C_{13}H_{12}O_9$	**312.23**	**311**		149; 221; 131	[6; 12; 41]
61	**Hydroxycinnamic acid**	**Fertaric acid** [Fertarate]	$C_{14}H_{14}O_9$	**326.2556**	**325**		193; 149; 134	[12]
62	**Hydroxycinnamic acid**	***p*-Coumaric acid-O-hexoside [**Trans-*p*-Coumaric acid 4-glucoside]	$C_{15}H_{18}O_8$	**326.2986**	**325**		193; 163; 119	[13; 37; 43]
63	**Hydroxycinnamic acid**	**1-Caffeoyl-beta-D-glucose** [Caffeic acid-glucoside]	$C_{15}H_{18}O_9$	**342.298**	**341**		179; 161; 135	[12; 31]
64	**Hydroxycinnamic acid**	**5-*O*-(4'-*O*-p-coumaroyl glucosyl)quinic acid**	$C_{22}H_{28}O_{13}$	**500.4499**		**501**	339; 277; 203	[18]
65	**Hydroxycinnamic acid**	***p*-Coumaroylcaffaoylquinic acid**	$C_{25}H_{24}O_{11}$	**500.4515**		**501**	355; 483; 281; 225; 193; 181	[49]
66	**Hydroxycinnamic acid**	**Coumaric acid derivative**	$C_{30}H_{30}O_7$	**502.5550**		**503**	457; 411; 391; 367; 382; 339; 293	[43]
67	**Hydroxybenzoic acid**	**Gallic acid**	$C_7H_6O_5$	**170.1195**		**171**	126	[12; 39]
68	**Hydroxybenzoic acid**	**Ellagic acid** [Benzoaric acid; Elagostasine; Lagistase; Eleagic acid]	$C_{14}H_6O_8$	**302.1926**		**303**	172; 158; 144; 127; 116	[12; 33; 47]
69	**Hydroxybenzoic acid**	**Salvianolic acid F**	$C_{17}H_{14}O_6$	**314.2895**		**315**	269; 243; 213; 207; 185; 181; 153; 144	[6]
70	**3,4-dihydroxybenzoic acid**	**Dihydroxybenzoyl-hexoside**	$C_{13}H_{16}O_9$	**316.2607**	**315**		153; 253; 284	[31]
71	**Hydroxybenzoic acid**	**Salvianolic acid G**	$C_{18}H_{12}O_7$	**340.2837**		**341**	323; 295; 255; 195; 159	[19; 56]
72	**Hydroxybenzoic acid**	**Salvianolic acid D**	$C_{20}H_{18}O_{10}$	**418.3509**	**417**		373; 329; 287; 209	[5; 6]
73	**Hydroxybenzoic acid**	**Dimethyl ellagic acid hexose**	$C_{22}H_{20}O_{13}$	**492.3864**		**493**	331; 299; 270; 242; 179; 150	[14]
		Others						

74	**2-hydroxycarboxylic acid**	**Malic acid** [DL-Malic acid]	$C_4H_6O_5$	**134.0874**	**133**		115	[6; 16; 43]
75	**Dicarboxylic acid**	**Tartaric acid**	$C_4H_6O_6$	**150.09**	**149**		131	[16]
76	**Auxin**	**Indole-3-carboxylic acid**	$C_{10}H_9NO_2$	**175.1840**		**176**	130	[36]
77	**Tricarboxylic acid**	**Citric acid** [Anhydrous; Citrate]	$C_6H_8O_7$	**192.1235**	**191**		111; 173; 143; 127	[28; 43; 47]
78	**Polyhydroxy carboxylic acid**	**Quinic acid**	$C_7H_{12}O_6$	**192.1666**	**191**		111; 173	[12; 13; 28]
79	**Propionic acid**	**Dihydroferulic acid**	$C_{10}H_{12}O_4$	**196.1999**	**195**		159; 129; 122; 113	[13]
80	**Gallate ester**	**Ethyl gallate**	$C_9H_{10}O_5$	**198.1727**	**197**		169; 125	[14]
81	**Amino acid**	**L-Tryptophan** [Tryptophan; (S)-Tryptophan]	$C_{11}H_{12}N_2O_2$	**204.2252**		**205**	188; 146; 170; 118	[31; 47]
82	**Carboxylic acid**	**Myristeolic acid**	$C_{14}H_{26}O_2$	**226.3550**		**227**	209; 199; 181; 155; 127	[13]
83	**Omega-3 acid**	**Linolenic acid** (Alpha-Linolenic acid; Linolenate)	$C_{18}H_{30}O_2$	**278.4296**		**279**	260; 176; 120	[4]
84	**Saturated long-chain fatty acid**	**9-Oxo-10E,12Z-octadecanoic acid** [9-Oxo-ODE]	$C_{18}H_{30}O_3$	**294.4290**		**295**	249; 220; 165; 125	[13]
85	**Phenolic glycoside**	**Protocatechuic acid-O-hexoside**	$C_{13}H_{16}O_9$	**316.2607**	**315**		153; 151; 298	[43; 50]
86	**Gallate ester**	**Galloyl glucose** [Beta-Glucogallin; 1-O-Galloyl-Beta-D-Glucose]	$C_{13}H_{16}O_{10}$	**332.2601**	**331**		313; 195; 166	[47]
87	**Gallic acid hexoside**	**Gallic acid hexoside**	$C_{13}H_{16}O_{10}$	**332.2601**	**331**		271; 169; 125	[37]
88	**Berberine alkaloid**	**Palmatine** [Berbericinine; Burasaine]	$C_{21}H_{22}NO_4$	**352.4037**		**353**	335; 317; 235; 137	[58]
89	**Amino sugar**	**Hexose-hexose-N-acetyl**	$C_{14}H_{25}NO_{10}$	**367.3490**	**366**		186; 142	[25]
90	**Sterol**	**Fucosterol** [Fucostein; Trans-24-Ethylidenecholesterol]	$C_{29}H_{48}O$	**412.6908**		**413**	395; 355; 297; 271; 199; 268; 194; 119	[13]
91	**Sterol**	**Stigmasterol** [Stigmasterin; Beta-Stigmasterol]	$C_{29}H_{48}O$	**412.6908**		**413**	301; 259; 189; 171	[13]
92	**Dihydrochalcone**	**Phlorizin** [Phloridzin; Phlorizoside; Floridzin: phlorrhizin; Phloretin 2'-Glucoside; Phloretin-*O*-hexoside]	$C_{21}H_{24}O_{10}$	**436.4093**		437	397; 377; 217	[12; 13]
93	**Triterpene**	**Oleanoic acid**	$C_{30}H_{48}O_3$	**456.7003**		**457**	439; 411; 365; 364; 337; 309; 293; 248; 205	[32; 49]
94	**Triterpene**	**Ursolic acid**	$C_{30}H_{48}O_3$	**456.7003**		**457**	411; 393; 365; 337; 279; 247; 219; 205	[32; 49; 56]

