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Characterization of phytochemical profile of rhizome of artificial cultured *Polygonatum sibiricum* with multiple rhizome buds

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Abstract

Rhizome of *Polygonatum sibiricum* is both a renowned traditional Chinese remedy and a commonly consumed delicacy. Due to the escalating demand and excessive overexploitation, there has been a growing interest in the artificial cultivation of this plant in recent years. To assess the therapeutic benefits of artificially cultivated *P. sibiricum*, it is crucial to identify and classify its phytochemical components, which are the primary bioactive compounds found in its rhizome. In this study, the phytochemical profile of an artificially cultivated *P. sibiricum* rhizomes with multiple rhizome buds (ACM) was characterized by using untargeted UHPLC-Q-Orbitrap-MS based approach. In addition, two-wild-types *P. sibiricum* rhizomes, namely the wild-type with multiple rhizome buds (WTM) and the wild-type with single rhizome bud (WTS), were used for comparison. A total of 183 phytochemicals, including 20 alkaloids, 48 flavonoids, 33 phenolic acids, and 82 terpenoids, were tentatively identified. Generally, the phytochemical profile of ACM was comparable to that of WTM and WTS. In specific, most of the identified alkaloids and phenolic acids, and approximately half of the identified terpenoids, were not significantly different. Notably, several phytochemicals with potent therapeutic properties, such as epiberberine, laetanine, sinapic acid, curcumenol, were present in ACM. Additionally, artificial cultivation increased the abundance of geniposide and naringenin, which have been linked to cardioprotective effects. These findings provide valuable insights for the future utilization of artificially cultivated *P. sibiricum*.

Keywords *Polygonatum sibiricum* rhizomes, Artificial cultivation, Wild-type, Phytochemicals, UHPLC-Q-Orbitrap-MS

Introduction

Polygonatum sibiricum, a member of the Liliaceae family, is cultivated extensively in China, Korean, Japan, and various other countries. The utilization of the rhizome of *P. sibiricum* as food has a lengthy history in many countries, attributed to its considerable abundance of starch (comprising 25.6–68.46% of dry mass) and pleasant taste [1]. Moreover, the rhizome of *P. sibiricum* has served as a therapeutic agent for more than two millennia, and is recorded in the renowned pharmaceutical book "Compendium of Materia Medica" [2]. The dried rhizome of *P. sibiricum* has been administered to treat diverse diseases, such as cough, fatigue, poor appetite, dizziness, and pulmonary afflictions [1, 3]. Scientific inquiry has corroborated that the dried rhizome of *P. sibiricum* possesses multiple pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anti-hyperglycemic, and anti-cancer effects [4–9]. Due to its dual function as both a dietary substance and a medicinal agent, China's Ministry of Health has thus approved it as a "medicine food homology", further emphasizing its notable status [10].

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As descripted by Tybjerg and Vestergaard [11], the rhizome of a mature plant of *Polygonatum* genus has a terminal bud. The above-ground foliage shoot grows from the terminal bud in the growth season. After growth season, the above-ground shoot dies and leaves behind a distinct scar in rhizome. The rhizome segment stretches to form a new rhizome segment from the scar area. Some cultivars of *Polygonatum* genus will grow one or multiple branch bud(s) next to the renewal bud in the next growth season to form new rhizome segment(s). New above-ground shoot(s) may grow from the new rhizome segment(s), or it may dormant for several years. In contrast, some cultivars have only one continuous rhizome segment and only one above-ground shoot, without any branch bud. The former cultivars with multiple rhizome buds normally have larger size of rhizome segments, and thus are considered as superior cultivars and thus are more favored in market.

The widely acknowledged nutritional and pharmacological benefits of the rhizomes of *P. sibiricum* has led to the increase of plant collection, thereby depleting the wild resources. At present, its wild resources have been significantly exhausted. Hence, one of the major solutions to meet the growing market demands is the development of artificial cultivation of *P. sibiricum* with superior properties, such as with multiple rhizome buds, to obtain a stable and increased yield. Previously, we developed a novel artificial cultivation of *P. sibiricum* with multiple rhizome buds, yielding approximately double the amount of rhizomes in comparison to the wild type. However, it remains uncertain whether the chemical constituents, specifically those with pharmacological activities, are altered. The investigation of the chemical constituents of this novel cultivation is of importance for comprehending its pharmacological activities and ensuring its quality control.

Several studies have indicated that the main chemical constituents of *Polygonatum* genus include phenolics, steroid saponins, alkaloids, lignins, amino acids, and carbohydrates [12–15]. Regarding the pharmacological properties of these compounds, it has been reported that a phenolic glycoside extracted from *P. sibiricum* possesses inhibitory effect on α-glucosidase [16]. Luo et al. reported a saponin extract of *P. sibiricum* had effects on alleviating diabetes and modulating gut microbiota [9]. Moreover, it has been summarized by Zhao and Li that phytochemicals, including steroidal saponins, flavonoids, and alkaloids, are the primary bioactive components in *Polygonatum* genus [3]. Given the potential bioactivities of phytochemical compounds like alkaloids, flavonoids, phenolic acids, and terpenoids, it is of great interest to characterize the phytochemical profile of the new cultivation of *P. sibiricum*.

In this study, we employed UHPLC-Q-Orbitrap-MS technique to analyze the metabolic profiles of rhizomes from the artificially cultivated *P. sibiricum*, as well as from two wild types of *P. sibiricum*, namely a wild type cultivar with a single rhizome bud and another with multiple rhizome buds, for the purpose of comparison. We aimed to identify the common and unique phytochemical compounds among these three cultivars. The findings of this study can offer valuable insights for the quality assurance of the artificial cultivation of *P. sibiricum*.

Materials and methods

Plant materials and chemicals

This study involved the analysis of rhizome segments obtained from three different types of *P. sibiricum* plants, namely, the artificial cultivation with multiple rhizome buds (ACM), the wild-type with single rhizome bud (WTS), and the wild-type with multiple rhizome buds (WTM). Five samples derived from five individual plants were collected for each type of *P. sibiricum*. The fertilization practices for ACM involve the application of ternary compound fertilizer ($N:P_2O_5:K_2SO_4 = 15:15:15$) at a rate of 37.5–45.0 g/m^2. This is done biannually, combined with weed control measures. The WTS and WTM plants were grown in the wild field in Shaowu city (Fujian, China), located at longitude 117°30′16.23″ and latitude 27°12′57.62″, with an average elevation of 514.2 m. The *P. sibiricum* plants grow on the southeast slope of the mountain, amidst a mixed forest of moso bamboo and evergreen broad-leaved trees. Annual precipitation on the slope is 1940 mm, with evaporation rates as follows: spring (March to May) 151.8 mm, summer (June to August) 327.3 mm, autumn (September to November) 231.0 mm, and winter (December to February) 93.4 mm. The average relative humidity is 82%, with an annual sunshine duration of 2187 h and an average annual temperature of 19.8 °C. The photos of these three plant materials were shown in Fig. 1. The basic information of the three plants, including the number of rhizome buds, yield, polysaccharide content, etc., were summarized in supplementary materials Additional file 1: Table S1.

