chemistry

MDPI

Article

Comprehensive Chemical Analysis of *Codonopsis lanceolata* Roots Using Ultra-High-Performance Liquid Chromatography–Quadrupole-Exactive–Orbitrap Mass Spectrometry

Chang Luo [1], Zenan Duan [1], Takashi Kikuchi [1,*], Kouharu Otsuki [1], Mi Zhang [1], Ryuichi Kambayashi [2], Katsuhiko Ito [3], Atsushi Sugiyama [2,*] and Wei Li [1,*]

[1] Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi 274-8510, Chiba, Japan; 3024005r@st.toho-u.ac.jp (C.L.)
[2] Department of Pharmacology, Faculty of Medicine, Toho University, 5-21-16 Omori-nishi, Ota-ku 143-8540, Tokyo, Japan
[3] Division of Industry-University Collaboration, Toho University, 5-21-16 Omori-nishi, Ota-ku 143-8540, Tokyo, Japan
* Correspondence: takashi.kikuchi@phar.toho-u.ac.jp (T.K.); atsushi.sugiyama@med.toho-u.ac.jp (A.S.); liwei@phar.toho-u.ac.jp (W.L.)

Abstract: The roots of *Codonopsis lanceolata* (Siebold & Zucc.) Benth. & Hook.f. ex Trautv. have been traditionally used for medicinal purposes across East Asia. However, their chemical constituents in Japanese-grown varieties remain uninvestigated. This study employed ultra-high-performance liquid chromatography–quadrupole–orbitrap mass spectrometry to perform a comprehensive chemical analysis of the roots of *C. lanceolata* cultivated in Nagano Prefecture, Japan, leveraging fragment pattern analysis of both isolated and commercially available compounds as references compounds. As a result, 27 compounds, including triterpenoid saponins (**19**–**22**), polyacetylenes (**6**, **15**, **18**), flavonoids (**16**, **17**), phenylpropanoids (**3**–**5**, **7**, **9**), a lignan (**10**), glycolipids (**8**, **11**–**14**), phospholipids (**23**–**27**), and amino acids (**1**, **2**), were identified. Notably, a triterpenoid saponin (**19**) was identified as a previously unreported compound, and ten compounds (**3**, **6**, **8**, **10**, **13**, **17**, and **23**–**27**) were identified from *C. lanceolata* roots for the first time. The ex vivo study revealed that lancemaside A (**22**) exhibited a time-dependent vasodilatory effect on rat aortic ring specimens. These findings not only advanced the understanding of the chemical constituents and biological activity of *C. lanceolata* roots but also provided valuable insights for their future applications and quality control.

Keywords: *Codonopsis lanceolata*; LC-MS; triterpenoid saponin; polyacetylene; vasodilatory effect

Academic Editor: Angelo Frongia

Received: 6 December 2024
Revised: 26 December 2024
Accepted: 2 January 2025
Published: 6 January 2025

Citation: Luo, C.; Duan, Z.; Kikuchi, T.; Otsuki, K.; Zhang, M.; Kambayashi, R.; Ito, K.; Sugiyama, A.; Li, W. Comprehensive Chemical Analysis of *Codonopsis lanceolata* Roots Using Ultra-High-Performance Liquid Chromatography–Quadrupole-Exactive–Orbitrap Mass Spectrometry. *Chemistry* **2025**, *7*, 4. https://doi.org/10.3390/chemistry7010004

1. Introduction

The *Codonopsis* genus (Campanulaceae) includes over 40 species distributed across West and Central Asia [1]. The roots of these plants, such as *C. pilosula*, *C. pilosula* var. *modesta*, and *C. tangshen*, have been widely used for enhancing the immune system, improving gastrointestinal function, alleviating gastric ulcers, stimulating appetite, and reducing blood pressure as part of traditional medicine for the treatment of various diseases and disorders in Japan, China, and Vietnam [2–4]. Additionally, some *Codonopsis* species are used as ingredients in tea, wine, and soups [3,4]. Previous phytochemical investigations have identified over one hundred compounds from plants of the *Codonopsis* genus, including

polyacetylenes (polyynes), flavonoids, alkaloids, phenylpropanoids, lignans, terpenoids, and steroids [2].

Codonopsis lanceolata is a herbaceous perennial plant distributed in East Asia whose roots are edible and also used in traditional Chinese medicine, with reported pharmacological properties such as anti-fatigue, antioxidant [5], blood pressure reduction [6], antidiabetes [7], antitussive, and expectorant effects [1]. Phytochemical investigations reported the isolation and structural determination of triterpenoid saponins, phenylpropanoids, glycolipids, ceramides, and lignans [2], while liquid chromatography (LC)–mass spectrometry (MS) analysis has revealed the presence of sesquiterpenes, triterpenoid saponins, phenylpropanoids, polyacetylenes, glycolipids, and amino acids [8,9]. However, the chemical constituents in Japanese-grown varieties remain uninvestigated.

In recent years, advancements in LC-MS and LC-MS/MS technology have greatly facilitated the analysis of natural products, proving particularly effectiveness for rapid structural identification [10,11]. In the present study, four compounds (**18**, **21**, **22**, and **27**) were isolated from the *C. lanceolata* roots using chromatographic methods, and their structures were confirmed by nuclear magnetic resonance (NMR) spectroscopic analysis. Based on characteristic ion fragmentation patterns of both isolated and commercially available compounds in LC-MS and MS/MS, the roots of *C. lanceolata* grown in Japan were analyzed using ultra-high-performance liquid chromatography (UHPLC) coupled with quadrupole–orbitrap mass spectrometry to investigate their chemical constituents. As a result, a total of 27 compounds were identified, including a previously unreported triterpenoid saponin (**19**). Among them, ten compounds (**3**, **6**, **8**, **10**, **13**, **17**, and **23**–**27**) were identified from *C. lanceolata* roots for the first time. The ex vivo vasodilatory effects of the fractions and two major compounds (**18** and **22**) were also evaluated on rat aortic ring specimens.

2. Materials and Methods

2.1. General Experimental Procedures

All LC-MS mobile phases were prepared using LC-MS-grade reagents from Kanto Chemical Co., Inc. (Tokyo, Japan). The mobile phases used for column chromatography (CC) and semi-preparative high-performance liquid chromatography (HPLC) were HPLC-grade and obtained from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The Diaion HP-20 resin used for CC was from Mitsubishi Chemical Corporation (Tokyo, Japan). For HPLC separations, an RP-C_{18} silica gel column (YMC Actus Triart C_{18}, 150 × 20 mm I.D., YMC. Co., Ltd., Kyoto, Japan) was used at a flow rate of 5.0 mL/min. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA-500 spectrometer (JEOL Ltd., Tokyo, Japan) with deuterated solvents as the internal references, and chemical shifts were reported in δ (ppm) units. Standards including hesperidin (90%) and chlorogenic acid hydrate (98%) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); L-tryptophan (99%), L-phenylalanine (99%), and syringin (98%) were purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Plant Materials

The roots of *Codonopsis lanceolata* (Siebold & Zucc.) Benth. & Hook.f. ex Trautv. used in this study were cultivated at Azumino city in Nagano Prefecture, Japan, and harvested in June 2021. The plant materials were identified by one of the authors, WL. A voucher specimen (TH-CLR-1) was deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University, Japan.

