Biological potential of methanol extracts from plants of the genus *Spiraea* L. growing in Russia

Anastasia Orlova [1]*, Alena Soboleva [1], Elena Tsvetkova [2, 3], Svetlana Silinskaia [1], Yana L. Esaulkova [4], Tatiana N. Veklich [5], Vladimir V. Zarubaev [4], Ksenia Fedoseeva [6], Ilya R. Akberdin [7] , Semyon K. Kolmykov [7], Vera A. Kostikova [8]*, Andrej Frolov [1]*

[1] Laboratory of Analytical Biochemistry and Biotechnology, K.A. Timiryazev Institute of Plant Physiology Russian Academy of Science, 35 Botanicheskaya str., Moscow, 127276, Russia

[2] Department of Biochemistry, St. Petersburg State University, 7-9-11 University Emb., St. Petersburg 199034, Russia

[3] Department of General Pathology and Pathological Physiology, Institute of Experimental Medicine, 197022 St. Petersburg, Russia

[4] The Laboratory of Experimental Virology, St. Petersburg Pasteur Institute, 14 Mira Str., St. Petersburg 197101, Russia

[5] Botany Laboratory, Amur Branch of Botanical Garden-Institute of the Far Eastern Branch Russian Academy of Science (AB BGI FEB RAS), Ignatievskoe Road, 2km, Blagoveshchensk, 675000, Russia

[6] Resource centre "Molecular and cell technologies", Universitetskaya emb. 7/9., St. Petersburg State University, 199034, St. Petersburg, Russia

[7] Department of Computational Biology, Sirius University, 1 Olimpiyskiy avenue, Sochi, Krasnodar territory, 354340, Russia

[8] Laboratory of Phytochemistry, Central Siberian Botanical Garden of Siberian Branch of Russian Academy of Sciences (CSBG SB RAS), 101 Zolotodolinskaya Str., Novosibirsk 630090, Russia

* Corresponding Authors:
Dr. Anastasia Orlova, Laboratory of Analytical Biochemistry and Biotechnology, K.A. Timiryazev Institute of Plant Physiology Russian Academy of Science, 35 Botanicheskaya str., Moscow, 127276, Russia.
Email address: orlova@ifr.moscow (A. Orlova)

Dr. Vera A. Kostikova, Laboratory of Phytochemistry, Central Siberian Botanical Garden of Siberian Branch of Russian Academy of Sciences (CSBG SB RAS), 101 Zolotodolinskaya Str., Novosibirsk 630090, Russia
Email address: serebryakovava@mail.ru (V.A. Kostikova)

Dr. Andrej Frolov, Laboratory of Analytical Biochemistry and Biotechnology, K.A. Timiryazev Institute of Plant Physiology Russian Academy of Science, 35 Botanicheskaya str., Moscow, 127276, Russia.
Email address: frolov@ifr.moscow (A. Frolov)

Abstract

The genus *Spiraea* is well-represented in the flora of Russian Federation which were only minimally addressed so far in respect of their metabolite profiles and characteristic biological activities. Therefore, phytochemical profiling of the major secondary metabolites in the first year *Spiraea* shoot extracts, accompanied with comprehensive screening of the activities exerted by these isolates, appears to be a remarkable step forwards. Obviously, this work will deliver new valuable information on the structure-activity relationships (SAR) for the major natural product constituents of these extracts. Indeed, although numerous phytochemical studies with *Spiraea* plants were reported so far, these works relied on limited numbers of species (or even on individual accessions), while, to the best of our knowledge, no full-scale screening with any logically completed set of *Spiraea* species was accomplished till now. Because of this, the data on the quantitative profiles of the principal secondary metabolites in the isolates prepared from different *Spiraea* species are quite fragmentary and require further supplementation and systematization. Therefore, our study aims filling this gap and covers the whole list of the *Spiraea* species naturally occurring in Russia. Moreover, here we successfully combined a metabolite profiling survey with a comprehensive screening of biological activities of the same extracts to address the Russian spirea species as promising sources of biologically active complexes for use in medical practice. For this, we address the relationships between the relative abundances of individual natural products occurring as their major extract constituents and the biological effects of the corresponding isolates in reliable laboratory models. Specifically, altogether 33 major components representing flavonoids (quercetin and kaempferol derivatives) and hydroxycinnamic acids (caffeic acid, ferulic acid, coumaric acid derivatives) were identified based on chromato-mass spectrometric (LC-MS) analysis. Further, the relative contents of these identified major components in the extracts, obtained from different *Spiraea* species, were addressed. The analysis of antioxidant potential revealed high activity of all extracts in the models of antiradical activity (DPPH assay), activity against cation radicals (TEAC assay) and superoxide anion radical (NBT assay). The screening of antiviral and antimicrobial activities of the same extracts revealed significant antiviral activity at a concentration of 2 μg/mL, and was assessed high (MIC < 1 mg/mL) or moderate (1 mg/mL $\leq$ MIC $\leq$ 4 mg/mL) antibacterial activity against the Gram-positive and Gram-negative bacteria strains.

Keywords: *Spiraea*, Rosaceae family, polyphenols, secondary metabolites, antioxidants, antimicrobial activity, antiviral activity.

1. Introduction

The potential of biological activity of medicinal plants relies on so-called biologically active complexes (BACs) – compounds that can have a pronounced effect on life processes at low concentrations [1]. Upon consumption by animals or humans, the constituents of these complexes are absorbed in gastrointestinal tract and transported to the target organs and cells to exert their therapeutic effect [2]. The knowledge about the composition of naturally occurring BACs, structures, properties and activity profiles of their constituents might give access to prototypes of

new prospective highly-efficient drugs. To gain this valuable information, in-depth analysis of chemical diversity of the plants with well-characterized biological activity is universally recognized as an efficient strategy for discovery of promising drug prototypes [3]. The other option is comprehensive evaluation of systematic and comprehensive of taxons, containing species with confirmed biological activity. Indeed, on one hand, new plants with unknown bioactivity profiles might have the chemical composition similar to that of their well-characterized relatives, that facilitates phytochemical studies. On the other, due to intra-taxon biochemical diversity, such valuable chemical information might essentially widen the scope of the drug candidate search [4].

The genus *Spiraea* L. (*Spiraea*) obviously represents one of such understudied taxons. It belongs to the tribe *Spiraeeae* of the subfamily *Amygdaloideae* (family *Rosaceae*) and includes about 90 species distributed in the temperate zone of the Northern Hemisphere. The center of species diversity of this genus is China, where up to 70 species can be found [5]. *Spiraea* species are typically deciduous shrubs, varying in shape and bush size from 0.15 to 2.50 m tall, with erect, spreading branches. The species comprising the genus *Spiraea* are light-loving shrubs, they develop better in open spaces, on stony mountain slopes, on screes, on river banks, in shrub steppes, where they are dominants and co-dominants [7].

Leaves are petiolate, simple, without leaflets, narrow-lanceolate to rounded, entire, 3-5-lobed, simple or double-toothed. Inflorescences of spring-flowering species are sessile or almost sessile umbrellas, or shield-shaped brushes with a rosette of leaves or scales at the base. The species flowering in June and July have simple or compound corymbs at the ends of the generative short, mostly leafy twigs or at the ends of shoots of the current year. Finally, the species flowering in July and August have narrow-cylindrical, broadly pyramidal or elliptical panicles developing at the ends of long leafy shoots of the current year. Flowers are oviparous, white, light or dark pink, red to purple and appear from April to October [8], [5]. The fruits of *Spiraea* are multi-seeded leaflets opening along the inner and later along the outer sutures. The seeds of *Spiraea* species are flat, lanceolate, brown, 1.5-2 mm long and 0.5 mm wide.

To date, different numbers of subgenera, sections and rows are distinguished in the structure of *Spiraea* genus system. According to the length of generative shoots, plants of this genus are divided into subgenera. Further, according to the structure of their inflorescences they are divided into sections. Based on genetical and morphological markers, some researchers distinguish rows within the sections [9].

In Russia, the genus *Spiraea* is represented with 20-25 species, which are distributed in four sections: *Spiraea* Ser., *Calospira* K. Koch, *Chamaedryon* Ser. and *Glomerati* Nakai. Remarkably, these species are unevenly distributed at the territory of the country. Thus, the most of the *Spiraea* species (20 – 23) are found in the Asian part of Russia [10][11][6], [12], whereas only 2 – 3 species were described in the European part of Russia [13].

Spiraea are widely used all over the world as ornamental shrubs for landscaping of cities and settlements, as fodder, melliferous and soil strengthening plants [11]. However, the species of the genus *Spiraea* are also of considerable interest as the plants used in folk medicine. Thereby, their impressing resource potential represents the principal advantage of spireas as the source of biologically active natural products. Various species of the genus *Spiraea* are used in folk medicine of Asian countries for the treatment of malaria and as anti-inflammatory agents [14] [15], [16]. In traditional Chinese medicine, young leaves, fruits and roots of *S. japonica* L. and its varieties are

used as a diuretic and analgesics [17], [18]. Decoctions and infusions of *S. salicifolia* L. are used for treatment of gastrointestinal diseases, rheumatism, helminthiasis, gynecological diseases and diabetes [19] [20]. The extracts obtained from the plants of the genus *Spiraea* show antioxidant [21], [22], antimicrobial [23], [24], antitumor [25][26], anti-inflammatory [27][28] and other types of biological activities.

According to literature data, several classes of bioactive natural products (i.e. secondary compounds with high biological activities) were identified in plants of the genus *Spiraea*: phenolic compounds (flavonoids, phenolic acids, tannins, coumarins), terpenoids, steroidal glycosides, cyanogenic glycosides, neolignanes, fatty acids, essential oils and others [29][35]. Accumulation of diterpene alkaloids was shown to be characteristic for some species of the genus *Spiraea* growing in Southeast Asia [36].

To date only limited information about the constituents of the spiraeas growing in Russia. Thus, the chemical composition of the most widespread *Spiraea* species – *S. salicifolia* and *S. media* Franz Schmidt – was most comprehensively studied, whereas the other Russian species are poorly studied in respect of the secondary metabolite profiles and associated biological activities. The biological activities of such *Spiraea* species as *S. humilis*, *S. elegans*, *S. sericea*, *S. trilobata* and *S. pubescens* were only minimally addressed in this respect, most likely due to their limited occurance [34]. Therefore, the aim of this work was a comparative phytochemical study of methanol extracts from shoots of the first year of 15 taxa of the genus *Spiraea* growing in Russia.

2. Materials and methods

2.1. Plant material

The plant material was obtained from 15 species of the genus *Spiraea* occurring in Russia - representatives of section *Chamaedryon* (*S. aquilegifolia, S. chamaedryfolia, S. crenata, S. elegans, S. flexuosa, S. media, S. pubescens, S. sericea, S. trilobata, S. ussuriensis*), section *Glomerati* (*S. hypericifolia*; section *Calospira* C. Koch: *S. betulifolia*) and section *Spiraria* Ser. Koch (*S. betulifolia* and sections *Spiraria* Ser.: *S. salicifolia, S. salicifolia* f. *alpestris* and *S. humilis*, Table 1). The plant material was collected from 10-15 specimens, localization of sampling is presented in Table 1. First-year shoots were collected from the middle part of the bush in the afternoon sunny time and dried in shadow in well-ventilated rooms. The dried material was crushed via ball mill (Retch MM400, Haan, Germany) and sieved through a sieve with a hole diameter of 2 mm.

Table 1 Sites of collection of the studied *Spiraea* specimens

ID	Species	Localization of sampling site
I	*S. media*	Amur Oblast, Zeya c. env.
II	*S. betulifolia*	Experimental field of CSBG SB RAS
III	*S. salicifolia* f. *alpestris*	Experimental field of CSBG SB RAS

IV	*S. pubescens*	Transbaikal region, Kalga settl. env.
V	*S. humilis*	Khabarovsk Krai, Selikhino vill. env.
VI	*S. flexuosa*	Experimental field of CSBG SB RAS
VII	*S. hypericifolia*	Novosibirsk Oblast, Steklyannoye vill. env.
VIII	*S. aquilegifolia*	Rep. of Buryatia, Ivolginsk vill. env.
IX	*S. sericea*	Amur Oblast, Sergeevka settl. env.
X	*S. trilobata*	Rep. of Altai, Ust-Koksa vill. env.
XI	*S. ussuriensis*	Amur Oblast, Sergeevka settl. env.
XII	*S. chamaedryfolia*	Experimental field of CSBG SB RAS
XIII	*S. crenata*	Novosibirsk Oblast, Shibkovo vill. env.
XIV	*S. elegans*	Zabaykalsky Krai, Mogocha c. env.
XV	*S. salicifolia*	Experimental field of CSBG SB RAS

2.2. Materials

Unless state otherwise, materials were obtained from the following manufacturers: Carl Roth GmbH + Co. KG (Karlsruhe, Germany): formic acid (p.a., ACS); Honeywell Riedel-de-Haën™ (Seelze, Germany): acetonitrile (LC-MS grade), methanol (LC-MS grade). All other chemicals were purchased from Merck KGaA (Darmstadt, Germany). Water was purified in house with a water conditioning and purification system Millipore Milli-Q Gradient A10 system (resistance 18 mΩ/cm, Merck Millipore, Darmstadt, Germany).

2.3. Preparation of extracts

Dried and crashed material of first-year shoots (5 g) were extracted with methanol (1:20 *w/v*) by double ultrasonic extraction at room temperature (RT). The extracts were evaporated to dryness on a rotary evaporator (DLAB CIENTIFIC CO., LTD., Beijing, China) under reduced pressure at the temperature not exceeding 40 °C. Afterwards, the dried extracts were re-suspended in water and lyophilized (Martin Christ Alpha 1-2 LD, Osterode am Harz, Netherlands). The residues were dissolved in DMSO to obtain the concentration of 50 mg/mL and stored at -20°C until used.

