applied sciences

MDPI

Article

Phenolic Compounds and Pyrrolizidine Alkaloids of Two North Bluebells: *Mertensia stylosa* and *Mertensia serrulata*

Nina I. Kashchenko [1,*], Daniil N. Olennikov [1] and Nadezhda K. Chirikova [2]

[1] Laboratory of Medical and Biological Research, Institute of General and Experimental Biology, Siberian Division of Russian Academy of Science, Sakh'yanovoy Street 6, 670047 Ulan-Ude, Russia
[2] Department of Biochemistry and Biotechnology, North-Eastern Federal University, 58 Belinsky Street, 677027 Yakutsk, Russia
* Correspondence: ninkk@mail.ru; Tel.: +7-983-421-73-40

Abstract: Two North bluebells, *Mertonian stylosa* and *M. serrulata*, are plants used in the traditional medicine of the Buryats as wound healing and antitumor remedies. Both mertensias have been used by local healers as substitutes for the rare Tibetan raw material *Cynoglossum amabile*. The lack of information on the chemical composition of *M. stylosa* and *M. serrulata* herbs has prompted the study of metabolites, in particular phenolic compounds and alkaloids, as components with high biological activity. In this study, the application of liquid chromatography–mass spectrometry for the metabolite profiling of both *Mertensia* species resulted in the identification of 30 compounds, including hydroxycinnamates, flavonoids, and pyrrolizidine alkaloids. In particular, lycopsamine *N*-oxide was the dominant alkaloid in *M. stylosa* (5.27 mg/g) and *M. serrulata* (2.14 mg/g) herbs, 5-*O*-caffeoylquinic acid (43.41 mg/g) and rutin (42.40 mg/g) prevailed among the phenolic compounds in *M. stylosa* herb, while rutin (25.72 mg/g) was the dominant compound of the *M. serrulata* herb. The investigated extracts of *M. stylosa* and *M. serrulata* herb revealed good scavenging capacity against DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and DMPD$^{\bullet+}$ radicals. To our knowledge, this is the first study of *M. stylosa* and *M. serrulata* alkaloids and phenolic compounds and antioxidativity.

Keywords: Boraginaceae; HPLC-MS; flavonoids; hydroxycinnamates; lycopsamine; antioxidativity

Citation: Kashchenko, N.I.; Olennikov, D.N.; Chirikova, N.K. Phenolic Compounds and Pyrrolizidine Alkaloids of Two North Bluebells: *Mertensia stylosa* and *Mertensia serrulata*. *Appl. Sci.* **2023**, *13*, 3266. https://doi.org/10.3390/app13053266

Academic Editor: Monica Gallo

Received: 10 February 2023
Revised: 28 February 2023
Accepted: 2 March 2023
Published: 3 March 2023

1. Introduction

Medicinal plants are significant resources for the health systems of traditional societies. Currently, approximately 70–80% of the rural population in developing countries in Asia has been found to be dependent on traditional medicine for primary health care [1]. Traditional medicine has no theoretical foundation or written sources and is transferred from one representative to another via a direct transfer of knowledge and skills within a limited population [2]. The nomadic peoples of Asia, in particular the Buryats, have had their own traditional medicine, which has existed since the 18th century in the Transbaikal region. The interpretation of traditional Tibetan medicine that arrived with Buddhism and the influence of the experience of Buryat folk medicine significantly changed the range of medicines, which led to the emergence of the Buryat branch of Tibetan medicine [3]. The Buryat emchi lamas often resorted to replacing raw materials of Tibetan and Indian origin with local plants in their medical practice because herbs, leaves, and flowers often deteriorated during transportation. In the selection of substitutes, the Buryat emchi lamas mainly focused on the descriptions of plants and pictures in Tibetan treatises. It has also been implied that the medicinal properties of substitute plants are similar or sufficiently similar to those of plants described in the treatises [4].

Species of the genus *Mertensia* (bluebells) are one of such substitute plants in Buryat traditional medicine. Emchi lamas replaced the Tibetan raw material *Cynoglossum amabile* Stapf & JR Drumm with the local species *Mertensia stylosa* and *M. serrulata* due to their similarity. Native healers used the *Mertensia* herb as a wound healing and antitumor

agent [5]. *Mertensia* and *Cynoglossum* belong to the Boraginaceae family, which includes approximately 2700 species [6]. Plants of the genus *Mertensia* grow predominantly in North America and Asia from western China to northeastern Russia [7]. *M. stylosa* (Fisch.) DC. is a perennial plant 20–50 cm in height with one or rarely several stems, oblong basal leaves that are 2–4 cm long, densely arranged lanceolate stem leaves, and umbellate inflorescence with blue–violet flowers on the pedicels. It grows in subalpine meadows along river banks and on wet rocks (Figure 1). *M. serrulata* (Turcz.) DC. is a perennial plant 20–35 cm in height with a simple stem that is branched at the base, ovate basal leaves that are 3–5 cm on long petioles, and sessile ovate stem leaves; the inflorescence is an umbrella-shaped curl with blue flowers on the pedicels. It grows on the rocky banks of streams and rivers and high mountains at an altitude of 450–1900 m [8].

(**a**) (**b**)

Figure 1. *Mertensia* species in their natural habitat: (**a**)—*M. stylosa* (Fisch.) DC.; (**b**)—*M. serrulata* (Turcz.) DC.

There is no scientific information on the chemical composition of *M. stylosa* and *M. serrulata*. The most studied species of the *Mertensia* genus is *M. maritima*, which has a high decorative value and is eaten fresh by the Iñupiat of Alaska [9]. It is known that carotenoids, phenolic acids, terpenoids, fatty acids, volatile compounds [10–12], as well as pyrrolizidine alkaloids, characteristic of plant objects of the Boraginaceae family [13,14], have been found in *M. maritima*. Because both alkaloids and phenolic compounds have a wide spectrum of biological activity [15,16], these two classes of compounds were chosen to study the chemical profiles of the *M. stylosa* and *M. serrulata* herbs.

In this work, for the first time, we performed qualitative and quantitative chromatographic analyses of pyrrolizidine alkaloids and phenolic compounds in herb extracts of *M. stylosa* and *M. serrulata* using high-performance liquid chromatography with photodiode array detection and electrospray ionization triple quadrupole mass spectrometric detection (HPLC-PDA-ESI-tQ-MS/MS).

2. Materials and Methods

2.1. Plant Material and Chemicals

Samples of *Mertensia serrulata* (herb) were collected during the flowering period in Republic of Buryatia, Kurumkansky District. The samples were collected in subalpine meadow in 5 locations, 10 samples from each (31.VII.2022, 54°18′59.4837″ N, 110°10′20.1782″ E, 1216 m a.s.l.; voucher No BKD/MSe0722/33-83). Samples of *M. stylosa* (herb) were collected during the flowering period in Irkutsk Oblast, Slyudyansky District. The samples were collected on the bank of the Slyudyanka river in 5 locations, 10 samples from each (11.VII.2022, 51°37′43.0338″ N, 103°40′28.5175″ E, 596 m a.s.l.; voucher No ISDMst-0722/42-92). The species were authenticated by Prof. Tamara A. Aseeva (IGEB SB RAS, Ulan-Ude, Russia).

Plant material was dried in the ventilated heat oven at 35 °C within 7–12 days and stored at 4–6 °C before analysis.