For the chromatogram analysis, UHPLC-grade organic solvents obtained from CNW Technologies, Inc. (Düsseldorf, Germany) were used. The internal standard 2-chlorophenylalanine was purchased from HC Biotech (Shanghai, China). Millipore Alpha-Q water purification system (Millipore, Billerica, MA, USA) was utilized to generate ultrapure water.

Sample extraction

The ACM, WTS, and WTM samples were subjected to freeze-drying and pulverization into powder form. The obtained powder (1 g) was then extracted with 5 mL of

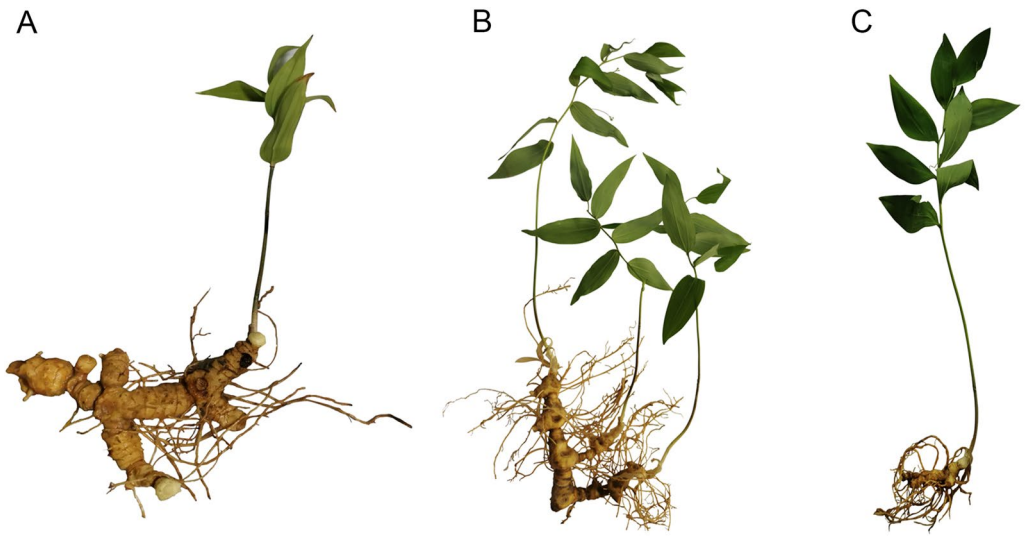

Fig. 1 The photos of the rhizomes of *P. sibiricum* from the artificial cultivation with multiple rhizome buds (**A**), the wild-type with multiple rhizome buds (**B**), and the wild-type with single rhizome bud (**B**)

25% methanol/water solution containing 1 μg/mL of internal standard of 2-chlorophenylalanine in an ultrasonic ice-water bath for 60 min. Following extraction, the resulting solution was filtered through a 0.22-μm filter and centrifuged at 13,800 × *g* for 15 min at 4 °C. A 300 μL supernatant aliquot was taken for LC–MS analysis. In addition, a quality control (QC) sample was prepared by mixing an equal volume of the supernatants from all samples.

Ultra-high performance liquid chromatography analysis

For the chromatographic analysis of the extracts of ACM, WTS, and WTM samples, an Agilent ultra-high-performance liquid 1290 UPLC system (Agilent, Santa Clara, USA) was utilized. This system consisted of a binary pump, split loop autosampler, column compartment and diode array detector (with a range 190–680 nm). Chromatographic separation was achieved using an Acquity UHPLC BEH C18 column (150 mm × 2.1 mm, 1.7 μm; Waters, Milford, MA). The mobile phases were 0.1% (v/v) formic acid in water (solvent A) and 0.1% (v/v) formic acid in acetonitrile (solvent B), with a flow rate of 0.4 mL/min. The gradient program was set as follows: solvent A from 95 to 85% (3.5 min), to 70% (6 min), to 30% (12 min), to 0% (18 min), maintained for 7 min, to 95% (26 min), maintained for 4 min. The injection volume was set at 5 μL. In order to ensure the stability of the instrument, QC sample was injected once at the starting, middle, and end of the sequence, thereby yielding three data points for monitoring.

Mass spectrometry analysis

Following chromatographic separation, high-resolution mass spectrometry data were recorded on an Q Exactive Focus Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with heated-ESI-II (HESI-II) ionization source, operating in both positive and negative electrospray ionization (ESI) modes. The source parameters were set as follows: positive and negative spray voltages of 4.0 kV and 3.6 kV, respectively; a nitrogen sheath gas flow rate of 45 arbitrary units (AU); a nitrogen auxiliary gas flow rate of 15 AU; and a capillary temperature of 400 °C. The acquisition scan range was 100–1500 m/z, with a full MS scan resolution of 70,000 full width at half maximum (FWHM) and a data-dependent MS/MS scan resolution of 17,500 FWHM. An external calibration for mass accuracy was performed before the analysis, following the manufacturer's guidelines. Data acquisition and processing were carried out by Xcalibur 4.0 software (Thermo Fisher Scientific, Bremen, Germany). Elemental composition prediction of the detected components was based on the following settings: elements in use, C0–80, H0–130, O 0–60; mass tolerance, < 5 ppm; and ring double-bond equivalent (RDBeq) ≥ 7.

Data processing

The data obtained from UHPLC-Q-Orbitrap-MS was first converted to the mzXML format using msConvert software (version 3, ProteoWizard). Following this, peak extraction, alignment, and integration across all raw data