2.4. Metabolite profiling

The profiling of secondary metabolites relied on the procedure of Leonova et al. (2020) [37] with minor changes. In detail, the 500 μL of lyophilized extracts of the first-year spiraea shoots were reconstituted in 1000 μL of methanol and analyzed by reversed phase-ultra-high performance liquid chromatography coupled online to quadrupole time-of-flight mass spectrometry (RP-UHPLC-QqTOF-MS). For this, the samples (2 μL) were injected (partial injection mode) in a

Waters ACQUITY I-Class UPLC System consisting of Binary Solvent Manager, FL Sample Manager, and separated at 400 μL/min on a Waters ACQUITY UPLC BEH C18 column (2.1 × 50 mm, particle size 1.7 μm, Waters GmbH, Eichborn, Germany) at 40 °C. The separation relied on the linear gradient elution mode, eluents A and B were ultrapure water and acetonitrile with 0.1% (*v/v*) formic acid. After a one-min wash-out of the unbound fraction with 5% eluent B, the samples were separated in a linear gradient to 95% eluent B within 10 min. Afterwards, the column was washed with 95% eluent B for 2 min and re-equilibrated at 5% eluent B for 4 min. The column effluents were infused on-line in a hybrid QqTOF mass spectrometer (Sciex TripleTOF 6600, AB Sciex, Darmstadt, Germany) operated in negative ion mode using a sequential window acquisition of all theoretical mass spectra (SWATH) algorithm. The nebulizer (GS1), drying (GS2) and curtain (CUR) gases were set to 60, 70 and 55 psig, respectively, while the ion spray voltage was set to −4500 V. The MS experiments were accomplished in the TOF-scan mode (accumulation time 100 ms) in the *m/z* range of 65–1250. The tandem mass spectrometric (MS/MS, MS2) experiments were accomplished in the SWATH mode. Thereby, the overall *m/z* range (65–1250 *m/z*) was split in 15 SWATH windows (Q1 mass ranges) of 81 *m/z* each with an overlap of 1 *m/z*. Each *m/z* window was acquired with 60 ms accumulation time at the collision potential (CE) of −45 V with a collision energy spread (CES) of 35 V and declustering potential (DP) of −35 V. Nitrogen was used as collision activated dissociation (CAD) gas. Annotation of the individual analytes relied on literature data and manual interpretation of the fragmentation patterns obtained in the MS/MS experiments

2.5. Targeted tandem mass spectrometry (MS/MS) experiments

For all features, which were annotated based on the signals of corresponding $[M-H]^-$ ions observed in the mass spectra (MS1) to polyphenolic structures with mass accuracy better than 10 ppm, but did not yield unambiguously interpretable fragmentation patterns in SWATH mode (typically due to simultaneous fragmentation of two or more intense *m/z*), additional targeted RP-UHPLC-MS/MS experiments were accomplished with a TripleTOF 6600 mass spectrometer (AB Sciex, Darmstadt, Germany) using the LC conditions summarized in Table S1 and source settings described in the previous section. The MS/MS conditions were set as follows: each analysis was performed with 50 ms accumulation time at the range of collision potential from −10 V to −82.5 V with collision energy spread (CES) of 0 V and declustering potential (DP) of −35 V. Nitrogen was employed as collisional activation dissociation (CAD) gas.

2.6. Antioxidant effects

The antioxidant effects of the plant isolates (fractions of total extract and individual compounds) were addressed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, Trolox equivalent antioxidant capacity (TEAC) and nitroblue tetrazolium (NBT) assays. They were done according to Orlova et al. [38] with minor modifications as follows.

2.6.1 DPPH free radical scavenging effect

Freeze-dried methanolic extracts were solubilized in DMSO at the concentration of 50 mg/mL. The aliquots of each sample were 50-fold diluted (final concentration 1 mg/mL) and 20 μL of these solutions (20 μg in total) were supplemented to 1 mL portions of 40 μmol/L methanolic solution

of stable nitrogen centered free radical DPPH$^{\bullet}$. The absorbance was monitored photometrically at 517 nm after 1 h incubation at RT. The capacity of the samples for scavenging of the DPPH$^{\bullet}$ radical was estimated from the difference in the absorbance acquired in presence and in absence of plant isolates. The corresponding values were expressed as the percentage of DPPH$^{\bullet}$ consumption as a function of the sample concentration. Thereby, the oxidant activities of the extracts were determined as relative values normalized to the antioxidant activity of ascorbic acid (taken as 100%).

2.6.2 Trolox equivalent antioxidant capacity (TEAC) assay

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was dissolved in water to obtain a 7 mmol/L solution which was further oxidized to corresponding radical cation ($ABTS^{+\cdot}$) in the presence of 2.45 mmol/L potassium persulfate ($K_2S_2O_8$) and incubation for 16 h at RT in the dark. The radical cation reagent ($ABTS^{+\cdot}$) was diluted with ethanol to achieve the absorbance of 0.70 ± 0.02 AU at 734 nm. Aliquots of samples diluted as described in the previous section were supplemented to 1 mL of the $ABTS^{+\bullet}$ solution. Absorbance was measured at 734 nm after six minutes of incubation in the dark at RT Antioxidant capacities of the samples were reported as Trolox equivalents.

2.6.3 Assessment of extract capacity to scavenge superoxide anion radicals (NBT assay)

The stock solutions (1 mmol/L) of phenazine methosulfate (PM) in ethanol, nitro blue tetrazolium chloride (NBT) in water, and β-NADH in 0.05 mol/L phosphate buffer (pH 7.4) were freshly prepared daily. The reaction mixtures contained 73 μmol/L β-NADH, 15 μmol/L PM, 50 μmol/L NBT, and 10 μg of samples dissolved in 1 mL of 0.02 mol/L Tris-HCl buffer, pH 8.0. The absorbance was determined at 560 nm immediately after mixing the reagents and later on after 15 sec of reaction. The change of absorbance in time (ΔAbs/min) and absorption coefficient of 1 μmol/L formazan solution 0.03 were used to calculate the rate of production of superoxide anion radical.

2.7. Cytotoxic assays

Cytotoxic effects of the methanol extracts prepared from the shoots of the 15 selected *Spiraea* species were evaluated in a MTT cell viability assay based MDCK cells culture (ATCC CCL-34). The cells were cultured in the growth medium containing DMEM/F12 with 10% fetal bovine serum and passaged twice a week with 0.25% trypsin in DPBS solution. For cytotoxicity experiments, cells were stripped off the plastic, concentrated by centrifugation, resuspended in the growth medium and the concentration of cell suspension was assessed with Neubauer chamber. The suspension was diluted to $0.25x10^6$ cells /mL and 100 μL of cells were seeded in each well of a 96-well plate. The cells were left in a CO_2 incubator for adhesion to the substrate for 24 hours. On the next day, a series of extract dilutions (4-300 μg/mL) in DMEM/F12 additive-free medium was prepared. Next, the growth medium was replaced for a plate with adhered cells and 100 μL of extract dilutions were added to each well of the plate in three repetitions. Incubation of cells with dilutions of substances was carried out for 72 hours. After incubation, 10 µg of 5 mg/mL 3-(4,5-dimethylthiazolyl-2) 2,5-diphenyltetrazolium bromide (MTT) solution was added to each well of the plate to obtain a concentration of 0.5 mg/mL and the plates were incubated at 37 °C for 2 hours.

After the incubation the medium was removed, the formazan crystals formed in the cells were dissolved in DMSO, and intensity of staining was quantified colorimetrically by a Multiscan FC plate analyser (Thermo Fisher Scientific, Whaltem, USA) at 540 nm. Based on the data obtained, the 50% cytotoxic concentrations (LD_{50}) were calculated.

2.8. Antiviral Assay

Viruses and cells. A/Puerto Rico/8/34 influenza virus (H1N1) was cultured in MDCK cells (ATCC CCL-34). The cells were seeded into 96-well plates at 10^4 cells/well and a volume of 100 μL/well of complete MEM medium. Incubation was performed overnight in a CO_2 incubator at 36°C in a 5% CO_2 atmosphere. Directly before the experiment, the cells were washed with MEM medium, and further manipulations were performed in serum-free medium.

Antiviral activity of the extracts. Aliquots of individual extracts (100 μL) were supplemented to the wells of a 96-well microtiter plate, which were pre-covered with a monolayer of MDCK cells. The plates with cells were incubated under the atmosphere of 5% CO_2 at 36°C for 1 h. Afterwards, 0.1 mL of the virus suspension (m.o.i. 0.01 TCID50 per cell) in alpha-MEM medium was added to each well and incubations were continued for 72 h under the atmosphere of 5% CO_2 at 37°C. After completence of the incubations, the cells were washed with MEM medium and cell viability was analyzed as described above. The 50% inhibitory concentration (IC_{50}), i.e. the concentration of the compound that resulted in a 50% reduction in the cytodestructive effect of the virus, was calculated from the data obtained.

2.9. Antibacterial Assay

The minimal inhibitory concentrations (MICs) of the extracts were determined by microdilution broth method, as recommended by Clinical Laboratory Standards Institute, USA with minor changes [38]. The following bacteria strains were cultured under aerobic conditions according to the approved standard protocol: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* EGD, *Staphylococcus aureus* ATCC 25923, *MRSA* ATCC 33591, *Micrococcus luteus* CIP A270. Strains *MRSA* ATCC 33591 and *Listeria monocytogenes* EGD were provided by Prof. R. Lehrer (University of California Los Angeles, USA); *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* CIP A270 and *Staphylococcus aureus* ATCC 25923 - Department of Molecular Microbiology, IEM. The microorganisms from an agar plate culture were incubated for 3-4 hours in 2.1% (*w/v*) Mueller-Hinton broth (MHB) (HiMedia, India) at 37° C on an orbital shaker at an agitation rate 150 rpm. After adjusting the turbidity to 0.5 McFarland (1,5x108 CFU/mL), suspensions were diluted in sterile 2.1% (*w/v*) MHB till the final bacterial concentration of 1.0 x 10^6 CFU/mL.

The extracts from fifteen species of the genus *Spiraea* were serially two-fold diluted (the initial concentration was 4000 μg/mL) with sterile 2.1% (*w/v*) MHB and 50 μL aliquots were added to the wells of a 96-well sterile polystyrene U-bottom plates (GreinerBio-one, Austria). Afterwards, 50 μL of bacterial suspension was added in each well. The controls of bacterial growth and viability and medium sterility were included. Since DMSO, used as the primary solvent for lyophilized extracts, has its own antibacterial activity, it was shown that in the concentrations used (not more than 1%) it did not inhibit the growth of all tested microorganisms. The microtiter plates were incubated aerobically without shaking at 37° C for 18 h. MICs were defined as the lowest extract

concentrations that inhibited the visual growth of microorganisms. The experiments were performed in triplicates, the MICs were calculated as the medians based on the data from three independent experiments, each accompanied with the complete set of the controls.

2.10. Statistical Analysis

The numerical results were expressed as the mean (mean ± standard deviation). Statistical significance of inter-group differences was assessed by the Mann–Whitney test ($p \leq 0.05$) with Bonferroni correction for multiple comparisons applied at the confidence level of $p \leq 0.05$

3. Results

3.1. Profiling of secondary metabolites in the methanolic first-year shoots extracts of *Spiraea* spp.

To date, the study of biological activity of plant isolates ultimately assumes at least qualitative analysis of plant secondary metabolome. Identification and unambiguous structural characterization of potential bioactives is universally recognized as the first step in the primary work on the primary development of promising pharmaceutical agents in terms of the evidence-based medicine. Indeed, since the last few decades, medicinal plants are considered not only as the sources of the preparations based on crude extracts, but also as potential producers of highly-active purified individual biologically active natural products and their complexes.

That is why, to address the pharmacological potential of the fifteen most widely occurring in Russia *Spiraea* species in first glance, we accomplished a comparative metabolomics profiling of the corresponding methanolic extracts of the first year shoots. Realizing that, most likely, the biological activities of the extracts are defined by the most abundant metabolites we focused on the most well-represented semi-polar secondary metabolites yielding the most intense signals even in the total ion current chromatograms (TICs), acquired in corresponding RP-UHPLC-QqTOF-MS/MS experiments (Figure 1). To get approximately two dozens of candidates per TIC, we set the intensity (peak intensity in corresponding extracted ion chromatograms, XICs) threshold value to $4.5*10^7$ counts in MS-spectra.