The reference compounds were purchased from BioCrick Co., Ltd. (Chengdu Tianfu, Sichuan, China): 7-acetyllycopsamine (Cat. No. BCN2000, ≥98%), 7-*O*-acetyllycopsamine *N*-oxide (Cat. No. BCN8935, ≥98%), 4-*O*-coumaroylquinic acid (Cat. No. BCX0010, ≥98%), 5-*O*-coumaroylquinic acid (Cat. No. BCX0028, ≥98%), 3-*O*-feruloylquinic acid (Cat. No. BCN3353, ≥98%), 4-*O*-feruloylquinic acid (Cat. No. BCN3352, ≥98%), 5-*O*-feruloylquinic acid (Cat. No. BCN3788, ≥98%); Sigma–Aldrich (St. Louis, MO, USA): acetonitrile for HPLC (Cat. No. 34851, ≥99.9%), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Cat. No. A1888, ≥ 98%), 3-*O*-caffeoylquinic acid (Cat. No. PHL89175, ≥95%), 4-*O*-caffeoylquinic acid (Cat. No. 65969, ≥98%), 5-*O*-caffeoylquinic acid (neochlorogenic acid; Cat. No. 94419, ≥98%), *N*,*N*-dimethyl-*p*-phenylenediamine (Cat. No. 193992, ≥ 97%), 2,2-diphenyl-1-picrylhydrazyl (Cat. No. D9132), 3,5-di-*O*-caffeoylquinic acid (Cat. No. SMB00131, ≥95%), 3,4-di-*O*-caffeoylquinic acid (Cat. No. SMB00224, ≥90%), 4,5-di-*O*-caffeoylquinic acid (Cat. No. SMB00221, ≥85%), caftaric acid (Cat. No. PHL89170, ≥95%), 3-*O*-coumaroylquinic acid (Cat. No. FC71593, ≥95%), kaempferol-3-*O*-rutinoside (nicotiflorin; Cat. No. 90242, ≥98%), kaempferol-3-*O*-glucoside (astragalin; Cat. No. 68437, ≥90%), lithium perchlorate (Cat. No. 205281, ≥95%), lycopsamine (Cat No. PHL89726, ≥95%), lycopsamine *N*-oxide (Cat. No. PHL83447, ≥90%), methanol (Cat. No. 322415, ≥99.8%), perchloric acid (Cat. No. 244252, ≥70%), rosmarinic acid (Cat. No. PHL89266, ≥95%), quercetin-3-*O*-glucoside (isoquercitrin; Cat. No. 16654, ≥98%), quercetin-3-*O*-rutinoside (rutin; Cat. No. CFN99642, ≥98%), trolox (Cat. No. 238813, ≥97%). 2-*O*-Caffeoyltartronic acid, 2-*O*-, 3-*O*-, 4-*O*-caffeoylthreonic acids, as well as 2-*O*-, 3-*O*-caffeoylglyceric acid were previously isolated from *Nonea rossica* and *Tournefortia sibirica* [17].

2.2. Plant Extracts Preparation

For preparation of plant extracts for HPLC analysis, the herb of *M. stylosa* and *M. serrulata* were crushed to particle size 0.125 μm. An amount of 200 mg of grounded plant samples were treated with 70% methanol (2 mL) three times using sonication (ultrasonic bath, 30 min, 50 °C, ultrasound power 100 W, frequency 35 kHz). The obtained liquid extracts were centrifuged at 20 °C (6000× *g*, 10 min), and the supernatants were filtered through 0.22 μm syringe filters into the measuring flask (10 mL). The final volume was 10 mL with 70% methanol.

2.3. High-Performance Liquid Chromatography with Photodiode Array Detection and Electrospray Ionization Triple Quadrupole Mass Spectrometric Detection (HPLC-PDA-ESI-tQ-MS) Metabolite Profiling

To analyze chemical profile of *M. stylosa* and *M. serrulata* herb extracts, high-performance liquid chromatography with photodiode array detection and electrospray ionization triple quadrupole mass spectrometric detection (HPLC-PDA-ESI-tQ-MS) was applied. Chromatographic separation of compounds was realized with liquid chromatograph LC-20 Prominence, column GLC Mastro C18 (2.1 × 150 mm, 3 μm). The detection was implemented on a photodiode array detector SPD-M30A and triple-quadrupole mass spectrometer LCMS 8050 (all Shimadzu, Columbia, MD, USA) according to a previously developed technique [18].

2.4. HPLC-PDA-ESI-tQ-MS Metabolite Quantification

For quantification of 28 compounds of *M. stylosa* and *M. serrulata* herb extracts with HPLC-PDA-ESI-tQ-MS, following reference compounds were used: lycopsamine, lycopsamine *N*-oxide, lycopsamine 7-*O*-acetate, lycopsamine *N*-oxide 7-*O*-acetate, 4-*O*-caffeoylquinic, 3-*O*-caffeoylthreonic, 2-*O*-caffeoylthreonic, 4-*O*-coumaroylquinic, 5-*O*-caffeoylquinic, 4-*O*-feruloylquinic, 2-*O*-caffeoylglyceric, 3-*O*-caffeoylquinic, 4-*O*-caffeoylthreonic, 3-*O*-caffeoylglyceric, 5-*O*-coumaroylquinic, 3-*O*-coumaroylquinic, 5-*O*-feruloylquinic, 2-*O*-caffeoyltartronic, 3-*O*-feruloylquinic, caftaric, 3,4-, 3,5-, 4,5-di-*O*-

caffeoylquinic, rosmarinic acids, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside. For the preparation of stock solutions (1000 µg/mL) applied for the calibration curve building, 10 mg of reference compounds were separately weighted and dissolved in the methanol-DMSO mixture (1:1) in volumetric flasks (10 mL). The calibration solution (1, 10, 25, 50 and 100 µg/mL) was chromatographed in known HPLC-PDA-ESI-tQ-MS conditions (Section 2.3). Determination coefficients (r^2), standard deviation (S_{YX}), limits of detection (LOD), limits of quantification (LOQ), and linear ranges as the main validation criteria were studied using the previously developed method [19] (Table 1). Five HPLC runs were satisfactory for the quantitative analyses, and the results were expressed as mean value ± standard deviation (S.D.).

Table 1. Regression equations, correlation coefficients (r^2), standard deviation (S_{YX}), limits of detection (LOD), limits of quantification (LOQ), and linear ranges for 28 reference standards.

