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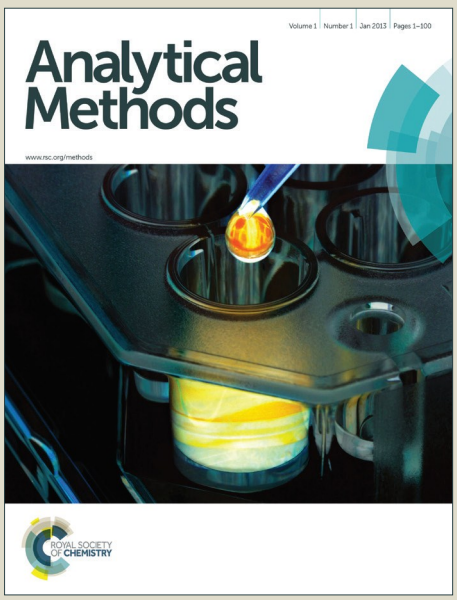

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Full paper

A strategy for quality control of the fruits of *Perilla frutescens* (L.) Britt based on antioxidant activity and fingerprint analysis

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Perilla frutescens (L.) Britt, widely used as an edible and medicinal plant in Asian countries, its fruits have been adopted in Chinese pharmacopoeia as a traditional Chinese medicine (TCM). To provide an efficient method for quality control, an attempt on antioxidant activity and fingerprint analysis was made in this work. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activities of the fruits of *P. frutescens* from different sources were measured firstly. Then, HPLC fingerprints of these samples were established and four active components were deduced by multiple correlation analysis. Combined with TLC bioautography assay and HPLC comparison, the four antioxidant components separated from silica gel chromatography and further purified by Sephadex LH-20 were determined to be caffeic acid, rosmarinic acid, luteolin and apigenin, respectively. Finally, total phenolic contents of the test samples were determined and their correlations with antioxidant activity were also investigated. The results indicated that most of the similarities of 22 batches of samples were more than 0.89 based on the four active components corresponding to the peaks 5, 10, 11 and 12 in the fingerprints and total phenolic contents correlated closely with antioxidant activity. Therefore the four components and total phenols are predominate contributors to the antioxidant activity of the fruits of *P. frutescens.* It is proposed that the fingerprint with the four characteristic peaks as common peaks and total phenolic contents determination were necessary for the quality control of this plant and should be adopted in its quality standards.

1. Introduction

Quality control of traditional Chinese medicines (TCMs) has gained more and more attention due to their long historical clinical practice, reliably therapeutic efficacy and especially complex chemical constituents. In recent years, some comprehensive methods which involve fingerprints with multi-component quantifications are recommended [1]. Although the characteristic constituents responsible for the activities of TCMs have not been fully elucidated, chemical markers which are uncharacteristic but easily obtained were selected instead for content determinations and chemical fingerprint profiles in most quality standards. Thus, a combination of biological activity with qualification or quantification for quality control of TCMs is necessary in order to guarantee efficacy and safety when they were utilized in clinical practices.

Perilla frutescens (L.) Britt belonging to the Labiatae family has been frequently used as an edible and medicinal plant in Asian countries. The fruits of *P. frutescens*, adopted in Chinese pharmacopoeia 2010, is employed for dyspnea and cough relief, phlegm elimination, and the bowel relaxation [2, 3]. In addition to α-linolenic acid from perilla oil, some components such as sterols, flavonoids and phenolic compounds have also been identified from the seeds of *P. frutescens* [4, 5]. However, in the pharmacopoeia, only content of rosmarinic acid was quantified. It still remains unclear which represents the quality of this herbal medicine that possesses various biological activities. Thus, we evaluated the antioxidant activity in terms of their abilities to protect against oxidative cell damage that can lead to various diseases, such as arthritis, cancer, diabetes, atherosclerosis, ischemia, failures in immunity and endocrine

functions [6]. Meanwhile, chromatographic fingerprinting with advantages in reflecting the overall chemical profile of TCMs, has been suggested to check for authenticity or provide quality evaluation and species differentiation [7, 8]. To our best knowledge, there have been no reports about the quality evaluation methods based on the fingerprints of the fruits of *P. frutescens*. Therefore, HPLC fingerprints of 22 batches of samples from different sources were established in this work. The relationships between fingerprints and antioxidant activities were also investigated.

2. Materials and Methods

2.1. Chemicals and materials

DPPH· (1,1-diphenyl-2-picrylhydrazyl radical) was purchased from Wako (Japan). Folin–Ciocalteu′s phenol reagent and sodium carbonate anhydrous were purchased from Sigma-Aldrich (UK). Gallic acid was purchased from Tianjin Guangfu Fine Chemical Industry Research Institute (China). TLC plates and silica gel were obtained from Qingdao Haiyang Chemical Co. (China). Sephadex LH-20 was obtained from Beijing Greenherbs Science and Technology (China). Acetonitrile and methanol of HPLC grade were purchased from Dikma Technology (USA). Caffeic acid, rosmarinic acid, luteolin and apigenin as reference standards were purchased from the Institute for the Control of Pharmaceutical and Biological Products of China. All other chemicals were of analytical grade without further purification.

2.2. Sample preparation

22 batches of the fruits of *P. frutescens* (L.) Britt (designated S1–S22) were collected from

Heilongjiang, Jilin, Neimeng, Hubei, Anhui, Jiangsu and Shanxi provinces in China (Table1). They were authenticated by Professor Xiuhua Wang from Northeast Forestry University (Harbin, China) and were examined to be qualified samples according to the quality standard of Chinese pharmacopoeia 2010. Voucher specimens were preserved at the Department of Pharmaceutical Engineering, Heilongjiang University.