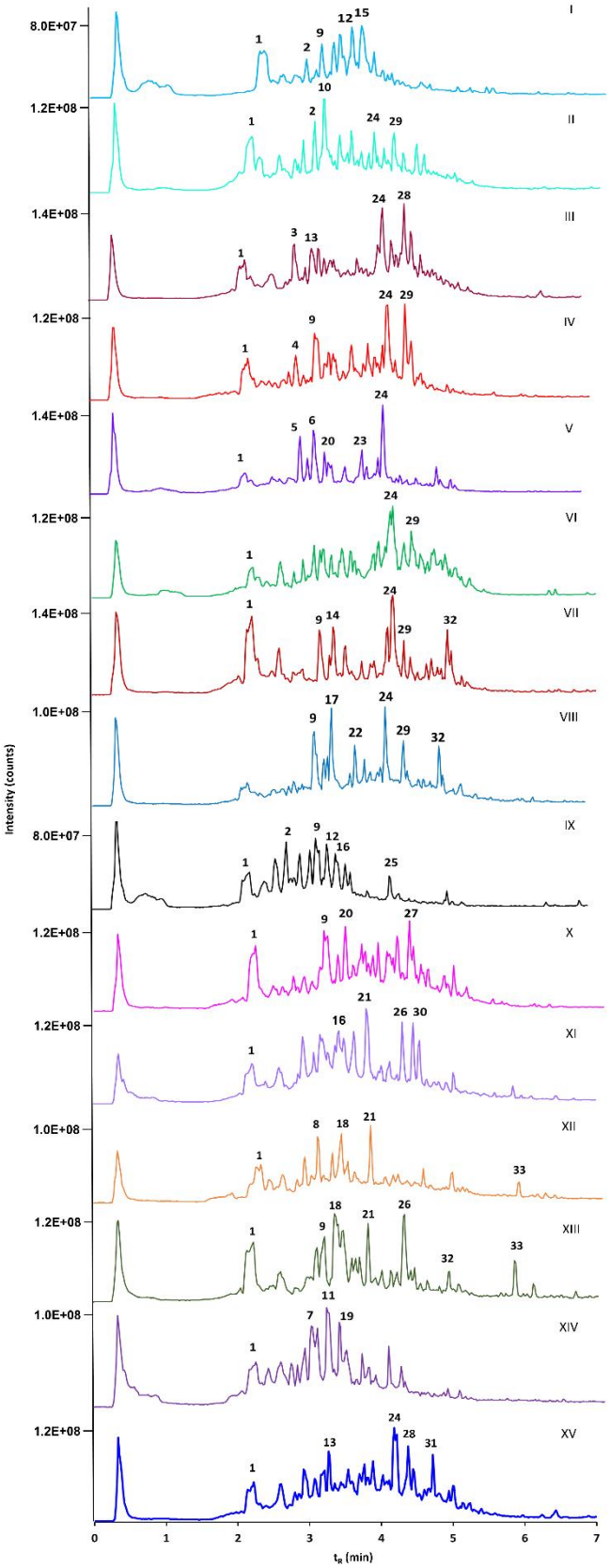

Figure 1. Total ion current chromatograms (TICs) of methanol extracts obtained from the first-year shoots of 15 species of the genus *Spireae* by reversed-phase ultrahigh-performance quadrupole-time-of-flight chromatography-mass spectrometry with electrospray ionization (RP-UHPLC-QqTOF-MS). The analyzed species of the genus *Spireae* are labeled as following: I - *S. media*, II - *S. betulifolia*, III - *S. salicifolia f. alpestris*, IV - *S. pubescens*, V - *S. humilis*, VI - *S. flexuosa*, VII - *S. hypericifolia*, VIII - *S. aquilegifolia*, IX - *S. sericea*, X - *S. trilobata*, XI - *S. ussuriensis*, XII - *S. chamaedryfolia*, XIII - *S. crenata*, XIV - *S. elegans*, XV – *S. salicifolia* (according to **Table 1**). Numbers **1-33** denote the semi-polar secondary metabolites, which were assigned as the major constituents of the extracts according to **Table 2**.

The interpretation of all TICs across the whole dataset in this way revealed, in total, 33 major semi-polar metabolites, which could be retained on the reversed phase. Among these, annotation of 17 metabolites relied on the SWATH-MS data (Supplementary Materials, Figure S1), whereas structural characterization of the remaining 16 compounds required additional tandem mass spectrometric (MS/MS) experiments (Supplementary Materials, Figure S2). Detailed related chromatographic and spectral information is summarized in Table 2.

Preliminary annotation of the major semi-polar constituents of the 15 prepared methanol extracts showed that polyphenols represented the main group of the principal extract components. Thereby, the most of the annotated polyphenols were represented by flavonoids (which dominated with the derivatives of quercetin and kaempferol) and hydroxycinnamic acids (caffeic and coumaric acids derivatives).

Among the annotated compounds, twelve compounds were featured with a common fragmentation pattern, dominated with the signals at *m/z* 300.0281 and characteristic for quercetin derivatives [38]. Thus, compound **4** (*m/z* 595.1358, t_R 2.9 min corresponding to the elemental composition $C_{26}H_{27}O_{16}^-$, 9.0 ppm (here and further practical mass-to-charge ratios are presented, theoretical ones could be found in Table 2)) was annotated as quercetin-hexosyl pentoside due to the presence of the characteristic *m/z* 300.0281 corresponding to quercetin resulting from the neutral loss of the hexosyl-pentoside moiety [M-H-295] and appeared to be a characteristic major metabolite of *S. salicifolia f. alpestris* (Figure S1-3). Further, compound **5** (*m/z* 609.1474, t_R 3.0 min corresponding to the elemental composition $C_{27}H_{29}O_{16}^-$, -2.1 ppm) was annotated as quercetin-hexosyl-deoxyhexoside by the presence of a characteristic ion *m/z* 300.0262 formed by neustral loss of hexosyl-deoxy-hexoside moiety [M-H-309] (Figure S1-4), and compound **6** (*m/z* 695.1402, t_R 3.1 min corresponding to the elemental formula $C_{30}H_{31}O_{19}^-$, 9.1 ppm) was annotated as acetyl-quercetin-hexosyl-deoxyhexoside due to the presence of the characteristic loss of the acetyl-hexosyl-deoxy-hexoside moiety [M-H-351] resulting in the fragment ion at *m/z* 300.0271 (quercetin aglycone) (Figure S2-2). These two conjugates were characteristic for the methanol extract of *S. humilis*. The compound **8** (*m/z* 787.1808, t_R 3.1 min corresponding to the elemental formula $C_{40}H_{47}O_{22}^-$, 10.4 ppm) was annotated as quercetin-trihexoside in *S. chamaedryfolia* extract by the presence of sequential neutral loss of one and two hexose residues (Figure S1-6), whereas the compound **9** (*m/z* 927.1833, t_R 3.2 min corresponding to the elemental formula $C_{42}H_{39}O_{24}^-$, 0.4 ppm) was annotated as quercetin-hexoside due to the presence of the characteristic loss of the hexoside moiety [M-H-163] in the extracts prepared from the shoots of *S. aquilegifolia, S. crenata, S. hypericifolia, S. media, S. pubescens, S. sericea, S. trilobata* (Figure S1-7). Also, compound **12** (*m/z* 679.1510, t_R 3.3 min corresponding to the elemental composition $C_{30}H_{31}O_{18}^-$, 0.9 ppm) was annotated as quercetin acetyl-ethyl-hexuronide-*O*-pentoside isomer 1 in the extracts of *S. media* and *S. sericea* (Figure S2-4), whereas compound **14** (*m/z* 1099.1840, t_R 3.4 min corresponding to the elemental formula $C_{48}H_{43}O_{30}^-$, 1.5 ppm) was annotated as quercetin-malonyl-hexoside by the presence of sequential neutral loss of malonic acid [M-H-86] and hexoside [M-H-163] moieties and detected in the extract of only one species, namely *S. hypericifolia* (Figure S1-10). In turn, compound **15** (*m/z* 679.1506, t_R 3.38 min corresponding to the elemental formula $C_{30}H_{31}O_{18}^-$, 1.5 ppm) was annotated as quercetin acetyl-ethyl-hexuronide-*O*-pentoside isomer 2 and characteristic for *S. media* extract (Figure S2-5) and compound **18** (*m/z* 771.1816, t_R 3.43 min corresponding to the elemental composition $C_{36}H_{35}O_{19}^-$, -4.9 ppm), which appeared to be characteristic for two

species - *S. schamaedryfolia* and *S. crenata,* and was annotated as quercetin-coumaroyl-dihexoside (Figure S1-12). All the above listed compounds were annotated as the quercetine derivative by the presence of the characteristic fragment at *m/z* 300.02 in their spectra, which was clear visible both in the SWATH and product ion spectra (Table 2, Figures S1-3, S1-4, S1-6, S1-7, S1-10, S1-12, S2-2, S2-4, S2-5).

Finally, the three major compounds of this group were annotated by the presence in their spectra a signal at *m/z* 301.0346 which correspondent to the fragment of the quercetin aglycon. This signal was present in the tandem mass spectra of the compound **13** (*m/z* 757.1708, t_R 3.3 min corresponding to the elemental formula $C_{35}H_{33}O_{19}^-$, -10.3 ppm), which was characteristic for the *S. salicifolia* and *S. salicifolia f. alpestris* extracts and was annotated as caffeoyl pentoside-quercetin hexoside (Figure S1-9). The same signal was found in the MS/MS spectra of the compound **16** (*m/z* 741.1661, t_R 3.4 min corresponding to the elemental composition $C_{35}H_{33}O_{18}^-$, 1.5 ppm), which was annotated as quercetin hexopyranosyl-*O*-p-coumaroyl-pentopyranoside and was characterized in the methanolic extracts of *S.sericea* and *S. ussuriensis* shoots (Figure S2-6). By the same fragment was recognized the compound **17** (*m/z* 867.1605, t_R 3.4 min corresponding to the elemental formula $C_{40}H_{35}O_{22}^-$, 2.3 ppm), annotated as quercetin-pentoside and found in the extracts of *S.aquilegifolia* (Figure S1-11). The compound **2** (*m/z* 465.0983, $\mathbf{t_R}$ 2.8 min corresponding to the elemental formula $C_{21}H_{21}O_{12}^-$, 10.8 ppm) was the last compound representing the group the quercetin derivatives. It was annotated as dihydroquercetin-hexoside and detected in the extracts of two species of the genus *Spiraea* (*S. media, S.sericea*), annotated by the presence of a dehydroxy-quercetin fragment ion with *m/z* 285.0373 in the corresponding target MS/MS spectrum (Figure S1-2).

The second group of the major compounds was represented also by flavonoids, namely - kaempferol derivatives. Annotation of these derivatives relied on the characteristic signal at *m/z* 285.0413 present in all corresponding MS/MS spectra and corresponding to the fragment ion of the kaempferol aglycon. The annotation of compound **10** (*m/z* 621.1452, t_R 3.2 min, $C_{28}H_{29}O_{16}^-$, 1.5 ppm), characteristic for the methanolic extracts prepared from the first year shoots of *S. betulifolia*, was confirmed by the presence of fragment signals corresponding to the sequential neutral losses of acetyl, pentose and hexose. Based on manual interpretation of the tandem mass spectrum, compound **10** was annotated as kaempferol acetyl-pentopyranoside-*O*-hexopyranoside (Figure S2-3). Compound **11** (*m/z* 1127.2890, t_R 3.2 min, $C_{52}H_{55}O_{28}^-$, -0.2 ppm), characteristic for *S. elegans*, was annotated by the presence of the signals corresponding to the neutral losses of the pentosyl moiety and the dehydroxy-hexoside. Based on this fragment pattern, this compound was annotated as kaempferol-pentosyl-deoxyhexoside (Figure S1-8).

The remaining 15 compounds were assigned to the class of hydroxycinnamic acids - derivatives of caffeic, *p*-coumaric, ferulic acids. The elemental composition of compounds **1** (*m/z* 707.1816, t_R 2.2 min, $C_{32}H_{35}O_{18}^-$, 4.5 ppm), characteristic for all studied extracts, **7** (*m/z* 879.2546, t_R 3.1 min, $C_{40}H_{47}O_{22}^-$, 2.1 ppm), found in *S. elegans* extract, **22** (*m/z* 537.1627, t_R 3.9 min, $C_{25}H_{29}O_{13}^-$, -2.4 ppm), characteristic for *S. aquilegifolia*, and **28** (*m/z* 491.1917, t_R 4.4 min, $C_{25}H_{31}O_{10}^-$, 1.2 ppm), characteristic for *S. salicifolia* and *S. salicifolia f. alpestris*, was determined from ESI-HR-MS data (Table 2). Based on the presence of caffeic acid fragment ion (*m/z* 179.0355) or neutral loss of caffeic acid in the fragmentation patterns of the studied compounds, these major constituents of the spirea extracts were assigned to the group of caffeic acid derivatives. Based on these data,

compound **1** was annotated as caffeoyl-quinic acid, compound **7** - as caffeoyl-hydroxy-methylbutyryl-pentoside, compound **22** as caffeoyl-loganic acid, and compound **28** was assigned as caffeoyl-hexopyranosy-dimethyl-octadienone (Figure S1-1, S1-5, S1-13, S2-12).

The compound **19** (*m/z* 847.2691, t_R 3.4 min, $C_{40}H_{47}O_{20}^-$, 2.9 ppm), found in extracts of *S. elegans*, **24** (*m/z* 1043.3410, t_R 4.2 min, $C_{50}H_{59}O_{24}^-$, -0.9 ppm), fairly widely distributed in extracts of the studied species, **26** (*m/z* 861.2806, t_R 4.30 min, $C_{41}H_{49}O_{20}^-$, 1.9 ppm), characteristic for *S. crenata* and *S. ussuriensis*, **29** (*m/z* 521.1639, t_R 4.42 min, $C_{25}H_{29}O_{12}^-$, 4.8 ppm), characteristic for *S. aquilegifolia, S. flexuosa, S. hypericifolia, S. pubescens, S. betulifolia* and **31** (*m/z* 475.1970, t_R 4.7 min, $C_{25}H_{31}O_9^-$, 0.8 ppm), detectable in *S. salicifolia* extract, could be annotated based on the ESI-HR-MS and MS/MS data (Table 2). The tandem mass spectra of all the analytes, with the exception of compound **26**, showed a characteristic signal *m/z* 163.0409 corresponding to the fragment ion of coumaric acid. Therefore, these major components could be assigned to the group of coumaric acid derivatives. The MS/MS spectra of compound **26** contained a characteristic fragment of dehydroxy-coumaric acid (*m/z* 147.0458). Because of this, this natural product was also assigned to the group of coumaric acid derivatives. The presence of the characteristic fragment *m/z* 325.0930 and neutral loss of 163 u allowed annotation of compound **19** as a *p*-coumaroyl-hexoside derivative, compound **24** as a *p*-coumaroyl-loganic acid isomer 1, compound **26** as a *p*-coumaroyl hexoside derivative, compound **29** as a *p*-coumaroyl-loganic acid isomer 2, and compound **31** as a phenylbytil-*O*-dihydrocoumaroyl-hexoside (Figures S2-7, S1-14, S2-10, S1-15, S2-13).