Compound	Ionization [a]	CE [b] (eV)	Regression Equation [c]		r^2	S_{yx}	LOD/LOQ (µg/mL)	Linear Range (µg/mL)
			a	b × 10^6				
Lycopsamine	P	+20	1.6726	−0.6389	0.9975	8.29×10^{-2}	0.16/0.50	0.5–100.0
Lycopsamine *N*-oxide	P	+20	1.5787	−0.3641	0.9981	6.48×10^{-2}	0.14/0.41	0.5–100.0
Lycopsamine 7-*O*-acetate	P	+20	1.4196	−0.4514	0.9989	5.76×10^{-2}	0.14/0.41	0.5–100.0
Lycopsamine *N*-oxide 7-*O*-acetate	P	+20	2.4561	−0.0171	0.9979	12.33×10^{-2}	0.17/0.50	0.6–100.0
4-*O*-Caffeoylquinic acid	N	−15	0.9217	−0.0437	0.9982	3.94×10^{-2}	0.14/0.43	0.5–100.0
3-*O*-Caffeoylthreonic acid	N	−20	1.3586	−0.0663	0.9987	9.69×10^{-2}	0.24/0.71	0.8–100.0
2-*O*-Caffeoylthreonic acid	N	−20	1.3722	−0.0829	0.9973	9.93×10^{-2}	0.24/0.72	0.8–100.0
4-*O*-Coumaroylquinic acid	N	−20	0.6284	−0.0517	0.9975	5.45×10^{-2}	0.29/0.87	0.9–100.0
5-*O*-Caffeoylquinic acid	N	−15	0.9406	−0.0497	0.9973	5.18×10^{-2}	0.18/0.55	0.6–100.0
4-*O*-Feruloylquinic acid	N	−20	0.9214	−0.0373	0.9997	2.10×10^{-2}	0.07/0.22	0.3–100.0
2-*O*-Caffeoylglyceric acid	N	−20	0.8115	−0.1006	0.9980	2.25×10^{-2}	0.10/0.28	0.3–100.0
3-*O*-Caffeoylquinic acid	N	−15	0.9320	−0.0523	0.9991	4.14×10^{-2}	0.15/0.44	0.5–100.0
4-*O*-Caffeoylthreonic acid	N	−20	1.3620	−0.0820	0.9961	9.91×10^{-2}	0.21/0.72	0.8–100.0
3-*O*-Caffeoylglyceric acid	N	−20	0.8523	−0.1004	0.9982	2.08×10^{-2}	0.08/0.24	0.3–100.0
5-*O*-Coumaroylquinic acid	N	−20	0.9911	−0.0379	0.9988	2.05×10^{-2}	0.07/0.21	0.3–100.0
3-*O*-Coumaroylquinic acid	N	−20	0.9804	−0.0210	0.9970	2.01×10^{-2}	0.06/0.21	0.3–100.0
5-*O*-Feruloylquinic acid	N	−20	1.8535	0.0761	0.9989	4.55×10^{-2}	0.08/0.25	0.3–100.0
2-*O*-Caffeoyltartronic acid	N	−15	1.5330	−0.0863	0.9985	4.15×10^{-2}	0.09/0.27	0.3–100.0
3-*O*-Feruloylquinic acid	N	−20	1.2416	−0.3615	0.9901	3.02×10^{-2}	0.08/0.24	0.3–100.0
Caftaric acid	N	−20	1.4238	−0.0891	0.9901	7.33×10^{-2}	0.17/0.52	0.6–100.0
Quercetin-3-*O*-rutinoside	N	−30	1.2716	−0.7389	0.9897	9.14×10^{-2}	0.23/0.72	0.8–100.0
Quercetin-3-*O*-glucoside	N	−30	1.8267	−0.4160	0.9990	11.73×10^{-2}	0.21/0.67	0.7–100.0
3,4-Di-*O*-caffeoylquinic acid	N	−20	1.6278	−0.0428	0.9990	7.11×10^{-2}	0.14/0.44	0.4–100.0
Kaempferol-3-*O*-rutinoside	N	−30	1.9634	−0.4511	0.9952	9.18×10^{-2}	0.15/0.46	0.5–100.0
3,5-Di-*O*-caffeoylquinic acid	N	−20	1.1105	−0.3211	0.9937	4.18×10^{-2}	0.12/0.38	0.4–100.0
4,5-Di-*O*-caffeoylquinic acid	N	−20	1.5632	−0.0376	0.9983	5.14×10^{-2}	0.11/0.33	0.4–100.0
Kaempferol-3-*O*-glucoside	N	−30	2.0859	−0.9171	0.9980	6.18×10^{-2}	0.03/0.09	0.1–100.0
Rosmarinic acid	N	−20	1.9610	−0.5271	0.9993	0.94×10^{-2}	0.02/0.05	0.5–100.0

[a] Ionization mode: N—negative, P—positive. [b] CE—collision energy. [c] Regression equation: y = ax + b.

2.5. Antioxidant Activity

To assess antioxidant activity of *M. stylosa*, *M. serrulata* herb extracts and dominant compounds (lycopsamine *N*-oxide, 5-*O*-caffeoylquinic acid, rutin) microplate spectrophotometric assays against 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH$^{\bullet}$) [20], 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation radicals (ABTS$^{\bullet+}$) [21], *N*,*N*-dimethyl-p-phenylenediamine radicals (DMPD$^{\bullet+}$) [22] were used. Trolox was applied as a reference standard in concentrations 1–100 µg/mL. The obtained results of DPPH$^{\bullet}$, ABTS$^{\bullet+}$, DMPD$^{\bullet+}$ methods were presented as IC_{50} and calculated graphically applying 'concentra-

tion (μg/mL)–antioxidant activity (%)' correlations. All the analyses were performed five times, and the data were expressed as the mean value ± standard deviation (S.D.).

2.6. Statistical Analysis

Data were first run for numerical normality and homogeneity of variance using the Shapiro–Wilk's and Levene's tests, respectively, and then, the analysis of variance was performed, and means were compared using Duncan's multiple range tests at $p \leq 0.05$. The results were presented as mean values ± standard deviations (S.D.). The linear regression analysis and generation of calibration graphs were conducted using Advanced Grapher 2.2 (Alentum Software Inc., Ramat-Gan, Israel).

3. Results and Discussion

3.1. Metabolites of Mertensia stylosa and M. serrulata: LC-MS Profile

3.1.1. Pyrrolizidine Alkaloids

Using high-performance liquid chromatography with photodiode array and electrospray ionization triple quadrupole mass spectrometric detection (HPLC-PDA-tQ-ESI-MS), six pyrrolizidine alkaloids were detected after a comparison of the retention times and mass spectra data with reference standards (Figure 2, Table 2). The derivatives of lycopsamine (**1**) were found in the studied species of the *Mertensia* herb, namely lycopsamine *N*-oxide (**2**), lycopsamine 7-*O*-acetate (**3**), and its isomer (**3***) and lycopsamine *N*-oxide 7-*O*-acetate (**4**) and its isomer (**4***) (Figure 3). Lycopsamine *N*-oxide was the dominant compound in the herbs of *M. stylosa* (5.27 mg/g) and *M. serrulata* (2.14 mg/g). Lycopsamine was found in the herbs of *M. stylosa* and *M. serrulata* at significantly lower levels of 0.94 and 0.30 mg/g, respectively. Isomers **3** and **4** were found in both studied species in trace amounts. The total content of pyrrolizidine alkaloids in the herb of *M. stylosa* exceeded the content of the same alkaloids in the herb of *M. serrulata* by 2.7 times. Previously, lycopsamine and lycopsamine *N*-oxide have been identified in *M. bakery*, *M. ciliata* [23], and *M. maritima* [24], whereas only lycopsamine has been found in *M. sibirica* [25]. Acetate derivatives of lycopsamine and lycopsamine *N*-oxide were revealed in the *Mertensia* genus for the first time.

