Quality analysis of Polygala tenuifolia root by ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry

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Abstract

Polygala tenuifolia root is usually used for functional food due to attractive health benefits. In this work, ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) were utilised to characterise the bioactive compounds in P. tenuifolia root. The UPLC-MS/MS information revealed 36 bioactive compounds, including oligosaccharide esters, polygalasaponins and polygalaxanthones. GC-MS identified 34 volatile compounds with fatty acids as the main chemicals. The leading compound judged by UPLC-MS/MS was tenuifoliside A, and oleic acid was the leading volatile from GC-MS profiles. All the samples showed similar bioactive compounds compositions, but the level of each compound varied. The results of principal component analysis revealed the principal bioactive compounds with significant level variations between samples. These principal chemicals could be used for quality judgement of P. tenuifolia root, instead of measuring the levels of all compositional compounds.

Full Text

Preamble

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Abstract

Polygala tenuifolia root is commonly used in functional foods due to its attractive health benefits. In this work, ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) were utilized to characterize the bioactive compounds in P. tenuifolia root. The UPLC-MS/MS analysis revealed 36 bioactive compounds, including oligosaccharide esters, polygalasaponins, and polygalaxanthones. GC-MS identified 34 volatile compounds with fatty acids as the predominant constituents. The leading compound identified by UPLC-MS/MS was tenuifoliside A, while oleic acid was the principal volatile component in the GC-MS profiles. All samples exhibited similar bioactive compound compositions, though the level of each compound varied. Principal component analysis revealed the principal bioactive compounds with significant level variations between samples. These key chemicals could be used for quality assessment of P. tenuifolia root, eliminating the need to measure all compositional compounds.

Keywords: Bioactive compound; GC-MS; Polygala tenuifolia; UPLC-MS/MS

1. Introduction

The genus Polygala includes herbaceous plants, shrubs, and small trees distributed worldwide. Some Polygala species are used in functional foods and folk medicines for treating inflammation and central nervous system disorders [**?**]. As a widely used medicinal plant, Polygala tenuifolia has attracted considerable attention due to its promising pharmaceutical properties against insomnia, neurosis, and dementia [**?**]. Moreover, P. tenuifolia root can attenuate cognitive dysfunction and relieve depressive disorders [**?**, **?**]. As these conditions are common in modern society, the preventive effects of P. tenuifolia root have generated significant interest among scientists in the functional food field, leading to the development of many functional food products formulated with this botanical.

Natural bioactive compounds, such as phenolics and carbohydrates, are responsible for the pharmaceutical activities of medicinal herbs [**?**, **?**]. It has been reported that oligosaccharides, saponins, and xanthones in P. tenuifolia root are critical chemicals involved in disease treatment and health promotion [**?**]. The antidepressant effect is mainly attributed to oligosaccharide esters, particularly 3,6' -disinapolysucrose. Polygalasaponins possess strong activity against cognitive impairments [**?**], while xanthones exhibit diverse biological profiles, including anticancer, antihypertensive, and antioxidant activities [**?**]. Clearly, the bioactive compound composition determines the quality of P. tenuifolia root. Although more than two hundred chemicals have been identified from the Poly-

gala genus, the chemical composition of P. tenuifolia root remains incompletely characterized. Determination of the phytochemical profile by ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) represents an excellent approach to understanding the chemical nature of this medicinal plant.

Metabolomics is increasingly utilized to gain insight into the chemical composition of biological materials [**?**]. Two types of analytical techniques based on MS and nuclear magnetic resonance spectroscopy (NMR) are widely applied in metabolomics, with MS demonstrating superior precision and resolution compared to NMR. Therefore, in this work, the bioactive compound profile of P. tenuifolia root was analyzed by both UPLC-MS/MS and GC-MS to evaluate the quality of commercial P. tenuifolia root.

2.1. Plant material

Four P. tenuifolia root samples were collected from Sanmenxia in Henan province (samples 1 and 2) and Yuncheng in Shanxi province (samples 3 and 4). Each sample was pulverized into powder and passed through a 60-mesh sieve.

