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## Original Article

# 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 used as a functional food due to its attractive health benefits. In this study, 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 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 samples tested showed similar bioactive compound compositions, but the level of each compound varied. Principal component analysis revealed the principal bioactive compounds with significant level variations between samples. These principal chemicals could be used for quality judgment of *P. tenuifolia* root, instead of measuring the levels of all compositional compounds.

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## 1. Introduction

The genus *Polygala* includes herbaceous plants, shrubs, and small trees, which are distributed over the world. Some *Polygala*

species are used in functional foods and folk medicines against inflammation and disorders of the central nervous system [1]. As a widely used medicinal plant, *Polygala tenuifolia* has attracted much attention due to its good pharmaceutical properties against insomnia, neurosis, and dementia [2].

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Moreover, *P. tenuifolia* root can attenuate cognitive dysfunction and relieve depression [3,4]. As cognitive dysfunction and depression are common disorders, the preventative effect of *P. tenuifolia* root has attracted much interest by scientists in the functional food field. Many functional food products formulated with *P. tenuifolia* root have been developed.

Natural bioactive compounds, such as phenolics and carbohydrates, are responsible for the pharmaceutical activities of medicinal herbs [5,6]. It has been reported that oligosaccharides, saponins, and xanthenes in *P. tenuifolia* root are critical chemicals involved in disease treatment and health benefits [7]. The antidepressant effect is mainly attributed to the occurrence of oligosaccharide esters, such as 3,6'-disinapolsucrose. Polygalasaponins possess good capability against cognitive impairments [8]. Xanthenes have diverse biological profiles, including anticancer, antihypertension, and antioxidation [9]. It is obvious that the bioactive compound composition determines the quality of *P. tenuifolia* root. More than 200 chemicals have been identified from the *Polygala* genus. However, the chemical composition of *P. tenuifolia* root is still not clear. Determination of phytochemical profile by ultrahigh-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) and gas chromatography–mass spectrometry (GC-MS) is a good choice to understand the chemistry nature of medicinal plant.

Metabolomics is being increasingly utilized to gain insight into the chemical composition of biological materials [10]. Two types of analytical techniques, based on MS and nuclear magnetic resonance spectroscopy (NMR), are widely applied in metabolomics. The MS technique shows better precision and resolution than NMR. Therefore, in this work, the bioactive compounds profile of *P. tenuifolia* root was analyzed using both UPLC-MS/MS and GC-MS. It is useful to judge the quality of commercial *P. tenuifolia* root.

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## 2. Materials and methods

### 2.1. Plant material

Four *P. tenuifolia* roots were collected from Sanmenxia in Henan province (Roots 1 and 2) and Yuncheng in Shanxi province (Roots 3 and 4). Each sample was pulverized into powder and screened 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 1 week in the dark at room temperature. The extract was centrifuged at  $8000 \times g$  for 10 minutes. Each sample was extracted in triplicate. The supernatant was collected and subjected to UPLC-MS/MS analysis. Acquity UPLC equipped with a BEH C18 column (1.7  $\mu\text{m}$ , 2.1 mm  $\times$  150 mm; Waters, Milford, MA, USA) was used for chemicals isolation. A gradient elution program was conducted as follows: 0–45 minutes, from 5% to 50% solvent B; 45–48 minutes, from 50% to 100% solvent B; 48–51 minutes, and from 100% to 5% solvent B; 51–53 minutes, 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°C; desolvation temperature, 500°C; 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 1 week in the dark at room temperature. The extracts were centrifuged at  $8000 \times g$  for 15 minutes. The supernatants were collected and subjected to trimethylsilyl derivatization due to the occurrence of fatty acids following the method of Yang et al [11]. A gas chromatography/mass spectrometer (GCMS-QP 2010; Shimadzu, Kyoto, Japan) was used to analyze the chemical composition. The derivatives were loaded into a RTX-5 capillary column. The temperature program was set as follows: the initial temperature of column was 50°C, holding for 1 minute, increasing to 250°C at 3°C/min, holding for 17 minutes, and increasing to 280°C at 10°C/min; injection temperature: 250°C. The ion source of the mass spectrometer was set at 250°C. The scanning  $m/z$  range was 20–550 atomic mass units. A 1- $\mu\text{L}$  aliquot of sample was injected and the split ratio was 10:1. The carrier gas was helium with a flow rate of 1.0 mL/min. The peaks were identified using the NIST database and retention index.