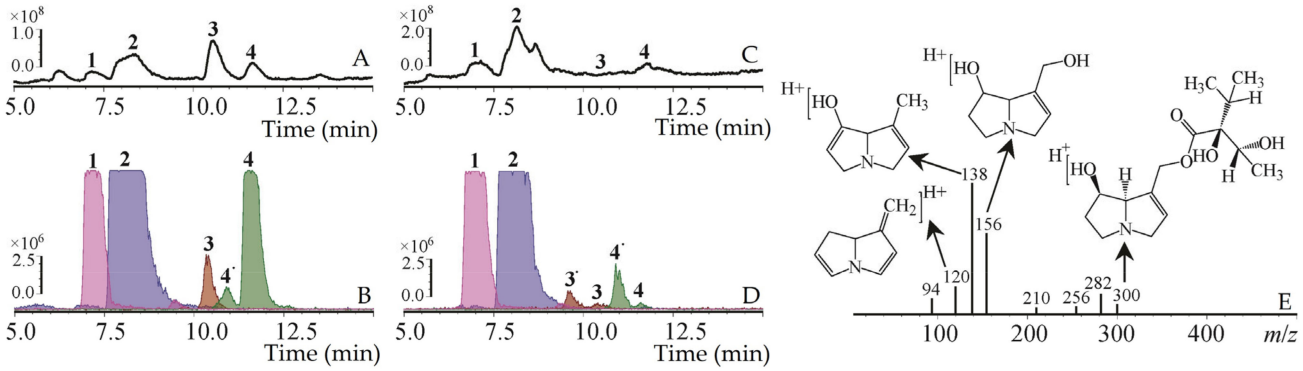

Figure 2. Fragments of HPLC-ESI-MS chromatograms of *Mertensia stylosa* (**A**,**B**) and *M. serrulata* (**C**,**D**) herb extracts (Total Ion Chromatogram or TIC mode, positive ionization; (**A**,**C**)) and (SIM mode, positive ionization, *m/z* 300, 316, 342 and 358; (**B**,**D**)). Compounds are numbered as listed in Table 2. (**E**) MS/MS spectrum of lycopsamine (**1**, positive ionization).

Table 2. Chromatographic (t_R), mass-spectrometric data (ESI-MS), and content of pyrrolizidine alkaloids in *Mertensia stylosa* и *M. serrulata* herb extracts.

No	tR, min	Compound [a]	ESI-MS, *m/z*		Content, mg/g of Dry Plant Weight ± S.D. [b]	
			$[M+H]^+$	MS/MS	*M. stylosa*	*M. serrulata*
1	7.11	Lycopsamine [S]	300	282, 256, 210, 156, 138, 120, 94	0.94 ± 0.02	0.30 ± 0.00
2	7.96	Lycopsamine *N*-oxide [S]	316	298, 272, 254, 210, 172, 138, 120, 94	5.27 ± 0.10	2.14 ± 0.04
3•	9.65	Isomer 3 [L]	342	324, 282, 256, 156, 138, 120, 94		trace [b]
3	10.31	Lycopsamine 7-*O*-acetate [S]	342	300, 324, 282, 256, 156, 138, 120, 94	0.11 ± 0.00	0.02 ± 0.00
4•	10.90	Isomer 4 [L]	358	340, 314, 298, 272, 254, 210, 172, 138, 120, 94	trace	trace
4	11.53	Lycopsamine *N*-oxide 7-*O*-acetate [S]	358	340, 314, 298, 272, 254, 210, 172, 138, 120, 94	0.37 ± 0.01	trace
		Total content **1–4**			6.69	2.46

[a] Compound identification was based on comparison of retention time and MS spectral data with reference standard ([S]) or interpretation of MS spectral data and comparison with literature data ([L]). [b] traces—<LOQ (limit of quantification).

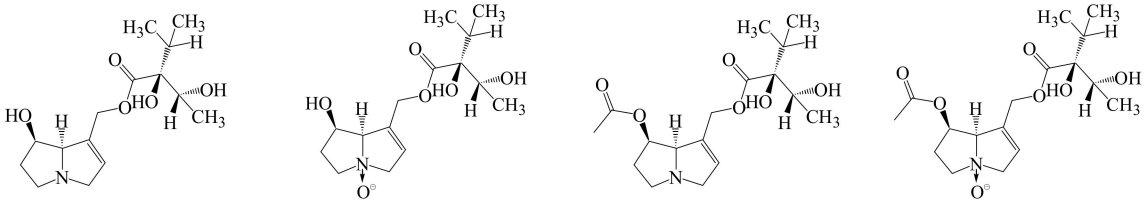

Figure 3. Structures of pyrrolizidine alkaloids (**1**–**4**) found in *Mertensia stylosa* and *M. serrulata*.

3.1.2. Phenolic Compounds

Using HPLC-PDA-tQ-ESI-MS, twenty-six phenolic compounds were detected in both *Mertensia* species (Figure 4, Table 3) and separated into hydroxycinnamates (22 compounds) and flavonoids (4 compounds) (Figure 5). The abundance of hydroxycinnamates was characteristic of the studied species of *Mertensia*. Derivatives of caffeic and quinic acids were authenticated by comparing the retention times and UV and MS spectral data with reference standards. Thus, derivatives of caffeic acid, including mono-caffeoylquinic (**5**, **9**, **12**), di-caffeoylquinic (**25**, **27**, **28**), caffeoylthreonic (**6**, **7**, **14**), caffeoylglyceric (**11**, **15**), caftaric (**22**), caffeoyltartronic (**20**), and rosmarinic (**30**) acids, were revealed. Derivatives of quinic acid, including feruloylquinic (**10**, **19**, **21**) and coumaroylquinic (**8**, **17**, **18**) acids, were also found. Components **13** and **16** produced a deprotonated ion with *m/z* 311 and daughter ions with *m/z* 179 and 161. The provisional structures of **13** and **16** were found to be *O*-caffeoyltartaric acid. Four flavonoid compounds were identified in the herbs of both *Mertensia* species using the reference standards. The found flavonoids belonged to the flavonol group depending on their aglycone structures and were derivatives of quercetin (**23**, **24**) and kaempferol (**26**, **29**).

Quantification of the principal compounds of *M. stylosa* and *M. serrulata* herbs was achieved using HPLC-MS data, which allowed determination of 26 phenolic compounds. 5-*O*-Caffeoylquinic acid and quercetin-3-*O*-rutinoside were dominating compounds of the *M. stylosa* herb while only quercetin-3-*O*-rutinoside dominated in the *M. serrulata* herb. Additionally, a high content of rosmarinic acid was noted for the *M. stylosa* herb. The total content of hydroxycinnamates in the *M. stylosa* herb exceeded the content of flavonoids by 1.2 times, and the total content of phenolic compounds was 116.27 mg/g. Moreover, the total content of flavonoids in the *M. serrulata* herb exceeded the total content of hydroxycinnamates by 2.54 times, and the total content of phenolic compounds was 41.90 mg/g. Earlier, rutin and rosmarinic acid were revealed in the seeds of *M. brevistyla*, *M. lanceolata*, *M. arizonica*, *M. macdougalii*, *M. viridis*, *M. ciliata*, *M. alpina*, *M. sibirica*, *M. virginica*, and *M. maritima* spp. *asiatica* [26]. Additionally, rosmarinic acid was found in the

callus culture of *M. maritima* [11]. Previously, quercetin-3-*O*-glucoside and kaempferol-3-*O*-rutinoside were detected in the shoots of *M. maritima* [24].