About 100 g of sample was extracted thrice using 10 times the amount of petroleum ether (60–90 °C) under reflux for 2 h. The supernatant was removed and the defatted fruits were re-extracted twice with 10 times the amount of 75% aqueous ethanol under reflux for 2 h. The 75% aqueous ethanol extract was combined and evaporated by rotary vaporization under reduced pressure, yielding 4.4 g of the ethanol extract which was stored in a refrigerator(4 °C) for subsequent analysis.

Table 1 should be here

2.3. Preparation of standard stock solutions

A reference standard mixture containing of four components (caffeic acid, rosmarinic acid, luteolin and apigenin) was accurately weighed and dissolved in methanol, then diluted to appropriate concentration for peaks confirmation in the fingerprints.

2.4. Determination of antioxidant activity

The antioxidant activity was measured by the DPPH method [9, 10] with slight modifications. Each extract dissolved in 2 mL of 95% ethanol (0.02–0.32 mg/mL) was mixed with 2 mL of 95% ethanol solution containing DPPH radicals, resulting in a final concentration of 2×10^{-4} mol/L DPPH ethanol solution. The mixture was shaken vigorously and left to stand for 30 min

in the dark, the absorbance was then measured at 517 nm against a blank using a UV–VIS spectrophotometer PC2501 model (Shimazu, Japan). The percentage scavenging effect was calculated as: scavenging rate = $[1 - (A_1 - A_2)/A_0] \times 100\%$, where A_0 was the absorbance of the control (without extract), A_1 was the absorbance in the presence of the extract, and A_2 was the absorbance without DPPH. The EC_{50} value (mg extract/mL) was the effective concentration at which DPPH radicals were scavenged by 50%. All determinations were performed in triplicate.

2.5. Determination of total phenolic contents

Total phenolic contents were determined by Folin–Ciocalteu method [11] with minor modifications. The mixture of 0.5 mL (0.005–0.16 mg/mL) gallic acid, 2.5 mL 10% Folin Ciocalteu and 2 mL 4% sodium carbonate were shaken vigorously for 3 min and then left to stand for 2 h in the dark. The absorbance was measured at 760 nm against a blank and the calibration curve was established using gallic acid. Then 0.5 mL of the diluted extract (0.5 mg/mL) was tested instead of gallic acid and the result was expressed as gallic acid equivalent (mg GAE/g). All determinations were performed in triplicate.

2.6. Chromatographic conditions

HPLC fingerprints were measured with a Hitachi L-2000 HPLC series equipped with L-2130 dual pump, 7725i injector with a 20 μL loop and UV detection. The extract of the sample was evaporated to dryness and the residue dissolved with methanol and filtered through a 0.45-μm filter to obtain the sample solution at the concentration of 1.0 mg/mL for HPLC fingerprints. An aliquot of the filtrate (10 μL) was injected into a Thermo ODS HYPERSIL C_{18} column

(250 × 4.6 mm i.d., 5 μm) and eluted with a linear gradient with a mobile phase containing solvent A (methanol: acetonitrile = 1: 1) and solvent B (0.5% acetic acid in water). The gradient elution program was: 10–40% A in 0–40 min, 40–60% A in 40–60 min. The flow rate was 0.8 mL/min and the effluent was monitored at 254 nm.

2.7. Mass spectra conditions

HPLC-MS analysis was performed on Thermo Scientific LTQ Orbitrap XL mass spectrometer and an ESI ion source. The ESI source was under the following conditions: capillary voltage of 27.00 V, spray voltage of 4.00 kV, capillary temperature of 275.00 °C, tube lens of 100.00 V. The mass spectra were collected in positive ion mode.

2.8. TLC bioautography analysis

The extract of the sample was subjected to column chromatography on silica gel which was preconditioned with chloroform. The column was gradiently eluted with chloroform–methanol (20: 1, 10: 1, 5: 1, 2: 1, 1: 1, 1: 2). The fractions were monitored by TLC plates which were developed in a presaturated solvent chamber with *n*-hexane–toluene–ethyl acetate–formic acid (4: 10: 5: 1) as developing reagents. The developed TLC plate was then removed from the chamber. After air-drying for 30 min, the TLC plate was sprayed with a 2.54-mM DPPH· methanol solution for derivatization. Spots with DPPH scavenging activities were observed as white yellow ones on a purple background. The fractions with DPPH scavenging activities were subsequently combined and applied to Sephadex LH-20 column using methanol as an elution for further purification, obtaining the active subfractions a, b, c and d.

2.9. Statistical analysis

Multiple correlation analysis was used for the study of relationship between EC_{50} values and peak areas in the HPLC fingerprints, using SPSS software (SPSS 17.0 for Windows, SPSS Inc., USA). Similarity analysis was performed by Excel 2003 according to the literature to calculate correlation coefficients between the chromatographic profiles and the reference chromatogram [12, 13].