### 2.4. Data analysis

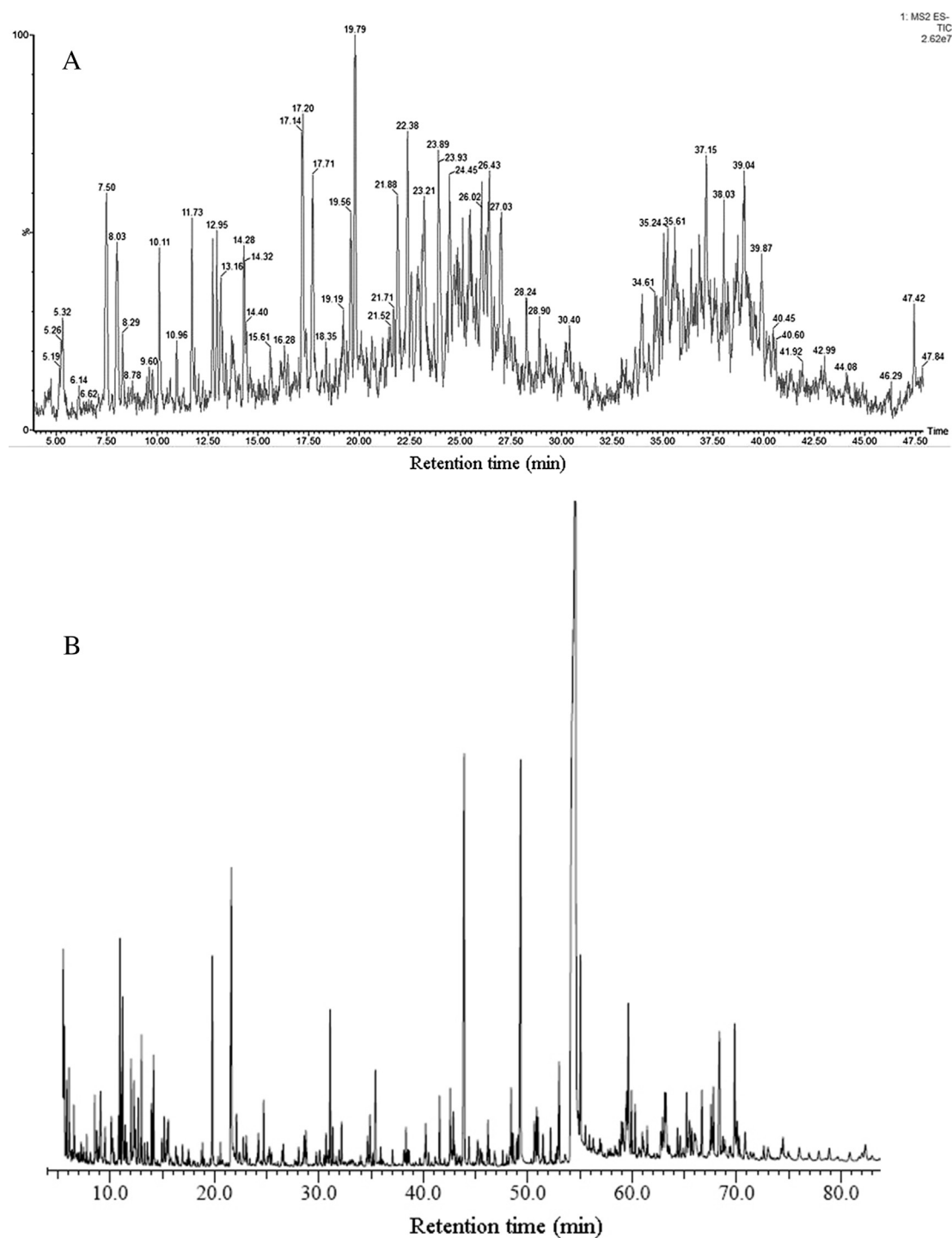
For each sample, the peak heights of metabolites in mass spectra were recorded and averaged over three replicates. An unsupervised multivariate statistical method, principal component analysis (PCA), was used on UV-scaling data by software SIMCA-P version 11.5 (Umetrics, Umeå, Sweden). PCA was performed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