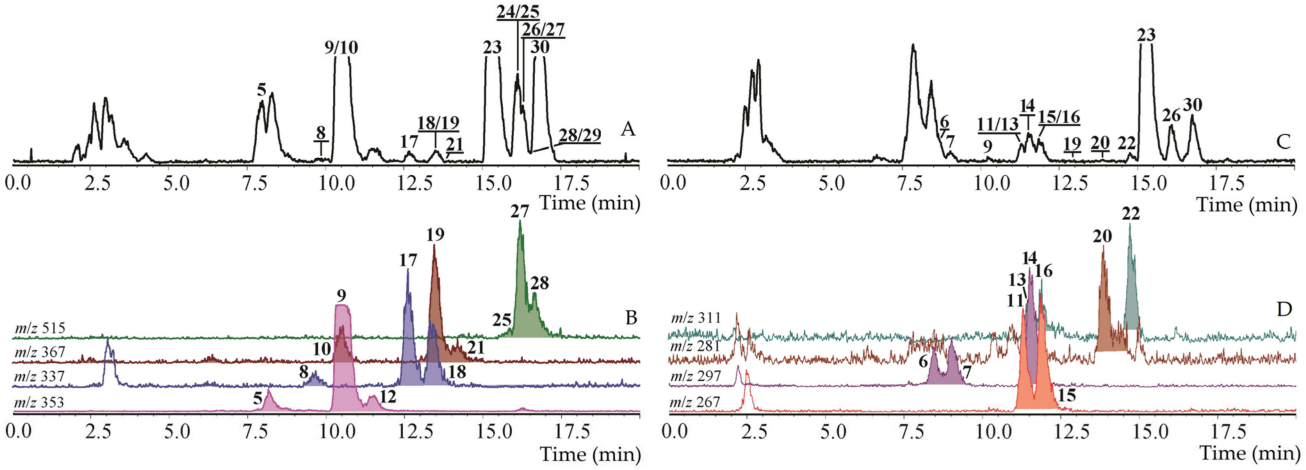

Figure 4. High-Performance Liquid Chromatography with Photodiode Array Detection and Electrospray Ionization Triple Quadrupole Mass Spectrometric Detection (HPLC-PDA-ESI-tQ-MS) chromatogram (Total Ion Chromatogram or TIC mode, negative ionization; (**A**,**C**)) or SIM-mode (**B**,**D**) of *Mertensia stylosa* (**A**,**B**) and *M. serrulata* extracts (**C**,**D**). Compounds are numbered as listed in Table 3.

Table 3. Chromatographic (t_R), mass-spectrometric data, and content of phenolic compounds in herb of *Mertensia stylosa* и *M. serrulata*.

No.	t_R, min	Compound [a]	ESI-MS, *m/z*		Content, mg/g of Dry Plant Weight ± S.D. [b]	
			[M–H]$^-$, *m/z*	MS/MS, *m/z*	*M. stylosa*	*M. serrulata*
5	8.14	4-*O*-Caffeoylquinic acid [S]	353	191, 179, 135	1.49 ± 0.03	
6	8.46	3-*O*-Caffeoylthreonic acid [S]	297	179, 161, 135		0.90 ± 0.02
7	9.04	2-*O*-Caffeoylthreonic acid [S]	297	179, 161, 135		1.15 ± 0.02
8	9.58	4-*O*-Coumaroylquinic acid [S]	337	191, 173, 163	trace [b]	
9	10.40	5-*O*-Caffeoylquinic acid [S]	353	191, 179, 135	43.41 ± 0.76	0.81 ± 0.02
10	10.51	4-*O*-Feruloylquinic acid [S]	367	193, 191, 149	trace	
11	11.25	2-*O*-Caffeoylglyceric acid [S]	267	179, 161, 135		trace
12	11.46	3-*O*-Caffeoylquinic acid [S]	353	191, 179, 135	trace	
13	11.48	*O*-Caffeoyltartaric acid [L]	311	179, 161, 135		trace
14	11.51	4-*O*-Caffeoylthreonic acid [S]	297	179, 161, 135		4.50 ± 0.09
15	11.81	3-*O*-Caffeoylglyceric acid [S]	267	179, 161, 135		2.53 ± 0.05
16	11.88	*O*-Caffeoyltartaric acid [L]	311	179, 161, 135		trace
17	12.60	5-*O*-Coumaroylquinic acid [S]	337	191, 173, 163	1.22 ± 0.03	
18	13.34	3-*O*-Coumaroylquinic acid [S]	337	191, 173, 163	trace	
19	13.47	5-*O*-Feruloylquinic acid [S]	367	193, 191, 149	1.57 ± 0.03	trace
20	13.80	2-*O*-Caffeoyltartronic acid [S]	281	179, 161, 135		trace
21	14.20	3-*O*-Feruloylquinic acid [S]	367	193, 191, 149	trace	
22	14.70	Caftaric acid [S]	311	179, 161, 135		trace
23	15.31	Quercetin-3-*O*-rutinoside [S]	609	463, 301	42.40 ± 0.85	25.72 ± 0.51
24	15.58	Quercetin-3-*O*-glucoside [S]	463	301	3.16 ± 0.06	
25	15.84	3,4-Di-O-caffeoylquinic acid [S]	515	353, 191, 173, 135	trace	
26	16.05	Kaempferol-3-*O*-rutinoside [S]	593	447, 285	6.52 ± 0.12	4.35 ± 0.08
27	16.19	3,5-Di-*O*-caffeoylquinic acid [S]	515	353, 191, 173, 135	3.54 ± 0.07	
28	16.23	4,5-Di-*O*-caffeoylquinic acid [S]	515	353, 191, 173, 135	trace	
29	16.46	Kaempferol-3-*O*-glucoside [S]	447	285	trace	
30	16.74	Rosmarinic acid [S]	359	197, 179, 161, 135	12.96 ± 0.25	1.94 ± 0.04
		Total content				
		hydroxycinnamates			64.19	11.83
		flavonoids			52.08	30.07
		phenolic compounds			116.27	41.90

[a] Compound identification was based on comparison of retention time, UV and MS spectral data with reference standard ([S]) or interpretation of UV and MS spectral data and comparison with literature data ([L]). [b] traces—<LOQ (limit of quantification).