2.3. LC-MS Analysis

2.3.1. Preparation of LC-MS Analysis Solution of *C. lanceolata*

The dried root powder (10 g) was extracted ultrasonically with methanol (MeOH) (100 mL) at room temperature. The extract was then placed on a Diaion HP-20 column and eluted with H_2O and MeOH, yielding a MeOH eluate fraction (308 mg). A portion of the MeOH eluate fraction (10 mg) was placed on a Sep-Pak C_{18} column Cartridge and eluted with MeOH to prepare the 1 mg/mL solution, filtered (0.2 μm), and subjected to LC-MS.

2.3.2. LC-MS Conditions

LC-MS analysis utilized a Vanquish UHPLC system coupled with a Q-Exactive hybrid quadrupole orbitrap high-resolution accurate mass spectrometer (Thermo Scientific, Waltham, MA, USA) with an electrospray ionization (ESI) source operated in both positive and negative ionization modes.

The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile (MeCN)). Gradient elution was performed as follows: 0–1 min, 5% B; 1–15 min, 5–80% B; 15–30 min, 100% B. The samples were injected into a TSKgel ODS-120H column (2.0 mm 1.D. × 10 cm, 1.9 μm, Tosoh Corporation, Tokyo, Japan) at a flow rate of 0.4 mL/min with a column oven temperature of 40 °C.

Mass spectrometer calibration solutions ensured high mass accuracy. Optimized parameters included a spray voltage of +3.5 kV (positive mode) or −2.5 kV (negative mode), a capillary temperature of 262.5 °C, and sheath, auxiliary, and sweep gas flow rates of 50, 12.5, and 25 units, respectively. The S-lens RF level was set at 50, and the probe heater temperature was maintained at 425 °C. Data collection included both full MS and data-dependent MS/MS (dd-MS/MS) modes. In-source collision-induced dissociation was set at 0 eV. The resolution was maintained at 70,000 for full MS and 35,000 for dd-MS/MS, with an AGC target of 1×10^6 and 1×10^5, respectively. The maximum injection times were 200 ms for full MS and 50 ms for dd-MS/MS. Scans covered an m/z range of 150–2000. Data-dependent scanning was performed using high-energy collision dissociation with normalized collision energies (NCEs) of 10 eV and 25 eV. Extracted ion chromatograms were generated with a mass tolerance of 5 ppm. All data were processed and analyzed using Thermo Xcalibur 5.1 software.

2.4. Extraction and Isolation

The dried roots of *C. lanceolata* (883 g) were extracted with MeOH at room temperature via ultrasonic treatment, yielding a crude extract (380 g). The resulting MeOH extract was partitioned sequentially between ethyl acetate (EtOAc), *n*-butanol *(n*-BuOH), and H_2O.

A portion (1 g) of the EtOAc soluble fraction (6.4 g), obtained after the concentration of the solvent, was fractionated to Diaion HP-20 CC. The MeOH-eluted fraction was separated using reversed-phase (RP)–HPLC under gradient conditions and isocratic preparative HPLC to obtain 1-lyso-palmitoylphosphatidylcholine (**27**, 2.5 mg) [60% MeCN].

The *n*-BuOH soluble fraction (32 g) underwent Diaion HP-20 CC, yielding the 80% MeOH elution (3.9 g) which was subsequently separated using gradient RP-HPLC (30–80% MeOH) and RP-HPLC to obtain lancemasides A (**22**, 67.6 mg) and B (**21**, 33.0 mg) and lobetyolin (**18**, 40.4 mg) [27% MeCN containing 0.06% trifluoro acetic acid (TFA), 60% MeOH containing 0.06% TFA, and 40% MeOH containing 0.06% TFA, respectively].

2.5. Evaluation of Vasodilatory Effects

2.5.1. Animals

The experiments were approved by the Toho University Animal Care and User Committee (No. 21-51-490 and No. 22-52-490) and performed according to the Guideline for

the Care and Use of Laboratory Animals of Toho University. Male SD rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). In the period between receiving the animals and starting the activity evaluation, they were quarantined and acclimatized for over a week to confirm that no abnormalities in appearance and behavior occurred, and the negative microbiological tests were accepted. The animals were reared in aluminum cages (2 to 3 animals/cage) with a temperature of 23 ± 1 °C, a relative humidity of 50±10%, and a lighting time from 6:00 to 18:00 in compliance with the light–dark cycle in the breeding room. Solid feed and purified water were available ad libitum.

2.5.2. Evaluation for Vasodilatory Effects on Rat Aortic Ring Specimens

The evaluation samples were dissolved and diluted to the optimum concentration using dimethyl sulfoxide (DMSO) for the administration specimen. The activity detection samples were the EtOAc fraction, the *n*-BuOH fraction, lobetyolin (**18**), and lancemaside A (**22**). Each sample was added to a final concentration of 1×10^{-5} g/mL in a magnus tube, and the vasodilation effect was evaluated.

Rats (388 ± 69 g) were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.). A midline incision was made on the chest and abdomen, and then the abdominal aorta was incised and bled. A ring-shaped specimen approximately 3 mm wide was created with the isolated thoracic aorta. The specimens were suspended using two stainless steel wires which were inserted into the lumen of the ring in a filled-nutrient solution magnus tube. A static tension (1.0 g) was applied, and the tension transducer measured the tension of the ring. Each specimen was contracted with phenylephrine (PE), and when the tension became constant, each sample was added. The tension changes in the specimen were recorded.