2.2. UPLC-MS/MS analysis

Two grams of P. tenuifolia root powder were extracted with 20 ml of methanol for one week in the dark at room temperature. The extract was centrifuged at 8000g for 10 min. Each sample was extracted in triplicate. The supernatant was collected and subjected to UPLC-MS/MS analysis. An AcquityTM UPLC system equipped with an AcquityTM UPLC BEH C18 column (1.7 m, 2.1×150 mm) was used for chemical separation. A gradient elution program was conducted as follows: 0-45 min, from 5% to 50% solvent B; 45-48 min, from 50% to 100% solvent B; 48-51 min, from 100% to 5% solvent B; 51-53 min, 5% solvent B. The flow rate was 0.25 ml/min. Solvent A was 0.1% formic acid in water, and solvent B was acetonitrile. Detection was performed on a triple quadrupole mass spectrometer using an electrospray ionization (ESI) interface. Ionization of the analytes was achieved using ESI in negative mode. The interface conditions were as follows: capillary voltage, 1.0 KV; cone voltage, 10 eV; collision voltage, 10 eV; source temperature, 150 ºC; desolvation temperature, 500 ºC; cone gas, nitrogen at a flow rate of 50 L/h; desolvation gas, nitrogen at a flow rate of 800 L/h; collision gas, argon at a flow rate of 0.2 mL/min. Mass scan was set in the range of m/z 50-2000. The daughter ion mode was monitored at a collision voltage of 10-40 eV.

2.3. GC-MS analysis

Two grams of P. tenuifolia root powder were extracted with 20 ml of hexane/acetone (7:3, v/v) for one week in the dark at room temperature. The extracts were centrifuged at 8000g for 15 min. The supernatants were collected and subjected to trimethylsilyl derivatization due to the presence of fatty acids.

The trimethylsilyl derivatization was conducted according to the method of Yang et al. [?]. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyze the chemical composition. The derivatives were loaded onto an RTX-5 capillary column. The temperature program was set as follows: initial column temperature 50 ºC, held for 1 min, increased to 250 ºC at 3 ºC/min, held for 17 min, then increased to 280 ºC at 10 ºC/min; injection temperature: 250 ºC. The ion source of the mass spectrometer was set at 250 ºC. The scanning m/z range was 20-550 amu. One microliter of sample was injected with a split ratio of 10:1. The carrier gas was helium with a flow rate of 1.0 ml/min. Peaks were identified using the NIST database and retention index.

2.4. Data analysis

For each sample, the peak heights of metabolites in mass spectra were recorded and averaged over three replicates. An unsupervised multivariate statistical method, principal component analysis (PCA), was applied to UV-scaling data using SIMCA-P version 11.5 software (Umetrics, Umeå, Sweden). PCA was performed to visualize the clustering of different samples without any prior knowledge of their group membership.

3.1. Semi-polar metabolites composition identified by UPLC-MS/MS

The methanolic extract of P. tenuifolia root was analyzed by UPLC-MS/MS to identify semi-polar metabolites. Figure 1 [Figure 1: see original paper] shows the full-scan ESI-MS spectra in negative mode. The identified chemicals of major peaks are listed in Table 1 . Metabolites with relatively high peaks are interpreted here. The leading peak was tenuifoliside A (retention time 19.79 min, Fig. 2 [Figure 2: see original paper]). The MS spectra showed an $[M\text{-}H]^-$ at m/z 680.7 and $[2M\text{-}H]^-$ at m/z 1362.7. The fragment ions were interpreted as follows: m/z 442.6 (loss of trimethoxycinnamoyl and hydroxyl group), 281.0 (loss of trimethoxycinnamoyl and fructose), 238.9 (cleavage of glucose), and 136.7 (p-hydroxybenzoic acid). The peak at 17.16 min gave $[M\text{-}H]^-$ and $[2M\text{-}H]^-$ at m/z 752.8 and 1506.9, identified as sibiricose A4. Its fragment ion at m/z 223.1 indicated synapic acid, and m/z 546.7 was due to the loss of a synapoyl group. The peak at 22.36 min gave $[M\text{-}H]^-$ and $[2M\text{-}H]^-$ at m/z 766.8 and 1535.8, respectively. Fragment ions at m/z 528.8 (loss of sinapoyl and hydroxyl), 237.3 (trimethoxycinnamic acid), and 222.6 (synapic acid) further confirmed this peak as tenuifoliside C.