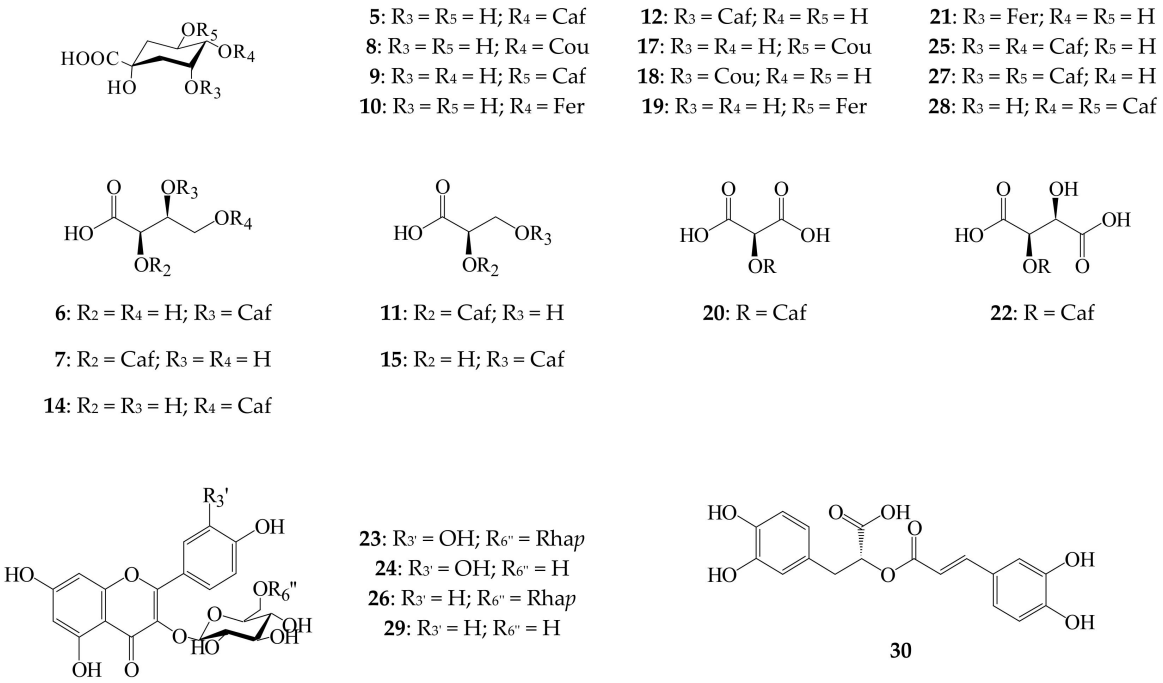

Figure 5. Structures of phenolic compounds found in *M. stylosa* and *M. serrulata*.

3.2. Antioxidant Activity

The antioxidant activity of *M. stylosa* and *M. serrulata* herb extracts and selected dominant compounds was estimated using well-known assays to explore the scavenging properties against 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH$^{\bullet}$), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radicals (ABTS$^{\bullet+}$), and *N*,*N*-dimethyl-*p*-phenylenediamine radicals (DMPD$^{\bullet+}$) (Table 4).

Table 4. Antioxidant activity of *M. stylosa*, *M. serrulata* herb extracts and selected compounds in three in vitro assays.

Object	Assay [a]		
	DPPH$^{\bullet}$ [b]	ABTS$^{\bullet+}$ [b]	DMPD$^{\bullet+}$ [b]
M. stylosa herb extract	21.17 ± 0.38 [d]	15.33 ± 0.31 [d]	79.34 ± 1.51 [d]
M. serrulata herb extract	37.95 ± 0.65 [e]	24.02 ± 0.46 [e]	96.11 ± 1.92 [e]
Lycopsamine *N*-oxide	397.45 ± 7.95 [f]	275.62 ± 5.24 [f]	572.86 ± 10.88 [f]
5-*O*-caffeoylquinic acid	8.14 ± 0.15 [b]	7.29 ± 0.15 [c]	21.14 ± 0.40 [a]
Rutin	9.54 ± 0.19 [c]	5.89 ± 0.11 [b]	38.19 ± 0.73 [b]
Trolox [c]	7.92 ± 0.16 [a]	3.41 ± 0.06 [a]	52.79 ± 1.06 [c]

[a] DPPH$^{\bullet}$—2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity; ABTS$^{\bullet+}$—2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical scavenging capacity; DMPD$^{\bullet+}$—*N*,*N*-dimethyl-*p*-phenylenediamine radical scavenging capacity. [b] IC_{50}, μg/mL. [c] Reference compound. Averages ± standard deviation (S.D.) were obtained from five different experiments. Values with different letters (a–f) in each column indicate statistically significant differences among groups at $p < 0.05$ by Duncan test.

The investigated herb extracts of *M. stylosa* and *M. serrulata* revealed good scavenging capacity against DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and DMPD$^{\bullet+}$ radicals. *M. stylosa* herb extract demonstrated the best values of half-maximal scavenging concentrations (IC_{50}) of 21.17, 15.33 and 79.34 μg/mL for DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and DMPD$^{\bullet+}$ radicals, respectively. The predominant phenolic compound, 5-*O*-caffeoylquinic acid, demonstrated superior scavenging activity against synthetic radicals while the alkaloid lycopsamine showed weak antiradical activity. Earlier antioxidant properties of *M. maritima* shoots extracts were evaluated with DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays (IC_{50} 0.57 and 0.78 mg/mL, respectively) [24].

Our studies have shown that two North mertensias, *M. stylosa* and *M. serrulata*, are able to accumulate various phytochemicals, such as alkaloids and phenolics, involved in the antioxidant potential of plant extracts. Pyrrolizidine alkaloids are usually esters formed from a necine base and necic acids [27]. Depending on the structure of the necine base, such alkaloids can be divided into four types: retronecine, otonecine, platynecine, and heliotridine [28]. According to chemotaxonomic data on the distribution of alkaloids in the Boraginaceae family, the species of genus *Mertensia* produce alkaloids of the retronecine type [29]. Pyrrolizidine alkaloids are known to have hepatotoxic and potentially carcinogenic properties [30–33]. However, because pyrrolizidine alkaloids are mainly present in the form of *N*-oxides, such compounds are considered less toxic than free components owing to their high water solubility [34]. Lycopsamine is an unsaturated monoester, and the monoester and *N*-oxide are less toxic than the diester or macrocyclic ester [35]. Some useful properties of lycopsamine are also known. For instance, lycopsamine significantly improved locomotory function and reduced apoptotic cell death following spinal cord injury in rats. Additionally, lycopsamine decreased the expression of tumor necrosis factor-α and upregulated the expression of interleukin-10 [36]. Thus, lycopsamine derivatives may be useful in the treatment of certain disorders, but more investigations are required.

The high biological activities of hydroxycinnamates and flavonoids are well known. In particular, 5-*O*-caffeoylquinic acid inhibits the invasion of non-small cell lung cancer cells through the inactivation of p70S6K and Akt activity [37], suppresses P-selectin expression on platelets by inhibiting cyclooxygenase enzymes [38], and possesses antimicrobial activity [39]. Quercetin-3-*O*-rutinoside or rutin has shown a number of pharmacological properties, including cytoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities [40–43]. Rosmarinic acid is a prospective therapeutic agent against a wide range of lifestyle-related diseases. Many anti-cancer mechanisms of action of rosmarinic acid have been confirmed: prevention of tumor formation development, such as reduction in lipid peroxidation byproducts [44]; inhibition of transcription factor HIF-1α expression [45]; and apoptosis induction [46]. Additionally, cardioprotective [47], antidiabetic [48], anti-inflammatory [49], and antidepressant [50] activities have been established for this compound. Thus, using HPLC-PDA-ESI-tQ-MS analysis, we found twenty-six phenolic compounds, including hydroxycinnamates and flavonoids. Some flavonoids and rosmarinic acid were previously found in other *Mertensia* species while the remaining hydroxycinnamates, derivatives of caffeic and quinic acids, and kaempferol-3-*O*-glucoside were found in this plant genus for the first time.

4. Conclusions

Chromatographic analysis of *Mertensia stylosa* and *M. serrulata* used in traditional medicine of Asian nomads revealed pyrrolizidine alkaloids and phenolic compounds, and a bioactivity study proved the antioxidant potential of the herb extracts. The presence of contradictory properties in these species is an interesting fact: lycopsamine alkaloid as an oncogenic substance and the abundance of hydroxycinnamates and flavonoids as antitumor components. Further studies of these plants are necessary for the wide introduction of new biologically active agents into medical and therapeutic practices.