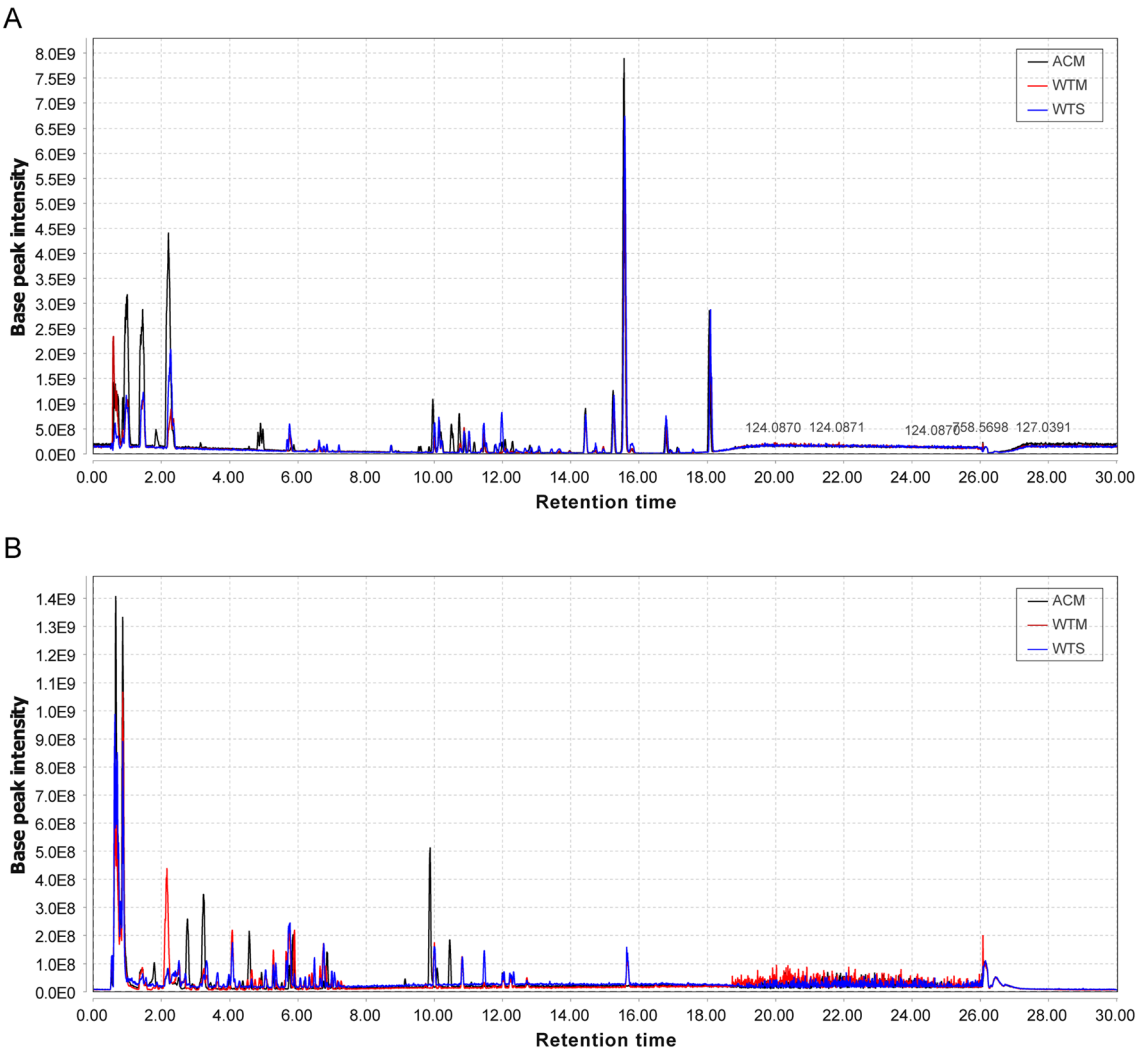

Fig. 2 The representative base peak chromatograms of the three types of *P. sibiricum* rhizomes acquired in positive (**A**) and negative (**B**) ionization modes

were executed using the XCMS package in R software (Version 3.6.1, R Core Team, New Zealand). Multivariate statistical analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were conducted using R software based on the output data from XCMS. For metabolite identification, the detected ion features were analyzed qualitatively based on the in-house metabolite database (Shanghai Biotree biotech Co., Ltd.) and public databases such as HMDB, METLIN, and M/Zcloud. Additionally, a volcano plot analysis was performed to identify the differential metabolites among the three types of plant materials.

Results and discussion

Validation of the UHPLC–Q-Orbitrap-MS method

To ensure the reproducibility and reliability of the untargeted metabolomic method employed in this study using UHPLC-Q-Orbitrap-MS, three injections of QC sample were executed. Additional file 1: Fig. S1 displays the base-peak-chromatogram (BPC) of the QC samples in positive and negative ionization modes. Additional file 1: Fig. S2 displays the extracted-ion-chromatograms (EIC) of the internal standard of 2-chlorophenylalanine in the QC sample for both positive and negative ionization

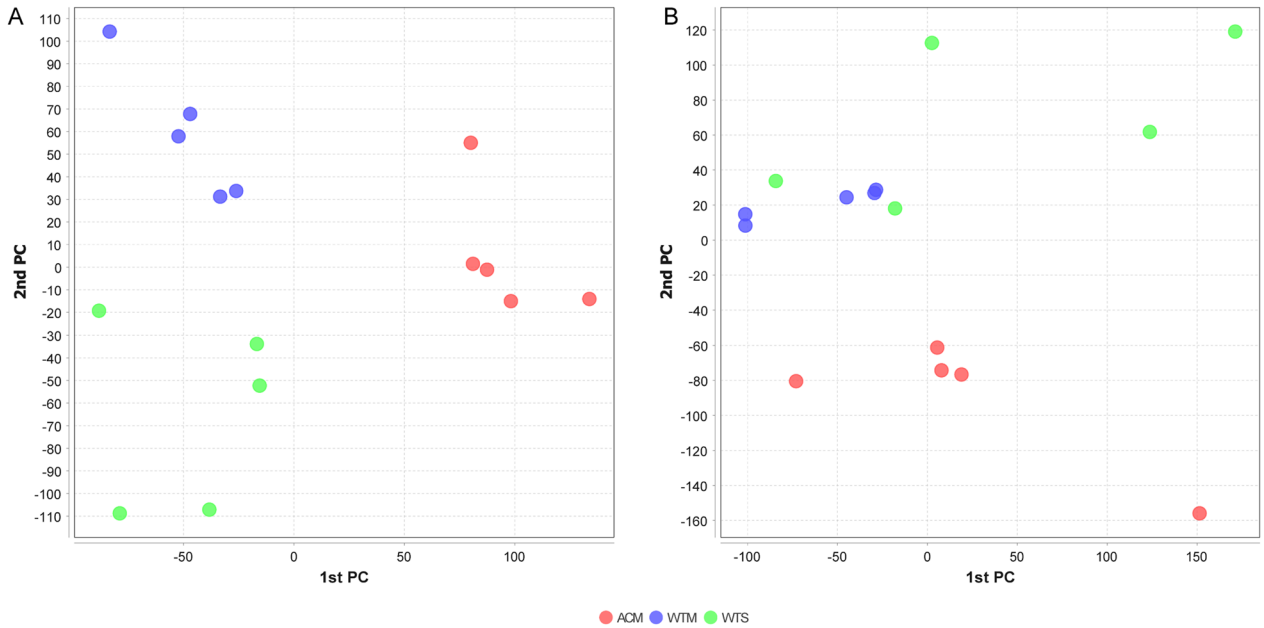

Fig. 3 PCA score plots of the metabolites acquired in positive (**A**) and negative (**B**) ionization modes

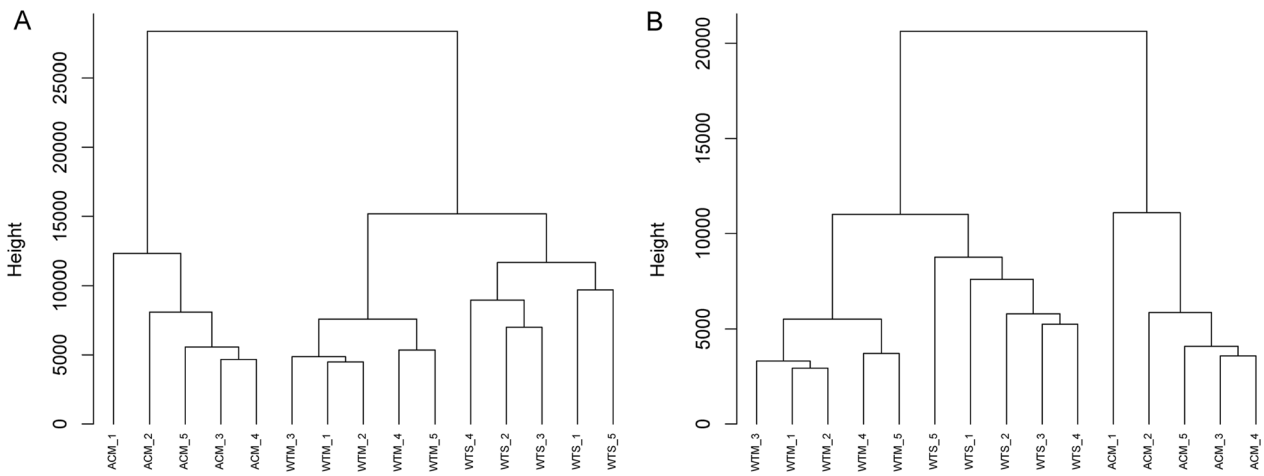

Fig. 4 HCA dendrograms of the metabolites acquired in positive (**A**) and negative (**B**) ionization modes