3. Results and Discussion

3.1. Identification of Compounds in C. lanceolata Roots Using LC-MS and LC-MS/MS Analyses

The solution obtained from *C. lanceolata* roots was analyzed using LC-MS and LC-MS/MS analyses (Figure 1). The retention time (t_R), molecular weight, molecular formula, and fragmentations both in the positive and negative ion modes were compared with the data of previous studies, and eight compounds, phenylalanine (**1**), tryptophan (**2**), syringin (**4**), chlorogenic acid (**5**), (*E*)-2-hexenyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**12**), lobetyolinin (**18**), lancemaside B (**21**), and lancemaside A (**22**), were easily identified [9,12,13]. To assist with LC-MS and LC-MS/MS analyses, phytochemical extraction and isolation were also carried out on *C. lanceolata* roots. As a result, four compounds (**18**, **21**, **22**, and **27**) were successfully isolated, and their chemical structures were determined through NMR data analysis and comparison with literature data [14–17]. These compounds, along with commercially available compounds, phenylalanine, tryptophan, chlorogenic acid, and hesperidin, were used as reference standards for LC-MS and LC-MS/MS analyses. Ultimately, through the examination of molecular formulas, retention times, and the regularity of fragmentation patterns, a total of 27 compounds were identified (Figure 2, Table 1).

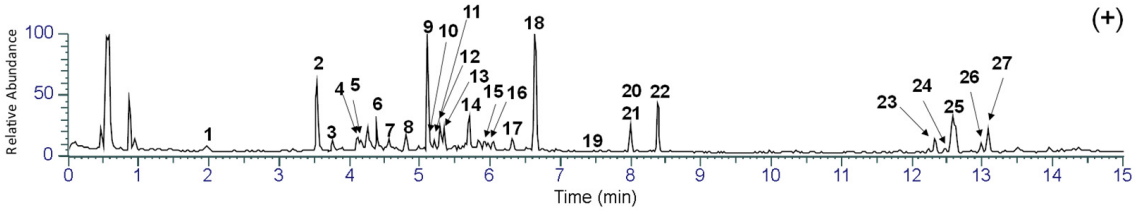

Figure 1. Total ion chromatogram (TIC) of *C. lanceolata* roots.

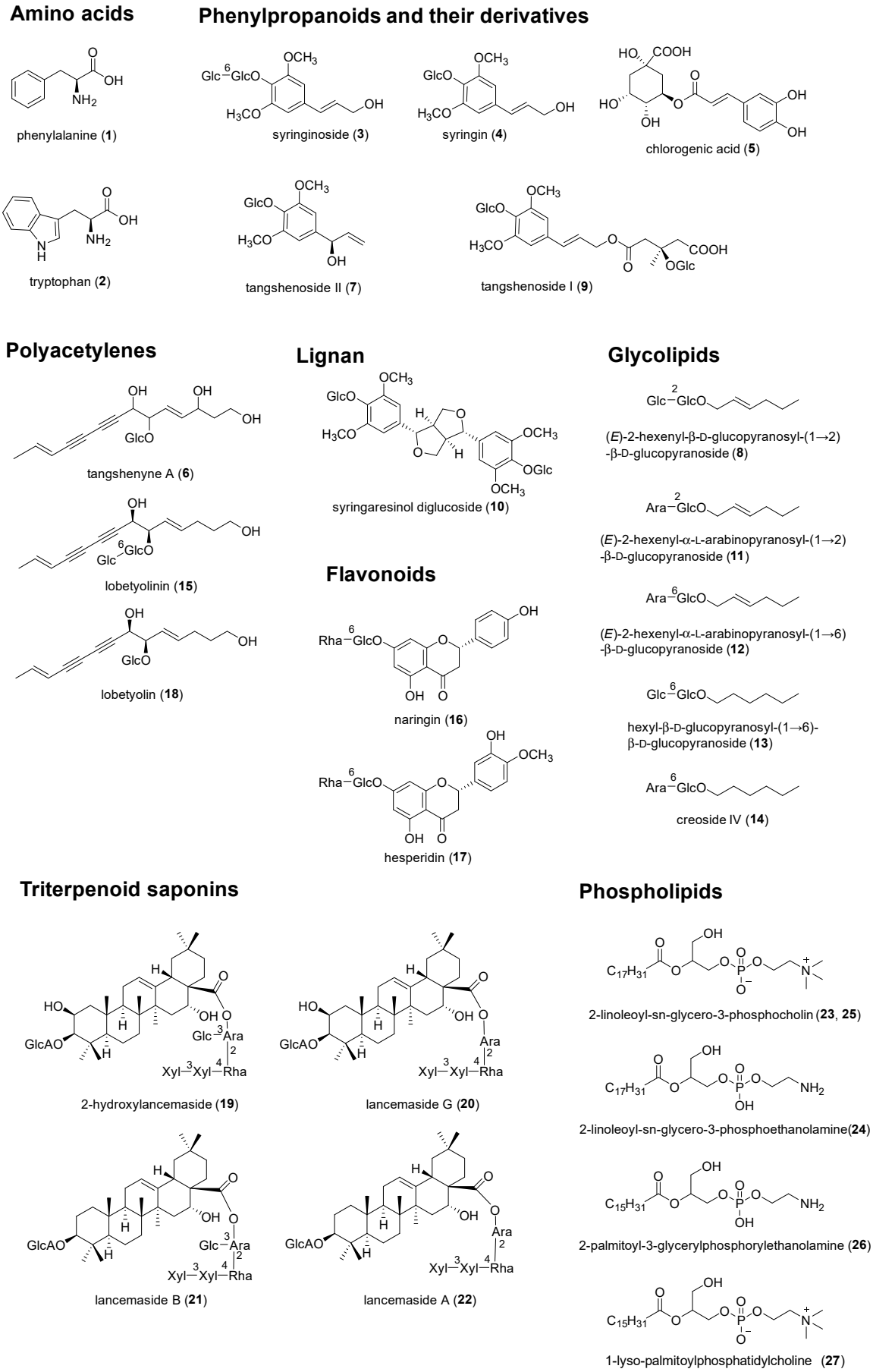

Figure 2. The chemical structures of compounds in *C. lanceolata* roots.

Table 1. Compounds identified from *C. lanceolata* roots by LC-MS.