3. Results and discussion

3.1. Determination of antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH), a paramagnetic compound with an odd electron, is one of the most popular radicals used to evaluate antioxidant activities of pure substances or complex samples. DPPH radical with the maximum absorption at 517 nm is reduced to non-colored DPPH·H when it reacts with antioxidants. Therefore, the decrease of the absorption can represent the antioxidant activity of the corresponding compounds [14–16]. Results of the DPPH· scavenging rates of the ethanol extracts from 22 batches of samples were given in Table 1. It was observed that the samples demonstrated significant ($p > 0.05$) antioxidant activities with EC_{50} values ranging from 32.66 to 63.55 μg/mL comparing with that of vitamin C as positive control (9.00 μg/mL). S4 and S18 from Hebei and Anhui provinces exhibited the strongest activity (EC_{50}＜35 μg/mL), about one third of vitamin C, while S2, S9 and S11 from Anhui and Heilongjiang provinces showed the weakest antioxidant activity (EC_{50}＞60 μg/mL). According to the production areas, EC_{50} values of the samples from Anhui and Heilongjiang provinces varied largely ranging from 32.66 to 63.55 μg/mL.

While those from Hebei and Jiangsu provinces demonstrated the moderate antioxidant activities and the qualities seemed to be more stable.

3.2. Total phenolic contents

Total phenolic contents were determined by Folin–Ciocalteu method. The values were obtained from the calibration curve $y = 0.0316 + 0.0095x$, where y is the absorbance and x is the concentration of gallic acid solution. Results of the total phenolic contents were shown in Table 1. The values ranged from 627.05 mg GAE/g for S11 from Heilongjiang province to 1308.16 mg GAE/g for S18 from Anhui province. A similar trend was observed as that of the EC_{50} values mentioned above and these results indicated that the antioxidant capacities of the ethanol extracts of the fruits of *P. frutescens* may be strongly correlated to total phenolic contents. Pearson correlation coefficient calculated by SPSS 17.0 showed that the total phenolic contents and EC_{50} values had a negative correlation and the correlation coefficient was 0.876. It means that the antioxidant property of *P. frutescens* has been attributed to its phenolic contents or structures of antioxidative capacities. And also, the results were in agreement with some reports in which correlations between the total phenolic contents and antioxidant capacities have been described [17, 18]

3.3. Fingerprint analysis

HPLC conditions were examined and compared using various columns (Thermo ODS HYPERSIL C_{18} 250 mm × 4.6 mm, 5 μm; Phenomenex Luna C_{18} 250 mm × 4.6 mm, 5 μm; YMC-Pack ODS-A 250 mm × 4.6 mm, 5 μm) and column temperatures (25, 30, 35, or 40 °C). Different mobile phases were also tried, viz. water– acetonitrile, water–methanol,

acetonitrile–methanol–0.5% acetic acid, acetonitrile–0.2% phosphoric acid and methanol–0.2% phosphoric acid. The optimized HPLC condition was established by comparing the resolution, baseline and elution time in each chromatogram after repeated testing. Figure 1 showed the typical HPLC fingerprints of 22 batches of samples from various sources under the optimal separation conditions. Twelve peaks with large areas and good segregation from consecutive peaks were selected as common peaks to study the relationships between antioxidant activity and peak areas.

Figure 1 should be here

The repeatability of the method was examined by the injection of six different samples prepared by the same sample preparation procedure. The relative standard deviation (RSD) of retention time (RT) and peak areas (PA) of 12 common peaks was used to estimate the repeatability. The results for analysis repeatability were shown in Table 2. RSD values for peak areas and retention time were all < 3.0%, which could meet the need of fingerprint analysis. The interday and intraday precisions were determined by repeated analysis for six times within a day or on five separate days. The RSD of retention time and peak areas was used to estimate the precision and the results were also shown in Table 2. RSD values for peak area and retention time were all < 3.0%. For the stability test, retention time and peak areas of 12 common peaks were analyzed every 4 h within 24 h, and the sample solution was found to be rather stable within 24 h (RSD values for peak areas and retention time were all < 3.0%, see Table 2). The limit of detection (LOD) and the limit of quantification (LOQ) values for caffeic acid, rosmarinic acid, luteolin and apigenin were determined at the signal-to-noise ratio (*S/N*) of 3 and 10, respectively. The LOD and LOQ of the four analytes were 1.00~1.50 μg/mL and 4.00~5.00 μg/mL, respectively (Table 3). It indicated that the analytical method was acceptable with

sufficient sensitivity.

Table 2 should be here

Table 3 should be here

Multiple correlation analysis is a special case of canonical correlation analysis when there is only one variable in one set of variables [19]. Most of information about the relationships between two sets of variables can be obtained through reasonable methods. We used multiple correlation analysis in this work to study the relationships between EC_{50} values and peak areas of 12 common peaks of 22 batches of samples in HPLC fingerprints. The results shown in Table 4 indicated that antioxidant activities of the ethanol extracts from the fruits of *P. frutescens* had a close correlation with peaks 5, 10, 11 and 12. These peaks, especially peak 10, may be the main antioxidant components with negative partial correlation coefficients greater than 0.40. Since similarity analysis has been adopted by the Chinese Pharmacopoeia as an evaluation standard for the quality control of injections, we choose similarity analysis to validate the established evaluation method. Similarity analysis based on the four characteristic peaks was performed by Excel 2003 and the results were shown in Table 1. It was observed that most of the similarities were greater than 0.89 except S15 from Anhui province. It indicated that the similarity based on the characteristic peaks could reveal the slight differences in internal qualities of qualified samples which had been evaluated by the quality standards of pharmacopoeia. The reasonable limit of similarity value for quality evaluation of *P. frutescens* still needs further investigations.

