Identification of phenolic constituents in *Lonicera caerulea* L. by HPLC with diode array detection electrospray ionisation tandem mass spectrometry

Mayya P. Razgonova [1,2*], *Nadezhda G.* Tikhonova[1], *Andrey S.* Sabitov[1], *Natalia M.* Mikhailova[1], *Svetlana R.* Luchko[1], *Alexander M.* Zakharenko [1,2], *Konstantin S.* Pikula [1], and *Kirill S.* Golokhvast [1,2,3,4]

[1] N.I. Vavilov All-Russian Institute of Plant Genetic Resources, B. Morskaya 42-44, 190000, Saint-Petersburg, Russian Federation
[2] Far Eastern Federal University, Sukhanova 8, 690950, Vladivostok, Russian Federation
[3] Siberian Federal Scientific Centre of Agrobiotechnology, 633501, Centralnaya, Presidium, Krasnoobsk, Russian Federation
[4] Pacific Geographical Institute, Far Eastern Branch of the Russian Academy of Sciences, Radio 7, 690041, Vladivostok, Russian Federation

Abstract: The purpose of this work was a comparative metabolomic study of extracts of Blue-berried honeysuckle *Lonicera caerulea* L.: №1043-11 (St. Petersburg); №1043-08 (St. Petersburg) №863; (Japan); №860 (Wild *Lonicera* from Amur river) from the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources. To identify target analytes in extracts HPLC was used in combination with a BRUKER DALTONIKS ion trap. The results showed the presence of 82 target analytes corresponding to family *Caprifoliaceae*. In addition to the reported metabolites, a number of metabolites were newly annotated in *Lonicera caerulea* L. There were flavonols: Dihydrokaempferol, Rhamnetin I, Rhamnetin II, Taxifolin-3-*O*-glucoside, Mearnsetin-hexoside, Horridin; flavones: Chrysoeriol, Apigenin-*O*-pentoside, Chrysoeriol-7-*O*-glucoside; flavanone Naringenin; flavan-3-ols: Catechin, Epicatechin, Biochanin A-7-*O*-glucoside; essential amino acids: L-Pyroglutamic acid, Tyrosine; polypeptide 5-Oxo-L-propyl-L-isoleucine; sterols: Ergosterol, Fucosterol, Beta-Sitosterin; triterpenoids: Betunolic acid, Oleanoic acid; anabolic steroid Vebonol, indole sesquiterpene alkaloid Sespendole; iridoids: Monotropein, *p*-Coumaroyl monotropein, *p*-Coumaroyl monotropein hexoside; Myristoleic acid, etc.

1 Introduction

Blue-berried honeysuckle *Lonicera caerulea* L., family *Caprifoliaceae* is known as a natural source of food, beverages and nutraceuticals due to its rich chemical composition, enriched with nutrient and biologically active compounds. The increased focus on these berries is due to their phenolic composition, antioxidant activity, and potential health benefits. The high content of phenols in *Lonicera caerulea* L. is directly related to their biological activity. Popularity of phenolic compounds has grown in recent years as they are excellent antioxidants. Antioxidant intake has been shown to be effective in preventing cancer, cardiovascular disease, osteoporosis, obesity, diabetes, and other health problems [Dias et al., 2017]. The antioxidant properties of plant phenolic compounds are relevant in the field of nutrition (inhibition of lipid oxidation), physiology (protection against oxidative stress) and cosmetology. Phenolic compounds provide antioxidant activity through direct reduction of reactive oxygen species (ROS), inhibition of enzymes involved in oxidative stress, binding of metal ions responsible for ROS production, and stimulation of endogenous antioxidant defense systems [Hossain et al., 2016]. The quality and quantity of phenolic compounds in plants usually depends on the stage of growth, the parts of the plant used and the growing conditions in the environment [Bujor O.-C., 2016].

In this regard, the purpose of this work is the simultaneous assessment of phenolic compounds in the berries of *Lonicera caerulea* L. of various species collected in different climatic-geographical zones of Russia. This study is a complete qualitative study of phenols and other compounds, leading to the identification of a large number of phenolic secondary metabolites isolated from *Lonicera caerulea* L. berries of various species.

* Corresponding author: razgonova.mp@dvfu.ru (M.R.)

Fig. 1. Polymorphism of wild *Lonicera caerulea* L. berries presented in the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources

Initial LC-MS/MS screening suggested that 82 target analytes detected in EtOH-extracts of Blue-berried honeysuckle. Therefore, tandem mass spectrometry was used in this study for comparative small molecule profiling of four *Lonicera* varieties cultivated in the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources.