Author Contributions: Conceptualization, N.I.K. and D.N.O.; methodology, D.N.O.; software, N.K.C.; validation, N.I.K., D.N.O. and N.K.C.; formal analysis, N.I.K.; investigation, D.N.O.; resources, N.K.C.; data curation, D.N.O.; writing—original draft preparation, N.I.K.; writing—review and editing, D.N.O.; visualization, N.K.C.; supervision, D.N.O.; project administration, N.I.K.; funding acquisition, N.K.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Education and Science of Russia, grant numbers 121030100227-7; FSRG-2023-0027.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The author is very thankful to colleagues for kindly providing the photos of plants: Irina Khan–*Mertensia stylosa* (https://www.plantarium.ru/page/image/id/66412.html; accessed on 6 February 2023); Daba Chimitov–*Mertensia serrulata* (https://www.plantarium.ru/page/image/id/753831.html; accessed on 6 February 2023). The authors acknowledge the Buryat Research Resource Center for the technical support in chromatographic and mass-spectrometric research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Oyebode, O.; Kandala, N.B.; Chilton, P.J.; Lilford, R.J. Use of traditional medicine in middle-income countries: A WHO-SAGE study. *Health Policy Plan.* **2016**, *31*, 984–991. [CrossRef] [PubMed]
2. Yuan, H.; Ma, Q.; Ye, L.; Piao, G. The Traditional medicine and modern medicine from natural products. *Molecules* **2016**, *21*, 559. [CrossRef] [PubMed]
3. Bolsokhoyeva, N. Tibetan medical schools of the Aga area (Chita region). *Asian Med.* **2007**, *3*, 334–346. [CrossRef]
4. Aseeva, T.A.; Dashiyev, D.B.; Dashiyev, A.D.; Nikolayev, S.M.; Surkova, N.A.; Chekhirova, G.V.; Yurina, T.A. *Tibetan Medicine of Buryats*; Publishing House SB RAS: Novosibirsk, Russia, 2008; pp. 5–27.
5. Batorova, S.M.; Yakovlev, G.P.; Aseeva, T.A. *Reference-Book of Traditional Tibetan Medicine Herbs*; Nauka: Novosibirsk, Russia, 2013; p. 207.
6. The Plant List. Available online: http://www.theplantlist.org/1.1/browse/A/Boraginaceae/ (accessed on 6 February 2023).
7. Nazaire, M.; Wang, X.-Q.; Hufford, L. Geographic origins and patterns of radiation of *Mertensia* (Boraginaceae). *Am. J. Bot.* **2014**, *101*, 104–118. [CrossRef]
8. Komarov, V.L. *Flora of the U.S.S.R.*; AN U.S.S.R.: Moscow, Russia, 1953; Volume XIX, pp. 248–255.
9. Gracie, C. *Spring Wildflowers of the Northeast*; Princeton University Press: Princeton, NJ, USA, 2012; pp. 222–227.
10. Delort, E.; Jaquier, A.; Chapuis, C.; Rubin, M.; Starken-mann, C.J. Volatile composition of oyster leaf (*Mertensia maritima* (L.) Gray). *J. Agric. Food Chem.* **2012**, *60*, 11681–11690. [CrossRef]
11. Fedoreyev, S.A.; Inyushkina, Y.V.; Bulgakov, V.P.; Veselova, M.V.; Tchernoded, G.K.; Gerasimenko, A.V.; Zhuravlev, Y.N. Production of allantoin, rabdosiin and rosmarinic acid in callus cultures of the seacoastal plant *Mertensia maritima* (Boraginaceae). *Plant Cell Tissue Organ Cult.* **2012**, *110*, 183–188. [CrossRef]
12. Park, H.Y.; Kim, D.H.; Saini, R.K.; Gopal, J.; Keum, Y.S.; Sivanesan, I. Micropropagation and quantification of bioactive compounds in *Mertensia maritima* (L.) Gray. *Int. J. Mol. Sci.* **2019**, *20*, 2141. [CrossRef]
13. Mädge, I.; Gehling, M.; Schöne, C.; Winterhalter, P.; These, A. Pyrrolizidine alkaloid profiling of four Boraginaceae species from Northern Germany and implications for the analytical scope proposed for monitoring of maximum levels. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2020**, *37*, 1339–1358. [CrossRef]
14. Damianakos, H.; Jeziorek, M.; Pietrosiuk, A.; Sykłowska-Baranek, K.; Chinou, I. Isolation of pyrrolizidine alkaloids from *Cynoglossum columnae* Ten. (Boraginaceae). *Planta Med.* **2013**, *79*, PI29. [CrossRef]
15. Zhang, Y.; Cai, P.; Cheng, G.; Zhang, Y. A Brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. *Nat. Prod. Commun.* **2022**, *17*, 1934578X211069721. [CrossRef]
16. Wei, X.; Ruan, W.; Vrieling, K. Current knowledge and perspectives of pyrrolizidine alkaloids in pharmacological applications: A mini-review. *Molecules* **2021**, *26*, 1970. [CrossRef]
17. Olennikov, D.N.; Kartashova, M.E.; Velichko, V.V.; Kruglov, D.S. New flavonoids from *Nonea rossica* and *Tournefortia sibirica*. *Chem. Nat. Compd.* **2022**, *58*, 1021–1025. [CrossRef]
18. Kashchenko, N.I.; Jafarova, G.S.; Isaev, J.I.; Olennikov, D.N.; Chirikova, N.K. Caucasian dragonheads: Phenolic compounds, polysaccharides, and bioactivity of *Dracocephalum austriacum* and *Dracocephalum botryoides*. *Plants* **2022**, *11*, 2126. [CrossRef]
19. Olennikov, D.N.; Chirikova, N.K.; Kashchenko, N.I.; Nikolaev, V.M.; Kim, S.-W.; Vennos, C. Bioactive phenolics of the genus *Artemisia* (Asteraceae): HPLC-DAD-ESI-TQ-MS/MS profile of the Siberian species and their inhibitory potential against α-amylase and α-glucosidase. *Front. Pharmacol.* **2018**, *9*, 756. [CrossRef]
20. Olennikov, D.N.; Chemposov, V.V.; Chirikova, N.K. Polymeric compounds of lingonberry waste: Characterization of antioxidant and hypolipidemic polysaccharides and polyphenol-polysaccharide conjugates from *Vaccinium vitis-idaea* press cake. *Foods* **2022**, *11*, 2801. [CrossRef]
21. Olennikov, D.N.; Khandy, M.T.; Chirikova, N.K. Oriental strawberry metabolites: LC–MS profiling, antioxidant potential, and postharvest changes of *Fragaria orientalis* fruits. *Horticulturae* **2022**, *8*, 975. [CrossRef]
22. Mori, K.; Kidawara, M.; Iseki, M.; Umegaki, C.; Kishi, T. A simple fluorimetric determination of vitamin C. *Chem. Pharm. Bull.* **1998**, *46*, 1474–1476. [CrossRef]
23. Li, Y.; Stermitz, F.R. Pyrrolizidine alkaloids from *Mertensia* species of Colorado. *J. Nat. Prod.* **1988**, *51*, 1289–1290. [CrossRef]
24. Song, K.; Sivanesan, I.; Ak, G.; Zengin, G.; Cziáky, Z.; Jekő, J.; Rengasamy, K.R.; Lee, O.N.; Kim, D.H. Screening of bioactive metabolites and biological activities of calli, shoots, and seedlings of *Mertensia maritima* (L.) Gray. *Plants* **2020**, *9*, 1551. [CrossRef]
25. Rizk, A.M. *Naturally Occurring Pyrrolizidine Alkaloids*; CRC Press: Boca Raton, FL, USA, 1991.