The elemental compositions of compounds **27** (*m/z* 505.1700, t_R 4.3 min, $C_{25}H_{29}O_{11}^-$, 2.9 ppm) and **32** (*m/z* 667.2029, t_R 4.9 min, $C_{34}H_{35}O_{14}^-$, 3.9 ppm) were predicted as $C_{25}H_{29}O_{11}^-$ and $C_{34}H_{35}O_{14}^-$, respectively on the basis of ESI-HR-MS data (Table 2). Further, based on the presence of the signals corresponding to the neutral loss of 270 u corresponding to the cleavage of ferulic acid moiety in the tandem mass spectra, these major constituents of the *S. trilobata*, *S.aquilegifolia, S. crenata, S. hypericifolia* extracts were assigned to the group of ferulic acid derivatives due to the. Thus, the tandem mass spectrum of compound **27** revealed a fragment at *m/z* 235.0616 corresponding to ferulic acid hydroxy-vinyl moiety (neutral loss of 270 u). Thus, compound **27** could be annotated as (hydroxy-methoxyphenyl)ethyl-*O*-feruloyl-hexopyranoside (Figure S2-11). The tandem mass spectrum of compound **32** revealed a fragment at *m/z* 265.0733 corresponding to hydroxy-oxopropyl ester of ferulic acid. Thus, based on the results of manual interpretation of the tandem mass spectrum, compound **32** was annotated as an unknown derivative of acetyl-di-feruloyl-glycerol (Figure S2-14).

Compound **3** (*m/z* 439.1823, t_R 2.8 min, $C_{21}H_{21}O_{12}^-$, 10.8 ppm), characteristic for the methanolic extracts prepared from the first year shoots of *S.pubescens*, was annotated by the presence of the $[M-H]^-$ signal at *m/z* 439.1823 in the mass spectrum, along with the fragment ion signals at *m/z* 221.0667 and at *m/z* 179.0561 (corresponding to the acetyl hexose and hexose fragments, respectively) in the tandem mass spectrum. Based on this fragmentation pattern, compound **3** was annotated as *O*-hexopyranosyl-*O*-hexanoyl-hexopyranose (Figure S2-1).

Compound **21** (*m/z* 893.2781, t_R 3.8 min, $C_{41}H_{40}O_{22}^-$, -6.7 ppm), characteristic for the methanolic extracts prepared from the first year shoots of *S. chamaedryfolia, S. crenata* and *S. ussuriensis*, was annotated by the presence of the $[M-H]^-$ signal at *m/z* 469.1416 in the mass spectrum, along with the fragment ion at *m/z* 309.1007 in the tandem mass spectrum obtained by

the neutral loss of glutaric acid and carbon monoxide. Based on this fragmentation pattern, compound **21** was annotated as (hydroxyphenyl)-oxopropenyl-glucopyranosyloxy-methylglutaric acid (Figure S2-8).

Compound **23** (*m/z* 493.2212, t_R 4.2 min, $C_{29}H_{33}O_7^-$, 3.9 ppm), characteristic for the methanolic extracts of *S. humilis*, was annotated by the presence of a fragment signal at *m/z* 447.2212 (characteristic for geranyl pentosyl-hexoside) and a fragment at *m/z* 315.1804 (characteristic for geranyl-hexoside) in the tandem mass spectrum. Based on this fragmentation pattern, compound **23** could be annotated as geranyl pentosyl-hexoside derivatives (Figure S2-9).

The relative recoveries of major components in the investigated methanol extracts of first year shoots of 15 plant species of the genus *Spiraea* were identified using extracted ion chromatogram for each of the major components considered. The results of the relative recoveries of the components are presented in Figure 2.

Table 2. Metabolites annotated in methanolic extracts of first-year shoots of fifteen species of plants of the Spirea genus by reversed phase ultra-high-performance liquid chromatography—tandem mass spectrometry (RP-UHPLC-QqTOF-MS/MS)

#	t_R, min [a]	$[M-H]^-$ observed (*m/z*) [b]	$[M-H]^-$ theoretical (*m/z*) [c]	Elemental composition[d]	Δm, ppm	Fragmentation patterns [e]	Annotation
1[f]	2.2	707.1816	707.1829	$C_{32}H_{35}O_{18}^-$	4.5	191.0618 (100), 353.0908 (60), 707.1861 (20)	Caffeoyl-quinic acid
2[f]	2.8	465.0983	465.1038	$C_{21}H_{21}O_{12}^-$	10.8	151.0042 (70), 285.0373 (50), 465.0983 (100)	Dihydroquercetin-hexoside
3[g]	2.9	439.1823	439.1762	$C_{18}H_{31}O_{12}^-$	0.5	131.0338 (15), 149.0442 (17), 165.0549 (10), 179.0554 (11), 191.0551 (8), 221.0658 (10), 251.0779 (5), 261.1331 (20), 311.1006 (5), 393.1762 (100), 439.1823 (40)	Hexopyranosyl-hexanoyl-hexopyranose
4[f]	2.9	595.1358	595.1305	$C_{26}H_{27}O_{16}^-$	9	300.0281 (20), 595.1358 (100)	Quercetin hexosyl-pentoside
5[f]	3.0	609.1474	609.1461	$C_{27}H_{29}O_{16}^-$	-2.1	300.0262 (40), 609.1474 (100)	Quercetin-hexosyl-deoxy-hexoside
6[g]	3.1	695.1402	695.1465	$C_{30}H_{31}O_{19}^-$	9.1	300.0271 (100), 651.1532 (70)	Quercetin acetyl-hexosyl-deoxy-hexoside

7 [f]	3.1	879.2546	879.2564	$C_{40}H_{47}O_{22}^-$	2.1	161.0251 (70), 179.0361 (20), 341.0881 (15), 439.1238 (100), 879.2546 (20)	Caffeoyl-hydroxy-methylbutyryl-pentoside
8 [f]	3.1	787.1808	787.1898	$C_{33}H_{39}O_{22}^-$	10.4	271.0258 (20), 300.0335 (70), 625.1462 (80), 787.1808 (100)	Quercetin-trihexoside
9 [f]	3.2	927.1833	927.1837	$C_{42}H_{39}O_{24}^-$	0.4	300.0298 (40), 463.0942 (100), 927.1833 (50)	Quercetin-hexoside
10 [g]	3.2	621.1452	621.1461	$C_{28}H_{29}O_{16}^-$	1.5	284.0336 (100), 285.0411 (40), 561.1245 (20), 579.1348 (30), 621.1452 (60)	Kaempferol-acetyl-pentopyranoside-hexopyranoside
11 [f]	3.2	1127.2887	1127.2891	$C_{52}H_{55}O_{28}^-$	-0.2	285.0413 (40), 431.1023 (70), 563.1459 (100), 1127.2887 (10)	Kaempferol-pentosyl-deoxyhexoside
12 [g]	3.3	679.1510	679.1516	$C_{30}H_{31}O_{18}^-$	0.9	300.0281 (10), 637.1401 (30), 679.1510 (100)	Quercetin acetyl-ethyl-hexuronide-pentoside isomer 1
13 [f]	3.3	757.1708	757.1622	$C_{35}H_{33}O_{19}^-$	-10.3	161.0255 (10), 301.0363 (70), 455.1219 (15), 595.1360 (95), 757.1708 (100)	Caffeoyl pentoside-quercetin hexoside
14 [f]	3.4	1099.1840	1099.1850	$C_{48}H_{43}O_{30}^-$	-0.5	300.0289 (40), 463.0878 (10), 505.1058 (100), 549.0901 (50), 1099.1840 (60)	Quercetin-malonyl-hexoside
15 [g]	3.4	679.1506	679.1516	$C_{30}H_{31}O_{18}^-$	1.5	300.0277 (10), 637.1398 (30), 679.1506 (100)	Quercetin acetyl-ethyl-hexuronide-pentoside isomer 2

16 [g]	3.4	741.1661	741.1672	$C_{35}H_{33}O_{18}^-$	1.5	161.0238 (20), 285.0401 (30), 301.0346 (10), 455.1181 (100), 579.1339 (60), 595.1287 (63), 741.1661 (70)	Quercetin hexopyranosyl-*O*-p-coumaroyl-pentopyranoside
17 [f]	3.4	867.1605	867.1625	$C_{40}H_{35}O_{22}^-$	2.3	301.0365 (55), 433.0817 (100), 867.1605 (30)	Quercetin-pentoside
18 [f]	3.4	771.1816	771.1778	$C_{36}H_{35}O_{19}^-$	-4.9	271.0255 (10), 300.0308 (90), 625.1417 (40), 771.1816 (100)	Quercetin-coumaroyl-dihexoside
19 [g]	3.4	847.2691	847.2663	$C_{40}H_{47}O_{20}^-$	2.9	145.0292 (10), 163.0398 (5), 325.0930 (15), 423.1296 (100), 847.2691 (3)	Coumaroyl hexoside derivatives
20	3.5	537.1627	537.1614	$C_{25}H_{29}O_{13}^-$	2.5	125.0241 (20), 145.0332 (100), 231.0661 (15), 357.0968 (5), 433.1120 (5), 519.1497 (30), 537.1627 (30)	Unknown
21 [g]	3.8	893.2781	893.2721	$C_{41}H_{49}O_{22}^-$	-6.7	147.0460 (20), 309.1000 (70), 423.1316 (20), 469.1369 (100), 893.2781 (3)	(Hydroxyphenyl)-oxopropenyl-glucopyranosyloxy-methylglutaric acid
22 [f]	3.9	537.1627	537.1614	$C_{25}H_{29}O_{13}^-$	-2.4	135.0405 (95), 161.0240 (40), 179.0379 (100), 357.0975 (20), 519.1506 (20), 537.1627 (80)	Caffeoyl-loganic acid
23 [g]	4.0	493.2212	493.2231	$C_{29}H_{33}O_7^-$	3.9	131.0349 (20), 161.0455 (18), 315.1804 (100), 447.2212 (20)	Geranyl pentosyl-hexoside derivatives

24 [f]	4.2	1043.3410	1043.3400	$C_{50}H_{59}O_{24}^-$	-0.9	163.0417 (60), 341.1044 (60), 503.1562 (60), 521.1708 (100), 1043.3412 (40)	*p*-Coumaroyl-loganic acid isomer 1
25	4.2	983.3212	983.3343	$C_{52}H_{55}O_{19}^-$	13.3	227.1286 (5), 285.0400 (10), 389.1801 (5), 593.1303 (100), 983.3212 (5)	Unknown
26 [g]	4.3	861.2806	861.2823	$C_{41}H_{49}O_{20}^-$	1.9	147.0458 (10), 407.1363 (20), 453.1416 (100), 861.2806 (3)	Coumaroyl hexoside derivatives
27 [g]	4.3	505.1700	505.1715	$C_{25}H_{29}O_{11}^-$	2.9	163.0401 (10), 179.0715 (40), 205.0509 (20), 235.0616 (25), 265.0717 (40), 307.0822 (100), 325.0930 (40), 487.1593 (95), 505.1700 (60)	Coumaroyl hexoside derivatives
28 [g]	4.4	491.1917	491.1923	$C_{25}H_{31}O_{10}^-$	1.2	179.0355 (10), 221.0462 (5), 251.0561 (10), 281.0675 (20), 341.0886 (3), 491.1917 (100)	Caffeoyl-hexopyranosy-dimethyl-octadienone
29 [f]	4.4	521.1639	521.1664	$C_{25}H_{29}O_{12}^-$	4.8	163.0389 (20), 341.1003 (15), 505.1668 (70), 521.1639 (100)	*p*-Coumaroyl-loganic acid isomer 2
30	4.5	819.2909	819.2870	$C_{43}H_{47}O_{16}^-$	-4.8	147.0422 (100), 161.0578 (70), 189.0527 (80), 249.0708 (20), 409.1458 (95), 819.2909 (40)	Unknown
31 [g]	4.7	475.1970	475.1974	$C_{25}H_{31}O_9^-$	0.8	163.0409 (10), 205.0516 (20), 325.0627 (40), 265.0727 (100), 325.0939 (80), 475.1970 (40)	Phenylbytil-*O*-dihydrocoumaroyl-hexoside

32 [g]	4.9	667.2029	667.2032	$C_{34}H_{35}O_{14}^-$	3.9	163.0407 (40), 177.0568 (100), 265.0733 (15), 307.0836 (20), 485.1469 (90), 667.2058 (30)	Unknown derivatives of acetyl-di-feruloyl-glycerol
33 [f]	5.8	599.1803	599.1770	$C_{30}H_{31}O_{13}^-$	-5.5	103.0558 (17), 147.0464 (100), 553.1734 (10), 599.1803 (30)	Trihydroxydihydrochalcone-siringoyl-hexoside

[a] the analytes are listed in the order of their elution; [b] the m/z values were derived from the acquired spectra; [c] the m/z values were calculated based on the predicted elemental composition (EC); [d] the elemental compositions were predicted with mass tolerance of 10 ppm; [e] MS/MS fragmentation patterns are provided as m/z values of the fragment ions (their relative intensities); [f] the list of genus representatives in which the compound was found; [f] Major metabolite annotation was performed using tandem mass spectrum acquired with a hybrid QqTOF mass spectrometer operated in the negative SWATH mode; [g] Major metabolite annotation was performed using tandem mass spectrum acquired with a hybrid QqTOF mass spectrometer operated in the negative product ion mode with unit Q1 resolution.