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## 3. Results

### 3.1. Semipolar metabolites composition identified by UPLC-MS/MS

The methanolic extract of *P. tenuifolia* root was analyzed using UPLC-MS/MS to identify the semipolar metabolites. Fig. 1 shows the full scan ESI-MS spectra in negative mode. The identified chemicals of main peaks are listed in Table 1. Those metabolites with relatively high peaks were interpreted here. The leading peak was tenuifoliside A (retention time, 19.79 minutes; Fig. 2). The MS spectra had an [M-H] at  $m/z$  680.7 and [2M-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 (breaking of glucose), and 136.7 (*p*-hydroxy benzoic acid). Peak at 17.16 minutes gave [M-H] and [2M-H] at  $m/z$  752.8 and  $m/z$  1506.9, respectively, which was identified as sibiricose



**Fig. 1** – Ultrahigh-performance liquid chromatography–tandem mass spectrometry and gas chromatography–mass spectrometry profiles of *Polygala tenuifolia* root. (A) Total ion chromatogram analyzed by ultrahigh-performance liquid chromatography–tandem mass spectrometry; (B) total ion chromatogram analyzed by gas chromatography–mass spectrometry.

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. Peak at 22.36 minutes gave [M-H] and [2M-H] at  $m/z$  766.8 and  $m/z$  1535.8, respectively. The fragment ions at  $m/z$  528.8 (loss of sinapoyl and hydroxyl),  $m/z$  237.3 (trimethoxy cinnamic acid), and  $m/z$  222.6 (synapic acid) further confirmed this peak as

tenuifoliside C. A peak at 12.96 minutes was polygalaxanthone XI (MW 568), a xanthone C-glycoside, which gave an [M-H] and [2M-H] at  $m/z$  567.0 and  $m/z$  1134.5, respectively. The fragment ions at  $m/z$  435.1 (loss of apiose),  $m/z$  416.9 (loss of apiose and  $H_2O$ ), and  $m/z$  344.8 (breaking of glucose at 0,3 bonds) further confirmed this structure. 1,2,3,7-Tetramethoxyxanthone (MW

**Table 1 – Semipolar metabolites found in the methanolic extract of *Polygala tenuifolia* root measured by ultrahigh-performance liquid chromatography–tandem mass spectrometry.**