2 Experimental

2.1 Materials

The object of the study was the four varieties of Blue-berried honeysuckle *Lonicera caerulea* L. of breeding varieties obtained as a result of many years of research from the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources. There were a varieties: №1043-11 (St. Petersburg); №1043-08 (St. Petersburg); №863 (Japan); №860 (Wild *Lonicera* from Amur river). The berries were harvested at the end of July 2020. All samples morphologically corresponded to the pharmacopoeial standards of the State Pharmacopoeia of the Russian Federation [SPh XIV, Russia, 2018].

2.2 Chemicals and Reagents

HPLC-grade acetonitrile was purchased from Fisher Scientific (Southborough, UK), MS-grade formic acid was from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water was prepared from a SIEMENS ULTRA clear (SIEMENS water technologies, Germany), and all other chemicals were analytical grade.

2.3 Fractional maceration.

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

2.4 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; 4-35 min, 100-25% CH_3CN; 35-50 min, 25-0% CH_3CN; control washing 50-60 min 0% CH_3CN. The entire HPLC analysis was done with a ESI detector at wavelengths of 230 ηm and 330 ηm; the temperature corresponded to 17°C. The injection volume was 1 ml.

2.5 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 four-stage ion separation mode (MS/MS mode) was implemented.

3 Results and discussion

Four of the most consumed extracts of *Lonicera caerulea* L. have been selected. All of them have a rich bioactive composition. There were four extracts from a varieties: №1043-11 (St. Petersburg); №1043-08 (St. Petersburg); №863 (Japan); №860 (Wild *Lonicera,* Amur river) from the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources.

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-positive ions. The four-stage ion separation mode (MS/MS mode) was implemented. The combination of both ionization modes (positive and negative) in MS full scan mode gave extra certainly to the molecular mass determination (*Fig. 2,3,4*). The positive-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 [Abeywickrama et al., 2016; Abu-Reidah et al., 2015; Rafsanjany et al., 2015; Goufo *et al.*, 2020; Paudel et al., 2013; Jaiswal et al., 2014; De Rosso et al., 2014; Marzouk et al., 2018; Barros et al., 2012; Pradhan & Saha, 2016; da Silva et al., 2019; Ruiz et al., 2013; Ruiz et al., 2010; Razgonova et al., 2020; Kajdzanoska et al., 2010] 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 *Lonicera caerulea* L. for

ease of identification (***Table 1***). The 82 target analytes shown in Table 1 belong to different polyphenolic families: flavones, flavonols, flavan-3-ols, flavanones, anthocyanins, hydroxycinnamic acids, hydroxybenzoic acids, stilbenes, proanthocyanidins and belong to others classes of compounds.

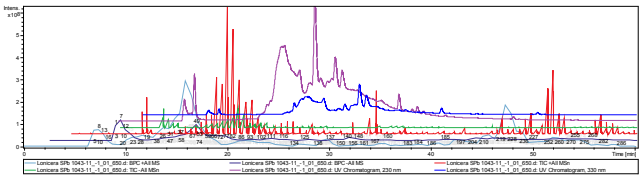

Fig.2. Chemical profiles of the *Lonicera caerulea* L. (variety SPb 1043-11) sample represented total ion chromatogram from MeOH-extract.

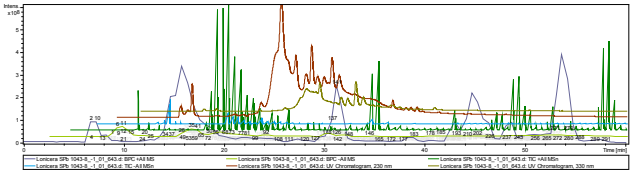

Fig.3. Chemical profiles of the *Lonicera caerulea* L. (variety SPb 1043-8) sample represented total ion chromatogram from MeOH-extract.

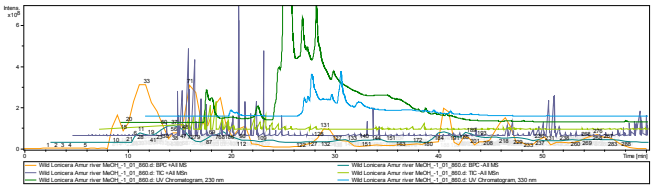

Fig.4. Chemical profiles of the *Lonicera caerulea* L. (variety Wild lonicera from Amur river) sample represented total ion chromatogram from MeOH-extract.

In addition to the reported metabolites, a number of metabolites were newly annotated in *Lonicera caerulea* L. There were flavonols: Dihydrokaempferol, Rhamnetin I, Rhamnetin II, Taxifolin-3-*O*-glucoside, Mearnsetin-hexoside, Horridin; flavones: Chrysoeriol, Apigenin-*O*-pentoside, Chrysoeriol-7-*O*-glucoside; flavanone Naringenin; flavan-3-ols: Catechin, Epicatechin, Biochanin A-7-*O*-glucoside; essential amino acids: L-Pyroglutamic acid, Tyrosine; polypeptide 5-Oxo-L-propyl-L-isoleucine; sterols: Ergosterol, Fucosterol, Beta-Sitosterin; triterpenoids: Betunolic acid, Oleanoic acid; anabolic steroid Vebonol, indole sesquiterpene alkaloid Sespendole; iridoids: Monotropein, *p*-Coumaroyl monotropein, *p*-Coumaroyl monotropein hexoside; Myristoleic acid, etc.