No.	t_R, min	Positive Mode		Negative Mode		Molecular Formula	Identification
		m/*z*	Adduct Ion	*m*/*z*	Adduct Ion		
1 [a]	2.07	166.0865	$[M+H]^+$	164.0718	$[M-H]^-$	$C_9H_{11}O_2N$	Phenylalanine
2 [a]	3.45	205.0973	$[M+H]^+$	203.0827	$[M-H]^-$	$C_{11}H_{12}O_2N_2$	Tryptophan
3	3.76	552.2290	$[M+NH_4]^+$	579.1938	$[M+HCOO]^-$	$C_{23}H_{34}O_{14}$	Syringinoside
4 [a]	4.08	390.1756	$[M+NH_4]^+$	417.1401	$[M+HCOO]^-$	$C_{17}H_{24}O_9$	Syringin
5 [a]	4.22	355.1021	$[M+H]^+$	353.0876	$[M-H]^-$	$C_{16}H_{18}O_9$	Chlorogenic acid
6	4.37	430.2068	$[M+NH_4]^+$	457.1715	$[M+HCOO]^-$	$C_{20}H_{28}O_9$	Tangshenyne A
7	4.53	390.1756	$[M+NH_4]^+$	417.1406	$[M+HCOO]^-$	$C_{17}H_{24}O_9$	Tangshenoside II
8	4.75	442.2281	$[M+NH_4]^+$	469.1925	$[M+HCOO]^-$	$C_{18}H_{32}O_{11}$	(*E*)-2-Hexenyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside
9	4.87	696.2704	$[M+NH_4]^+$	677.2294	$[M-H]^-$	$C_{29}H_{42}O_{18}$	Tangshenoside I
10	5.22	760.3021	$[M+NH_4]^+$	787.2679	$[M+HCOO]^-$	$C_{34}H_{46}O_{18}$	Syringaresinol diglucoside
11	4.97	412.2179	$[M+NH_4]^+$	439.1820	$[M+HCOO]^-$	$C_{17}H_{30}O_{10}$	(*E*)-2-Hexenyl-α-L-arabinopyranosyl-(1→2)-β-D-glucopyranoside
12 [a]	5.15	412.2173	$[M+NH_4]^+$	439.1820	$[M+HCOO]^-$	$C_{17}H_{30}O_{10}$	(*E*)-2-Hexenyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside
13	5.21	444.2440	$[M+NH_4]^+$	471.2083	$[M+HCOO]^-$	$C_{18}H_{34}O_{11}$	Hexyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside
14	5.66	414.2332	$[M+NH_4]^+$	441.1793	$[M+HCOO]^-$	$C_{17}H_{32}O_{10}$	Creoside IV
15	5.88	576.2651	$[M+NH_4]^+$	603.2303	$[M+HCOO]^-$	$C_{26}H_{38}O_{13}$	Lobetyolinin
16	5.92	581.1865	$[M+H]^+$	579.1727	$[M-H]^-$	$C_{27}H_{32}O_{14}$	Naringin
17 [a]	6.30	611.1968	$[M+H]^+$	609.1829	$[M-H]^-$	$C_{28}H_{34}O_{15}$	Hesperidin
18 [c]	6.50	414.2118	$[M+NH_4]^+$	441.1761	$[M+HCOO]^-$	$C_{20}H_{28}O_8$	Lobetyolin
19 [b]	7.59	1386.6553	$[M+NH_4]^+$	1367.6113	$[M-H]^-$	$C_{63}H_{100}O_{32}$	2-Hydroxylancemaside B
20	7.96	1207.5736	$[M+NH_4]^+$	1205.5582	$[M-H]^-$	$C_{59}H_{90}O_{27}$	Lancemaside G
21 [c]	7.96	641.3328	$[M+NH_4]^+$	1351.6165	$[M-H]^-$	$C_{63}H_{100}O_{31}$	Lancemaside B
22 [c]	8.20	583.3271	$[M+NH_4]^+$	1189.5635	$[M-H]^-$	$C_{57}H_{92}O_{26}$	Lancemaside A
23	12.33	615.3323	$[M+H]^+$	564.3309	$[M+HCOO]^-$	$C_{26}H_{50}O_7NP$	2-Linoleoyl-sn-glycero-3-phosphocholine
24	12.43	547.3271	$[M+H]^+$	476.2783	$[M-H]^-$	$C_{23}H_{45}O_7NP$	2-Linoleoyl-sn-glycero-3-phosphoethanolamine
25	12.52	643.3469	$[M+H]^+$	564.3309	$[M+HCOO]^-$	$C_{26}H_{50}O_7NP$	2-Linoleoyl-sn-glycero-3-phosphocholine
26	12.88	585.3420	$[M+H]^+$	452.2782	$[M-H]^-$	$C_{21}H_{45}O_7NP$	2-Palmitoyl-3-glycerylphosphorylethanolamine
27 [c]	12.94	759.3731	$[M+H]^+$	540.3307	$[M+HCOO]^-$	$C_{24}H_{50}O_7NP$	1-Lyso-palmitoylphosphatidylcholine

[a] Identifications were confirmed by comparison of retention times and product ion spectra with reference compounds or standards. [b] Previously unreported compounds. [c] Compounds isolated in this study.

3.1.1. Triterpenoid Saponins

Lancemaside A (**22**) and lacemaside B (**21**), which have the same aglycone but different sugar chains, were highly characteristic saponin markers of *C. lanceolata*. In the positive ion mode of full MS of **22** and **21**, ammonium adduct ions $[M+NH_4]^+$ and aglycone dehydration ions $[Aglycone-H_2O+H]^+$ were observed. In the MS/MS analysis with $[Aglycone–H_2O+H]^+$ as the precursor ion at an NCE = 25 eV, product ions which were interpreted as the dissociation of the hydroxyl group at the C-16 position and the carboxyl group at the C-18 position were observed. The product ions in the low mass-to-charge ratio region (*m*/*z* 190–270) were interpreted as those generated through the retro-Diels–Alder (rDA) reaction, specifically product ions resulting from the cleavage of the C ring, as well as product ions derived from the D/E rings formed sequentially through decarboxylation and dehydration, and product ions originating from the A/B rings formed via dehydration (Figure 3A). In the MS/MS analysis of **22** with $[M+NH_4]^+$ as the precursor ion at an NCE = 10 eV, the sequential loss of two xylose (Xyl), a rhamnose (Rha), an arabinose (Ara), and a glucuronic acid (GlcA) moieties was observed. A characteristic product ion at *m*/*z* 795 $C_{57}H_{92}O_{26}^+$ was attributed to the incomplete cleavage of the rhamnose moiety, likely

resulting from an rDA reaction and the elimination of CO (Figure 3B). In lancemaside B (**21**), a similar MS/MS fragment pattern was observed, with the additional detection of product ions derived from the detachment of the glucose moiety. Furthermore, the precursor ion *m*/*z* 1221 $[M-Xyl+H]^+$ produced *m*/*z* 1089 $[M-2Xyl+H]^+$ and *m*/*z* 1059 $[M-Xyl-Glc+H]^+$ product ions, confirming that the glucose moiety was attached to the arabinose moiety (Figures S7–S10).