No.	Retention time (min)	MW	[M-H] <sup>-</sup> (m/z)	[2M-H] <sup>-</sup> (m/z)	Fragment ions (m/z)	Chemicals
1	5.29	462	460.9	922.9	299.1, 136.9	Sibiricoses A3
2	7.5	518	516.7	1034.9	340.6, 192.8	Arillalose B
3	7.96	548	546.8	1094.8	222.6, 204.9	Sibiricoses A6/A1
4	10.11	548	546.9	1094.3	222.8, 204.8	Sibiricoses A6/A1
5	10.96	568	566.8		435.2, 314.5	Polygalaxanthone III or VIII
6	11.73	538	536.7		386.7, 266.7	Sibiricaxanthone B
7	12.75	568	566.5		434.8, 314.9	Polygalaxanthone III or VIII
8	12.96	568	567.0	1134.5	435.1, 416.9, 344.8	Polygalaxanthone XI
9	13.16	562	560.7	1123.2	399.0, 341.0, 236.8	Sibiricoses A2
10	13.69	256	255.0	511.0	212.9, 168.5	1-Hydroxy-2,3-methylenedioxyxanthone
11	13.77	550	548.8		467.3, 254.9	—
12	14.3	668	666.7	1334.9	461.1, 280.8, 239.1	Tenuifoliside B
13	14.4	754	752.7	1506.7	705.2, 528.5, 205.0	—
14	16.3	582	580.9		549.0, 323.0, 272.7	Polygalaxanthone V
15	16.45	754	752.9	1507.1	705.0, 692.5, 630.8	Telephiose E
16	17.16	754	752.8	1506.9	546.7, 223.1	Sibiricoses A4
17	17.71	724	722.7	1447.3	696.5, 546.8, 516.6	Arillanin A
18	19.21	652	650.7		528.8, 222.5, 204.5	Acylsucroses 3'-O-(4-hydroxy-3,5-dimethoxy-E-cinnamoyl), 6-O-benzoyl
19	19.56	1484	1482.8			Tenuifolioses G <sup>a</sup>
20	19.79	682	680.7	1362.7	442.6, 281.0, 238.9, 136.7	Tenuifoliside A
21	21.85	1526	1525.4		688.7	Tenuifolioses F <sup>a</sup>
22	21.9	1496	1494.9			Tenuifolioses L <sup>a</sup>
23	22.36	768	766.8	1535.8	528.8, 237.3, 222.6	Tenuifoliside C
24	22.87	1660	1659.1			Polygalasaponin XLV <sup>a</sup>
25	23.12	1296	1294.8			Tenuifolioses C <sup>a</sup>
26	23.19	738	736.9	1477.5	704.8	Reinioses A <sup>a</sup>
27	23.94	1308	1306.8		1160.8, 163.3, 145.0	Tenuifolioses I
28	24.45	1338	1336.9		894.5, 320.6	Senegoses L
29	24.69	1296	1294.9		1118.5, 881.4	Senegoses K
30	25.48	1308	1306.8		964.7	Reiniosides B <sup>a</sup>
31	26.07	1338	1336.8			Tenuifolioses B <sup>a</sup>
32	26.38	1350	1348.9		1308.4, 1250.1	Polygalasaponin XXXV
33	26.47	1526	1525.2			Tenuifolioses F <sup>a</sup>
34	26.99	1380	1379.0		1336.6, 390.8	Tenuifolioses A
35	39.91	1732	1731.2		1587.8, 425.4	Onjisaponin W
36	47.40	316	314.9		214.4, 116.8	1,2,3,7-Tetramethoxyxanthone

— = unknown; a = possible structure; MW = molecular weight.