modes (*m/z* 200.04728 for positive ionization mode; *m/z* 198.03273 for negative ionization mode; mass tolerance < 5 ppm). The well overlapping BPCs and EICs of the internal standard indicated a satisfactory stability of the analytical method. The RSD values of peak intensities for the internal standard were computed as 8.1% for positive ionization mode and 3.4% for negative ionization mode, signifying a commendable precision and stability of the methodology.

General metabolic profiles comparison

Untargeted metabolomic approaches, involving high-resolution mass spectrometry and chemometric tools, have been utilized in the analysis of the chemical constituents of diverse plant materials [17]. In the present study, the untargeted metabolomic approach based on UHPLC-Q-Orbitrap-MS was used to comprehensively profile the chemical constituents of all the samples. Firstly, a 25% aqueous methanol solution was utilized for the extraction

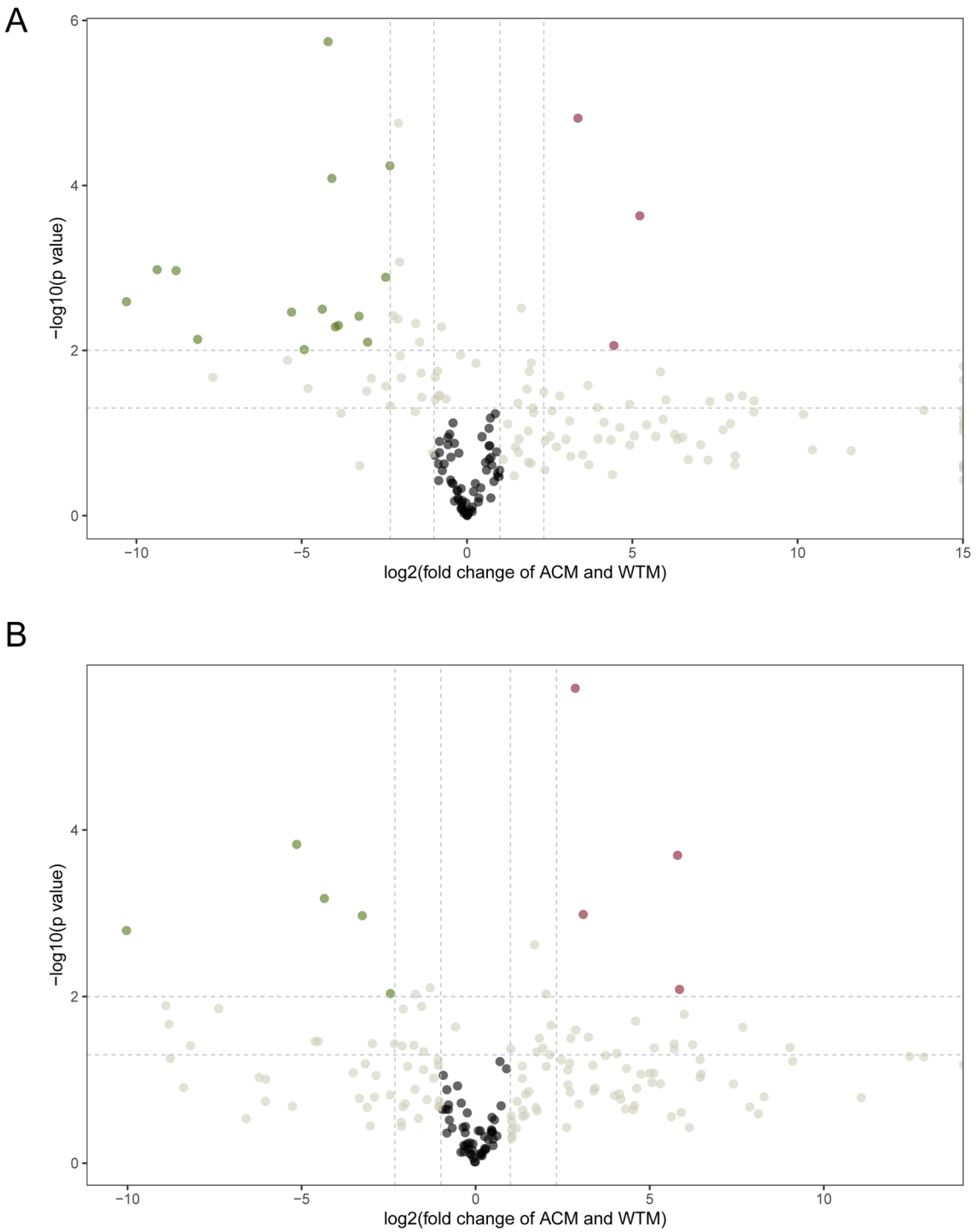

Fig. 5 Volcano plots representing the comparison of the phytochemicals between ACM and WTM (**A**), and the comparison of the phytochemicals between ACM and WTS (**B**)

of both polar and nonpolar compounds from dried rhizome samples. Subsequently, the samples were subjected to UHPLC-Q-Orbitrap-MS analysis. The representative BPCs of the three types of samples in both positive and negative ionization modes were demonstrated in Fig. 2. In general, comparable metabolic profiles among the samples were observed in both ionization modes, with most peaks overlapping but varying in intensities.

Subsequently, peak extraction, alignment, and integration were carried out, which resulted two data matrixes containing 9508 and 7173 ion features for the positive and negative modes, respectively. To compare the general metabolic profiles among the three types of samples, chemometric tools, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were employed. The ion features acquired from UHPLC-Q-Orbitrap-MS in both ionization modes were introduced to R software for PCA and HCA analyses. To visualize the distribution of the data from all samples, an unsupervised PCA was performed as the first step. The score plots from both positive and negative ionization modes, depicted in Fig. 3A, B, revealed that all samples were categorized into distinct groups that were consistent with their respective sample types. In general, the wild-type samples were clustered more closely. Next, HCA was carried out to group the samples with similar metabolic profiles. As shown in Fig. 4, the dendrograms generated from the results obtained through positive and negative ionization modes revealed a clear clustering pattern that was consistent with their respective types. Taken together, the chromatograms indicated that the metabolic profile of the artificially cultivated *P. sibiricum* with multiple rhizome buds was analogous to those of the wild-types of *P. sibiricum*, including those with multiple and single rhizome bud(s). As revealed by PCA and HCA, differences in metabolic profiles among them could be observed, suggesting variations in some metabolites existed. More details on the metabolites, particularly those belonging to phytochemicals, were presented and discussed in below.