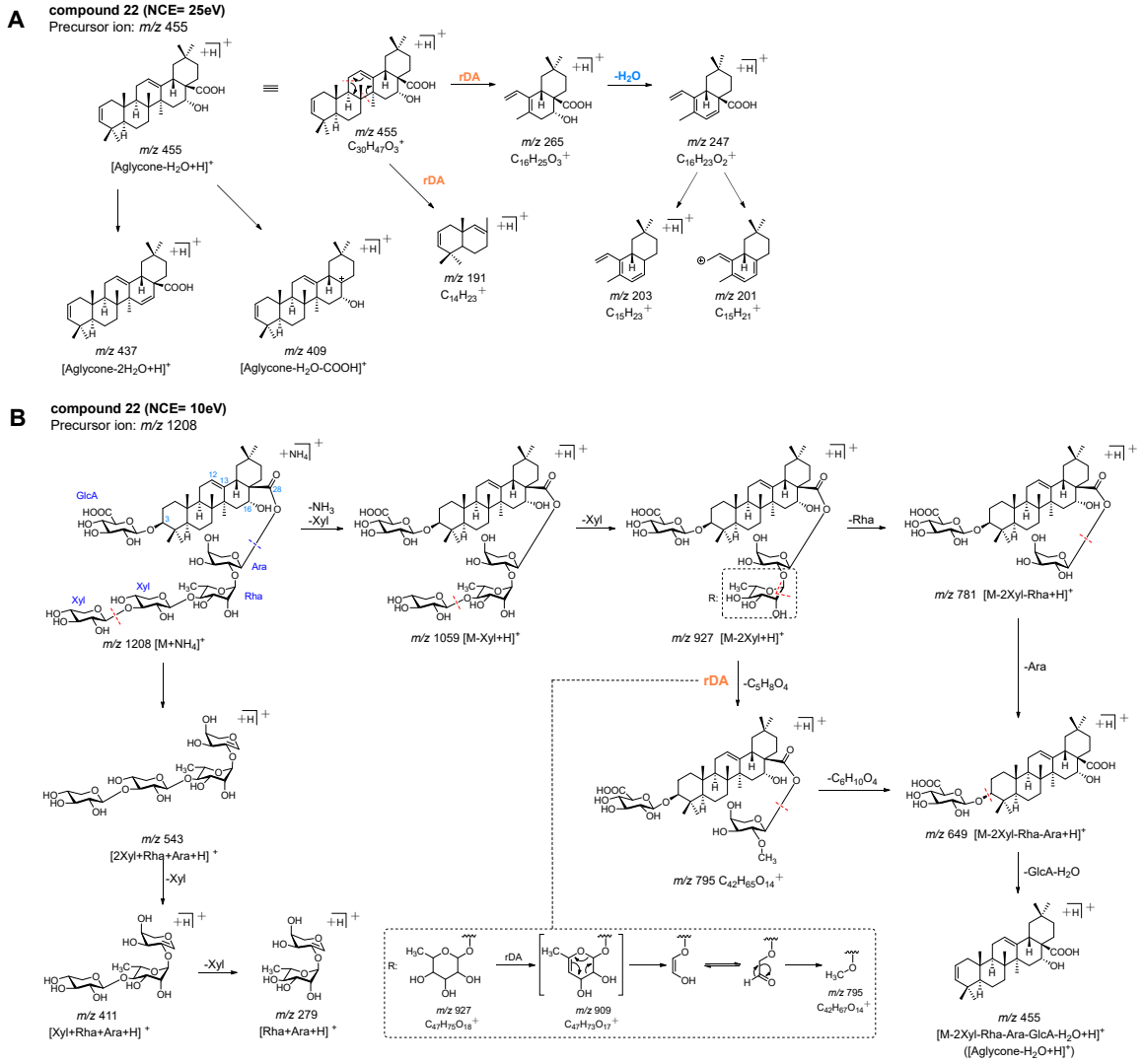

Figure 3. Fragmentation pathway of lancemaside A (**22**) using precursor ions of *m*/*z* 455 (NCE = 25 eV) (**A**) and *m*/*z* 1208 (NCE = 10 eV) (**B**).

Through full MS analysis, the molecular formulas and precursor ions were determined, and by logically examining the fragmentation pathways under varying voltages in MS/MS, two more saponins **19** and **20** in *C. lanceolata* were identified. Saponin **19** has the same sugar chain as **21**, while **20** has the same sugar chain as **22**, as suggested by the product ion spectra at an NCE = 10 eV using $[M+NH_4]^+$ as the precursor ion. Meanwhile, in comparison to **21** and **22**, the MS/MS spectra of $[Aglycone-H_2O+H]^+$ in **19** and **20** showed product ions derived from the A/B rings, with the loss of an additional H_2O molecule, suggesting the presence of 2,3-dihydroxy substituents in the A ring. Consequently, **20** was identified as lancemaside G, a previously reported saponin from *C. lanceolata* [9], and **19** was identified as 2-hydroxylancemaside B, a previously unreported compound.

3.1.2. Polyacetylenes

Lobetyolin (**18**) showed product ions by the losses of the glucose moiety, H_2O molecules, and sequential carbon eliminations from the long-chain aglycone in MS/MS analysis using $[M+NH_4]^+$ as the precursor ion at an NCE of 25 eV (Figure 4). Compound **15** was identified as lobetyolinin, a previously reported polyacetylene from *C. lanceolata* [9], based on the MS/MS analysis where product ions were observed due to the sequential elimination of two glucose moieties, along with sequential carbon eliminations from the long-chain aglycone similar to those observed for **18**. In compound **6**, the presence of a hydroxyl group at the C-12 position was suggested by the presence of the product ion *m*/*z* 171 ($C_{12}H_{11}O^+$). Tanshenine A (**6**) was found for the first time in *C. lanceolata*, although it has been reported from other *Codonopsis* species [18].

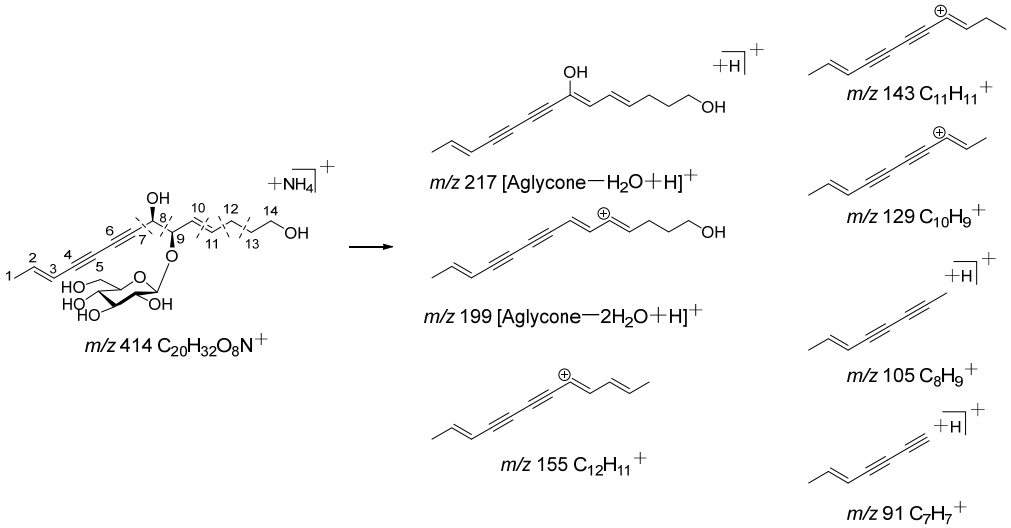

Figure 4. Interpretable product ions revealed by MS/MS of lobetyolin (**18**).