A peak at 12.96 min was polygalaxanthone XI (MW 568), a xanthone C-glycoside, which gave $[M\text{-}H]^-$ and $[2M\text{-}H]^-$ at m/z 567.0 and 1134.5, respectively. Fragment ions at m/z 435.1 (loss of apiose), 416.9 (loss of apiose and H O), and 344.8 (cleavage of glucose at 0,3 bonds) further confirmed the structure. 1,2,3,7-Tetramethoxyxanthone (MW 316) was detected at 47.40 min

with $[M-H]^-$ at m/z 314.9 and fragment ions at m/z 214.4 (loss of C H O) and 116.8 (loss of C H O). The chemical structures of these compounds are shown in Fig. 2. Additionally, other chemicals including polygalasaponins, xanthones, and sucrose esters were identified, as reported in the literature [**?**].

3.2. Volatile metabolites composition identified by GC-MS

Volatile metabolites were extracted by hexane/acetone, trimethylsilylated, and determined by GC-MS, yielding 34 volatile compounds including alcohols, carboxylic acids, esters, and terpenoids (Table 2). The leading volatile chemical in all samples tested was oleic acid. Other volatile compounds present at percentages higher than 1% included 2,5-dihydroxy-3,6-bis(hydroxymethyl)-1,4-dioxane, hexadecanoic acid, 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid, stearic acid, 11-eicosenoic acid, eicosanoic acid, and eicosanoic acid 2-glycerol ester.

3.3. Principal component analyses of P. tenuifolia root based on their metabolites profiles

Principal component analysis was conducted on methanolic extracts of P. tenuifolia root samples. The first two principal components explained 75.9% of the total variance, with the first principal component accounting for 45.8% and the second for 30.1%. The loading plot (Fig. 3A [Figure 3: see original paper]) revealed the main compounds responsible for sample differences. The first component was represented by arillalose B, sibiricoses A1 and A6, tenuifolisides B and C, arillanin A, 6-O-benzoyl 3'-O-(4-hydroxy-3,5-dimethoxy-E-cinnamoyl) acylsucroses, tenuifolioses A, E, and L, polygalasaponin XLV, and reinioside B, characterized as saponins and oligosaccharide esters. The second component was primarily defined by 1-hydroxy-2,3-methylenedioxyxanthone, sibiricose A2, and tenuifolioses B, F, and G, characterized as xanthones and oligosaccharide esters. The score plot showed statistical similarities between samples. Sample 1# P. tenuifolia root was characterized by large amounts of arillalose B, sibiricose A2, and telephiose E, while sample 3# showed high levels of tenuifoliose B and I. These samples were located in opposite quadrants in the score plot, indicating significant differences. Sample 2# root was positioned near sample 1# in the score plot.

Figure 3B shows the results of principal component analysis on volatile metabolite levels. The first two principal components explained 81.5% of the total variance, with 51.9% accounted for by the first component and 29.6% by the second. Major metabolites defining the first component were propanedioic acid, 2-hydroxybenzoic acid, hexadecanoic acid, palmitelaidic acid, 9-octadecenoic acid methyl ester, stearic acid, 11-eicosenoic acid, eicosanoic acid, tetracosanoic acid, and eicosanoic acid 2-glycerol ester. The second component was mainly characterized by 5-ethyl-m-xylene, 1,2,3,4-tetramethylbenzene, and 2,3-dihydroxypropanoic acid. Sample 1# P. tenuifolia root was characterized

by high levels of 5-ethyl-m-xylene and 1,2,3,4-tetramethylbenzene, while sample 3# was characterized by high levels of 2,3-dihydroxypropanoic acid.