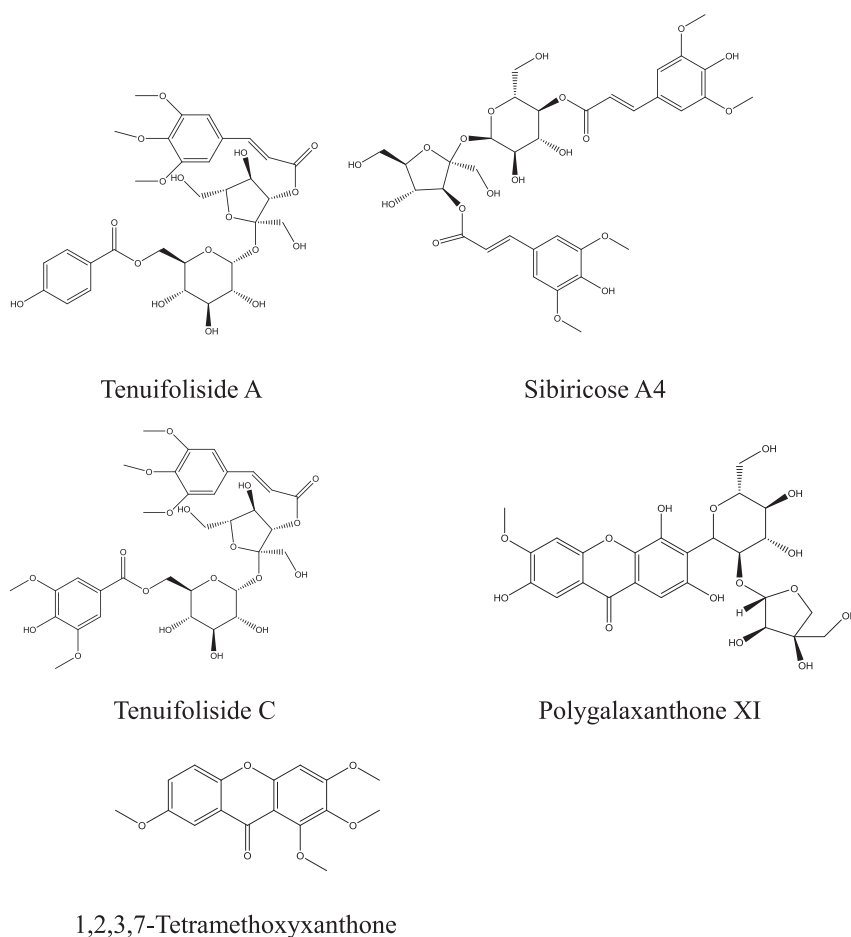
316) was detected at 47.40 minutes with [M-H] at  $m/z$  314.9, and fragment ions at  $m/z$  214.4 (loss of C<sub>5</sub>H<sub>9</sub>O<sub>2</sub>) and  $m/z$  116.8 (loss of C<sub>10</sub>H<sub>14</sub>O<sub>4</sub>). The chemical structures of the above compounds are shown in Fig. 2. Some other chemicals, including polygalasaponins, xanthenes, and sucrose esters, were also identified, as has been described previously [12–15].

### 3.2. Volatile metabolites composition identified by GC-MS

The volatile metabolites were extracted by hexane/acetone, trimethylsilylated, and determined by GC-MS, which gave 34 volatile compounds, including alcohol, carboxylic acids, esters, and terpenoids (Table 2). The leading volatile chemical was oleic acid in all samples tested. Other volatile compounds with percentage higher than 1% were 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. PCA of *P. tenuifolia* root based on their metabolites profiles

PCA was conducted on methanolic extract of *P. tenuifolia* root samples. The first two principal components accounted for 75.9% of the total variance, in which the first principal component accounted for 45.8% of the total variance and the second principal component 30.1%. The loading plot (Fig. 3A) revealed the main compounds responsible for the sample difference. The first component was represented by arillalose B, sibiricoses A1, A6, tenuifolisides B, C, arillanin A, 6-O-benzoyl 3'-O-(4-hydroxy-3,5-dimethoxy-E-cinnamoyl) acylsucroses, tenuifolioses A, E, L, polygalasaponin XLV, and reinioside B, which are characterized as saponins and oligosaccharide esters. The second component was mainly defined by 1-hydroxy-2,3-methylenedioxyxanthone, sibiricoses A2, tenuifolioses B, F, and G, which were characterized as xanthone and oligosaccharide esters. Score plot showed the statistical similarity between samples. *P. tenuifolia* Root 1 was



**Fig. 2 – Structures of characteristic metabolites in *Polygala tenuifolia* root.**

characterized by large amounts of arillalose B, sibiricose A2, and telephiose E, while Root 3 showed high levels of tenuifoliside B and I. They were located in two opposite quadrants in the score plot, which indicated significant differences. Root 2 was located in the proximity of Root 1 in the score plot.