3.1.3. Flavonoids

Hesperidin (**17**) and compound **16** exhibited product ions due to the loss of rhamnosyl and glucosyl moieties in the MS/MS analysis using $[M+NH_4]^+$ as the precursor ion at an NCE of 10 eV. In the MS/MS analysis using $[Aglycone+H]^+$ as the precursor ion at an NCE of 25 eV, the two compounds showed identical product ions derived from the A-ring via the cleavage of the C-ring, but differences in those derived from the B-ring. Namely, in compound **16**, a product ion originating from the B-ring was observed with one less methoxy group than **17**. Therefore, compound **16** was identified as naringin [19].

3.1.4. Phenylpropanoids and Lignans

Compound **3** was identified as syringinoside based on its MS/MS fragmentation patterns, which were similar to those of syringin (**4**), except for the loss of an additional glucosyl moiety [20]. Compound **7** has the same molecular formula as **4** and exhibited a similar MS/MS fragmentation pattern; however, based on the chromatographic behavior reported in the literature [15], it was identified as tangshenoside II. Compound **9** exhibited similar product ions as **4** in the MS/MS analysis using $[M+NH_4]^+$ as the precursor ion at an NCE of 25 eV. Meanwhile, the MS/MS analysis using $[M-H]^-$ as the precursor ion showed product ions derived from eliminations of the glucosyl moiety, CO_2, and the 3-methylglutaric acid moiety. Therefore, compound **9** was identified as tangshenoside I [15]. While compounds **7** and **9** are known in *C. lanceolata* [9], compound **3** has not been previously reported from the *Codonopsis* genus.

Compound **10** is a dihydrofuranofuran-type lignan, and in the MS/MS analysis using $[M-H]^-$ as the precursor ion, the product ions derived from the aglycone and the C_6-C_1 unit matched the spectrum reported in the literature [21]. Compound **10** was identified as syringaresinol diglucoside, which has been reported in *C. convolvulacea* [22].

3.1.5. Phospholipids

1-Lyso-palmitoylphosphatidylcholine (**27**), using MS/MS analysis (NCE = 25 eV) with $[M+H]^+$ as the precursor ion, was interpreted to generate product ions corresponding to the phosphocholine moiety resulting from the loss of palmitic acid, as well as a choline moiety and its dehydration ion. In addition, the MS/MS analysis using $[M+HCOO]^-$ as the precursor ion detected ions derived from the palmitic acid moiety. Compounds **23** and **25** have the same molecular formula and exhibited similar MS/MS fragmentation patterns to **27**. However, in the MS/MS analysis using $[M+HCOO]^-$ as the precursor ion, ions derived from the linolenic acid moiety were observed. Compounds **23** and **25** were considered to be 2-linoleoyl-sn-glycero-3-phosphocholine [23,24], likely isomers with different stereochemical structures or positions of the double bonds. In the MS/MS spectra of compounds **24** and **26** with $[M+H]^+$ as the precursor ion, product ions derived from the ethanolamine moiety, rather than the choline moiety, were observed. Additionally, in the MS/MS spectra with $[M-H]^-$ as the precursor ion, compound **24** exhibited ions derived from the linolenic acid moiety, identical to **23**, while compound **26** showed ions derived from palmitic acid, identical to **27**. Therefore, compound **24** was identified as 2-linoleoyl-sn-glycero-3-phosphoethanolamin [25], and **26** was identified as 2-palmitoyl-3-glycerylphosphorylethanolamine [26].

3.1.6. Glycolipids

Compounds **11**, **12**, and **14**, based on their molecular formulas and chromatographic behaviors reported in the literature, were identified as (*E*)-2-hexenyl-α-L-arabinopyranosyl-(1→2)-β-D-glucopyranoside (**11**), (*E*)-2-hexenyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**12**), and creoside IV (**14**), which have been reported in *C. lanceolata* [9,27]. In the MS/MS using $[M+NH_4]^+$ as the precursor ion, these compounds exhibited product ions derived from the arabinopyranosyl glucopyranoside moiety as well as from the hexenyl or hexyl parts. Compound **8**, also reported in *C. lanceolata*, is (*E*)-2-hexenyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**8**) [27], and in the MS/MS with $[M+NH_4]^+$ as the precursor ion, ions from the hexenyl moiety and glucopyranosyl glucopyranoside moiety were observed. On the other hand, compound **13**, which exhibited product ions from the hexyl moiety, is inferred to be hexyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**13**), which has been reported in *C. tangshen* [28].

3.2. Evaluation of Vasodilatory Effect

The vascular dilator effects of the EtOAc fraction, the *n*-BuOH fraction, lancemaside A (**22**), and lobetyolin (**18**) were evaluated at a final concentration of 1×10^{-5} g/mL using rat aortic ring specimens (Figure 5). Lanceocide A (**22**) showed a significant time-dependent vasodilatory effect. It has been reported that **22** can activate nitric oxide (NO) synthase [29], suggesting that its vasodilatory action was likely due to an increase in endothelial-derived NO.

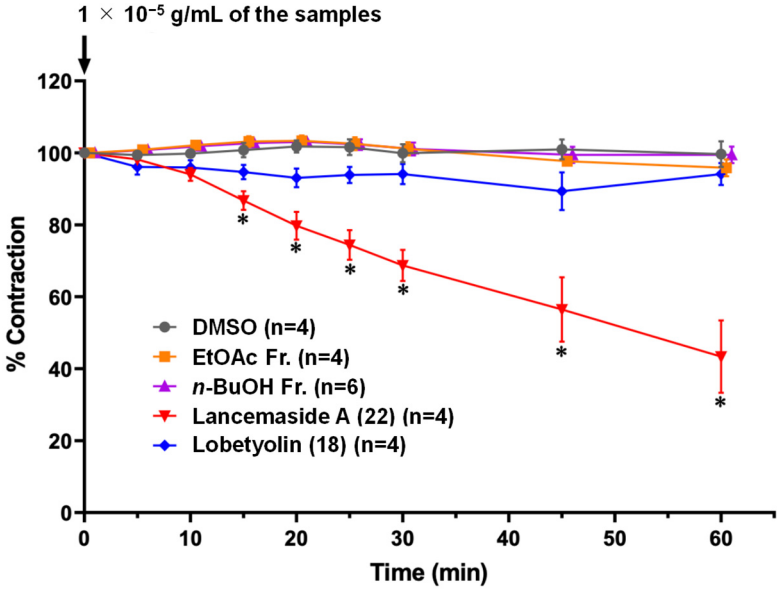

Figure 5. The vasodilatory effect of each sample on the aortic ring preparation. This figure shows the changes in contraction of the aortic ring preparation precontracted by phenylephrine after treatment with EtOAc Fr., BuOH Fr., lancemaside A (**22**), lobetyolin (**18**), and the vehicle: DMSO. * $p < 0.05$ vs. DMSO.