Table 1. Identified target analytes in MeOH extracts of berries of *Lonicera caerulea* L.

№	№ of collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources	Class of compounds	Identification	Formula	Calculated mass	Observed mass [M-H]-	Observed mass [M+H]+	MS/MS Stage 1 fragmentation	MS/MS Stage 2 fragmentation	MS/MS Stage 3 fragmentation
		POLYPHENOLS								
1	**SPb 1043-11; SPb 1043-8**	**Flavonol**	**Kaempferol**	**$C_{15}H_{10}O_6$**	**286.2363**		**287**	269; 149	239; 181	
2	**863; SPb 1043-8**	**Flavonol**	**Dihydrokaempferol**	**$C_{15}H_{12}O_6$**	**288.2522**		**289**	176; 144; 272	144	116
3	**863; 860; SPb 1043-8**	**Flavonol**	**Quercetin**	**$C_{15}H_{10}O_7$**	**302.2357**		**303**	257; 146	229	201; 145
4	**SPb 1043-11**	**Flavonol**	**Rhamnetin I** [beta-Rhamnocitrin; Quercetin 7-Methyl ether]	**$C_{16}H_{12}O_7$**	**316.2623**		**317**	299; 213	267	239
5	**863**	**Flavonol**	**Rhamnetin II**	**$C_{16}H_{12}O_7$**	**316.2623**		**317**	302	274; 153; 121	229; 153; 121
6	**863; SPb 1043-11**	**Flavonol**	**Isorhamnetin** [Isorhamnetol; Quercetin 3'-Methyl ether; 3-Methylquercetin]	**$C_{16}H_{12}O_7$**	**316.2623**	**315**		283	255; 211	227
7	**SPb 1043-8**	**Flavonol**	**Kaempferol-3-*O*-hexoside**	**$C_{21}H_{20}O_{11}$**	**448.3769**		**449**	287	213	213
8	**863**	**Flavonol**	**Quercetin-3-(3-O-arabinosyl)glucoside**	**$C_{26}H_{28}O_{16}$**	**596.4909**		**597**	303; 465	257; 165	229
9	**863; 860**	**Flavonol**	**Quercetin 3-*O*- glucoside** [Isoquercitrin; Hirsutrin; Quercetin-3-O-Glucopyranoside; 3-Glucosylquercetin]	**$C_{21}H_{20}O_{12}$**	**464.3763**		**465**	303	229; 165	201; 161
10	**863; SPb 1043-11; SPb 1043-8; 860**	**Flavonol**	**Taxifolin-3-*O*-glucoside**	**$C_{21}H_{22}O_{12}$**	**466.3922**		**467**	449; 287	377; 279	345; 283
11	**860**	**Flavonol**	**Kaempferol acetyl hexoside**	**$C_{23}H_{22}O_{12}$**	**490.4136**		**491**	257	183	
12	**SPb 1043-11**	**Flavonol**	**Mearnsetin-hexoside**	**$C_{22}H_{22}O_{13}$**	**494.4023**		**495**	477; 387	387; 315; 199	
13	**863**	**Flavonol**	**Horridin** [Quercetin 3-Rhamnosyl-(1->2)-Rhamnoside]	**$C_{27}H_{30}O_{15}$**	**594.5181**		**595**	463; 432; 301	301	286
14	**SPb 1043-11**	**Flavonol**	**Kaempferol 3-O-(6-O-rhamnosyl-glucoside)**	**$C_{27}H_{30}O_{15}$**	**594.5181**		**595**	287	213	185