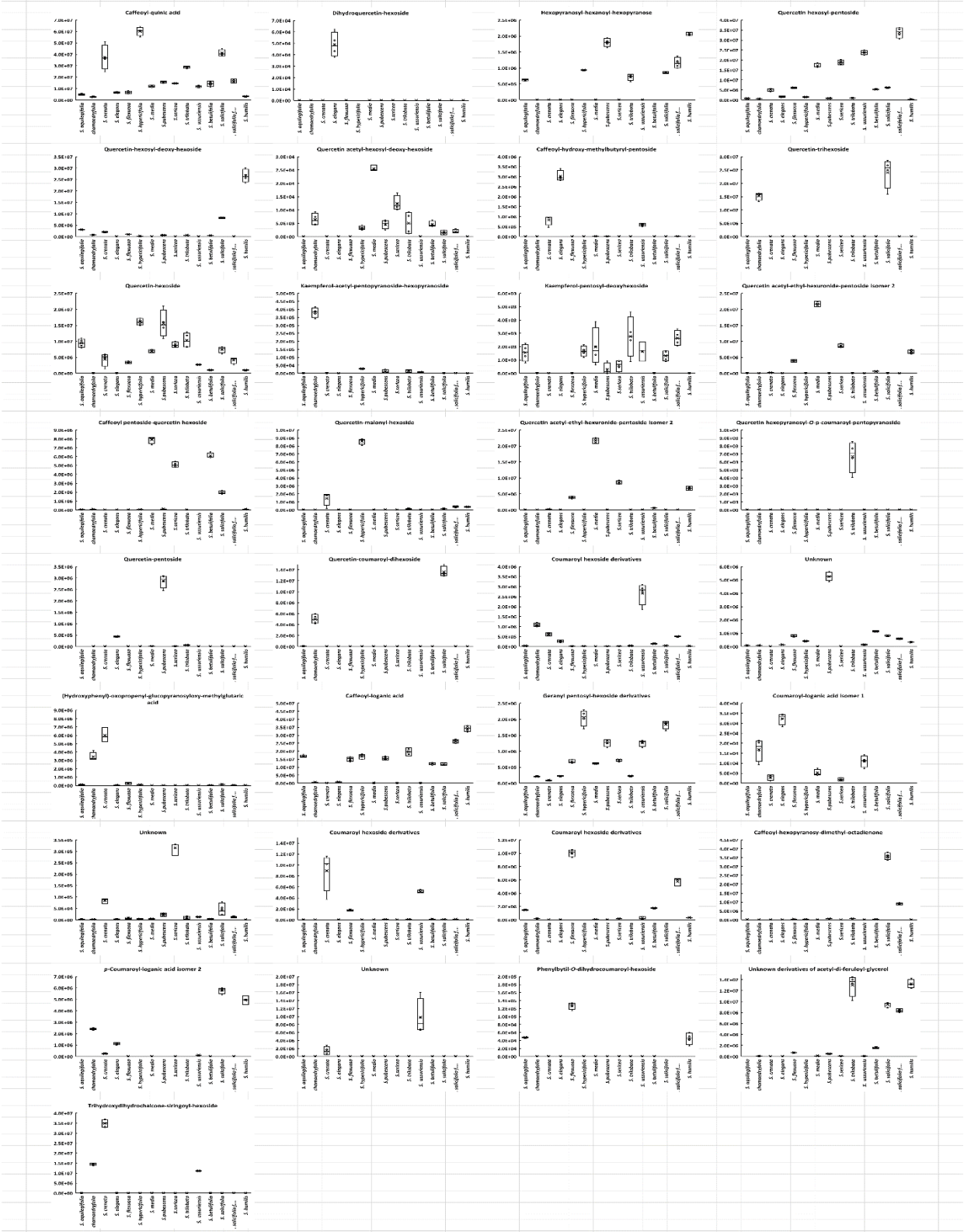

Figure 2. Relative recoveries of the major components in methanol extracts of first-year shoots of 15 plant species of the genus *Spiraea*, expressed as boxplots. Major components are named according to Table 2.

3.2 Antioxidant effects of the methanolic shoot extracts of *Spiraea* spp.

The antioxidant effects of the *Spiraea* shoot extracts were addressed by three independent tests. Thus, the DPPH assay showed that the shoot extracts of *S. humilis* extract appeared to be the strongest antioxidant (99.4% of the normalized to ascorbic acid activity, Table 3), whereas the extracts of *S. aquilegifolia* (85.1%), *S. betulifolia* (84.9%) and *S. media* (84.7%) were slightly less active. The lowest reactivity towards the DPPH free radical showed *S. flexuosa* and *S. ussuriensis* (31.8% and 34.1%, respectively).

Table 3. Antioxidant potential of the methanolic extracts obtained from the first-year shoots of *Spiraea* plants

Plant species	DPPH normalized activity, %	TEAC, μmol/L eq. Trolox/μg	NBT assay, nmol $O_2^{-\bullet}$/min
S. humilis	99,470±0,001	26,725±0,007	21,240±0,012
S. aquilegifolia	85,141±0,002	19,678±0,018	25,107±0,006
S. chamaedryfolia	43,172±0,002	14,218±0,007	23,960±0,016
S. trilobata	79,691±0,001	21,127±0,019	24,320±0,019
S. betulifolia	84,957±0,004	21,020±0.007	21,342±0,019
S. ussuriensis	34,062±0,001	13,915±0,005	21,693±0,003
S. media	84,733±0,001	21,664±0,003	25,404±0,011
S. salicifolia	73,227±0,009	21,161±0,009	22,724±0,013
S. pubescens	56,639±0,006	16,919±0,008	22,920±0,013
S. crenata	44,607±0,001	14,793±0,007	28,169±0,016
S. flexuosa	31,811±0,001	14,749±0,005	24,071±0,005
S. hypericifolia	74,069±0,001	20,466±0,015	29,969±0,034
S. elegans	36,721±0,001	14,329±0,010	25,942±0,007
S. sericea	56,337±0,001	16,917±0,005	27,204±0,008
S. salicifolia f. alpestris	58,548±0,002	18,752±0,006	27,849±0,011
DMSO (control)	-	-	30,169±0,041

DPPH – 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay; TEAC – Trolox equivalent antioxidant capacity; NBT (nitroblue tetrazolium) assay – assessment of capacity to scavenge superoxide anion radicals. 20 μg of each lyophilized extracts was used.

The Trolox equivalent antioxidant capacity (TEAC) assay revealed the extract from the first year shoots of *S. ussuriensis* as the most active against the Trolox cation radical (13.9 μmol/L eq.

Trolox/µg of the extract), while *S. humilis* extract showed the least pronounced activity of 26.7 µmol/L eq. Trolox/µg. The highest activity against the superoxide radical was shown by the *S. humilis, S. betulifolia* and *S. ussuriensis* extracts with 21.2, 21.3 and 21.7 nmol $O_2^{-\bullet}$/min, respectively. The extract of *S. hypericifolia* showed the least activity (29.9 nmol $O_2^{-\bullet}$/min) with an activity level close to that of the blank sample.

3.3 Antiviral effects of the methanolic shoot extracts of *Spiraea* spp.

Methanolic extracts obtained from the first-year shoots of 15 *Spiraea* species were screened for antiviral activity against influenza A (H1N1) virus. For this, the extracts were lyophilized and reconstituted in DMSO (concentration of the stock solutions 50 mg/mL) and then diluted in the culture medium to obtain the final concentrations of 4 – 300 μg/mL. Cytotoxicity of the extracts was investigated and the most selective dry extracts were found (Table 4).

Table 4. Antiviral activities of the methanolic extracts obtained from the first-year shoots of *Spiraea* plants

Plant species	LD_{50}, µg/mL	IC_{50}, µg/mL	SI
S. humilis	59.2	3.8	16
S. aquilegifolia	53.6	5	11
S. chamaedryfolia	27.4	2	14
S. trilobata	68.6	>33	2
S. betulifolia	50.9	13.8	4
S. ussuriensis	19	1	19
S. media	43.6	>33	1
S. salicifolia	31.1	2	16
S. pubescens	48.9	12.1	4
S. crenata	45.2	14.1	3
S. flexuosa	25.3	>11	2
S. hypericifolia	45.3	19.2	2
S. elegans	47.5	17.7	3
S. sericea	44.4	13.8	3
S. salicifolia f. alpestris	48.3	4	12

LD_{50} – 50% cytotoxic concentration, leading to the death of half of the cells in culture; IC_{50} – 50% effective concentration, which halves viral activity; SI – selectivity, the ratio of LD_{50} to IC_{50}.

All the analyzed *Spiraea* extracts showed antiviral activity against the influenza A virus. Based on the IC_{50} values, the extracts of *S. ussuriensis, S. chamaedryfolia* and *S. salicifolia* with the IC_{50} values of 1, 2 and 2 μg/mL, respectively, appeared to have the highest antiviral activities. In contrast, the *S. trilobata* and *S. media* extracts yielded the highest IC_{50} values (> 33 μg/mL), i.e. these extract were the least active.

Of course, the applicability of individual extracts as potential antiviral agents can be considered only in the context of the cytotoxicity data. Thus, when the cytotoxic concentration leading to the death of half of the cells in culture (LD_{50}) was addressed, it was found that the most efficient

extract of *S. ussuriensis* was also the most cytotoxic (LD_{50} = 19 μg/mL). On the other hand, some extracts that showed the least activity were found to be the least cytotoxic. For example, the extract from the first-year shoots of *S. trilobata* showed the least cytotoxicity (LD_{50} = 68.6 μg/mL). In terms of selectivity (SI = LD_{50}/IC_{50}), *Spiraea* specimens were arranged as follows: *S. media* < *S. flexuosa* = *S. hypericifolia* = *S. trilobata* < *S. crenata* = *S. elegans* = *S. sericea* < *S. pubescens* = *S. betulifolia* < *S. aquilegifolia* < *S. salicifolia f. alpestris* < *S. chamaedryfolia* < *S. salicifolia* < *S. humilis* < *S. ussuriensis*. Thus, *S. ussuriensis* (SI = 19) and *S. salicifolia* and *S. humilis* (SI = 16) showed the highest selectivity against influenza A virus. Due to this, these spirea species can be considered as the most promising candidates as a potential source of antiviral agents.

3.4 Antibacterial activity of methanolic shoot extracts of *Spiraea* spp.

The antibacterial activities of the extracts prepared from first-year shoots of the 15 *Spiraea* species were investigated *in vitro* by broth microdilution assay, and the corresponding MICs were determined for each of the six test microorganisms (Table 5). The assay revealed either high (MIC < 1 mg/mL) or moderate (1 mg/mL ≤ MIC ≤ 4 mg/mL) antibacterial activity against the Gram-positive and Gram-negative bacteria strains. The extracts obtained from *S. humilis* showed the strongest antibacterial activity – the corresponding MICs obtained with all tested microorganisms differed from the MICs acquired for the other extracts by 1-2 orders of magnitude. The extract from *S. media* also demonstrated high antibacterial activity with the MICs 2-4 times higher in comparison to those of *S. humilis*. Interestingly, the spectra of the antibacterial activity of the extracts from these two *Spiraea* species turned out to be similar: the microorganisms *P. aeruginosa* and *MRSA* (Methicillin-resistant *Staphylococcus aureus*) were the most resistant to the action of these extracts, and both extracts most efficiently inhibited the growth of *M.luteus*, while the MICs against this microorganism were the highest for most of the other extracts. The extracts from *S.aquilegifolia*, *S.betulifolia*, *S.salicifolia S.pubescens*, *S.crenata* and *S.salicifolia f. alpestris* exhibited high activity against *E.coli.* and *S.aureus,* with only moderate effect observed against the other microorganisms. Finally, the last group of the extracts – the isolates from *S.chamaedryfolia*, *S.trilobata*, *S.ussuriensis*, *S.flexuosa*, *S.hypericifolia*, *S.elegans* and *S.sericea,* showed high antibacterial activity only against *E.coli.* The antibacterial activities against the other tested microorganisms, observed with the extracts from these species appeared to be moderate.

Table 5 Antibacterial activities of the methanolic extracts obtained from the first-year shoots of *Spiraea* plant

Plant species	Activity (MICs, µg/mL)					
	Microorganism strain					
	E.c. ATCC 25922	*P.a.* ATCC 27853	*S.a.* ATCC 25923	*MRSA* ATCC 33591	*M.l.* CIP A270	*L.m.* EGD
S. humilis	62.5	500	125	500	31.2	125
S.aquilegifolia	125	2000	500	1000	1000	1000
S.chamaedryfolia	125	2000	1000	1000	2000	4000
S.trilobata	125	1000	1000	1000	2000	2000

S.betulifolia	125	1000	500	2000	1000	2000
S.ussuriensis	250	2000	1000	2000	4000	2000
S.media	125	1000	250	1000	125	500
S.salicifolia	125	1000	250	2000	1000	2000
S.pubescens	125	1000	250	1000	4000	2000
S.crenata	125	1000	500	1000	1000	2000
S.flexuosa	125	1000	1000	2000	4000	4000
S.hypericifolia	250	1000	1000	2000	4000	4000
S.elegans	125	2000	1000	1000	4000	2000
S. sericea	125	1000	1000	2000	4000	4000
S.salicifolia f. alpestris	125	1000	250	2000	1000	1000

Antimicrobial activities were expressed as minimal inhibitory concentrations, MICs. *E.c.* – *Escherichia coli*, *P.a.* – *Pseudomonas aeruginosa*, *L.m.* – *Listeria monocytogenes*, *S.a.* – *Staphylococcus aureus*, *MRSA* – Methicillin-resistant *Staphylococcus aureus*, *M.l.* – *Micrococcus luteus*.