Research carried out using tandem mass spectrometry showed the presence of 94 biologically active compounds corresponding to the species *V. amurensis*, moreover, salvianolic acids F, D and G, oleanolic, ursolic, myristoleic acids, berbericinin, mearnsetin, esculin, nevadensin, stigmasterol, fucosterol, phlorizin, L-tryptophan were identified for the first time in V. amurensis.

Anthocyanins have been identified in extracts: Malvidin-3-*O*-glucoside, Pelargonidin-3-*O*-glucoside (callistephin), Peonidin-3-*O*-glucoside, Cyanidin-3,5-dihexoside, Cyanidin-3,5-diglucoside, Peonidin-3,5-diglucoside, Malvidin 3-(6-*O*-coumaroyl) glucoside, Petunidin-3-*O*-glucoside-5-*O*-glucoside, Malvidin 3-(6'-*p*-caffeoyl glucoside), Malvidin 3,5-*O*-diglucoside. A large group of flavonoids identified; flavonols: Kaempferol, Aromadendrin, Kaempferide, Quercetin, Dihydroquercetin, Kaempferol-3-*O*-galactoside, Quercetin 3-*O*-galactoside, Taxifolin-3-*O*-glucoside, Quercetin-3-*O*-glucuronide, Isorhamnetin-3-*O*-rhamonoside, Isorhamnetin-3-*O*-glucoside, Myricetin-3-*O*-galactoside, Kaempferol-3,7-Di-*O*-glucoside; flavones: Apigenin, Luteolin, Nevadensin, Apigenin-7-*O*-glucoside, Isovitexin 6"-O-deoxyhexoside, Vitexin 2"-*O*-glucoside, Apigenin 6-*C*-[6"-acetyl-2"-*O*-deoxyhexoside]-glucoside; flavanones: Naringenin, Hesperitin, Eriodictyol-7-*O*-glucoside; flavan-3-ols: Catechin, Epicatechin.

Glycosylated coumarins have also been identified: Umbelliferone, Esculin, Fraxin, Fraxetin-7-*O*-beta-glucuronide, berberine Palmatine, stilbenes Polydatin and trans-Resveratrol, sterols: Fucosterol, Stigmasterol, dihydrochalcone Phlorizin.

Отдельно необходимо отметить, что такие соединения, как кумарины Umbelliferone, Fraxin и Esculin, флавон Nevadensin, флаван-3-ол Epicatechin, стерол Fucosterol, флаванол Taxifolin-3-O-glucoside были идентифицированы с помощью масс-спектрометрического исследования только в островных образцах дикого винограда *V. amurensis* (острова Пахтусова и остров Рикорда, залив Петра Великого, Японское море).

Separately, it should be noted that compounds such as coumarins Umbelliferone, Fraxin and Esculin, flavone Nevadensin, flavan-3-ol Epicatechin, sterol Fucosterol, flavanol Taxifolin-3-O-glucoside were identified by mass spectrometric studies only in island samples of wild *V. amurensis* grapes (Pakhtusov Islands and Rikord Island, Peter the Great Bay, Japanese Sea).

4 Conclusions

Amur grape *V. amurensis* Rupr. contains a large number of polyphenolic complexes, which are biologically active compounds. In this work, we have tried for the first time to carry out a comparative metabolic study of biologically active substances of wild grapes obtained from five different places in the Primorsky and Khabarovsk territories. High performance liquid chromatography (HPLC) in combination with a BRUKER DALTONIKS ion trap (tandem mass spectrometry) was used to identify target analytes in extracts. The results showed the presence of 94 biologically active compounds corresponding to the species V. amurensis, and salvianolic acids F, D and G, oleanolic, ursolic, myristeolic acids, berbericinin, mearnsetin, esculin, nevadensin, stigmasterol, fucosterol, phlorizin, L-tryptophan were identified for the first time in *V. amurensis*.

The findings may support future research into the production of various pharmaceutical and dietary supplements containing *V. amurensis* extracts. A wide variety of biologically active polyphenolic compounds opens up rich opportunities for the creation of new drugs and biologically active additives based on extracts from this family of grapes (*Vitaceae*).

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