15	**863; SPb 1043-11**	**Flavonol**	**Rutin** (Quercetin 3-*O*-rutinoside)	**$C_{27}H_{30}O_{16}$**	**610.5175**		**611**	303; 197	285; 229; 195	229
16	**860**	**Flavan-3-ol**	**Catechin** [D-Catechol]	**$C_{15}H_{14}O_6$**	**290.2681**		**291**	289; 159	230; 127	
17	**SPb 1043-11**	**Flavan-3-ol**	**Epicatechin**	**$C_{15}H_{14}O_6$**	**290.2681**		**291**	273; 137		
18	**863**	**Flavan-3-ol**	**Biochanin A-7-*O*-glucoside**	**$C_{22}H_{22}O_{10}$**	**446.4041**		447	245; 187	217	148; 182
19	**SPb 1043-11; SPb 1043-8**	**Flavone**	**Apigenin** [5,7-Dixydroxy-2-(40Hydroxyphenyl)-4H-Chromen-4-One]	**$C_{15}H_{10}O_5$**	**270.2369**		**271**	225	179	
20	**SPb 1043-11**	**Flavone**	**Chrysoeriol** [Chryseriol]	**$C_{16}H_{12}O_6$**	**300.2629**		**301**	255; 157	209	135
21	**SPb 1043-11**	**Flavone**	**Apigenin-O-pentoside**		**448**		449	403; 287; 216	347; 137	291
22	**863; 860; SPb 1043-11**	**Flavone**	**Luteolin 7-*O*-glucoside** [Cynaroside]	**$C_{21}H_{20}O_{11}$**	**448.3769**		**449**	287	269; 241; 132	133
23	**860**	**Flavone**	**Chrysoeriol-7-O-glucoside**	**$C_{22}H_{22}O_{11}$**	**462.4035**		**463**	301; 243	183	
24	**SPb 1043-11**	**Flavone**	**Diosmin** [Diosmetin-7-*O*-rutinoside; Barosmin; Diosimin]	**$C_{28}H_{32}O_{15}$**	**608.5447**		**609**	591; 531	531	487
25	**863**	**Flavanone**	**Naringenin** [Naringetol; Naringenine]	**$C_{15}H_{12}O_5$**	**272.5228**		**273**	147; 246		
26	**SPb 1043-11**	**Anthocyanin**	**Delphinidin**	**$C_{15}H_{11}O_7$**	**303.2436**		**304**	212; 149	212; 145	
27	**SPb 1043-11**	**Anthocyanin**	**Petunidin**	**$C_{16}H_{13}O_{7+}$**	**317.2702**		**318**	256	238; 113	238
28	**863**	**Anthocyanin**	**Cyanidin-pentoside**	**$C_{20}H_{19}O_{10}$**	**419.3589**		419	287	259; 188; 133	160
29	**863; SPb 1043-11**	**Anthocyanin**	**Cyanidin-3-O-glucoside [Cyanidin 3-O-beta-D-Glucoside]**	**$C_{21}H_{21}O_{11}$ +**	**449.3848**		**449**	287	287; 213;	185; 141
30	**SPb 1043-11**	**Anthocyanin**	**Peonidin-3-O-galactoside**	**$C_{22}H_{23}O_{11}$ +**	**463.4114**		**463**	301	286	258; 150
31	**SPb 1043-8**	**Anthocyanin**	**Peonidin-3-O-glucoside**	**$C_{22}H_{23}O_{11}$ +**	**463.4114**		**463**	301	286	258; 200
32	**863**	**Anthocyanin**	**Peonidin 3-O-acetyl hexoside**	**$C_{24}H_{25}O_{12}$**	**505.4481**		**506**	303; 487	303; 229; 165	201; 159
33	**SPb 1043-11; SPb 1043-8;**	**Anthocyanin**	**Delphinidin 3-*O*-Beta-*D*-sambubioside**	**$C_{26}H_{29}O_{16}$**	**597.4989**		**597**	303; 465; 229	229; 165	201; 172
34	**SPb 1043-11; SPb 1043-8;**	**Anthocyanin**	**Peonidin 3-*O*-rutinoside**	**$C_{28}H_{33}O_{15}$**	**609.5526**		**609**	301; 463	286	258
35	**863; 860; SPb 1043-11; SPb 1043-8**	**Anthocyanin**	**Cyanidin 3,5-*O*-diglucoside**	**$C_{27}H_{31}O_{16}$**	**611.5335**		**611**	287; 449	287; 213	185