Table 4 should be here

3.4. Confirmation of the four active components

In order to confirm the main active components deduced from fingerprint analysis, TLC bioautography was performed on the basis of activity-guided purification. In the method, the components with DPPH· scavenging activity could be observed visually as white yellow spots on a purple background [20]. The fractions with DPPH· scavenging activities isolated from silica gel column were combined and subjected to Sephadex LH-20 column chromatography to obtain subfractions a–d. Figure 2A showed the TLC plate of subfractions a–d and reference standard mixture containing of four components (caffeic acid, rosmarinic acid, luteolin and apigenin) inspected under UV (254 nm). Figure 2B showed the profile of the same plate of antioxidant components under visible light. It was observed that the spots from bottom to top were caffeic acid, rosmarinic acid, luteolin and apigenin respectively. They were the main components of subfractions a–d and also showed the obvious DPPH· scavenging activities.

Figure 2 should be here

Meanwhile, subfractions a–d and reference standard mixture were injected into the HPLC system with the same conditions as the fingerprinting. Comparing with the retention time (RT), caffeic acid (RT = 18.44 min), rosmarinic acid (RT = 35.28 min) luteolin (RT = 47.13 min) and apigenin (RT = 53.41 min) corresponded to the main peaks of subfractions a–d respectively (Figure 3). The results were consistent with that of TLC bioautography and therefore the four active components in subfractions a–d were confirmed. It was also found that the order of elution of peaks 5, 10, 11, and 12 in the fingerprint of sample was same as that of reference standard mixture. Mass spectra also showed the pseudo-molecular ion peaks

of luteolin (*m/z*: 287.05524 $[M+H]^+$) and apigenin (*m/z*: 271.06039 $[M+H]^+$). Their fragment ions at *m/z*: 153.02 and 135.04, 153.02 and 119.05 respectively were well in accordance with the regular pattern of the flavonoids.[21] Figure 4 showed the mass fragmentation patterns of identified compounds of luteolin and apigenin by HPLC-MS analysis in positive ion mode.

Figure 3 should be here

Figure 4 should be here

Thus the peaks 5, 10, 11 and 12 were confirmed to be caffeic acid, rosmarinic acid, luteolin and apigenin respectively. Moreover, the EC_{50} values of the four active compounds were determined by DPPH assay and listed in Table 1. Among them, Apigenin showed the weakest activity with the EC_{50} value of 26.27 μg/mL and this illustrated the poor visibility of subfraction d in Figure 2B. However, rosmarinic acid, caffeic acid and luteolin demonstrated significant DPPH· scavenging activities with EC_{50} value of 3.56 ± 0.02 μg/mL, 4.02 ± 0.01 μg/mL and 6.77 ± 0.04μg/mL respectively, which was comparable to that of vitamin C (9.00 μg/mL). Based on above TLC bioautography together with HPLC comparison, it concluded that four active components corresponding to peaks 5, 10, 11 and 12 respectively in the fingerprint contribute to the antioxidant activity of the fruits of *P. frutescens*.

4. Conclusions

In order to provide an efficient method for quality control of the fruits of *P. frutescens,* fingerprints of 22 batches of samples were established and their antioxidant activities were evaluated by DPPH assay. With the help of multiple correlation analysis, four potential active peaks in the fingerprints were deduced and then confirmed by TLC bioautography combined

with HPLC comparison with reference standards. Total phenolic contents were determined and they correlated closely with antioxidant activities. Therefore, the four components and total phenols are predominate contributors to the antioxidant activity of the fruits of *P. frutescens*. It is proposed that the fingerprint with the four active components as common peaks combined with total phenolic contents determination were necessary for the quality control and should be adopted in the present quality standards of the fruits of *P. frutescens*. The results also suggested that the method established provided an example to correlate chemical fingerprint with active components using chemometrics and TLC chromatography. It offered a practical and objective criterion for quality control of the fruits of *P. frutescens* and also simplified the process of screening active ingredients by investigation *in vivo*.

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Table Headings and Tables

Table 1. EC_{50} values by DPPH, total phenolic contents (n = 3) and similarities from various sources of the fruits of *P. frutescens* (L.) Britt