Identification of the phytochemicals

Tentative identification of 106 and 77 phytochemical compounds was achieved in the positive and negative modes of the mass spectra, respectively. These phytochemicals were classified into four categories: alkaloids (20), flavonoids (48), phenolic acids (33), and terpenoids (82). For further details, including their compound names, composite score, molecular formula, class, accurate mass, retention time, and integrated peak areas in all samples, refer to Additional file 2: Table S2.

To date, several studies have reported the metabolic profiles of the genus *Polygonatum.* For example, Qi et al. [18] recently conducted a UPLC-QTOF-MS/MS based untargeted metabolomic method to compare the metabolites of four species from *Polygonatum* genus, including *P. sibiricum, P. cyrtonema, P. kingianum,* and *P. macropodium.* In that study, a total of 185 flavonoids, 127 lipids, 105 phenolic acids, and 36 steroids were identified. Furthermore, it was reported that flavonoids, steroids, and terpenoids were the main differentially abundant compounds among the four different seed samples. In another study, the metabolic profiles of *Polygonatum* species, including *P. sibiricum*, *P. cyrtonema*, and *P. kingianum* was compared by using UPLC-QTOF-MS/MS [19]. It was reported in this study that 25 constituents, including adenosine, citric acid, guanine, L-pipecolic acid, L-tryptophan, pyroglutamic acid, and sucrose, were identified as biomarkers for distinguishing the three *Polygonatum* species. It was reviewed by Zhao and Li [3] that steroidal saponins and flavonoids were the primary bioactive compounds in this genus [3]. Due to the potential bioactivities of the phytochemical compounds, the present study mainly focused on the variations in phytochemical profiles in the three studied cultivars of *P. sibiricum*.

Comparison of the phytochemicals

In order to comprehensively compare the phytochemicals among the three sample types, Student's *t*-test analyses were performed to compare ACM with WTM, and ACM with WTS, based on the peak areas of each identified phytochemicals. Furthermore, fold changes were calculated for ACM vs WTM, and ACM vs WTS. The results of Student's t-test analyses and fold changes were visualized as volcano plots in Fig. 5. In the volcano plot, the vertical axis represents the negative logarithm of the p-value (base 10) derived from the t-test analyses, and the horizontal axis represents the logarithm of the fold change (base 2) between artificial cultivation and wild-type samples. As shown in Fig. 5, each point on the plot represents an individual phytochemical detected in UHPLC-Q-Orbitrap-MS. Phytochemical compounds with a fold change ≥ 5.0 or ≤ 0.2 and p-value < 0.01 were regarded as differentially altered compounds, and marked in red (significantly higher in artificial cultivation, fold change ≥ 5.0 and p-value < 0.01) or green (significantly higher in wild-type samples, fold change ≤ 0.2 and p-value < 0.01). Phytochemical compounds with a fold change ≥ 0.5 and ≤ 2, and p-value > 0.05 were regarded not significantly changed compounds, and marked in black. Figure 5A displays the results of the comparison of the phytochemicals between ACM and WTM, while Fig. 5B displays the results of the comparison between ACM and WTS.

As shown in Fig. 5A, 62 compounds were found to have no differential detection in ACM and WTM samples. These compounds included 14 alkaloids, five flavonoids, 12 phenolic acids, and 31 terpenoids. It was also noticed that 18 phytochemicals were significantly altered in artificial cultivation compared to wild-type with multiple rhizome buds. In the comparison between ACM and WTS, 52 compounds were found to be at similar levels in these two types of samples (Fig. 5B). These compounds included 10 alkaloids, one flavonoid, 14 phenolic acids, and 27 terpenoids. As for the differential compounds,

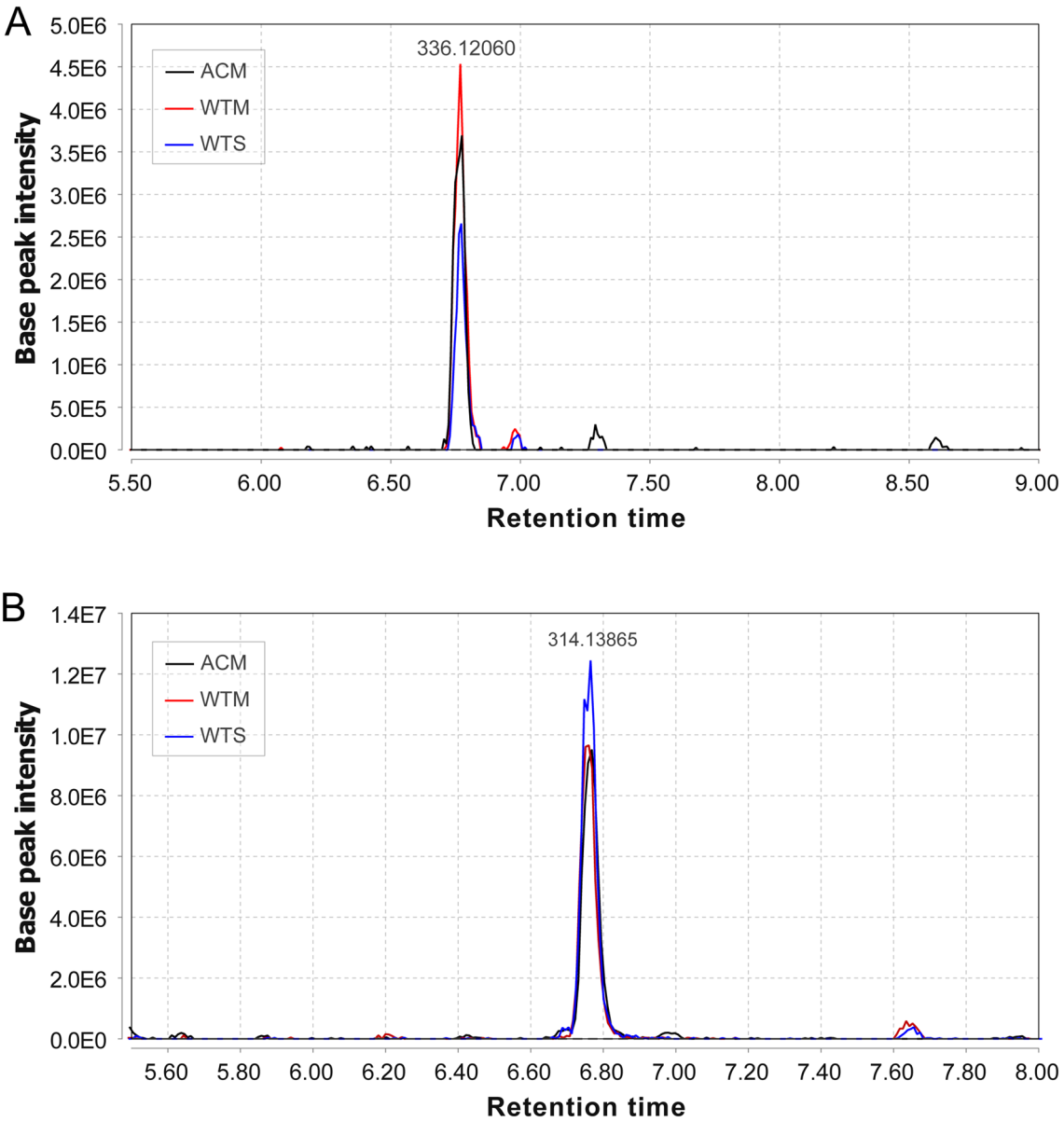

Fig. 6 The extract ion chromatogram of epiberberine at m/z 336.12060 in positive ionization mode (**A**), and laetanine at m/z 314.13865 in positive ionization mode (**B**)

five compounds were found to be significantly lower in abundance in ACM, and four compounds were significantly higher in abundance in WTS. Details of these compounds are listed in Additional file 2: Table S2.