3.3. Compound Distribution in Different Parts of C. lanceolata

This study identified 27 compounds contained in the roots of *C. lanceolata*, while the compounds in the aerial parts of this plant have also been reported in a previous study [30]. From the aerial parts, one alkaloid, one amino acid derivative, ten flavonoids, one phenylpropanoid derivative, and one polyacetylene have been isolated. Among these, the phenylpropanoid and polyacetylene, identified as tangshenoside I (**9**) and lobetyolin (**15**), respectively, were common to both the roots and aerial parts. On the other hand, while only two flavonoids were detected in the roots, various flavonoid compounds were isolated from the aerial parts. However, triterpenoid saponins, which were the major compounds of the roots, were not isolated from the aerial parts. The abundance of triterpenoid saponins in the roots may contribute to the medicinal effects of the roots used in traditional medicines.

4. Conclusions

This study employed ultra-high-performance liquid chromatography coupled with quadrupole exactive–orbitrap mass spectrometry to conduct a comprehensive chemical analysis of the *C. lanceolata* cultivated in Nagano prefecture, Japan. LC-MS analysis was conducted using nine compounds, including four isolated ones, as reference standards. Based on chromatographic behavior and MS/MS fragmentation patterns, a total of twenty-seven compounds were identified, including four triterpenoid saponins, three polyacetylenes, two flavonoids, five phenylpropanoids and their derivatives, one lignan, five glycolipids, five phospholipids, and two amino acids. Among these, compound **19** is a previously unreported compound, and a phenylpropanoid (**3**), a polyacetylene (**6**), a lignan (**10**), a flavonoid (**17**), two glycolipids (**8** and **13**) and phospholipids (**23**–**27**) have not been previously reported in *C. lanceolata* roots. The ex vivo study revealed that lancemaside A (**22**) exhibits a time-dependent vasodilatory effect. These discoveries not only enhance our understanding of the chemical constituents and biological activities of *C. lanceolata* but also offer valuable insights into its future pharmacological applications and quality evaluations.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/chemistry7010004/s1: Tables S1–S4: NMR data of isolated com-

pounds; Figures S1–S106: LC-MS analysis of compounds. Figures S107–S114: ^{1}H and ^{13}C NMR spectra of isolated compounds.

Author Contributions: Conceived and designed the experiments, K.I., A.S. and W.L.; performed the experiments, C.L., Z.D., T.K., K.O., M.Z. and R.K.; writing—original draft preparation, C.L.; writing—review and editing, T.K., A.S. and W.L. All authors have read and agreed to the published version of the manuscript.

Funding: The investigation was supported by the Japan Society for the Promotion of Science KAKENHI 24K09867 (W. Li).

Data Availability Statement: The data presented in this study and the samples of the compounds are available upon request from the corresponding author.

Acknowledgments: The authors would like to express their gratitude to Wataru Hama (Azumino Choseikai Co., Ltd.) for kindly providing the plant materials of *Codonopsis lanceolata* roots.

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

References

1. Dong, J.; Na, Y.; Hou, A.; Zhang, S.; Yu, H.; Zheng, S.; Lan, W.; Yang, L. A review of the botany, ethnopharmacology, phytochemistry, analysis method and quality control, processing methods, pharmacological effects, pharmacokinetics and toxicity of Codonopsis radix. *Front. Pharmacol.* **2023**, *14*, 1162036. [CrossRef] [PubMed]
2. Dar, A.A.; Abrol, V.; Singh, N.; Gashash, E.A.; Dar, S.A. Recent bioanalytical methods for the isolation of bioactive natural products from genus *Codonopsis*. *Phytochem. Anal.* **2023**, *34*, 491–506. [CrossRef] [PubMed]
3. Liang, W.; Sun, J.; Bai, G.; Qiu, D.; Li, Q.; Dong, P.; Chen, Y.; Guo, F. Codonopsis radix: A review of resource utilisation, postharvest processing, quality assessment, and its polysaccharide composition. *Front. Pharmacol.* **2024**, *15*, 1366556. [CrossRef] [PubMed]
4. Chu, R.; Zhou, Y.; Ye, C.; Pan, R.; Tan, X. Advancements in the investigation of chemical components and pharmacological properties of *Codonopsis*: A review. *Medicine* **2024**, *103*, e38632. [CrossRef]
5. Jang, G.Y.; Lee, Y.R.; Song, E.M.; Jeong, H.S. Physicochemical characteristics and antioxidant activities of Deoduck (*Codonopsis lanceolata*). *Korean J. Food Nutr.* **2018**, *31*, 258–263.
6. Shin, Y.K.; Han, A.Y.; Hsieh, Y.S.; Kwon, S.; Kim, J.; Lee, K.-W.; Seol, G.H. Lancemaside A from *Codonopsis lanceolata* prevents hypertension by inhibiting NADPH oxidase 2-mediated MAPK signalling and improving NO bioavailability in rats. *J. Pharm. Pharmacol.* **2019**, *71*, 1458–1468. [CrossRef]
7. Zhang, Y.; Wang, H.; Zhang, L.; Yuan, Y.; Yu, D. *Codonopsis lanceolata* polysaccharide CLPS alleviates high fat/high sucrose diet-induced insulin resistance via anti-oxidative stress. *Int. J. Biol. Macromol.* **2020**, *145*, 944–949. [CrossRef]
8. Wang, M.-C.; Wu, Y.-F.; Yu, W.-Y.; Yu, B.; Ying, H.-Z. Polyacetylenes from *Codonopsis lanceolata* root induced apoptosis of human lung adenocarcinoma cells and improved lung dysbiosis. *BioMed Res. Int.* **2022**, *2022*, 7713355. [CrossRef]
9. Xia, Y.; Liu, F.; Feng, F.; Liu, W. Characterization, quantitation and similarity evaluation of *Codonopsis lanceolata* from different regions in China by HPLC-Q-TQF-MS and chemometrics. *J. Food Compos. Anal.* **2017**, *62*, 134–142. [CrossRef]
10. Stavrianidi, A. A classification of liquid chromatography mass spectrometry techniques for evaluation of chemical composition and quality control of traditional medicines. *J. Chromatogr. A* **2020**, *1609*, 460501. [CrossRef]
11. Ma, B.; Yang, S.; Li, J.; Ouyang, H.; He, M.; Feng, Y.; Tan, T. A four-step filtering strategy based on ultra-high-performance liquid chromatography coupled to quadrupole-time-of-flight tandem mass spectrometry for comprehensive profiling the major chemical constituents of Akebiae fructus. *Rapid Commun. Mass Spectrom.* **2019**, *33*, 1464–1474. [CrossRef] [PubMed]
12. He, J.-Y.; Ma, N.; Zhu, S.; Komatsu, K.; Li, Z.-Y.; Fu, W.-M. The genus *Codonopsis* (Campanulaceae): A review of phytochemistry, bioactivity and quality control. *J. Nat. Med.* **2015**, *69*, 1–21. [CrossRef] [PubMed]
13. Hossen, M.J.; Kim, M.-Y.; Kim, J.-H.; Cho, J.Y. *Codonopsis lanceolata*: A review of its therapeutic potentials. *Phytother. Res.* **2016**, *30*, 347–356. [CrossRef] [PubMed]
14. Ishimaru, K.; Yonemitsu, H.; Shimomura, K. Lobetyolin and lobetyol from hairy root culture of *Lobelia inflata*. *Phytochemistry* **1991**, *30*, 2255–2257. [CrossRef]
15. Ushijima, M.; Komoto, N.; Sugizono, Y.; Mizuno, I.; Sumihiro, M.; Ichikawa, M.; Hayama, M.; Kawahara, N.; Nakane, T.; Shirota, O.; et al. Triterpene glycosides from the roots of *Codonopsis lanceolata*. *Chem. Pharm. Bull.* **2008**, *56*, 308–314. [CrossRef]
16. Shirota, O.; Nagamatsu, K.; Sekita, S.; Komoto, N.; Kuroyanagi, M.; Ichikawa, M.; Ohta, S.; Ushijima, M. Preparative separation of the saponin lancemaside A from *Codonopsis lanceolata* by centrifugal partition chromatography. *Phytochem. Anal.* **2008**, *19*, 403–410. [CrossRef]