4. Discussion

Here we present a comprehensive phytochemical screening of the major secondary metabolites in the extracts prepared from the first year shoots of 15 *Spiraea* species occurring in Russian Federation. We are convinced that our study is a significant step forward in respect of the current state of research in this field. Indeed, to the best of our knowledge, it is the first study, considering such a representative set of *Spiraea* species, which were selected according to clear and straightforward logics. Moreover, here we connect a metabolite profiling survey with a comprehensive study of biological activities to address the Russian spirea species as promising sources of biologically active complexes for use in medical practice.

Having in mind the pharmaceutical context of this work, we focused on the structural annotation of only major secondary metabolites with further interpretation of their prospective roles as bioactive natural products based on the activity data. The selection of the major metabolites relied on $4.5*10^7$ counts in corresponding mass spectra as the threshold value of their signal intensity. On the other hand, we did not go in much detail to comprehensively characterize the interspecies metabolic differences at the level of minor compounds, although we realize that multiple variations in their relative contents might be found, due to high chemical diversity of the selected set of the *Spiraea* species, which is clearly seen from the relative abundances of the top-constituents (Figure 2). In this decision we relied on the general assumption that the major components of the extracts should contribute most essentially to the formation of its biological activity profile. On the other hand, cutting off the low-abundant species might essentially simplify the overall picture and might essentially facilitate understanding of the structure-activity relationships (SAR) at the follow up steps of this study.

Expectedly, the obtained here results did not conflict with the previously published data, which indicated polyphenols as the principal group of semi-polar secondary metabolites constituting methanolic extracts of *Spiraea* shoots **Ошибка! Источник ссылки не найден.**9-[42]. Thus, when applying the selected criteria of secondary metabolites extracted from the first-year shoots of the fifteen *Spiraea* species considered, we annotated in total 33 major components. Among

them, 15 metabolites represented the class of flavonoids, namely quercetin derivatives (**2, 4-6, 8-9,12-18**) and kaempferol derivatives (**10-11**). The presence of quercetin and kaempferol derivatives has been described earlier, and both flavonoid aglycones and their derivatives (mainly *O*-glycosides) were characterized in the spirea parts as one of the major compounds [34], [29], [44]-[45]. On the other hand, no previously undescribed compounds were found in the studied *Spiraea* species. In addition, a wide variety of apigenin, luteolin and isorhamnetin derivatives were reported in leaves and shoots of various spirea species [41]1, [46]6, [47]. However, these metabolites, as well as some isoflavones, reported earlier in *Spiraea* plants [32], [48], were not discovered as the major extract components here.

The further group of the annotated major extract constituents included 15 secondary metabolites which could be attributed to hydroxycinnamic acids. This group of compounds appeared to be not less diverse and abundant (at least based on the intensity of the corresponding chromatographic signals) than flavonoids. Among the major metabolites of the studied species, derivatives of caffeic (**1, 7, 22, 28**), coumaric (**19, 24, 26, 29, 31**) and ferulic (**27, 32**) acids were tentatively annotated. A wide variety of derivatives of this group were previously reported [49]9-[51]. Also, among the major components, we found derivatives of glutaric acid and geraniol alcohol, which, to the best of our knowledge, were not reported previously. In addition, three components that could not be annotated using the chromatography-mass-spectrometric method were found during the study of the composition of the major components of the extracts. Apparently, additional studies are needed to identify these compounds, which include obtaining them individually and establishing their structure by nuclear magnetic resonance spectroscopy.

When comparing the profiles of the major extract components, it was found that only a minor part of them were common for all, or almost all species (Figure 2). Thus, only caffeoyl-quinic acid (**1**) appeared to be the common component for all extracts, while quercetin hexoside (**9**) occured in seven and coumaroyl-loganic acid (**24**) in eight out of fifteen extracts. The other 30 identified major constituents were distributed among all studied extracts with minimal overlap in component composition and relative abundance (Figures 1, 2).

High-resolution mass spectrometry (HR-MS) as a profiling method used in this study allowed making only a preliminary qualitative and semi-quantitative assessment of the composition of the major metabolites in the studied extracts. For comprehensive characterization of the major components, it is critically important to identify them unambiguously. This can be achieved by two strategies: (*i*) isolation of pure compounds from the extracts and unambiguous structure assignment by NMR spectroscopy or (*ii*) by co-elution with authentic standards. At the next step, the isolated compounds (or commercially available authentic compounds) need to be used as the standards for absolute quantification by the standard addition approach [38]. However, the process of isolation and working up individual compounds is a very labour-, time-consuming and rather expensive procedure. Because of this, it can be justified, only when the first data on the principal extract composition and profiles of biological activity are acquired and the species of interest are selected based on some correlations built between chemical composition and biological activity.

Therefore, here we make this first and absolutely mandatory step, and implement this logic to the study of a highly diverse taxonomic group – the genus *Spiraea*. The further in-depth investigation of the *Spiraea* species will rely on the results of this study. The rationales for selection the species for the comprehensive studies might include (*i*) annotation of new natural products as

the major extract constituents, (*ii*) the presence of significant biological activities of the extracts and (*iii*) high natural occurrence to access sufficient resource base or (*iv*) the availability of technologies for introduction into culture (at the cell, tissue or agricultural levels). The latter requirement could be addressed already at the step of selection the species for the study. Taking into account these facts, the preliminary selection of fifteen spirea species of the 15 confirmed species was made taking into account the high prevalence of these species on the territory of the Russian Federation and the possibility of their introduction into culture. To identify the most promising species for further research, the biological activity of their methanol extracts was screened on several relevant *in vitro* models.

Since the main group of biologically active plant compounds are representatives of the group of polyphenols, the choice of activities was addressed in favour of models, the mechanism of which is presumably related to the antioxidant properties of the studied components. This key property of the phenolics in general and polyphenols in particular allows targeting oxidative stress, which is known to be associated with multiple pathologies including age-related diseases, like type 2 diabetes mellitus (T2DM) and neurodegenerative diseases [52]2-[54]. Thus, antioxidant properties of phenolics are tightly connected with an array of other biological activities – anti-inflammatory, antidiabetic, neuroprotective and anti-ageing [55]5-[58].

Taking this fact into account, analysis of antioxidant activity of polyphenol-containing plant extracts became a gold standard in the activity-driven phytochemical research. Therefore, much data on the antioxidant properties of plant extracts are available [59]9-[61], that allows cross-verification of the newly acquired information. Finally, due to their relative simplicity and reliability, antioxidant tests can be easily performed in a high-throughput format, being a convenient methodology for screening large amounts of biological material. In this study, we used three antioxidant activity assays (DPPH assay, TEAC assay and NBT assay) to evaluate the activity profile of the extracts in the most comprehensive way. The choice of these three models was based on their complementarity and the possibility of using different mechanisms to understand their effects on different pathways of ROS metabolism. The data obtained showed that the most pronounced antioxidant properties in all three experiments (99.5% in the DPPH test, 26.7 μmol/L eq. Trolox/μg in TEAC test and 21.2 nmol $O_2^{-\bullet}$/min in NBT test) were shown by the extract of *S. humilis*, which contained several derivatives of quercetin, caffeic acid, coumaric acid and geraniol as the major constituents. Based on their activity, the rest of the analyzed extracts could be divided into two groups - the extracts with high activity and the extracts with moderate antioxidant activity. The methanol extracts of *S. aquilegifolia, S. trilobata, S. betulifolia, S. media, S. salicifolia* can be classified as the first group. Extracts of other species of the genus - *S. ussuriensis, S. chamaedryfolia, S. pubescens, S. crenata, S. flexuosa, S. hypericifolia, S. elegans, S. sericea, S. salicifolia f. alpestris* - were classified as moderately active.

In general, these results are consistent with the published data. Indeed, high antioxidant activity of some species of the genus has been noted earlier [23] [62]. At the same time, no general patterns in the qualitative and relative quantitative composition of major components in the studied extracts were revealed, which suggests that their unique complexes, characteristic of a particular plant species, rather than individual major compounds present in the extracts, are responsible for their activity (Figure 2). The data obtained by us agree with earlier studies describing high antioxidant potential, positively correlating with the content of polyphenolic compounds (total polyphenols,

flavonoids and phenolic acids), as well as linking the manifestation of antioxidant activity of plants of this genus with the accumulation of a complex of compounds of unique composition with a different number of hydroxyl groups directly involved in radical quenching [62]2-[64]. The correlation analysis of the dependence of antioxidant activity on the content of metabolites carried out in our analysis showed a significant contribution of phenolic acid derivatives (geranyl pentosyl hexoside, caffeoyl-loganic acid, hexopyranosyl-hexanoyl-hexapyranose) and some quercetin derivatives (quercetin acetyl-hexosyl-deoxy-hexoside) to the manifestation of different types of antioxidant activity. Thus, geranyl-pentosyl-hexoside, caffeoyl-loganic acid, quercetin acetyl-hexosyl-deoxy-hexoside, hexopyranosyl-hexanoyl-hexapyranose make the greatest contribution to the display of activity against cation radical in the TEAC test (Pearson correlation coefficients are 0.69, 0.68, 0.66, 0.64 and 0.64, respectively), geranyl-pentosyl-hexoside and hexopyranosyl-hexanoyl-hexapyranose contributed most to the free radical activity in the DPPH test (Pearson correlation coefficients are 0.63 and 0.62, respectively), and quercetin malonyl hexoside contributed most to the superoxide anion radical activity in the NBT test (Pearson correlation coefficient is 0.59) (Figure S3). Consequently, the high content of these components in the extracts of *S. humilis, S. hypericifolia, S. elegans* and *S. trilobata* may account for the high antioxidant potential of these plant species.

At the next step, we established the antiviral activity profiles of the spirea extracts against influenza A virus. Influenza is one of the most common viral pathologies with high mortality and risk of systemic complications [66]. It should be noted that there is currently a wide range of drugs available at the pharmaceutical market for the prevention and treatment of viral diseases. However, the vast majority of the medicines presented belong to the group of symptomatic and immunomodulatory preparations, while there are unjustifiably few direct-acting antiviral agents with proven efficiency. Therefore, research aiming development of new efficient drugs for individual and combination therapy of influenza remains an important public health challenge. Earlier studies demonstrate high activity of polyphenol-rich extracts of various plant species against influenza virus types A and B [67].

Typically, such antiviral activity relies on several types of mechanisms. For example, polyphenolic compounds prevent virus attachment to the cell membrane by binding to surface receptors, block translocation of viral ribonculeoproteins and reduce viral protein expression, as in the case of resveratrol, or inhibit replication in the case of quercetin and its derivatives [70]. In our study, all the extracts under consideration had moderate and pronounced antiviral activity against influenza A virus, but the highest activity was characteristic of *S. ussuriensis, S. salicifolia* and *S. humilis* extracts. These extracts were characterized by the presence of a large number of compounds from the phenolic acid group among the major metabolites. Thus, the high contents of caffeic acid, coumaric acid and their derivatives in the extracts, along with representatives of the class of polyphenolic compounds, is capable of exerting a pronounced anti-influenza effect both when used individually and in combination with other pharmaceutical agents due to inhibition of neuraminidase [71]. In addition, based on our correlation analysis, it was shown that antiviral activity correlates with antioxidant activity inherent in the studied extracts, especially with activity against free radical (Pearson correlation coefficient is 0.66) and activity against cation radical (Pearson correlation coefficient is 0.60). These correlations may indicate the contribution of the antioxidant potential of the studied extracts to the mechanism of their antiviral action, which does

not contradict the available literature data (Figure S4) [74]. However, to establish the exact mechanism of action of extracts and major components of spirea extracts, it is necessary to accomplish additional studies.

The uncontrolled use of antibiotics by consumers, their improper disposal, their widespread use in agricultural practice, and technogenic accidents at production facilities expands the problem of microbial resistance formation to a global scale [75]. Polyphenolic compounds are widely known antimicrobial agents due to their ability to both directly affect pathogenic and opportunistic microbes and inhibit microbial virulence factors [76]. That is why the study of antimicrobial agents of plant origin for the creation of new antimicrobial therapy agents, as well as auxiliary agents of classical antibiotic therapy, remains a steadily relevant task. In spite of a rather long-established idea about the prevalence of polyphenolic compounds in plants of the genus *Spiraea*, studies of their antimicrobial activity are rather scarce. Thus, to date, data on the antimicrobial activity of species of the genus *Spiraea* are limited to screening the activity of the shoots of *S. chamaedryfolia* against *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 29213), *S. pneumoniae* (ATCC 49619), *M. catarrhalis* (ATCC 25238) and MRSA (ATCC 43300), demonstration of moderate activity of the seed extract of *S. tomentosa* against *S. aureus* (ATCC 12600), *E. coli* (ATCC 8677), *P. aeruginosa* (ATCC 9721) and *C. albicans* (ATCC 10231), and high activity of *S. thunbergii* leaf extract against *E. coli* [23][24] [34][80].

Screening of methanol extracts of 15 species of the genus *Spiraea* in this study showed on average high to moderate antimicrobial activity against Gram-positive and Gram-negative bacteria. The lowest MICs, and hence the highest antimicrobial activities against all the microorganisms tested, were possessed by extracts of *S. humilis* and *S. media*, the extracts of which are enriched with quercetin derivatives. In addition, the high antimicrobial activity of *S. humilis* may be related to the presence of geraniol derivative in the major components, which according to literature data has a pronounced antibacterial and antifungal activity [81]. It is worth noting that among the extracts studied, the geraniol derivative (compound **23** - geranyl 6-*O*-pentopyranosyl-hexopyranoside) was annotated only in the methanol extract of *S. humilis*.