In the following sessions, we mainly focus on the phytochemical compounds that were in similar abundance between artificial cultivation and wild-types, as well as the distinguishingly different phytochemicals. The comparison of several specific compounds is presented according to their chemical classes.

Alkaloids

Alkaloids are a widespread group of bioactive plant phytochemicals that contain at least one nitrogen atom. Due to their enormous bioactivities, various plant alkaloids, such as berberine, harmaline, taxol, quinidine, efedrina, have been used to counteract various diseases [20, 21]. In this study, 20 alkaloids were identified. Of these, 14 alkaloids were found to be at similar level in both the artificial cultivation and the wild-type with multiple rhizome buds. And 10 alkaloids were found to be at the similar level in the artificial cultivation and wild-type

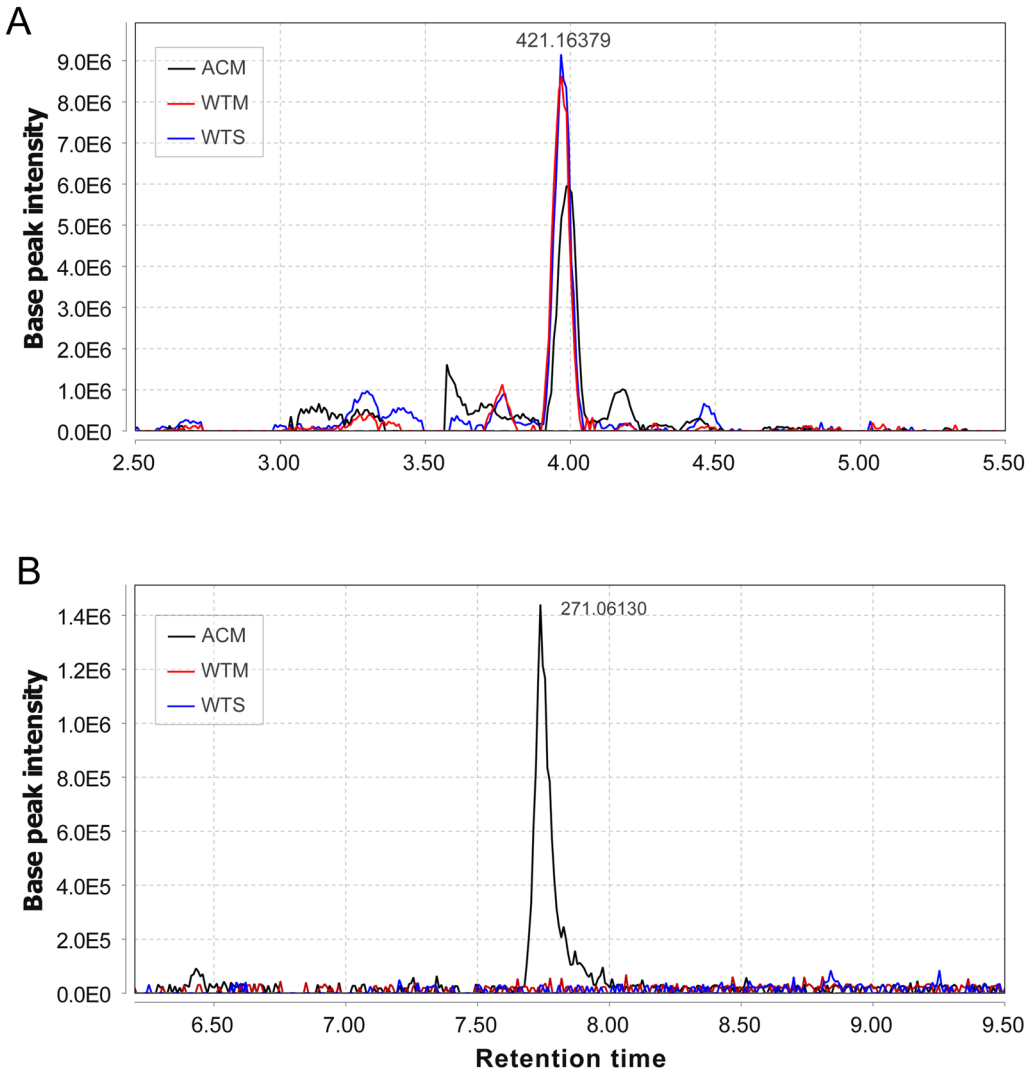

Fig. 7 The extract ion chromatogram of mulberrin at m/z 421.16378 in negative ionization mode (**A**), and naringenin at m/z 271.06130 in negative ionization mode (**B**)

with single rhizome bud. Of these alkaloids, nine compounds, namely 3-formylindole, biotin, DL-coniine, histamine, nicotinamide, laetanine, 1-methyl-2-[(6Z)-6-undecen-1-yl]-4(1H)-quinolinone, epiberberine, and choline, were the overlapping alkaloids, suggesting that these compounds were present at similar level in all samples. Several pharmacological studies have demonstrated the physiological functions of these compounds. For example, epiberberine, a multi-target small molecule with low toxicity, has been reported to possess various health benefits and therapeutic effects, such as antioxidant and anti-Alzheimer activities [22, 23]. Notably, it has been reported by Xiao et al. [24] that due to the Agt, TGFβ1, and Smad2 expression inhibitory effect of epiberberine, it could be a potential drug for the treatment of diabetic nephropathy dependent on the Agt-TGFβ/Smad2 pathway. Similarly, it has been reported that laetanine, a noraporphine alkaloid, possesses antiplasmodial effect [25]. The representative EICs of epiberberine (*m/z* 336.12060) and laetanine (*m/z* 314.13865) were shown in Fig. 6A, B, respectively. The comparable peak areas in all three sample types suggest that artificial cultivation did not significantly alter the abundance of these two compounds. Regarding the differential alkaloids, based on our criteria, including a fold change ≥ 5.0 or ≤ 0.2 and p-value < 0.01, no alkaloid was identified in both comparisons.