4.1. Phytochemical profile of P. tenuifolia root

Phytochemical analysis provides an effective approach for evaluating the quality of commercial biological resources such as medicinal herbs and fruits. Currently, LC-MS and GC-MS are two efficient and precise techniques for resolving the phytochemical profile of plant resources [**?**, **?**]. The former technique can identify semi-polar metabolites with advantages of high precision and short analysis time, and is widely applied in characterizing plant secondary metabolites. GC-MS reveals the chemical nature of volatile metabolites [**?**], which define plant flavor [**?**], and provides complementary information to LC-MS analysis for obtaining a complete phytochemical profile. Based on the bioactive compound profiles obtained in this work, four samples showed similar metabolite profiles, though the level of each metabolite varied.

More than seventy metabolites were identified from P. tenuifolia root in this study. Three sucrose esters—sibiricose A4, tenuifoliside A, and tenuifoliside C—were detected as the leading metabolites in the UPLC-MS/MS profile. These have been reported as bioactive compounds responsible for the pharmaceutical effects of P. tenuifolia root against depression [**?**]. Several saponins and xanthones were also detected as important constituents, including 1,2,3,7-tetramethoxyxanthone, 1-hydroxy-2,3-methylenedioxyxanthone, polygalaxanthones III, V, and VIII, as well as polygalasaponins XLV and XXXV. Polygalasaponins play a critical role in the characteristic bioactivities of P. tenuifolia root, such as neuroprotection and memory improvement. Cognitive dysfunction is a symptom of central nervous system disorder that endangers quality of life. Xue et al. demonstrated that polygalasaponins significantly prevent scopolamine-induced cognitive impairments in mice [**?**]. Although the numbers and levels of saponins and xanthones in the UPLC-MS profile were lower than those of oligosaccharide esters (Table 1 and Figure 1), they serve as prerequisites for the health-beneficial effects of P. tenuifolia root. GC-MS analysis showed that the major volatile compounds were fatty acids, consistent with the findings of Wang et al. [**?**]. Among these, oleic acid was the leading volatile metabolite and is also a pharmacologically active compound. While volatile compounds contribute less to the pharmaceutical activities of P. tenuifolia root than non-volatile compounds, they influence flavor characteristics to some extent.

Based on the levels and importance of pharmacological functions, oligosaccharide esters, polygalasaponins, and xanthones identified in the UPLC-MS profile could be selected to evaluate the quality of P. tenuifolia root. A sample with relatively high levels of these bioactive compounds is considered to be of good quality. ANOVA analysis of representative metabolite levels (data not shown), including tenuifoliside A, tenuifoliose A, sibiricose A4, and onjisaponin W, indicated that four P. tenuifolia roots showed no significant differences in the levels

of tenuifoliside A, tenuifoliose A, and onjisaponin W. For sibiricose A4, sample 2# showed a significantly ($p < 0.05$) higher level than sample 1#, but no significant ($p > 0.05$) differences were observed among samples 2#, 3#, and 4#. These results indicated that sample 1# had inferior quality compared to the other three samples.

4.2. Multivariate statistical analysis of P. tenuifolia root metabolites

PCA is an unsupervised clustering technique for identifying patterns in data, expressing the data in a way that emphasizes similarities and differences. By reducing the number of dimensions, it can define a limited number of principal components that describe independent variation in the results [**?**, **?**]. In this work, PCA was performed on semi-polar and volatile metabolites of P. tenuifolia root to identify principal variables defining pharmacological quality. The loading plot displays the influence of individual spectral peaks in each principal component and describes the separation shown in the score plot [**?**]. Points in the loading plot far from the zero point represent characteristic markers with the highest confidence for each group. Figure 3A shows the characteristic compounds for each sample. The first two principal components explained most of the total variance. The first principal component was defined by tenuifolioses (A, E, and L) and polygalasaponin XLV. The second principal component was defined by tenuifolioses (B, F, and G) and 1-hydroxy-2,3-methylenedioxyxanthone. Surprisingly, although tenuifoliside A showed the leading peak in the UPLC-MS profile, it was not a representative chemical in the first two principal components due to insignificant variance. Only metabolites with significant variance are calculated in the PCA model.