Fig. 3B shows the results of PCA on volatile metabolites levels. The first two principal components accounted for 81.5% of the total variance, 51.9% by the first component and 29.6% by the second component. Major metabolites defining the first component were propanedioic acid, 2-hydroxyl benzoic 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-tetramethyl-benzene, and 2,3-dihydroxyl-propionic acid. *P. tenuifolia* Root 1 was characterized by high levels of 5-ethyl-*m*-xylene and 1,2,3,4-tetramethyl-benzene. Root 3 was characterized by high level of 2,3-dihydroxyl-propanoic acid.

## 4. Discussion

### 4.1. Phytochemical profile of *P. tenuifolia* root

Phytochemical analysis is a good way to evaluate the quality of commercial biological resources, such as medicinal herbs and

fruits. At present, LC-MS and GC-MS are two efficient and precise techniques to resolve the phytochemical profile of plant resources [16,17]. The former technique can identify semipolar metabolites with advantages of high precision and short time consumed. It is widely applied to the characterization of plant secondary metabolites. GC-MS can reveal the chemistry nature of volatile metabolites [18], which define the flavor of the plant [19]. GC-MS also provides complementary information to LC-MS analysis to obtain a complete phytochemical profile. From the bioactive compounds profile obtained in this work, it can be concluded that four samples had similar metabolites profiles, but the level of each metabolite varied.

More than 70 metabolites were identified from *P. tenuifolia* root in this study. Three sucrose esters, including sibiricose A4 and tenuifoliside A and C, were detected as the leading metabolites in the UPLC-MS/MS profile. They have been reported as the bioactive compounds responsible for the pharmaceutical effect of *P. tenuifolia* root against depression [20]. Several saponins and xanthones were also detected as important constituents, including 1,2,3,7-tetramethoxyxanthone, 1-hydroxyl-2,3-methylenedioxyxanthone, and 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, which endangers the life quality of

**Table 2 – Volatile metabolites occurred in hexane/acetone extract of *Polygala tenuifolia* root determined by gas chromatography–mass spectrometry.**

No.	Retention time (min)	Chemicals
1	5.86	isobutanol
2	6.55	2-ethoxyethyl acetate
3	9.10	2,2',5,5'-tetrahydro-2,2'-bifuran
4	10.95	octyl-3-ol
5	11.44	phenol
6	12.02	propanedioic acid
7	12.29	4-oxo-pentanoic acid
8	12.71	o-cymene
9	13.00	benzenamine
10	13.98	5-ethyl-m-xylene
11	14.16	1,2,3,4-tetramethyl-benzene
12	15.19	2,3-dihydroxyl-propanoic acid
13	22.11	2-benzyl-1,3-dioxolane
14	24.72	decanoic acid
15	31.08	2-hydroxyl benzoic acid
16	34.89	2,3-dimethyl-3-hydroxyglutaric acid
17	38.35	9H-carbazole
18	41.56	undecanedioic acid
19	42.58	cinoxate
20	43.89	2,5-dihydroxy-3,6-bis(hydroxymethyl)-1,4-dioxane
21	48.41	palmitelaidic acid
22	49.29	hexadecanoic acid
23	50.87	9-octadecenoic acid, methyl ester
24	54.34	9,12-octadecadienoic acid
25	54.52	oleic acid
26	55.06	stearic acid
27	59.62	11-eicosenoic acid
28	60.29	eicosanoic acid
29	63.12	isooctyl phthalate
30	65.22	tetracosanoic acid
31	66.68	thymol-glucoside
32	68.38	eicosanoic acid,2-glycerol ester
33	69.82	squalene
34	89.42	22,23-dibromostigmasterol acetate

people. Xue et al [8] pointed out that polygalasaponins can significantly prevent scopolamine-induced cognitive impairments in mice. Even though the numbers and levels of saponins and xanthenes in the UPLC-MS profile were less than oligosaccharide esters (Table 1 and Fig. 1), they work as prerequisites to the health-beneficial effects of *P. tenuifolia* root. GC-MS analysis showed that the major volatile compounds were fatty acids, consistent with the determinations of Wang et al [21]. Among these, oleic acid was the leading volatile metabolite. It is also a pharmacologically active chemical. Although the contribution of volatile compounds to the pharmaceutical activities of *P. tenuifolia* root is much less than nonvolatile compounds, they influence the flavor characteristics to some extent.