Sample Code	Collected Location	Harvesting Time	EC_{50} (μg/mL, mean ± SD)	Total Phenolic contents (mg GAE/g, mean ± SD)	Similarities
S1	Anhui	2012.01	48.57 ± 0.26	932.98 ± 3.14	0.9999
S2	Anhui	2012.07	60.29 ± 0.49	768.48 ± 4.82	0.9796
S3	Heilongjiang	2012.09	39.57 ± 0.30	1199.80 ± 0.20	0.9538
S4	Hebei	2012.03	34.39 ± 0.61	1124.13 ± 3.62	0.8910
S5	Heilongjiang	2012.08	43.08 ± 0.39	1031.50 ± 3.10	0.9799
S6	Jiangsu	2012.03	35.31 ± 0.29	1122.60 ± 4.20	0.9354
S7	Heilongjiang	2011.08	53.78 ± 0.69	975.91 ± 4.20	0.9972
S8	Neimeng	2012.03	51.13 ± 0.76	957.14 ± 2.06	0.9789
S9	Heilongjiang	2012.05	63.54 ± 0.43	892.60 ± 5.78	0.9683
S10	Heilongjiang	2012.04	39.13 ± 0.19	1131.17 ± 2.08	0.9874
S11	Heilongjiang	2012.11	63.55 ± 0.56	627.05 ± 0.15	0.9956
S12	Hebei	2012.08	44.31 ± 0.47	1058.74 ± 3.12	0.9873
S13	Jiangsu	2013.05	39.83 ± 0.05	1201.30 ± 3.10	0.9989
S14	Hebei	2013.01	51.29 ± 0.12	882.67 ± 1.06	0.9163
S15	Anhui	2011.02	51.46 ± 0.37	815.31 ± 1.05	0.8620
S16	Hebei	2013.06	47.97 ± 0.46	942.40 ± 4.22	0.9944
S17	Hebei	2013.07	45.69 ± 0.10	927.72 ± 2.10	0.9603
S18	Anhui	2012.08	32.66 ± 0.42	1308.16 ± 3.62	0.9999
S19	Hebei	2012.12	41.44 ± 0.10	1051.55 ± 4.25	0.9991
S20	Jiangsu	2011.07	47.35 ± 0.37	1075.80 ± 2.66	0.9906
S21	Anhui	2012.03	54.51 ± 0.03	954.90 ± 2.10	0.9572
S22	Shanxi	2012.01	37.64 ± 0.76	1089.15 ± 3.43	0.9997
Rosmarinic acid			3.56 ± 0.02		
Caffeic acid			6.77 ± 0.04		
Luteolin			4.02 ± 0.01		
Apigenin			26.27 ± 0.09		
Vitamin C			9.00 ± 0.01		

Table 2. Precision, repeatability and stability data of the fruits of *P. frutescens* (L.) Britt fingerprints (n = 6)

Peak no.	Precision				Repeatability		Stability	
	Interday		Intraday					
	RT（mean ± RSD%）	PA（mean ± RSD%）	RT（mean ± RSD%）	PA（mean ± RSD%）	RT（mean ± RSD%）	PA（mean ± RSD%）	RT（mean ± RSD%）	PA（mean ± RSD%）
1	6.358 ± 0.34	67145 ± 1.49	6.442 ± 0.08	67001 ± 1.05	6.435 ± 0.38	66978 ± 1.46	6.440 ± 0.35	66970 ± 0.79
2	8.535 ± 0.04	6945 ± 1.92	8.616 ± 0.11	7026 ± 0.66	8.611 ± 0.08	7238 ± 2.86	8.609 ± 0.05	7123 ± 0.99
3	10.123 ± 0.54	1538 ± 1.30	9.978 ± 0.11	1522 ± 0.20	9.971 ± 0.22	1502 ± 2.85	9.968 ± 0.27	1533 ± 2.10
4	15.134 ± 0.16	6539 ± 1.54	15.068 ± 0.08	6498 ± 1.24	15.059 ± 0.15	6511 ± 1.65	15.054 ± 0.05	6523 ± 2.64
5	18.443 ± 0.16	7639 ± 1.78	18.720 ± 0.04	7708 ± 0.33	18.706 ± 0.12	7692 ± 1.48	18.715 ± 0.27	7690 ± 1.86
6	29.444 ± 0.10	55518 ± 1.59	29.667 ± 0.05	54096 ± 1.49	29.651 ± 0.09	54113 ± 1.91	29.634 ± 0.11	54120 ± 1.64
7	30.481 ± 0.16	56379 ± 2.12	30.575 ± 0.15	56092 ± 2.02	30.561 ± 0.06	56210 ± 2.45	30.539 ± 0.02	56331 ± 1.31
8	31.735 ± 0.01	29443 ± 0.89	31.658 ± 0.04	29082 ± 0.30	31.644 ± 0.05	29100 ± 2.58	31.621 ± 0.17	29044 ± 1.83
9	34.023 ± 0.09	49622 ± 1.19	33.982 ± 0.04	50338 ± 1.98	33.967 ± 0.07	50114 ± 2.16	33.943 ± 0.03	50210 ± 1.97
10	35.276 ± 0.01	428929 ± 0.01	35.207 ± 0.01	428036 ± 0.01	35.192 ± 0.01	428304 ± 0.01	35.168 ± 0.01	428409 ± 0.01
11	47.123 ± 0.05	337297 ± 1.65	47.431 ± 0.08	331128 ± 2.68	47.423 ± 0.09	331110± 2.64	47.377 ± 0.11	331234 ± 0.84
12	53.406 ± 0.08	56100 ± 2.35	53.619 ± 0.05	56323 ± 1.59	53.631 ± 0.08	56331 ± 2.39	53.583 ± 0.08	56311 ± 1.12

Table 3. LODs and LOQs of caffeic acid, rosmarinic acid, luteolin and apigenin.

Analytes	LOD (μg/mL)	LOQ (μg/mL)
Caffeic acid	1.00	4.50
Rosmarinic acid	1.50	4.00
Luteolin	1.00	4.50
Apigenin	1.50	5.00

Table 4. The partial correlation coefficients between the EC_{50} values and the area values of 12 common peaks by multiple correlation analysis.