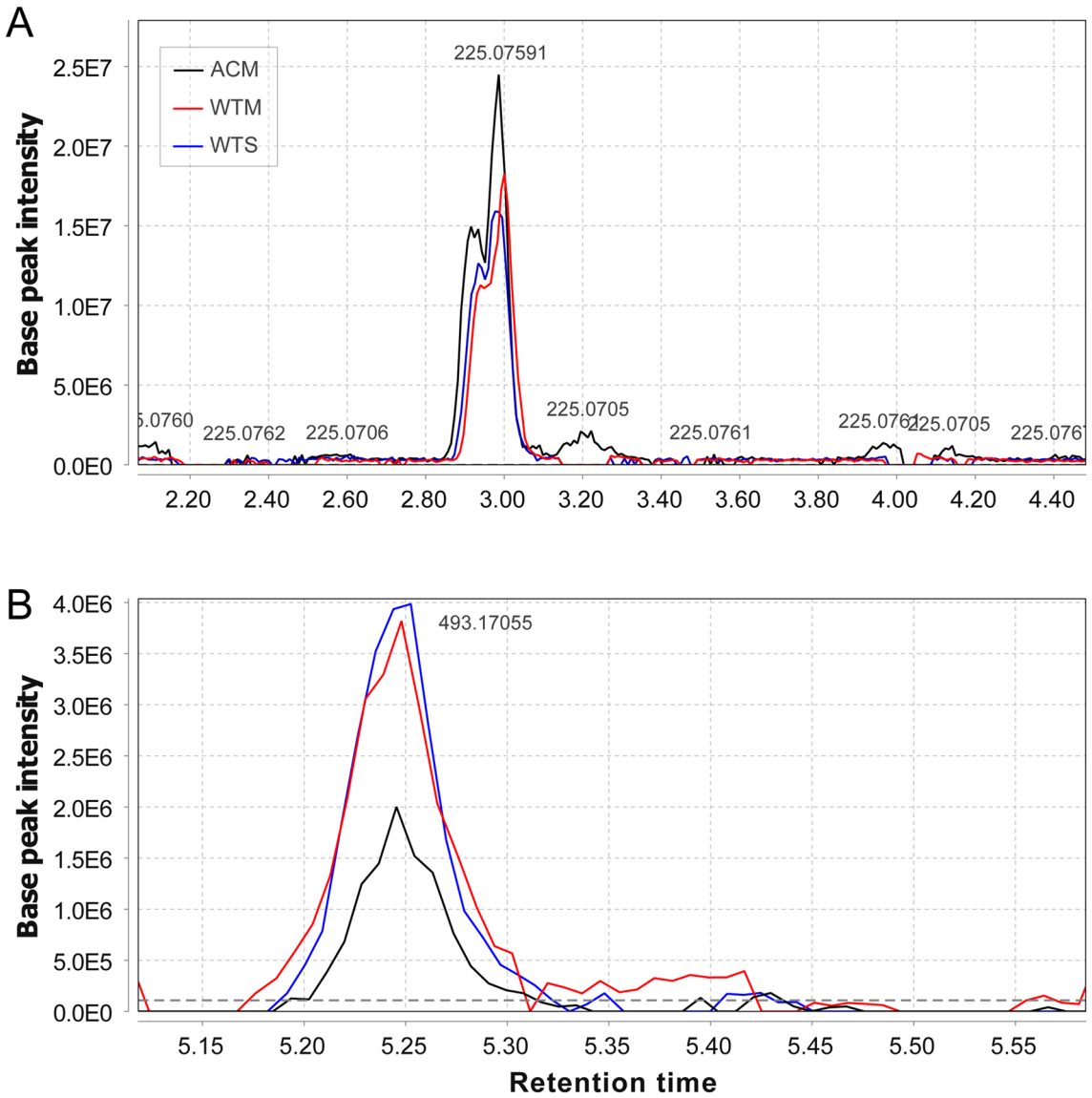

Fig. 8 The extract ion chromatogram of sinapic acid at m/z 225.07591 in positive ionization mode (**A**), and picroside I at m/z 493.17055 in positive ionization mode (**B**)

Flavonoids

Flavonoids, which are a group of secondary metabolites occurring in both forms of glycoside-bound and free aglycone, are present in various fruits, vegetables, and herbal materials. These plant metabolites are renowned for their antioxidant properties, which may aid in managing symptoms of cardiovascular disease and diabetes [26]. The *Polygonatum* genus contains a high amount of flavonoids [4]. Tao has revealed that eight species of the *Polygonatum* genus contain a total of 54 flavonoids, which belong to six subtypes [27]. In the present study, we identified 48 flavonoids from the rhizomes of artificially cultured and two wild-type *P. sibiricum*. Among the identified flavonoids, four, namely formononetine, carpachromene, kaempferol-3-O-glucoside-7-O-rhamnoside, mulberrin, were found to have similar abundance between ACM and WTM ($p > 0.05$), whereas only mulberrin and formononetine were found to have similar abundance between ACM and WTS. The representative EICs of mulberrin (*m/z* 421.16378) were shown in Fig. 7A. Recently, it was reported by Ge et al. that mulberrin exerted hepatic fibrosis amelioration effect via suppressing the TGF-β1/SMAD2/3 signaling pathway [28]. The relatively high abundance of mulberrin in all three types of samples suggests their therapeutic potential against liver fibrosis.