Based on the level and importance to the pharmacological functions, the oligosaccharide esters, polygalasaponins and xanthenes identified in the UPLC-MS profile could be selected to evaluate the quality of *P. tenuifolia* root. A *P. tenuifolia* root with relatively high levels of bioactive compounds is considered as having good quality. Through analysis of variance of representative metabolites levels (data not shown), the results indicate that four *P. tenuifolia* roots had no significant differences in the levels of

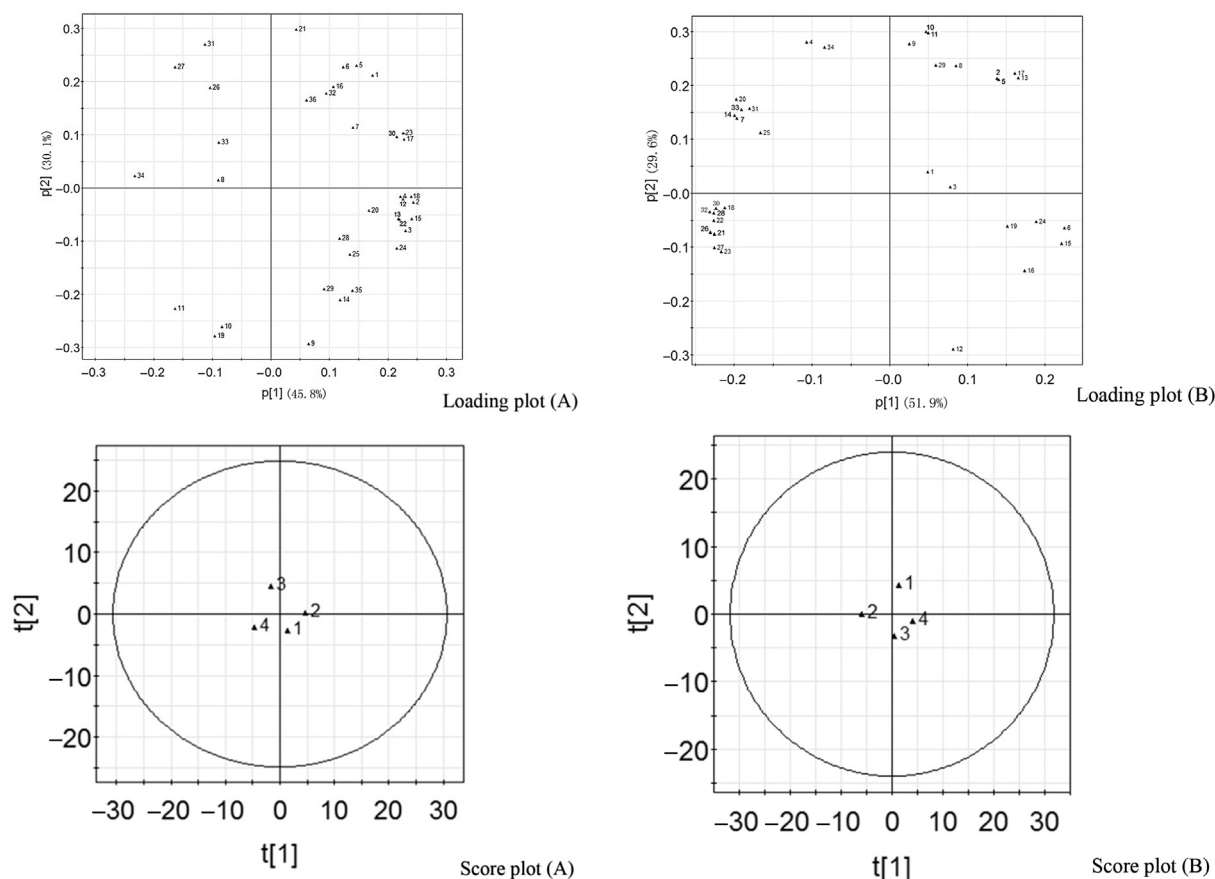
tenuifoliside A, tenuifolioside A, and onjisaponin W. For sibiricoside A4, Root 2 sample showed a significantly ( $p < 0.05$ ) higher level than Root 1, but no significant ( $p > 0.05$ ) differences between Roots 2, 3, and 4 were observed. The results showed that Root 1 had a worse quality compared with 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, and expressing the data in a way to emphasize their similarities and differences. Through reducing the number of dimensions, it can define a limited number of principal components that describe independent variation in the results [10,22]. In this study, PCA was carried out on semipolar and volatile metabolites of *P. tenuifolia* root to find principal variables defining the pharmacological quality. In the loading plot, it displays the influence of individual spectral peaks in each principal component and describes the separation shown in the score plot [23]. The points in the loading plot far away from zero point represent characteristic markers with most confidence to each group. Fig. 3A shows the characteristic compounds for each sample. The first two principal components accounted for most of the total variance. The first principal components were labeled by tenuifolioses (A, E, and L) and polygalasaponin XLV. The second principal components were labeled by tenuifolioses (B, F, and G) and 1-hydroxy-2,3-methylene-dioxyxanthone. It was unexpected that tenuifoliside A had the leading peak in the UPLC-MS profile. However, it was not the representative chemical in the two first principal components due to the insignificant variance. Only those metabolites having