Peak No.	Coefficients
1	-0.1200
2	-0.1561
3	0.0265
4	0.1302
5	-0.2593
6	-0.2066
7	-0.0215
8	0.1560
9	0.2130
10	-0.4128
11	-0.2706
12	-0.2187

Figure Legends

Figure 1. HPLC fingerprints of 22 batches of the fruits of *P. frutescens* (L.) Britt from various sources

Figure 2. TLC plate visualized **(A)** under UV 254 nm, **(B)** stained with 2.54 mM DPPH solution in ethanol and visualized under visible light. Tracks 1, 2, 4 and 5 are subfractions a–d respectively eluted from silica gel column and Sephadex LH-20 column. Track 3 is the reference standards mixture of caffeic acid, rosmarinic acid, luteolin and apigenin with spots from bottom to top respectively

Figure 3. The HPLC correlation chromatograms between fingerprints of *P. Frutescens* (L.) Britt and subfractions a–d with reference standards mixture (*S* sample of *P. Frutescens* (L.) Britt; *m* mixture of reference standards: (5) caffeic acid, (10) rosmarinic acid, (11) luteolin and (11) apigenin)

Figure 4. Mass fragmentation patterns of identified compounds: (A1) pseudo-molecular ion peak of luteolin, (A2) fragmentation patterns of luteolin, (B1) pseudo-molecular ion peak of apigenin, (B2) fragmentation patterns of apigenin

Figure 1. HPLC fingerprints of 22 batches of the fruits of *P. frutescens* (L.) Britt from various sources

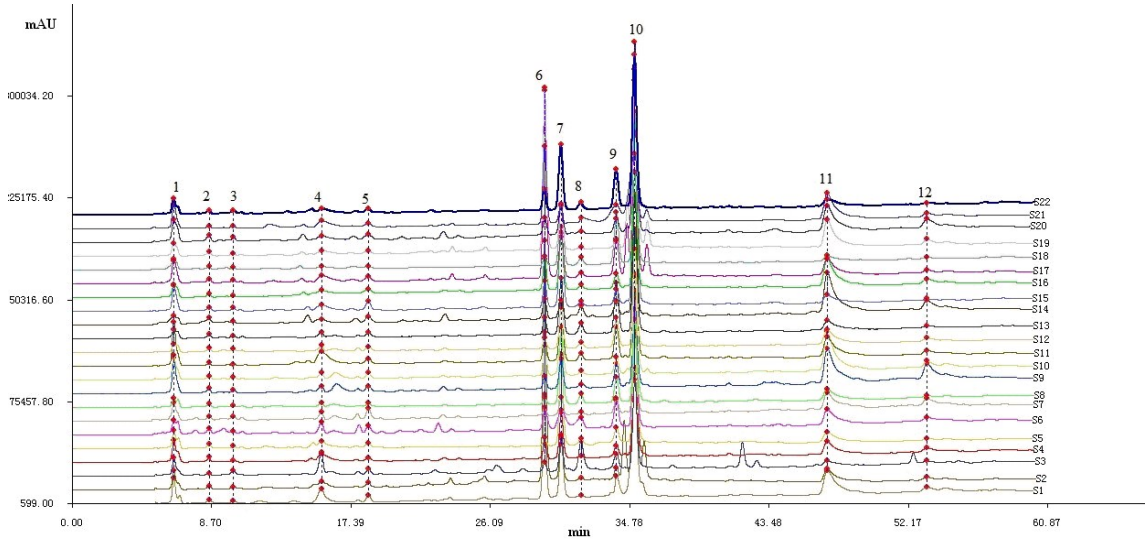


Figure 2. TLC plate visualized **(A)** under UV 254 nm, **(B)** stained with 2.54 mM DPPH solution in ethanol and visualized under visible light