Further, correlation analysis revealed that antimicrobial activity against selected microbial strains correlated with high contents of dihydroquercetin hexoside, quercetin malonyl hexoside and coumaroyl hexoside derivatives in the extracts. Based on these data, it is possible to explain the relatively high activity of *S. elegans* (the species characterized by the highest dihydroquercetin hexoside content), *S.hypericifolia* (the species characterized by the highest quercetin malonyl hexoside content) against *Escherichia coli* (MIC 125 and 250 μg/mL, respectively). However, on the other hand, the *S. ussuriensis* extract characterized by the highest amount of coumaroyl-hexoside derivatives responsible for antimicrobial activity against *Pseudomonas aeruginosa* showed no significant activity against this strain (MIC 2000 μg/mL) (Figure S3).

In addition, based on our correlation analysis, it was shown that antimicrobial activity against *Pseudomonas aeruginose* and *Micrococcus luteus* strains correlates with antioxidant activity. Thus, Figure S4 shows the possible correlation of antimicrobial activity in respect of *Pseudomonas aeruginose* with activity against cation radical (Pearson correlation coefficient is -0.61), and the correlation of the activity against *Micrococcus luteus* strain with activity against free radicals and cation radical (Pearson correlation coefficient is -0.66 and -0.63, respectively). The data obtained are interesting in terms of selective correlation of antimicrobial activity of the extracts with

antioxidant activity and require further verification. However, further studies are needed to better substantiate the mechanism of antimicrobial activity of major extract compounds, activity of this extract component as well as possible synergistic and antagonistic interactions of the extract components.

5. Conclusions

To conclude, in this study we screened major secondary metabolites extracted with methanol from the first-year shoots of fifteen species representing the genus *Spiraea*. It was shown that the main group of metabolites of representatives of this genus are polyphenolic compounds, in particular derivatives of quercetin, kaempferol, hydroxycinnamic acids (caffeic, coumaric, ferulic, etc.). As a result of phytochemical and pharmacological screening, we have identified the most promising species of the genus *Spiraea* for further study, including *S. humilis*, which has a marked complex antioxidant, antimicrobial and antiviral effect, *S. media* with marked antioxidant, antimicrobial and cytotoxic properties, *S. ussuriensis*, a strong anioxidant and cytotoxic species and *S. trilobata* with antioxidant and antiviral properties. Further in-depth phytochemical studies, as well as evaluation of the pharmacological activity of individual metabolites of these plant species and their purified complexes will help to form a holistic understanding of the prospects for further industrial development and application of the components of various spiraea species as biologically active additives and medicinal preparations.

CRediT authorship contribution statement

Anastasia Orlova: Writing–review & editing, Writing–original draft, Investigation, Supervision, Conceptualization. **Alena Soboleva:** Investigation, Writing–review & editing. **Elena Tsvetkova:** Investigation, Writing–review & editing. **Svetlana Silinskaia**: Investigation. **Yana L. Esaulkova**: Investigation. **Tatiana Veklich**: Investigation. **Vladimir V. Zarubaev**: Investigation. **Ksenia Fedoseeva**: Project administration, Funding acquisition. **Ilya R. Akberdin**: Investigation. **Semyon K. Kolmykov**: Investigation. **Vera A. Kostikova**: Project administration, Funding acquisition, Conceptualization. **Andrej Frolov**: Writing review & editing, Supervision, Project administration, Conceptualization.

Ethical approval

This article does not include any studies that use human or animal tissues.

Data availability statement

The data presented in this study are available on request from the corresponding authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements:

The work was infrastructurally supported by the Ministry of Science and Higher Education of the Russian Federation (theme № 122042700045-3). We thank the core facilities of the St. Petersburg State University "Molecular and cell technologies" (Contract No. 01/1-69-46-SPbSU (NP ID: C-111/30))

Funding:

This work was financial support from the Russian Science Foundation (grant RSF 23-24-00310 and grant RSF 23-15-00142).

Supplementary Materials:

Supplementary data to this article can be found online at

References:

[1] B. Godlewska-Żyłkiewicz, R. Świsłocka, M. Kalinowska, A. Golonko, G. Świderski, Ż. Arciszewska, E. Nalewajko-Sieliwoniuk, M. Naumowicz, W. Lewandowski. Biologically Active Compounds of Plants: Structure-Related Antioxidant, Microbiological and Cytotoxic Activity of Selected Carboxylic Acids. Materials, 2020, 13(19), 4454. https://doi.org/10.3390/ma13194454

[2] D. Olender, J. Żwawiak, L. Zaprutko. Multidirectional Efficacy of Biologically Active Nitro Compounds Included in Medicines. Pharmaceuticals, 2018, 11(2), 54. https://doi.org/10.3390/ph11020054

[3] D. Sinha, U.E. Odoh, S. Ganguly, M. Muhammad, M. Chatterjee, I. Chikeokwu, C. Egbuna. Phytochemistry, History, and Progress in Drug Discovery. Phytochem. Comput. Tools, Databases Drug Discov. 2023, 1-26. https://doi.org/10.1016/B978-0-323-90593-0.00001-0

[4] O. Bender, E.J. Llorent-Martínez, G. Zengin, A. Mollica, R. Ceylan, L. Molina-Garcia, M.L. Fernández-de Córdova, A. Atalay. Integration of in vitro and in silico perspectives to explain chemical characterization, biological potential and anticancer effects of Hypericum salsugineum: A pharmacologically active source for functional drug formulations. PLoS One, 2018, 13(6), e0197815. https://doi.org/10.1371/journal.pone.0197815

[5] L.T. Lu, A. Crinan. Spiraea Linnaeus. In Flora of China; Wu, Z.Y., Raven, P.H., Eds.; Science Press: Beijing, China; Missouri Botanical Garden Press: St. Louis, MI, USA, 2003; Volume 9, pp. 47-73.

[6] The Plant List (2013) Version 1.1. Available online: https://www.theplantlist.org (accessed on 1 May 2021).
[7] T.I. Slavkina. Vidy roda *Spiraea*, introduzcirovannye Botanicheskim sadom AN UzSSR [Species of the genus *Spiraea* introduced in the Botanical Garden of UzSSR] // Dendrologiya Usbekistana [Dendrology of Uzbekistan]. Tashkent, 1972, 196-304 [in Russian].
[8] V.V. Shulgina. Genus Spiraea L. In Trees and Shrubs of the USSR – Native, Cultivated or Promising for Introduction; Sokolov, S.Y., Ed.; Akademija Nauk SSSR: Moskow/Leningrad, Russia, 1954; Volume 3, 269-331. (In Russian)
[9] T.T. Yü, K.C. Kuan. Taxa nova Rosacearum sinicarum // Acta phytotaxonomica Sinica. 1963. – V. 8 (3). – 214-215.
[10] A.I. Pojarkova. Sorbaria. A.Br. In: Komarov, V.L & Yuzepchuk, S.V. (Eds.) Flora of the U.S.S.R., Vol. 4. Department of Commerce, U.S. Virginia, 1971, 240-243.
[11] T.N. Vstovskaya, I. Yu. Koropachinsky. Woody plants of the Central Siberian Botanical Garden. – Novosibirsk: SB RAS Publishing House, «Geo» affiliation, 2005, 235 p. [in Russian].
[12] K.S. Baikov. Conspectus Florae Rossiae Asiaticae: Plantae Vasculares; Publishing house of the Siberian Branch of the Russian Academy of Sciences: Novosibirsk, Russia, 2012. ISBN 9785769212130. (In Russian)
[13] P.F. Mayevsky. Flora of the middle zone of the European part of Russia. 10th ed. ispr. and add. Moscow: Association of Scientific publications KMK, 2006, 600 p.
[14] X.S. Zhang, B.D. Wang. Chinese Medicine Dictionary: Shanghai Science and Technology Publishing House, Shanghai, 1986. p. 1117, 1978.
[15] T.S. Wu, C.C. Hwang, P.C. Kuo, T.H. Kuo, A.G. Damu, C.R. Su. New neolignans from Spiraea formosana // Chem. Pharm. Bull. 2004, Vol. 52, N10, 1227-1230. https://doi.org/10.1248/cpb.52.1227
[16] S.W. Khan, S. Khatoon. Ethanobotanical studies on useful trees and shrubs of Haramosh and Bugrote valleys, in Gilgit northern area of Pakistan // Pak. J. Bot. 2007, Vol. 39, 699-710.
[17] Z.Y. Wu. Index florae yunnannensis (tomus I). Kuming, Yunnan, China: The People's Publishing House, 1984, 524 pp.
[18] Z.W. Xie. Quanguo Zhongcaoyao Huibian (A Collection of Chinese Herbal Drugs), 2nd edn., People's Hygenic Publishing House, Beijing, 1996, P. 514, 767.
[19] V.K. Lavrenov, G.V. Lavrenova. Complete Encyclopedia of Medicinal Plants, Olma-Press, Moscow, Russia, 1999, Volume 2, pp. 337-340. (In Russian)
[20] S.M. Batorova. Guide to Traditional Tibetan Medicinal Plants; Nauka, Novosibirsk, Russia, 2013. (In Russian)
[21] B.W. Lee, J.H. Ha, H.G. Shin, S.H. Jeong, D.B. Jeon, J.H. Kim, J.Y. Park, H.J. Kwon, K. Jung, W.S. Lee, H.Y. Kim, S.H. Kim, H.J. Jang, Y.B. Ryu, I.C. Lee. *Spiraea prunifolia* var. *simpliciflora* attenuates oxidative stress and inflammatory responses in a murine model of lipopolysaccharide-induced acute lung injury and TNF-stimulated NCI-H292 cells. Antioxidants 2020, 9, 198. https://doi.org/10.3390/antiox9030198
[22] V.A. Kostikova, N.V. Petrova, T.M. Shaldaeva, V.V. Koval, A.A. Chernonosov. Non-Targeted Screening of Metabolites in Aqueous-Ethanol Extract from *Spiraea hypericifolia* (Rosaceae)

Using LC-HRMS. International Journal of Molecular Sciences, 2023, 24(18), 13872. https://doi.org/10.3390/ijms241813872
[23] J.R. Borchardt, D.L. Wyse, C.C. Sheaffer, K.L. Kauppi, R.G. Fulcher, N.J. Ehlke, D.D. Biesboer, R.F. Bey. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. J. Med. Plant. Res. 2008, 2, 81-93.
[24] J. Lee, J. Lee, J. Lim, S. Sim, D. Park. Antibacterial effects of S-(-)-tulipalin B isolated from *Spiraea thunbergii* Sieb. on Escherichia coli, major food borne pathogenic microorganism. J. Med. Plant. Res. 2008, 2, 59-65.
[25] Q. Kong. Application of *Spiraea alpina* Extract in Preparing Anticancer Drugs. Chinese Patent 103585310 (CN), 19 February 2014 (In Chinese).
[26] O.A. Kaidash, V.A. Kostikova, E.V. Udut, V.V. Shaykin, D.R. Kashapov. Extracts of *Spiraea hypericifolia* L. and *Spiraea crenata* L.: The phenolic profile and biological activities //Plants. 2022, T. 11, №. 20, C. 2728. https://doi.org/10.3390/plants11202728
[27] V.M. Mirovich, I.M. Krivosheev, V.V. Gordeeva, A.V. Tsyrenzhapov. Method for Producing Agent Possessing Anti-Inflammatory, Diuretic and Antioxidant Activity. Russian Patent RU 2542493C1, 8 November 2013. (In Russian).
[28] M.O. Sim, H.J. Lee, J.H. Jang, H.E. Lee, H.K. Jung, T.M. Kim, J.H. No, J. Jung, D.E. Jung, H.W. Cho. Anti-inflammatory and antioxidant effects of *Spiraea prunifolia Sieb*. et *Zucc*. var. *simpliciflora Nakai* in RAW 264.7 cells. Korean J. Plant Res. 2017, 30, 335-342. (In Korean)
[29] L. Hörhammer, R. Hänsel, W. Endres. Über die flavonglykoside der gattungen Filipendula und Spiraea. Arch. Pharm. 1956, 289, 133-140. (In German)
[30] T. Bodalski, W. Cisowski. Flavonoids in the inflorescence of *Spiraea media* Schm. Diss. Pharm. Pharmacol. 1969, 21, 443-447.
[31] A.L. Budantsev. Plant Resources of Russia: Wild Flowering Plants, Their Component Composition and Biological Activity. Families Caprifoliaceae-Lobeliaceae; KMK: Saint Petersburg, Russia; Moscow, Russia, 2011, Volume 4, 630 p. (In Russian)
[32] U.R. Mughal, R. Mehmood, A. Malik, B. Ali, R.B. Tareen. Flavonoid constituents from *Spiraea brahuica*. Helv. Chim. Acta 2012, 95, 100-105.
[33] H. Cheng, F.H. Zeng, X. Yang, Y.J. Meng, L. Xu, F.P. Wang. Collective total syntheses of atisane-type diterpenes and atisine-type diterpenoid alkaloids: (–)-spiramilactone B, (–)-spiraminol, (–)-dihydroajaconine and (–)-spiramines C and D. Angew. Chem. 2016, 128, 400-404.
[34] V.A. Kostikova, N.V. Petrova. Phytoconstituents and bioactivity of plants of the genus *Spiraea* L. (Rosaceae): A review //International Journal of Molecular Sciences, 2021, T. 22, №. 20, C. 11163.
[35] S.Y. Keskin, A. Avcı, H.F.F. Kurnia. Analyses of phytochemical compounds in the flowers and leaves of *Spiraea japonica var. fortunei* using UV-VIS, FTIR, and LC-MS techniques //Heliyon, 2024, T. 10, №. 3. https://doi.org/10.1016/j.heliyon.2024.e25496
[36] D. Luo, M.S. Song, B. Xu, Y. Zhang, J.W. Zhang, X.G. Ma, X.J. Hao, H. Sun. A clue to the evolutionary history of modern East Asian flora: Insights from phylogeography and diterpenoid alkaloid distribution pattern of the *Spiraea japonica* complex. Molecular Phylogenetics and Evolution, 2023, 184. https://doi.org/10.1016/j.ympev.2023.107772

[37] T. Leonova, V. Popova, A. Tsarev, C. Henning, K. Antonova, N. Rogovskaya, M. Vikhnina, T. Baldensperger, A. Soboleva, E. Dinastia, M. Dorn, O. Shiroglasova, T. Grishina, G.U. Balcke, C. Ihling, G. Smolikova, S. Medvedev, V.A. Zhukov, V. Babakov, I. Tikhonovich, A. Frolov. Does Protein Glycation Impact on the Drought-Related Changes in Metabolism and Nutritional Properties of Mature Pea (*Pisum sativum* L.) Seeds? International Journal of Molecular Sciences, 2020б 21(2), 567. https://doi.org/10.3390/ijms21020567

[38] A. Orlova, E. Kysil, E. Tsvetkova, D. Meshalkina, A. Whaley, A.O. Whaley, A. Laub, A. Francioso, O. Babich, L.A. Wessjohann, L. Mosca, A. Frolov, M. Povydysh. Phytochemical Characterization of Water Avens (*Geum rivale* L.) Extracts: Structure Assignment and Biological Activity of the Major Phenolic Constituents. Plants, 2022, 11, 2859. https://doi.org/10.3390/plants11212859

[39] M.H. Yean, J.S. Kim, S.S. Kang, Y.S. Kim. A new megastigmane glucoside and three new flavonoid glycosides from *Spiraea prunifolia var. simpliciflora*. Helv. Chim. Acta 2014, 97, 1123-1131.