36	**SPb 1043-11**	**Anthocyanin**	**Peonidin-3,5-*O*-diglucoside** [Peonin; Peonidin 3-Glucoside-5-Glucoside]	**$C_{28}H_{33}O_{16}$**	**625.5520**		**625**	301; 463	286	258
37	**863**	**Hydroxybenzoic acid (Phenolic acid)**	**4-Hydroxybenzoic acid** [PHBA; Benzoic acid; p-Hydroxybenzoic acid]	**$C_7H_6O_3$**	**138.1207**		**139**	121		
38	**863; 860**	**Hydroxybenzoic acid (Phenolic acid)**	**Gallic acid**	**$C_7H_6O_5$**	**170.1195**		**171**	139	111	
39	**863; SPb 1043-11**	**Hydroxybenzoic acid (Phenolic acid)**	**Ellagic acid** [Benzoaric acid; Elagostasine; Lagistase; Eleagic acid]	**$C_{14}H_6O_8$**	**302.1926**	**301**		257	229	201
40	**863**	**Hydroxybenzoic acid (Phenolic acid)**	**Salvianolic acid D**	**$C_{20}H_{18}O_{10}$**	**418.3509**	**417**		373	347	303
41	**SPb 1043-11; SPb 1043-8**	**Methylbenzoic acid**	**Methylgallic acid** [Methyl gallate]	**$C_8H_8O_5$**	**184.1461**		**185**	139	111	
42	**863**	**Hydroxycinnamic acid**	**Cinnamic acid** (Trans-cinnamic acid; Phenylacrylic acid)	**$C_9H_8O_2$**	**148.1586**	**147**		129		
43	**SPb 1043-11**	**Hydroxycinnamic acid**	**Caffeic acid** [(2E)-3-(3,4-Dihydroxyphenyl)acrylic acid]	**$C_9H_8O_4$**	**180.1574**		**181**	135	119	
44	**863; 860; SPb 1043-11; SPb 1043-8**	**Hydroxycinnamic acid**	**Chlorogenic acid** [3-O-Caffeoylquinic acid]	**$C_{16}H_{18}O_9$**	**354.3087**	**353**		191	127	
45	**860**	**Hydroxycinnamic acid**	**Feruloylquinic acid**	**$C_{17}H_{20}O_9$**	**368.3353**	**367**		179; 135	135	
46	**863**	**Hydroxycinnamic acid**	**5-*O*-Feruloylquinic acid**	**$C_{17}H_{20}O_9$**	**368.3353**		**369**	163	145	117
47	**SPb 1043-11; SPb 1043-8**	**Hydroxycinnamic acid**	**3-*O*-Hydroxydihydrocaffeoylquinic acid**	**$C_{16}H_{20}O_{10}$**	**372.3240**	**371**		191	127	
48	**863; 860;**	**Hydroxycinnamic acid**	**5-O-(4'-O-Feruloyl glucosyl)quinic acid**	**$C_{23}H_{30}O_{14}$**	**530.4759**		**531**	299; 245	281; 167	149
49	**863; SPb 1043-11**	**Cinnamic acid**	**Ferulic acid**	**$C_{10}H_{10}O_4$**	**194.184**	**193**		161	133	
50	**860**	**Stilbene**	**Resveratrol** [trans-Resveratrol; 3,4',5-Trihydroxystilbene; Stilbentriol]	**$C_{14}H_{12}O_3$**	**228.2433**		**229**	211	183; 127	138
51	**863**	**Stilbene**	**Oxyresveratrol**	**$C_{14}H_{12}O_4$**	**244.2427**		**245**	220; 173; 112		
52	**860;**	**Oligomeric proanthocyanidins**	**Epiafzelechin** [(epi)Afzelechin]	**$C_{15}H_{14}O_5$**	**274.2687**		**275**	185; 244	157	

53	**SPb 1043-11**	**Oligomeric proanthocyanidins**	**(*Epi*)Catechin-A-(*epi*)afzelechin**	**$C_{30}H_{24}O_{11}$**	**560.5050**		**561**	399; 278; 201	325; 255; 191; 132	
54	**SPb 1043-11**	**Oligomeric proanthocyanidins**	**(Epi)catechin-(4,8'/2,6')-(epi)catechin**		**576**		**577**	559; 447; 377; 306; 265; 179	247; 121	175
55	**SPb 1043-11**	**Oligomeric proanthocyanidin**	**3-O-Galloyl (epi)catechin-(4,8)-(epi)gallocatechin**		**746**		**748**	575; 466; 379; 270	318; 264; 175	235; 161
56	**SPb 1043-11; SPb 1043-8**	**Aryl-beta-glycoside**	**Arbutin**	**$C_{12}H_{16}O_7$**	**272.2512**		**273**	227	181	135
		OTHERS								
57	**SPb 1043-8**	**Non-proteinogenic L-alpha-amino acid**	**L-Pyroglutamic acid** [Pidolic acid; 5-Oxo-L-Proline]	**$C_5H_7NO_3$**	**129.1140**		**130**	111		
58	**SPb 1043-11**	**Amino acid**	**Leucine [**(S)-2-Amino-Methylpentanoic acid]	**$C_6H_{13}NO_2$**	**131.1729**		**132**	130	112	
59	**860; SPb 1043-8**	**Amino acid**	**Phenylalanine** [L-Phenylalanine]	**$C_9H_{11}NO_2$**	**165.1891**		**166**	120		
60	**SPb 1043-11; SPb 1043-8; 860**	**Cyclohexenecarboxylic acid**	**Shikimic acid** [L-Schikimic acid]	**$C_7H_{10}O_5$**	**174.1513**		**175**	128	111	
61	**863**	**Amino acid**	**Tyrosine [**(2S)-2-Amino-3-(4-Hydroxyphnyl)Propanoic acid]	**$C_9H_{11}NO_3$**	**181.1885**		**179**	133	115	
62	**SPb 1043-8**	**Propenyl**	**Methyl eugenol**	**$C_{11}H_{14}O_2$**	**178.2277**		**179**	151	123	
63	**863**	**Dicarboxylic acid**	**Azelaic acid [**Nonanedioic acid; Anchoic acid; Finacea]	**$C_9H_{16}O_4$**	**188.2209**		**189**	171	139	111
64	**863; 860; SPb 1043-8**	**Tricarboxylic acid**	**Citric acid** [Anhydrous; Citrate]	**$C_6H_8O_7$**	**192.1235**	**191**		111; 173		
65	**SPb 1043-8**	**Polyhydroxycarboxylic acid**	**Quinic acid**	**$C_7H_{12}O_6$**	**192.1666**	**191**		111; 173	111	
66	**863**	**Propanoic acid**	**Dihydroferulic acid**	**$C_{10}H_{12}O_4$**	**196.1999**		**197**	127		
67	**863; SPb 1043-8**	**Carboxylic acid**	**Myristoleic acid** [Cis-9-Tetradecanoic acid]	**$C_{14}H_{26}O_2$**	**226.3550**		**227**	209; 165	121	
68	**SPb 1043-11; SPb 1043-8**	**Polypeptide**	**5-Oxo-L-propyl-L-isoleucine**	**$C_{11}H_{18}N_2O_4$**	**242.2716**		**243**	196; 137	151	
69	**863**	**Medium-chain fatty acid**	**Hydroxy dodecanoic acid**	**$C_{12}H_{22}O_5$**	**246.3001**		**247**	229	187	