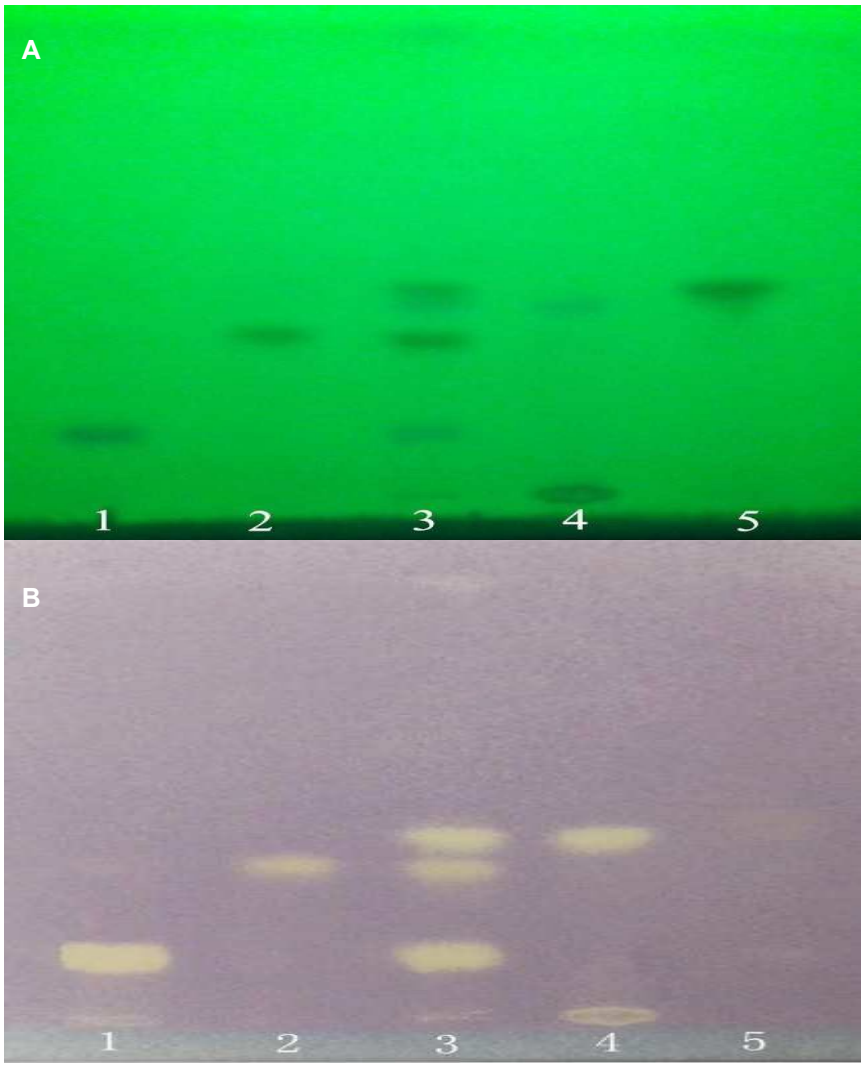


Figure 3. The HPLC correlation chromatograms between fingerprints of *P. Frutescens* (L.) Britt and subfractions a–d with reference standards mixture

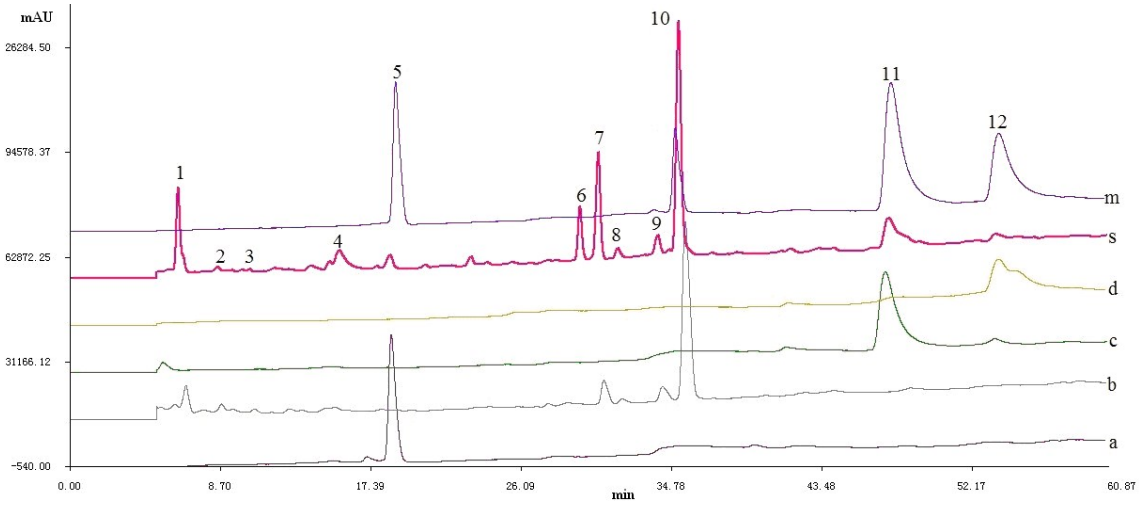
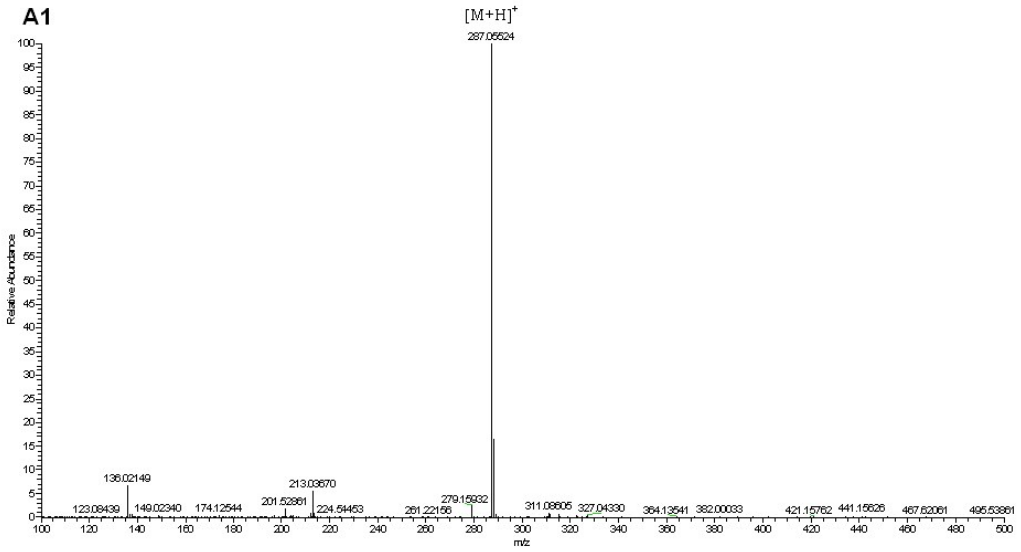
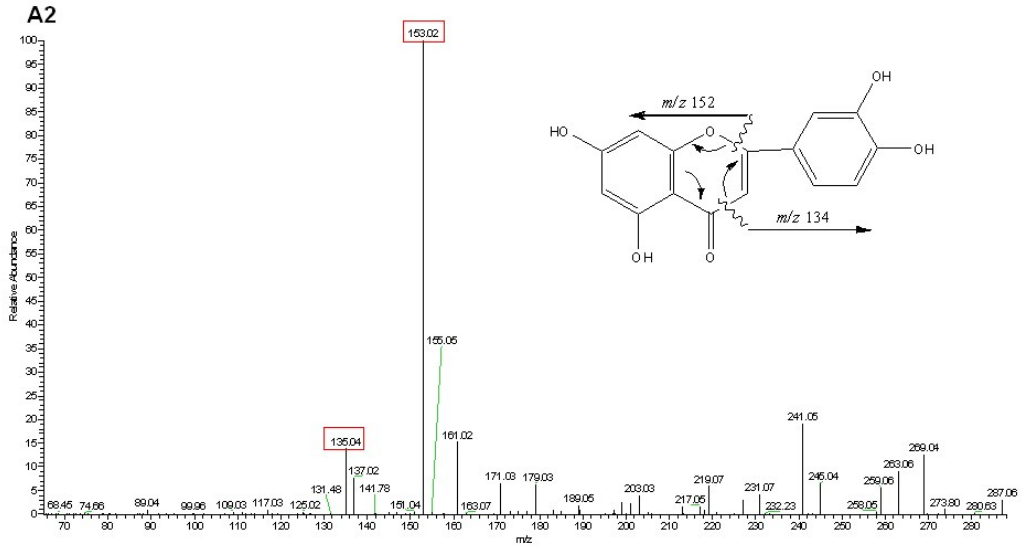
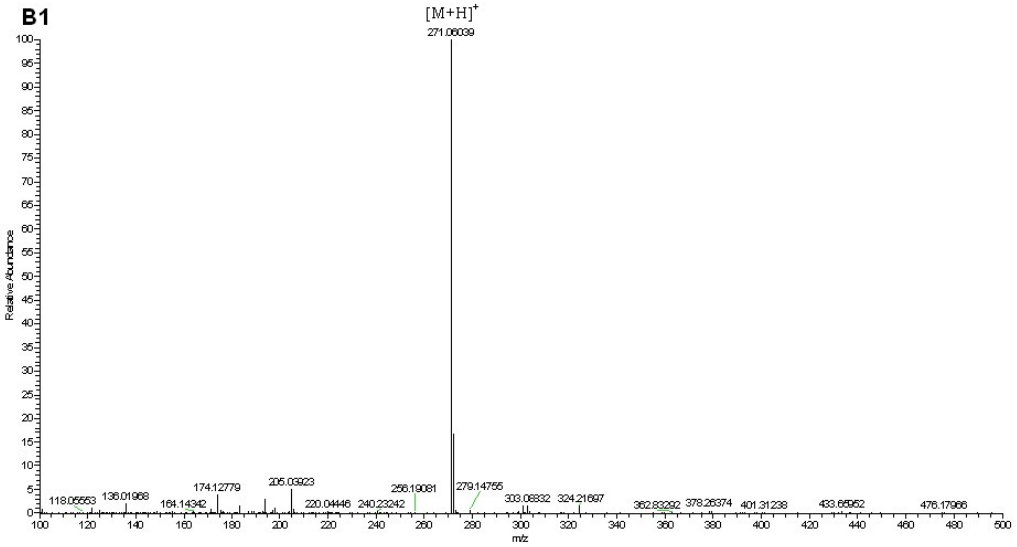