[40] S.D. Yan, H.L. Yao, Y.H. Zhang, H. Gao, K. Liu, Y. Liu, F.Y. Dong, W. Wang. Chemical constituents from *Spiraea salicifolia*. Chin. Trad. Herb. Drugs. 2016, 47, 2806-2811.

[41] T.K. Chumbalov, L.T. Pashinina, N.D. Storozhenko, Flavones and their 5-glycosides from *Spiraea hypericifolia*. Chem. Nat. Compd. 1975, 11, 440.

[42] R.R. Paris, L. Murgu. Polyhenolic compounds of *Spiraea crenata*. Plant Med. Phytother. 1970, 4, 138-149.

[43] V.A. Kostikova, A.A. Kuznetsov, E.D. Tishchenko, A.N. Fayzylkhakova. Chemotaxonomic study of *Spiraea aemiliana* compared to the closely species *S. betulifolia* and *S. beauverdiana*. Acta Biol. Sib. 2019, 5, 15-21. (In Russian)

[44] I.M. Krivosheev. Pharmacognostic Study of *Spiraea salicifolia* L. Growing in Eastern Siberia. Ph.D. Thesis, Irkutsk State Medical University, Ulan-Ude, Russia, 2014. (In Russian).

[45] E.A. Karpova, O.V. Imetkhenova. Phenolic compounds of representatives of sect. Glomerati of genus *Spiraea* L. of the flora of Siberia. Turczaninowia 2015, 18, 108-115. (In Russian)

[46] M.I. Choudhary, N. Naheed, A. Abbaskhan, S. Ali. Hemiterpene glucosides and other constituents from *Spiraea canescens*. Phytochemistry 2009, 70, 1467-1473.

[47] I.M. Krivosheev, V.M. Mirovich. The study of chemical composition of *Spiraea salicifolia* L. overground organs by Highly Effective Liquid Chromatography method. Sib. Med. J. 2012, 2, 104-105. (In Russian)

[48] S. Keskin, Y. Sirin, H.E. Cakir, G. Kaya, M. Keskin. Phenolic composition and antioxidant properties of *Spiraea nipponica*. Int. J. Sci. Technol. Res. 2019, 5, 87-92.

[49] E.A. Karpova, T.A. Polyakova. Seasonal dynamics of the composition of phenolic compounds of the leaves of *Spiraea media var. sericea* (Turcz.) Regel. Chem. Plant Raw Mater. 2014, 3, 145-149. (In Russian)

[50] E.C. Bate-Smith. The phenolic constituents of plants and their taxonomic significance. Bot. J. Linn. Soc. 1962, 58, 95-173.

[51] B.T. Ahn, K.J. Oh, S.K. Park, S.G. Chung, E.H. Cho, J.G. Kim, J.S. Ro, K.S. Lee. Phenolic compounds from leaves of *Spiraea salicifolia*. Kor. J. Pharmacogn. 1996, 27, 178-183.

[52] L. Piconi, L. Quagliaro, A. Ceriello. Oxidative Stress in Diabetes. 2003, 41(9), 1144-1149. https://doi.org/10.1515/CCLM.2003.177

[53] D.M. Teleanu, A.-G. Niculescu, I.I. Lungu, C.I. Radu, O. Vladâcenco, E. Roza, B. Costăchescu, A.M. Grumezescu, R.I. Teleanu. An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. Int. J. Mol. Sci. 2022, 23, 5938. https://doi.org/10.3390/ijms23115938
[54] G. Tomaselli, A. Barth. Sudden Cardio Arrest: Oxidative stress irritates the heart. Nat Med 16, 2010, 648-649. https://doi.org/10.1038/nm0610-648
[55] E. Frecska, P. Bokor, M. Winkelman. The Therapeutic Potentials of Ayahuasca: Possible Effects against Various Diseases of Civilization. Frontiers in pharmacology, 2016, 7, 35. https://doi.org/10.3389/fphar.2016.00035
[56] M.C. Sabu, R. Kuttan. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Journal of ethnopharmacology, 2002, 81(2), 155-160. https://doi.org/10.1016/s0378-8741(02)00034-x
[57] M. Lalkovičová, V. Danielisová. Neuroprotection and antioxidants. Neural Regeneration Research, 2016, 11(6), 865-874. doi: 10.4103/1673-5374.184447
[58] M.E. Rusu, I. Fizeșan, L. Vlase, D.S. Popa. Antioxidants in Age-Related Diseases and Anti-Aging Strategies. Antioxidants (Basel, Switzerland), 2022, 11(10), 1868. https://doi.org/10.3390/antiox11101868
[59] G. Chiva-Blanch, F. Visioli. Polyphenols and health: Moving beyond antioxidants. Journal of Berry Research, 2012, 2, 63-71.
[60] A. Scalbert, I.T. Johnson, M. Saltmarsh. Polyphenols: antioxidants and beyond. The American journal of clinical nutrition, 2005, 81(1 Suppl), 215S–217S. https://doi.org/10.1093/ajcn/81.1.215S
[61] L. Warren, P.D. Manos, T. Ahfeldt, Y.H. Loh, H. Li, F. Lau, W. Ebina, P.K. Mandal, Z.D. Smith, A. Meissner, G.Q. Daley, A.S. Brack, J.J. Collins, C. Cowan, T.M. Schlaeger, D.J. Rossi. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell stem cell, 2010, 7(5), 618-630. https://doi.org/10.1016/j.stem.2010.08.012
[62] V.A. Kostikova, T.M. Shaldaeva. The antioxidant activity of the Russian Far East representatives of the *Spiraea* L. genus (Rosaceae Juss.). Russ. J. Bioorg. Chem. 2017, 43, 790-794
[63] T.I. Shirshova, K.V. Bezmaternykh, I. Beshley, A. Smirnova, O. Oktyabr'skii. Antioxidant Properties of Extracts of Leaves and Inflorescences of *Spiraea Media Franz Schmidt* from the Flora of Komi Republic. Pharmaceutical Chemistry Journal. 2020, 54. 622-625. 10.1007/s11094-020-02246-3
[64] A.A. Kudaibergen, M.A. Dyusebaeva, A. Ydyrys, Y. Feng, J. Jenis. Investigation of Chemical Constituents of Medicinal Plant *Spiraea Hypericifolia* L. International Journal of Biology and Chemistry. 2019, 12(1), 128-134. https://doi.org/10.26577/ijbch-2019-1-i17.
[65] R.L. Kradin, A. Jay. Fishman. Influenza Virus. Viruses and the Lung. Springer Berlin Heidelberg, 2014,79-86
[66] W.W. Thompson, L. Comanor, D.K. Shay. Epidemiology of seasonal influenza: use of surveillance data and statistical models to estimate the burden of disease. J Infect Dis 2006, 194(Suppl 2), S82-91

[67] M. De Angelis, D. Della-Morte, G. Buttinelli, A. Di Martino, F. Pacifici, P. Checconi, L. Ambrosio, P. Stefanelli, A.T. Palamara, E. Garaci, C. Ricordi, L. Nencioni. Protective Role of Combined Polyphenols and Micronutrients against Influenza A Virus and SARS-CoV-2 Infection In Vitro. Biomedicines 2021, 9, 1721. https://doi.org/10.3390/biomedicines9111721

[68] R.R. He, M. Wang, C.Z. Wang, B.T. Chen, C.N. Lu, X.S. Yao, J.X. Chen, H. Kurihara. Protective effect of apple polyphenols against stress-provoked influenza viral infection in restraint mice. Journal of agricultural and food chemistry, 2011, 59(8), 3730-3737. https://doi.org/10.1021/jf104982y

[69] M. Nakayama, K. Suzuki, M. Toda, S. Okubo, Y. Hara, T. Shimamura. Inhibition of the infectivity of influenza virus by tea polyphenols. Antiviral research, 1993, 21(4), 289-299. https://doi.org/10.1016/0166-3542(93)90008-7

[70] R. Bahramsoltani, H.R. Sodagari, M.H. Farzaei, A.H. Abdolghaffari, M. Gooshe, N. Rezaei. The preventive and therapeutic potential of natural polyphenols on influenza. Expert review of anti-infective therapy, 2016, 14(1), 57-80. https://doi.org/10.1586/14787210.2016.1120670

[71] Y. Kashiwada, F.A. Ahmed, S. Kurimoto, S.Y. Kim, H. Shibata, T. Fujioka, Y. Takaishi. New α-glucosides of caffeoyl quinic acid from the leaves of *Moringa oleifera* Lam. Journal of natural medicines, 2012, 66(1), 217-221. https://doi.org/10.1007/s11418-011-0563-5

[72] K.C. Liu, J.M. Fang, J.T. Jan, T.J. Cheng, S.Y. Wang, S.T. Yang, Y.S. Cheng, C.H. Wong. Enhanced anti-influenza agents conjugated with anti-inflammatory activity. Journal of medicinal chemistry, 2012, 55(19), 8493-8501. https://doi.org/10.1021/jm3009844

[73] Y. Xie, B. Huang, K. Yu, F. Shi, T. Liu, W. Xu. Caffeic acid derivatives: a new type of influenza neuraminidase inhibitors. Bioorganic & medicinal chemistry letters, 2013, 23(12), 3556-3560. https://doi.org/10.1016/j.bmcl.2013.04.033

[74] R. Naithani, L.C. Huma, L.E. Holland, D. Shukla, D.L. McCormick, R.G. Mehta, R.M. Moriarty. Antiviral activity of phytochemicals: a comprehensive review. Mini reviews in medicinal chemistry, 2008, 8(11), 1106-1133. https://doi.org/10.2174/138955708785909943

[75] T. Manso, M. Lores, T. de Miguel. Antimicrobial Activity of Polyphenols and Natural Polyphenolic Extracts on Clinical Isolates. Antibiotics (Basel, Switzerland), 2021, 11(1), 46. https://doi.org/10.3390/antibiotics11010046

[76] M. Daglia. Polyphenols as antimicrobial agents. Current opinion in biotechnology, 2012, 23(2), 174-181. https://doi.org/10.1016/j.copbio.2011.08.007

[77] V. Katalinic, S.S. Mozina, D. Skroza, I. Generalic, H. Abramovic, M. Milos, I. Ljubenkov, S. Piskernik, I. Pezo, P. Terpinc, M. Boban. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia) Food Chem. 2010, 119, 715-723. doi:10.1016/j.foodchem.2009.07.019

[78] B. Radovanović, A. Milenkovic Andjelkovic, A.B. Radovanović, M. Andjelkovic. Antioxidant and Antimicrobial Activity of Polyphenol Extracts from Wild Berry Fruits Grown in Southeast Serbia. Tropical Journal of Pharmaceutical Research, 2013, 12, 813-819. 10.4314/tjpr.v12i5.23

[79] L. Othman, A. Sleiman, R.M. Abdel-Massih. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Frontiers in microbiology, 2019, 10, 911. https://doi.org/10.3389/fmicb.2019.00911

[80] T. Kiss, K.B. Cank, O. Orbán-Gyapai, E. Liktor-Busa, Z.P. Zomborszki, S. Rutkovska, I. Pučka, A. Németh, D. Csupor. Phytochemical and pharmacological investigation of *Spiraea chamaedryfolia*: A contribution to the chemotaxonomy of *Spiraea genus*. BMC Res. Notes, 2017, 10, 762-768.

[81] Y. Lei, P. Fu, X. Jun, P. Cheng. Pharmacological Properties of Geraniol - A Review. Planta medica, 2019, 85(1), 48-55. https://doi.org/10.1055/a-0750-6907

[82] M.H.P. de Lira, F.P. de Andrade Júnior, G.F.Q. Moraes, G. da S. Macena, F. de O. Pereira, I.O. Lima. Antimicrobial activity of geraniol: an integrative review. Journal of Essential Oil Research, 2020, 32(3), 187-197. https://doi.org/10.1080/10412905.2020.1745697