Tenuifolioses are characteristic oligosaccharide esters in P. tenuifolia root [**?**], constructed from pentasaccharide, phenolic acid, and acetyl moieties. These oligosaccharides are very rare in other plant resources and exhibit good neuroprotective and antioxidant activities, making P. tenuifolia root a valuable traditional folk medicine. Seven xanthones were detected in the UPLC-MS profile, but only 1-hydroxy-2,3-methylenedioxyxanthone was included in the principal component. Xanthones show good potential for treating inflammation, asthma, and allergy [**?**], and also exhibit bioactivity against neuroblastoma [**?**]. Polygalaxanthones are a type of xanthone characterized by a C-glycoside moiety that partially contributes to the bioactivity of P. tenuifolia root.

Through PCA calculation, several oligosaccharide esters, xanthones, and saponins were selected as principal components. These metabolites can be used to determine the quality of commercial P. tenuifolia roots, eliminating the need to measure all metabolites. This result also demonstrated that UPLC-MS/MS and GC-MS are efficient tools for quality evaluation of medicinal plants [**?**].

5. Conclusions

Analysis of the bioactive compound profile is an efficient method to define the quality of P. tenuifolia root. Using UPLC-MS/MS and GC-MS, semi-polar and volatile metabolites were identified and their levels were statistically compared. Tenuifoliside A was the leading metabolite in P. tenuifolia root, while xanthones and saponins represent two other important metabolite classes. PCA plots identified representative metabolites for each sample, which could be utilized to judge the commercial quality of P. tenuifolia root.

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References

[1] El Sayah M, Cechinel V, Pinheiro TR, et al. In vitro effect of the extract and the 1,7-dihydroxy-2,3-dimethoxy xanthone from Polygala cyparissias on the contractions induced by inflammatory mediators and ovalbumin in normal and actively sensitised trachea from guinea pig. Inflamm. Res. 1999;48:218-223.

[2] Park HJ, Lee K, He H, et al. Effects of Polygala tenuifolia root extract on proliferation of neural stem cells in the hippocampal CA1 region. Phytother. Res. 2008;22:1324-1329.

[3] Hu Y, Liao HB, Liu P, et al. A bioactive compound from Polygala tenuifolia regulates efficiency of chronic stress on hypothalamic-pituitary-adrenal axis. Pharmazie 2009;64:605-608.

[4] Ikeya Y, Takeda S, Tunakawa M, et al. Cognitive improving and cerebral protective effects of acylated oligosaccharides in Polygala tenuifolia. Biol. Pharm. Bull. 2004;27:1081-1085.

[5] Yang B, Chen F, Hua YL, et al. Prooxidant activities of quercetin, p-courmaric acid and their derivatives analysed by quantitative structure-activity relationship. Food Chem. 2012;131:508-512.

[6] Zhu QQ, Jiang YM, Lin S, et al. Structural identification of (1→6)- -D-glucan, a key responsible for the health benefits of longan, and evaluation of anticancer activity. Biomacromolecules 2013;14:1999-2003.

[7] Klein LC, de Andrade SF, Cechinel V. A pharmacognostic approach to the Polygala genus: Phytochemical and pharmacological aspects. Chem. Biodivers. 2012;9:181-209.

[8] Xue W, Hu JF, Yuan YH, et al. Polygalasaponin XXXII from Polygala tenuifolia root improves hippocampal-dependent learning and memory. Acta Pharm. Sinic. 2009:30:1211-1219.

[9] Na Y. Recent cancer drug development with xanthone structures. J. Pharm. Pharmacol. 2009;61:707-712.

[10] Bernillon S, Biais B, Deborde C, et al. Metabolomic and elemental profiling of melon fruit quality as affected by genotype and environment. Metabolomics 2013;9:57-77.

[11] Yang B, Jiang YM, Zhao MM, et al. Structural characterisation of polysaccharides purified from longan (Dimocarpus longan Lour.) fruit pericarp. Food Chem. 2009;115:609-614.

[12] Dao TT, Dang TT, Nguyen PH, et al. Xanthones from Polygala karensium inhibit neuraminidases from influenza A viruses. Bioorg. Med. Chem. Lett. 2012;22:3688-3692.