17. Kim, Y.-A.; Park, M.-S.; Kim, Y.H.; Han, S.-Y. Synthesis of 1-lyso-2-palmitoyl-rac-glycero-3-phosphocholine and its regioisomers and structural elucidation by NMR spectroscopy and FAB tandem mass spectrometry. *Tetrahedron* **2003**, *59*, 2921–2928. [CrossRef]
18. Sun, J.; Wang, L.; Wang, M.; Wang, Z.; Li, F. Two new polyacetylene glycosides from the roots of *Codonopsis tangshen* Oliv. *Nat. Prod. Res.* **2016**, *30*, 2338–2343. [CrossRef]
19. Nishiura, M.; Esaki, S.; Kamiya, S. Flavonoids in *Citrus* and related genera. I. Distribution of flavonoid glycosides in *Citrus* and *Poncirus*. *Agric. Biol. Chem.* **1969**, *33*, 1109–1118.
20. Niwa, M.; Iwadare, Y.; Wu, Y.-C.; Hirata, Y. Two new phenylpropanoid glycosides from *Wikstroemia sikokiana*. *Chem. Pharm. Bull.* **1988**, *36*, 1158–1161. [CrossRef]
21. Baygildieva, D.I.; Braun, A.V.; Stavrianidi, A.N.; Rodin, I.A. Determination of eleutheroside B and eleutheroside E in extracts from eleutherococcus senticosus by liquid chromatography/mass spectrometry. *J. Anal. Chem.* **2020**, *75*, 1832–1837. [CrossRef]
22. Jinan Haoyu Qingtian Pharmaceutical Technology Co., Ltd. Pharmaceutical Composition for Preventing and Treating Pharyngitis. CN Patent 106727840A, 31 May 2017.
23. Slotboom, A.J.; De Haas, G.H.; Burbach-Westerhuis, G.J.; Van Deenen, L.L.M. Hydrolysis of phosphoglycerides by purified lipase preparations. II. Preparation of unsaturated 2-monoacyl choline phosphoglycerides. *Chem. Phys. Lipids* **1970**, *4*, 30–36. [CrossRef]
24. Akesson, B.; Arner, A.; Sundler, R. Metabolism of different monoacylphospholipids in isolated hepatocytes and the intact rat. *Biochim. Biophys. Acta Lipids Lipid Metab.* **1976**, *441*, 453–464. [CrossRef] [PubMed]
25. Akama, K.; Awai, K.; Yano, Y.; Tokuyama, S.; Nakano, Y. *In vitro* and *in vivo* stability of polymerized mixed liposomes composed of 2,4-octadecadienoyl groups of phospholipids. *Polym. Adv. Technol.* **2000**, *11*, 280–287. [CrossRef]
26. Matsumoto, M. Phospholipides. III. Enzymic formation of lysophosphatidylethanolamine. *J. Biochem.* **1961**, *49*, 32–37.
27. Du, Y.E.; Lee, J.S.; Kim, H.M.; Ahn, J.-H.; Jung, I.H.; Ryu, J.H.; Choi, J.-H.; Jang, D.S. Chemical constituents of the roots of *Codonopsis lanceolata*. *Arch. Pharmacal Res.* **2018**, *41*, 1082–1091. [CrossRef]
28. Yuda, M.; Ohtani, K.; Mizutani, K.; Kasai, R.; Tanaka, O.; Jia, M.; Ling, Y.; Pu, X.; Saruwatari, Y. Neolignan glycosides from roots of *Codonopsis tangshen*. *Phytochemistry* **1990**, *29*, 1989–1993. [CrossRef]
29. Lee, Y.S.; Kim, H.; Kim, J.; Seol, G.H.; Lee, K.-W. Lancemaside A, a major triterpene saponin of *Codonopsis lanceolata* enhances regulation of nitric oxide synthesis via eNOS activation. *BMC Complement. Altern. Med.* **2019**, *19*, 110. [CrossRef]
30. Noh, Y.D.; Son, S.-R.; Jun, M.G.; Jang, D.S. Phytochemical study on the aerial parts of *Codonopsis lanceolata*. *Nat. Prod. Sci.* **2023**, *29*, 200–205. [CrossRef]