26. Lyashenko, S.; González-Fernández, M.J.; Borisova, S.; Belarbi, E.H.; Guil-Guerrero, J.L. *Mertensia* (Boraginaceae) seeds are new sources of γ-linolenic acid and minor functional compounds. *Food Chem.* **2021**, *350*, 128635. [CrossRef]
27. Bruneton, J. *Farmacognosia*, 2nd ed.; Acribia: Zaragoza, Spain, 2008; ISBN 978-1-84585-006-7.
28. Gottschalk, C.; Ronczka, S.; Preiß-Weigert, A.; Ostertag, J.; Klaffke, H.; Schafft, H.; Lahrssen-Wiederholt, M. Pyrrolizidine alkaloids in natural and experimental grass silages and implications for feed safety. *Anim. Feed Sci. Technol.* **2015**, *207*, 253–261. [CrossRef]
29. El-Shazly, A.; Wink, M. Diversity of pyrrolizidine alkaloids in the Boraginaceae structures, distribution, and biological properties. *Diversity* **2014**, *6*, 188–282. [CrossRef]
30. Wiedenfeld, H. Plants containing pyrrolizidine alkaloids: Toxicity and problems. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2011**, *28*, 282–292. [CrossRef] [PubMed]
31. Moreira, R.; Pereira, D.M.; Valentao, P.; Andrade, P.B. Pyrrolizidine alkaloids: Chemistry, pharmacology, toxicology and food safety. *Int. J. Mol. Sci.* **2018**, *19*, 1668. [CrossRef] [PubMed]
32. Neuman, M.G.; Cohen, L.; Opris, M.; Nanau, R.M.; Hyunjin, J. Hepatotoxicity of pyrrolizidine alkaloids. *J. Pharm. Pharm. Sci.* **2015**, *18*, 825–843. [CrossRef]
33. Wang, Z.; Han, H.; Wang, C.; Zheng, Q.; Chen, H.; Zhang, X.; Hou, R. Hepatotoxicity of pyrrolizidine alkaloid compound intermedine: Comparison with other pyrrolizidine alkaloids and its toxicological mechanism. *Toxins* **2021**, *13*, 849. [CrossRef]
34. Schramm, S.; Köhler, N.; Rozhon, W. Pyrrolizidine alkaloids: Biosynthesis, biological activities and occurrence in crop plants. *Molecules* **2019**, *24*, 498. [CrossRef]
35. Mattocks, A.R. *Chemistry and Toxicology of Pyrrolizidine Alkaloids*; Academic Press: London, UK, 1986; p. 41.
36. Jin, J.; Li, H.; Zhao, G.; Jiang, S. Lycopsamine exerts protective effects and improves functional outcome after spinal cord injury in rats by suppressing cell death. *Med. Sci. Monit.* **2018**, *24*, 7444–7450. [CrossRef]
37. In, J.K.; Kim, J.K.; Oh, J.S.; Seo, D.W. 5-Caffeoylquinic acid inhibits invasion of non-small cell lung cancer cells through the inactivation of p70S6K and Akt activity: Involvement of p53 in differential regulation of signaling pathways. *Int. J. Oncol.* **2016**, *48*, 1907–1912. [CrossRef]
38. Park, J.B. 5-Caffeoylquinic acid and caffeic acid orally administered suppress P-selectin expression on mouse platelets. *J. Nutr. Biochem.* **2009**, *20*, 800–805. [CrossRef]
39. Bajko, E.; Kalinowska, M.; Borowski, P.; Siergiejczyk, L.; Lewandowski, W. 5-*O*-Caffeoylquinic acid: A spectroscopic study and biological screening for antimicrobial activity. *LWT Food Sci. Technol.* **2016**, *65*, 471–479. [CrossRef]
40. Javed, H.; Khan, M.M.; Ahmad, A.; Vaibhav, K.; Ahmad, M.E.; Khan, A.; Ashafaq, M.; Islam, F.; Siddiqui, M.S.; Safhi, M.M.; et al. Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. *Neuroscience* **2012**, *17*, 340–352. [CrossRef]
41. Trumbeckaite, S.; Bernatoniene, J.; Majiene, D.; Jakstas, V.; Savickas, A.; Toleikis, A. The effect of flavonoids on rat heart mitochondrial function. *Biomed. Pharmacother.* **2006**, *60*, 245–248. [CrossRef]
42. Richetti, S.K.; Blank, M.; Capiotti, K.M.; Piato, A.L.; Bogo, M.R.; Vianna, M.R.; Bonan, C.D. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behav. Brain Res.* **2011**, *217*, 10–15. [CrossRef]
43. Ganeshpurkar, A.; Saluja, A.K. The pharmacological potential of rutin. *Saudi Pharm. J.* **2017**, *25*, 149–164. [CrossRef]
44. Venkatachalam, K.; Gunasekaran, S.; Namasivayam, N. Biochemical and molecular mechanisms underlying the chemopreventive efficacy of rosmarinic acid in a rat colon cancer. *Eur. J. Pharmacol.* **2016**, *791*, 37–50. [CrossRef]
45. Xu, Y.; Han, S.; Lei, K.; Chang, X.; Wang, K.; Li, Z.; Liu, J. Anti-Warburg effect of rosmarinic acid via miR-155 in colorectal carcinoma cells. *Eur. J. Cancer Prev. Off. J. Eur. Cancer Prev. Organ.* **2016**, *25*, 481–489. [CrossRef]
46. Heo, S.K.; Noh, E.K.; Yoon, D.J.; Jo, J.C.; Koh, S.; Baek, J.H.; Park, J.H.; Min, Y.J.; Kim, H. Rosmarinic acid potentiates ATRA-induced macrophage differentiation in acute promyelocytic leukemia NB4 cells. *Eur. J. Pharmacol.* **2015**, *747*, 36–44. [CrossRef]
47. Karthik, D.; Viswanathan, P.; Anuradha, C.V. Administration of rosmarinic acid reduces cardiopathology and blood pressure through inhibition of p22phox NADPH oxidase in fructose-fed hypertensive rats. *J. Cardiovasc. Pharmacol.* **2011**, *58*, 514–521. [CrossRef]
48. Jayanthy, G.; Roshana Devi, V.; Ilango, K.; Subramanian, S.P. Rosmarinic Acid Mediates Mitochondrial Biogenesis in Insulin Resistant Skeletal Muscle Through Activation of AMPK. *J. Cell. Biochem.* **2017**, *118*, 1839–1848. [CrossRef]
49. Liang, Z.; Xu, Y.; Wen, X.; Nie, H.; Hu, T.; Yang, X.; Chu, X.; Yang, J.; Deng, X.; He, J. Rosmarinic acid attenuates airway inflammation and hyperresponsiveness in a murine model of asthma. *Molecules* **2016**, *21*, 769. [CrossRef] [PubMed]
50. Jin, X.; Liu, P.; Yang, F.; Zhang, Y.H.; Miao, D. Rosmarinic acid ameliorates depressive-like behaviors in a rat model of CUS and Up-regulates BDNF levels in the hippocampus and hippocampal-derived astrocytes. *Neurochem. Res.* **2013**, *38*, 1828–1837. [CrossRef] [PubMed]