[13] Fu J, Zuo L, Yang JZ, et al. Oligosaccharide polyester and triterpenoid saponins from the roots of Polygala japonica. Phytochemistry 2008;69:1617-1624.

[14] Zhang DM, Miyase T, Kuroyanagi M, et al. Polygalasaponins XLII-XLVI from roots of Polygala glomerata. Phytochemistry 1998;47:459-466.

[15] Ling Y, Li ZX, Chen MC, et al. Analysis and detection of the chemical constituents of Radix polygalae and their metabolites in rats after oral administration by ultrahigh-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J. Pharmaceut. Biomed. 2013;85:1-13.

[16] Mari A, Lyon D, Fragner L, et al. Phytochemical composition of Potentilla anserina L. analyzed by an integrative GC-MS and LC-MS metabolomics platform. Metabolomics 2013;9:599-607.

[17] Chen RC, Wei KJ, Wang TM, et al. Simultaneous quantification of antibiotic dyes in aquatic products and feeds by liquid chromatography-tandem mass spectrometry. J. Food Drug Anal. 2013; 21:339-346.

[18] Chen HC, Peng LW, Sheu MJ, et al. Effects of hot water treatment effects on the essential oils of calamondin. J. Food Drug Anal. 2013;21:363-368.

[19] Besada C, Sanchez G, Salvador A, et al. Volatile compounds associated to the loss of astringency in persimmon fruit revealed by untargeted GC-MS analysis. Metabolomics 2013;9:157-172.

[20] Hu Y, Liao HB, Dai-Hong G, et al. Antidepressant-like effects of 3,6-disinapoyl sucrose on hippocampal neuronal plasticity and neurotrophic signal pathway in chronically mild stressed rats. Neurochem. Int. 2010;56:461-465.

[21] Wang Y, Chang L, Zhao X, et al. Gas chromatography-mass spectrometry analysis on compounds in volatile oils extracted from Yuan Zhi (Radix Polygalae) and Shi Chang Pu (Acorus Tatarinowii) by supercritical CO . J. Trad. Chinese Med. 2012;32:459-464.

[22] Park MK, Cho IH, Lee S, et al. Metabolite profiling of Cheonggukjang, a fermented soybean paste, during fermentation by gas chromatography-mass spectrometry principal component analysis. Food Chem. 2010;122:1313-1319.

[23] Yuk J, McIntyre KL, Fischer C, et al. Distinguishing Ontario ginseng landraces and ginseng species using NMR-based metabolomics. Anal. Bioanal. Chem. 2013;405:4499-4509.

[24] Miyase T, Iwata Y, Ueno A. Tenuifolioses A-F, oligosaccharide multi-esters from roots of Polygala-tenuifolia Willd. Chem. Pharm. Bull. 1991;39:3082-3084.

[25] Mak NK, Li WK, Zhang M, et al. Effects of euxanthone on neuronal differentiation. Life Sci. 2000;66:347-354.

[26] Yang B, Yang HS, Chen F, Hua YL, Jiang YM. Phytochemical analyses of Ziziphus jujuba Mill. var. spinosa seed by ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. Analyst 2013;138:6881-6888.

Table 1. Semi-polar metabolites occurring in the methanolic extract of P. tenuifolia root measured by UPLC-MS/MS.

Retention (min)	$[M-H]^-$ (m/z)	$[2M-H]^-$ (m/z)	Fragment ions (m/z)	Chemicals
-	299.1, 136.9	-	-	Sibiricose A3
-	340.6, 192.8	-	-	Arillalose B
-	222.6, 204.9	-	-	Sibiricose A6/A1
-	222.8, 204.8	-	-	Sibiricose A6/A1
-	435.2, 314.5	-	-	Polygalaxanthone III or VIII
-	386.7, 266.7	-	-	Sibiricaxanthone B
-	434.8, 314.9	-	-	Polygalaxanthone III or VIII