significant variance will be calculated in the PCA model. Tenuifolioses are the characteristic oligosaccharide esters in *P. tenuifolia* root [24], which are constructed by pentasaccharide, phenolic acid, and acetyl moieties. It is very rare to find these oligosaccharides in other plant resources. They show good neuroprotective and antioxidant activities, which make *P. tenuifolia* root a good choice for traditional folk medicine. Seven xanthenes were detected in the UPLC-MS profile, and only 1-hydroxy-2,3-methylenedioxyxanthone was included among the principal components. Xanthenes show good potential for treatment against inflammation, asthma, and allergy [1]. They also exhibit bioactivity against neuroblastoma [25]. Polygalaxanthenes are a type of xanthone characterized by a C-glycoside moiety, which contribute partially to the bioactivity of *P. tenuifolia* root. Through PCA calculation, some oligosaccharide esters, xanthenes, and saponins were selected as principal components. These metabolites can be chosen to determine the quality of commercial *P. tenuifolia* roots, instead of measuring the levels of all metabolites. This result also proved that UPLC-MS/MS and GC-MS were efficient for quality evaluation of medicinal plants [26].

## 5. Conclusion

Analysis of bioactive compounds profile was an efficient way to define the quality of *P. tenuifolia* root. By using UPLC-MS/MS



**Fig. 3** – Plots of PCA on semipolar and volatile metabolites of *Polygala tenuifolia* root. (A) Loading plot and score plot are the results of semipolar metabolites; (B) loading plot and score plot are the results of volatile metabolites.

and GC-MS, the semipolar and volatile metabolites were identified and their levels were statistically compared. Tenifoliside A was the leading metabolite in *P. tenuifolia* root. Xanthones and saponins are two other important types of metabolite found in *P. tenuifolia* root. PCA plot indicated the representative metabolites for each sample, and these metabolites could be utilized to judge the commercial quality of *P. tenuifolia* root.

### Conflicts of interest

All authors declare no conflicts of interest.

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