70	**863**	**Terpenoid trilactone**	**Bilobalide** [(-)-Bilobalide]	**$C_{15}H_{18}O_8$**	**326.2986**	**325**		183;	119;	
71	**860**	**Iridoid**	**Monotropein**	**$C_{16}H_{22}O_{11}$**	**390.3393**		391	219; 372	202; 148	160
72	**863**	**Phytosterol**	**Ergosterol** [Provitamin D2; Ergosterin]	**$C_{28}H_{44}O$**	**396.6484**		**397**	379; 291; 206	291; 223	139
73	**860**	**Sterol**	**Fucosterol** [Fucostein; Trans-24-Ethylidenecholesterol]	**$C_{29}H_{48}O$**	**412.6908**		**413**	395; 324; 219	329	
74	**863; SPb 1043-11**	**Sterol**	**Beta-Sitosterin** [Beta-Sitosterol]	**$C_{29}H_{50}O$**	**414.7067**		**415**	216; 312	159; 115	
75	**SPb 1043-11**	**Anabolic steroid**	**Vebonol**	**$C_{30}H_{44}O_3$**	**452.6686**		453	435	336; 226	209; 139
76	**860**	**Triterpenoid**	**Betunolic acid**	**$C_{30}H_{46}O_3$**	**454.6844**		455	437; 345; 247	326; 283	303; 239; 199
77	**863**	**Triterpenic acid**	**Oleanoic acid**	**$C_{30}H_{48}O_3$**	**456.7003**		**457**	425; 295; 225	167	
78	**863; SPb 1043-11**	**Thromboxane receptor antagonist**	**Vapiprost**	**$C_{30}H_{39}NO_4$**	**477.6350**		**478**	337	263; 121	119
79	**863; SPb 1043-11**	**Indole sesquiterpene alkaloid**	**Sespendole**	**$C_{33}H_{45}NO_4$**	**519.7147**		**520**	184	125	
80	**863**	**Iridoid glucoside**	***p*-Coumaroyl monotropein**	**$C_{25}H_{28}O_{13}$**	**536.4820**		**537**	375; 256; 185		
81	**863; SPb 1043-11**	**Iridoid**	**p-Coumaroyl monotropein hexoside**		**698.8810**		**699**	537; 347; 259	375; 259; 185	
82	**SPb 1043-11**	**Steroidal alkaloid**	**Alpha-chaconine**	**$C_{45}H_{73}NO_{14}$**	**852.0594**		**852**	706	560; 398	398; 204

The CID-spectrum (collision induced dissociation spectrum) in positive ion modes of Dihydrokaempferol from extracts of *Lonicera caerulea* L. (variety SPb 1043-8) is shown in *Fig. 5*. The $[M + H]^+$ ion produced three fragment ions at *m/z* 270.99, *m/z* 193.01, *m/z* 127.03 (*Fig. 5*). It was identified in the bibliography in extracts from *Potato* [Oertel et al., 2017]*; F. glaucescens* [Hamed et al., 2020]; *Echinops* [Seukep et al., 2020]; *Rhodiola rosea* [Lee et al., 2016]; *Rhodiola crenulata* [Daikonya et al., 2011].