Figure 4. Mass fragmentation patterns of identified compounds: (A1) pseudo-molecular ion peak of luteolin, (A2) fragmentation patterns of luteolin, (B1) pseudo-molecular ion peak of apigenin, (B2) fragmentation patterns of apigenin







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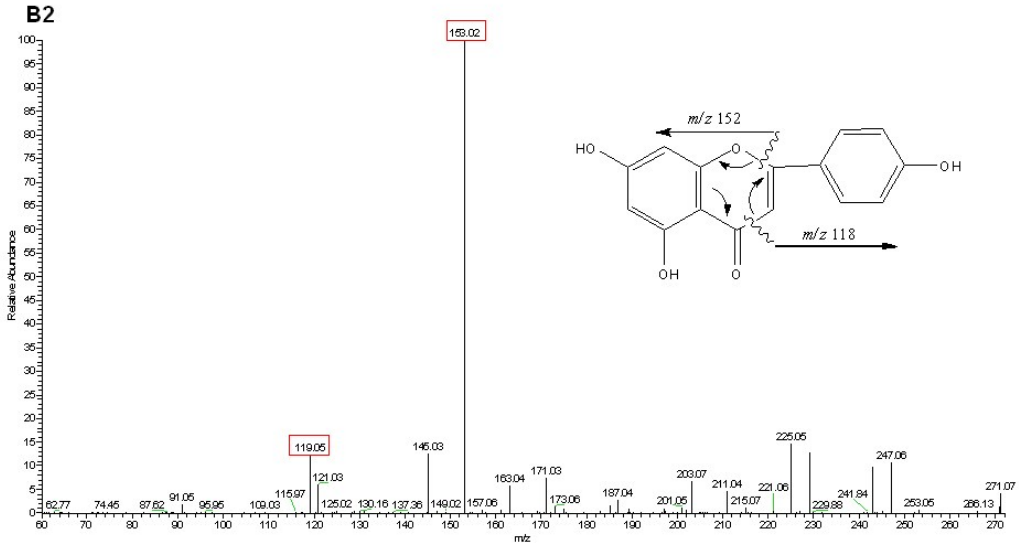

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A strategy for quality control of the fruits of Perilla frutescens (L.) Britt based on antioxidant activity and fingerprint analysis