Retention (min)	$[M-H]^-$ (m/z)	$[2M-H]^-$ (m/z)	Fragment ions (m/z)	Chemicals
12.96	435.1, 416.9, 344.8	567.0	1134.5	Polygalaxanthone XI
-	399.0, 341.0, 236.8	-	-	Sibiricose A2
-	212.9, 168.5	-	-	1-Hydroxy-2,3-methylenedioxyxanthone
-	467.3, 254.9	-	-	Tenuifoliside B
-	461.1, 280.8, 239.1	-	-	Polygalaxanthone V
-	705.2, 528.5, 205.0	-	-	Telephiose E
17.16	546.7, 223.1	752.8	1506.9	Sibiricose A4
-	549.0, 323.0, 272.7	-	-	Arillanin A
-	705.0, 692.5, 630.8	-	-	Acylsucroses 6-O-benzoyl 3’ -O-(4-hydroxy-3,5-dimethoxy-E-cinnamoyl)
-	696.5, 546.8, 516.6	-	-	Tenuifoliose G
19.79	442.6, 281.0, 238.9, 136.7	680.7	1362.7	Tenuifoliside A
-	528.8, 222.5, 204.5	-	-	Tenuifoliose F
-	-	-	-	Tenuifoliose L
22.36	528.8, 237.3, 222.6	766.8	1535.8	Tenuifoliside C
-	1160.8, 163.3, 145.0	-	-	Polygalasaponin XLV
-	894.5, 320.6	-	-	Tenuifoliose C
-	1118.5, 881.4	-	-	Reiniose A

Retention (min)	$[M-H]^-$ (m/z)	$[2M-H]^-$ (m/z)	Fragment ions (m/z)	Chemicals
-	1308.4, 1250.1	-	-	Tenuifoliose I
-	1336.6, 390.8	-	-	Senegose L
-	1587.8, 425.4	-	-	Senegose K
-	214.4, 116.8	-	-	Reinioside B
-	-	-	-	Tenuifoliose B
-	-	-	-	Polygalasaponin XXXV
-	-	-	-	Tenuifoliose F
-	-	-	-	Tenuifoliose A
-	-	-	-	Onjisaponin W
47.40	214.4, 116.8	314.9	-	1,2,3,7-Tetramethoxyxanthone

Note: a, possible structure; -, unknown.

Table 2. Volatile metabolites occurring in hexane/acetone extract of P. tenuifolia root determined by GC-MS.

Retention time (min)	Chemicals
-	isobutanol
-	2-ethoxyethyl acetate
-	2,2' ,5,5' -tetrahydro-2,2' -bifuran
-	octyl-3-ol
-	phenol
-	propanedioic acid
-	4-oxo-pentanoic acid
-	o-cymene
-	benzenamine
-	5-ethyl-m-xylene
-	1,2,3,4-tetramethyl-benzene
-	2,3-dihydroxyl-propanoic acid
-	2-benzyl-1,3-dioxolane
-	decanoic acid

Retention time (min)	Chemicals
-	2-hydroxyl benzoic acid
-	2,3-dimethyl-3-hydroxyglutaric acid
-	9H-carbazole
-	undecanedioic acid
-	cinoxate
-	2,5-dihydroxy-3,6-bis(hydroxymethyl)-1,4-dioxane
-	palmitelaidic acid
-	hexadecanoic acid
-	9-octadecenoic acid, methyl ester
-	9,12-octadecadienoic acid
-	oleic acid
-	stearic acid
-	11-eicosenoic acid
-	eicosanoic acid
-	isooctyl phthalate
-	tetracosanoic acid
-	thymol-glucoside
-	eicosanoic acid, 2-glycerol ester
-	squalene
-	22,23-dibromostigmasterol acetate

Figure legends

Figure 1. UPLC-MS and GC-MS profiles of P. tenuifolia root. (A) Total ion chromatogram analyzed by UPLC-MS; (B) Total ion chromatogram analyzed by GC-MS.

Figure 2. Structures of characteristic metabolites in P. tenuifolia root.

Figure 3. PCA plots of semi-polar and volatile metabolites of P. tenuifolia root. Loading plot (A) and score plot (A) show results for semi-polar metabolites; loading plot (B) and score plot (B) show results for volatile metabolites.

Note: Figure translations are in progress. See original paper for figures.