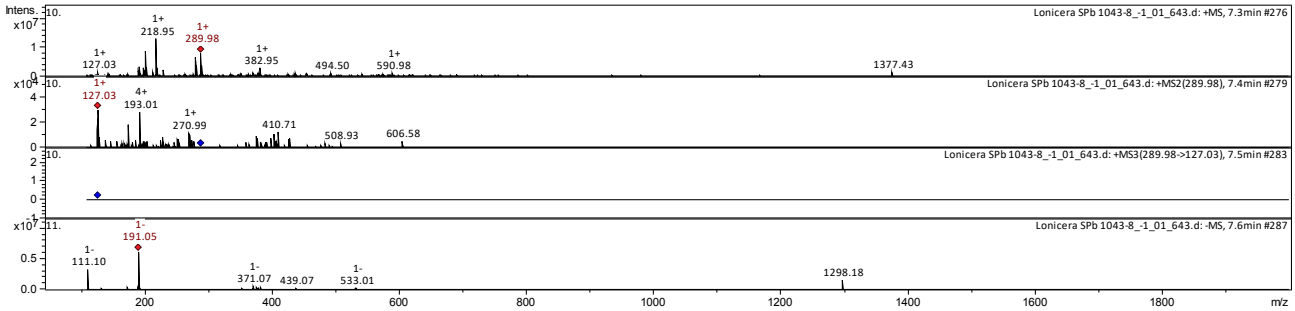

Fig.5. CID-spectrum of dihydrokaempferol from extracts of *Lonicera caerulea* L. (variety SPb 1043-8), *m/z* 289.98.

The CID-spectrum in positive ion modes of Dihydrokaempferol from extracts of *Lonicera caerulea* L. (variety Wild *Lonicera* from Amur river) is shown in *Fig. 6*. The $[M + H]^+$ ion produced one fragment ion at *m/z* 448.92 (*Fig. 6*). The fragment ion with *m/z* 448.92 yields three daughter ions at *m/z* 376.96, *m/z* 344.93, and *m/z* 286.95. The fragment ion with *m/z* 376.96 yields two daughter ions at *m/z* 344.92, and *m/z* 286.99. It was identified in the bibliography in extracts from *Rubus ulmifolius* [da Silva et al., 2019]*; Vitis vinifera* [Goufo et a., 2020]

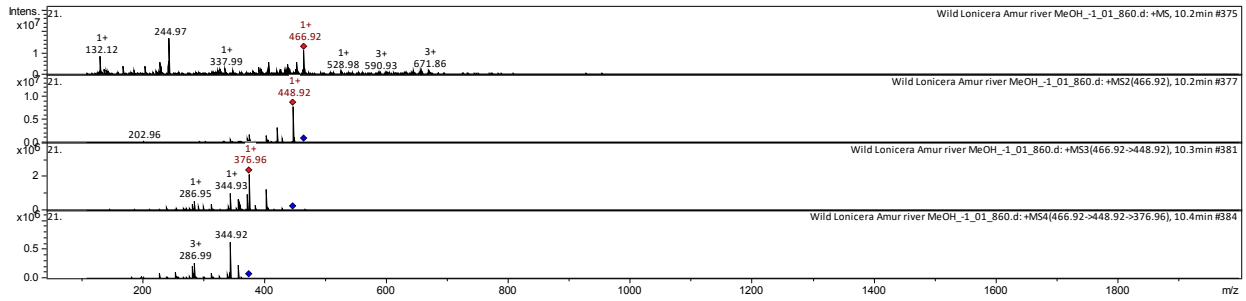

Fig.6. CID-spectrum of Taxifolin 3-*O*-glucoside from extracts of *Lonicera caerulea* L. (variety Wild *Lonicera* from Amur river), *m/z* 466.92.

4. Conclusions

Blue-berried honeysuckle *Lonicera caerulea* L. contains a large number of polyphenolic compounds and other biologically active substances. In this work, we first tried to conduct a comparative metabolomic study of biologically active substances of wild Blue-berried honeysuckle obtained from locations in Khabarovsk territory and from the collection of N.I. Vavilov All-Russian Institute of Plant Genetic Resources (St.-Petersburg). 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 82 biologically active compounds corresponding to the Blue-berried honeysuckle *Lonicera caerulea* species. In addition to the reported metabolites, a number of metabolites were newly annotated in blue-berried honeysuckle. There were flavonols: Dihydrokaempferol, Rhamnetin I, Rhamnetin II, Taxifolin-3-*O*-glucoside, Mearnsetin-hexoside, Horridin; flavones: Chrysoeriol, Apigenin-*O*-pentoside, Chrysoeriol-7-*O*-glucoside; flavanone Naringenin; flavan-3-ols: Catechin, Epicatechin, Biochanin A-7-*O*-glucoside; essential amino acids: L-Pyroglutamic acid, Tyrosine; polypeptide 5-Oxo-L-propyl-L-isoleucine; sterols: Ergosterol, Fucosterol, Beta-Sitosterin; triterpenoids: Betunolic acid, Oleanoic acid; anabolic steroid Vebonol, indole sesquiterpene alkaloid Sespendole; iridoids: Monotropein, *p*-Coumaroyl monotropein, *p*-Coumaroyl monotropein hexoside; Myristoleic acid, etc.