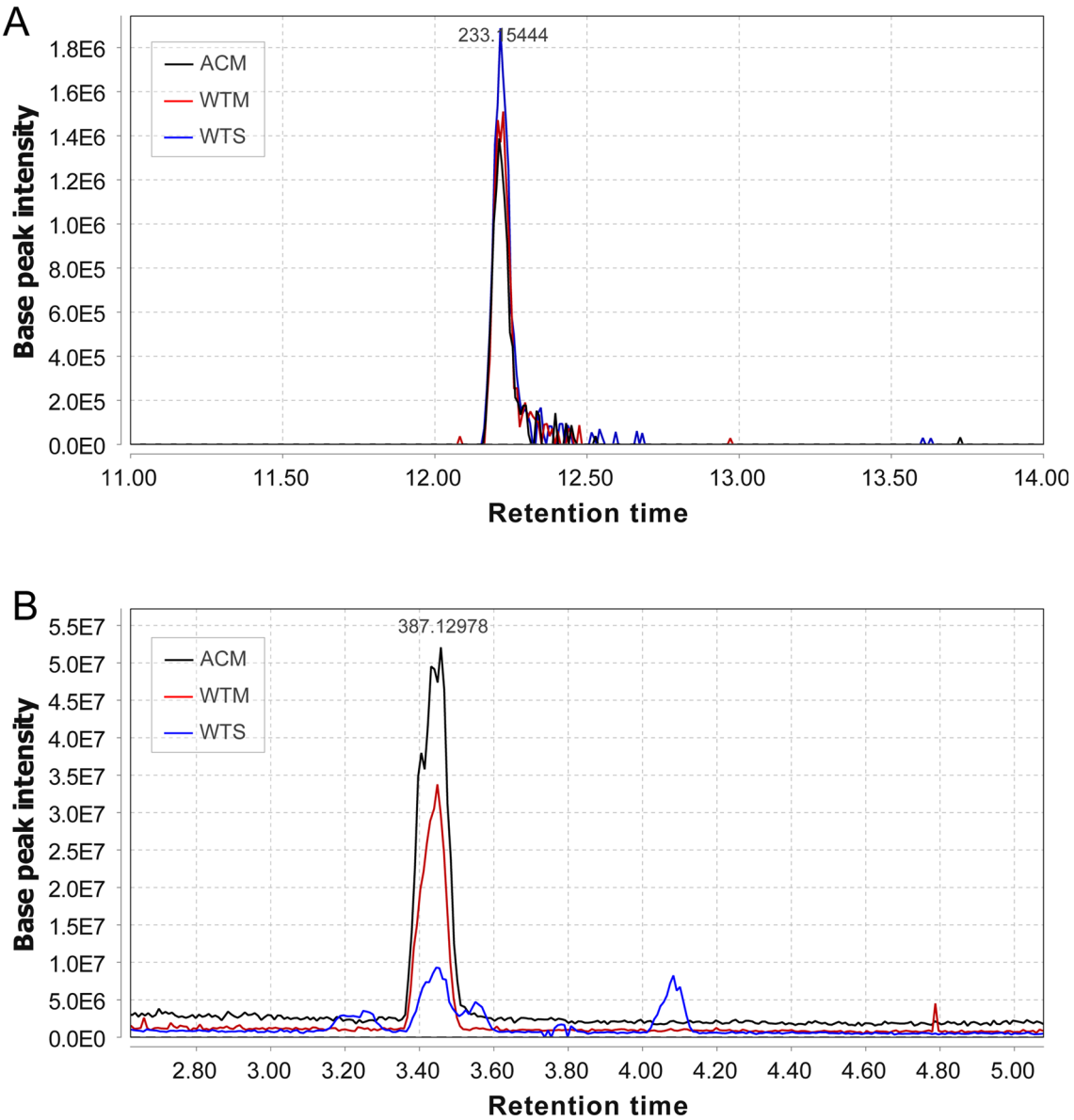

Fig. 9 The extract ion chromatogram of curcumenol at m/z 233.15444 in negative ionization mode (**A**), and geniposide at m/z 387.12978 in negative ionization mode (**B**)

Apart from the five aforementioned flavonoids, most of the detected flavonoids exhibited differing abundances in the three sample types. Taken naringenin as an example, Fig. 7B demonstrated its representative EICs. This compound is one of the most important naturally-occurring flavonoids, predominantly found in some edible fruits, like citrus and tomatoes [29]. It has also been reported that naringenin exerts various beneficial effects, such as antioxidant, antitumor, antiviral, antibacterial, anti-inflammatory, antiadipogenic and cardioprotective effects [29]. Notably, it has been observed that naringenin exerts anti-diabetic and anti-dyslipidemic effects by inhibition of gluconeogenesis through upregulations of AMPK, similar to metformin [30]. The comparison of naringenin abundance in the rhizomes of three types of *P. sibiricum* revealed that artificially cultivated plants had significantly higher levels of naringenin. Additionally, several flavonoids, such as biochanin A, kaempferol, loureirin B, were found to be more abundant in the artificially cultivated samples than in the wild-type samples. These compounds have been well documented to exhibit antidiabetic and antihyperlipidemic effects [31–33], suggesting the potential pharmacological application of the artificial cultivation.

Phenolic acids

Phenolic acids, comprising hydroxybenzoic acids and hydroxycinnamic acids, a major group of plant phenolic compounds that are widely distributed across the plant kingdom. These acids exhibit exceptional antioxidant activity and a wide range of health benefits, making them popular in the industries of therapeutics, cosmetics, and food [34]. In the present study, 33 phenolic acids were identified. Among them, 12 and 14 phenolic acids were found to be in the similar levels in the comparison of ACM against WTM, and ACM against WTS, respectively. There were 11 compounds, including rosmarinic acid, *p*-coumaroyl-beta-D-glucose, cinnamic acid, caffeic acid, dimethylcaffeic acid, sinapic acid, sinapoyl aldehyde, trans-ferulic acid, methyl vanillate, *p*-coumaroyl-glucose, ferulic acid, overlapped in the two pairs of comparisons. Taken sinapic acid as an example, as demonstrated in Fig. 8A**,** it can be seen that the level of sinapic acid remained relatively constant between the two types of samples. Sinapic acid is one of the most common hydroxycinnamic acids, and is widespread in plant kingdom [35]. This phenolic acid is known to exhibit antioxidant, anti-inflammatory, anticancer, antimutagenic, antiglycemic, neuroprotective, and antibacterial activities [35]. It was reported that sinapic acid could ameliorate the progression of diabetic nephropathy via NRF2/HO-1 mediated pathways [36]. Notably, we observed a significant reduction in the abundance of picroside I in the ACM samples compared to the wild-type samples, as evidenced by a significantly lower peak area (Fig. 8B).

Terpenoids

Terpenoids are a structurally diverse group of plant secondary metabolites, contributing largely to the aroma and taste of various plants [37]. In addition, numerous terpenoids exhibit significant pharmacological bioactivity, including anti-oxidant, anti-inflammatory, anti-cancer, and anti-ulcer properties [38]. Thus, characterizing the terpenoid profiles of herbal materials like *P. sibiricum* is crucial in understanding their potential pharmacological applications. Across all samples, a total of 82 terpenoids were identified in the rhizomes of *P. sibiricum*, representing the largest phytochemical class. The comparison of terpenoids abundance in the rhizomes of artificial cultivation and two wild-types of *P. sibiricum* revealed that 39 terpenoids were found to have similar levels (fold change ≥ 0.5 and ≤ 2, and p-value > 0.05), while 13 terpenoids were found to be significantly different (fold change ≥ 5.0 or ≤ 0.2 and p-value < 0.01).

Among the 39 terpenoids with similar abundance across all samples, 19 compounds were identified as overlapping terpenoids. For example, the peak areas of curcumenol exhibited similarity in the three representative chromatograms (Fig. 9A). Curcumenol, a sesquiterpene widely existing in edible plants, is renowned for its diverse health and medicinal benefits, including neuroprotection, anti-inflammatory, anti-tumor, and hepatoprotective activities [39]. On the contrary, ACM had higher levels of geniposide compared to WTM and WTS, as shown in Fig. 9B. Geniposide is an iridoid monoterpene that contains a glycosyl moiety attached to its iridoid skeleton, and has been detected in over 40 plant species, many of which are used in herbal medicine [40]. Geniposide has been experimentally proved to possess therapeutic effects in diabetes mellitus [41, 42]. In addition to the anti-diabetic effect, Zhou et al. [43] have systematically reviewed the various medicinal benefits of geniposide. These include antioxidant, anti-inflammatory, immune-regulatory, cardioprotective, anti-diabetic effects. In the present study, the rhizomes of artificially cultivated *P. sibiricum* displayed significantly higher abundance of geniposide than the wild-types, indicating the potential pharmacological applications of artificially cultivated *P. sibiricum*.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00792-4.

Additional file 1. The basic information of the artificially cultured and wild-type *P. sibiricum* samples were listed in **Table S1**. The BPCs of QC samples were shown in **Figure S1**. The EICs of 2-chlorophenylalanine in QC samples were shown in **Figure S2**.

Additional file 2. The tentatively identified phytochemical compounds were listed in **Table S2**.

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Not applicable.

Author contributions

WC: writing—original draft, performed experiments and sorted the data. WC, ZP, HZ and ZL: conceptualization, analyzed the validation data. WC: data curation. WC, ZP, GL, and SX: performed experiments and analyzed the data. ZL and JL: writing—review and editing, conceptualization, supervision. All authors read and approved the final manuscript.

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Availability of data and materials

Data will be provided upon request.

Declarations

Competing interests

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.

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