The findings may support future research into the production of various pharmaceutical and dietary supplements containing blue-berried honeysuckle *Lonicera caerulea* L. extracts. A wide variety of biologically active compounds opens up rich opportunities for the creation of new drugs and biologically active additives based on extracts from this family *Caprifoliaceae*.

References

1. G. Abeywickram; S.C. Debnath, P. Ambigaipalan, F. Shahidi, *J. Agric. Food Chem,* **64(49)** (2016)
2. I.M. Abu-Reidah, M. S. Ali-Shtayeh, R. M. Jamous, D. Arraes-Roman, A. Segura-Carretero, Food Chem, **166** 179-191 (2015).

3. L. Barros, M. Duenas, A. M. Carvalho, I.C.F.R. Ferreira, C. Santos-Buelga, Food and Chem, **50(5)** 1576-1582 (2012)
4. O.-C. Bujor *Extraction, identification and antioxidant activity of the phenolic secondary metabolites isolated from the leaves, stems and fruits of two shrubs of the Ericaceae family* (PhD THESIS, 2016)
5. A. Daikonya, S. Kitanaka, J Food Chem Safety, **18(3)** 183-190 (2011)
6. L.P. Da Silva, E. Pereira, T.C.S.P. Pires, M.J. Alves, O.R. Pereira, L. Barros I.C.F.R. Ferreira, Food Res. Int., **119** 34-43 (2019)
7. M. De Rosso, L. Tonidandel, R. Larcher, G. Nicolini, A. Dalla Vedova, F. De Marchi, M. Gardiman, M. Giust, R. Flamini, Food Chem, **163** 244-251 (2014)
8. T.R. Dias, M.G. Alves, S. Casal, P.F. Oliveira, B.M. Silva, Current Med. Chem., **24** 334-354 (2017)
9. P. Goufo, R.K. Singh, I. Cortez, Antioxidants, **9** 398 (2020)
10. A.R. Hamed, S.S .El-Hawary, R.M. Ibrahim, U.R. Abdelmohsen, A.M. El-Halawany, J. Chrom. Sci., 1-9 (2020)
11. M.K. Hossain, A.A. Dayem, J. Han, Y. Yin, K. Kim, S.K. Saha, G.-M. Yang, H.Y.Choi, S.-G. Cho, Int. J. Mol. Sci., **17** 569 (2016)
12. R. Jaiswal, H. Muller, A. Muller, M.G.E. Karar, N. Kuhnert, Phytochem. **108** 252-263 (2014)
13. M. Kajdzanoska, V. Gjamovski, M.Stefova, Macedonian J. of Chemistry and Chemical Engr., **29(2)** 181-194 (2010)
14. T.H. Lee, C.C. Hsu, G. Hsiao.; J.Y. Fang, W.M. Liu, C.K. Lee, Planta Med., **82(8)** 698-704 (2016)
15. M.M. Marzouk, S.R. Hussein, A. Elkhateeb, M. El-shabrawy, E.-S. S. Abdel-Hameed, S.A. Kawashty, J. Applied Pharm. Sci., **8(08)** 116–122 (2018)
16. A. Oertel, A. Matros, A. Hartmann, P. Arapitsas K.J. Dehmer, S. Martens, H.P. Mock, Planta, **246** 281-297 (2017)
17. P.C. Pradhan, S .Saha, J Food Sci. Technol., **53(2)** 1205-1213 (2016)
18. L. Paudel, F.J. Wyzgovski, J.C. Scheerens, A.M. Chanon, R.N. Reese, D. Smiljanic, C. Wesdemiotis, J.J. Blakeslee, K.M. Riedl, P.L. Rinaldi, J. Agricult. Food. Chem., **61** 12032–12043 (20130
19. N. Rafsanjany, J. Senker, S. Brandt, U. Dobrindt, A. Hensel, J. Agric. Food Chem., **63** 8804-8818 (2015)
20. M.P. Razgonova, A.M. Zakharenko, V. Grudev, S. Ercisli, K.S. Golokhvast, Molecules **25** 3774 2020
21. A. Ruiz, I. Hermosin-Gutierrez, C. Mardones, C. Vergara, E. Herlitz, M. Vega, C. Dorau, P. Winterhalter, von D. Baer, Agric. & Food Chem., **51** 706-713 (2010)
22. A. Ruiz, I. Hermosin-Gutierrez, C. Vergara, D. von Baer, M. Zapata, A. Hitschfild, L. Obando, C. Mardones, Food Res. Int., **51** 706-713 (2013)
23. A.J. Seukep, Y.-L. Zhang, Y.-B .Xu, M.-Q. Guo, Pharmaceut., **13** 59 (2020)
24. *State Pharmacopeia XIV* (in Russ., 2018)