JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** 53, 8141–8147 **8141**

Determination of Phenolic Compounds in *Perilla frutescens* L. by Capillary Electrophoresis with Electrochemical Detection

YOUYUAN PENG,[†,§] JIANNONG YE,[#] AND JILIE KONG*,[†]

Department of Chemistry, Fudan University, Shanghai 200433, China; Department of Chemistry, Quanzhou Normal University, Quanzhou 362000, China; and Department of Chemistry, East China Normal University, Shanghai 200062, China

A method based on capillary electrophoresis with electrochemical detection has been developed to analyze flavonoids and phenolic acids in *Perilla frutescens* L. for the first time. Catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid are major important active ingredients in the plant. Operated in a wall-jet configuration, a 300 μm diameter carbon-disk electrode was used as the working electrode, which exhibits a good response at 0.90 V (versus saturated calomel electrode) for the analytes. Under the optimum conditions, the analytes were baseline separated within 20 min in a 100 mmol/L borax buffer (pH 8.7). Notably, excellent linearity was obtained over 3 orders of magnitude with detection limits (S/N = 3) ranging from 2×10^{-7} to 1×10^{-6} g/mL for all analytes. This proposed method has been successfully applied to monitor the flavonoids and phenolic acids contents in the leaves and seeds of *P. frutescens* L. at different growth stages with relatively simple extraction procedures, and the assay results were satisfactory.

KEYWORDS: ***Perilla frutescens*** **L.; capillary electrophoresis; electrochemical detection; phenolic acids; flavonoids**

INTRODUCTION

Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants as a result of plant adaptation to biotic and abiotic stress conditions (infection, wounding, water stress, cold stress, high visible light). Protective phenylpropanoid metabolism in plants has been well documented (*1*−*4*). In recent years phenolic compounds have attracted great interest from researchers because polyphenols are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (*5*, *6*). Many epidemiological studies have shown that consumption of edible plants rich in phenolic compounds is associated with a lowered risk of degenerative diseases such cancers (*7*), cardiovascular diseases (*8*), and immune dysfunctions (*9*). These epidemiological results are corroborated by many in vitro and in vivo studies demonstrating the impact of phenolic compounds on mammalian biology (*10*) and displaying the remarkable scope of biochemical and pharmacological actions of these compounds, among others, their antiviral (*11*), antiinflammatory (*12*), and antiallergic (*13*) properties.

Perilla frutescens L., referred to as “zi-su” in China, belongs to the family Labiatae (*14*). *P. frutescens* L. is an edible plant frequently used as one of the most popular garnishes and food colorants in some Asian countries such as China and Japan. The leaves of *P. frutescens* L., shown to be detoxicant, antitussive, antibiotic, and antipyretic (*15*, *16*), are also utilized as a folk medicine for treating intestinal disorders and allergies, particularly in the traditional Chinese medical practice (*17*). The main phenolic compound has been proven to be rosmarinic acid, and there are small amounts of flavonoids and phenolic acids such as catechin, apigenin, luteolin, caffeic acid, and ferulic acid found in the leaves and seeds of *P. frutescens* L. (*18*−*20*).

Although the biological activity of *P. frutescens* L. as well as its superior safety is well documented, very few studies have been published on the qualitative and quantitative presence of flavonoids and phenolic acids in *P. frutescens* L. (*21*, *22*). Hence, it is necessary and interesting to establish some simple, economical, and accurate methods for the determination of flavonoids and phenolic acids in the leaves and seeds of *P. frutescens* L. However, it is often a challenging task to do so because of the diversity of compositions and the significant concentration difference of active ingredients as well as effects of many factors such as climate, region of growth, and season of harvest on the contents of active ingredients in medicinal herbs (*23*).

The most widely used method for the analysis of phenolic compounds such as rosmarinic acid, luteolin, and apigenin is high-performance liquid chromatography (HPLC) (*21*, *22*, *24*), but HPLC has some shortcomings including time-consuming sample pretreatment and short column lifetime owing to numerous coexistent interferences in herbs. Now, capillary electrophoresis (CE) is becoming increasingly recognized as an important analytical separation technique for charged com-

* Corresponding author (e-mail jlkong@fudan.edu.cn).
[†] Fudan University.
[§] Quanzhou Normal University.
[#] East China Normal University.

10.1021/jf051360e CCC: $30.25 © 2005 American Chemical Society
Published on Web 09/20/2005

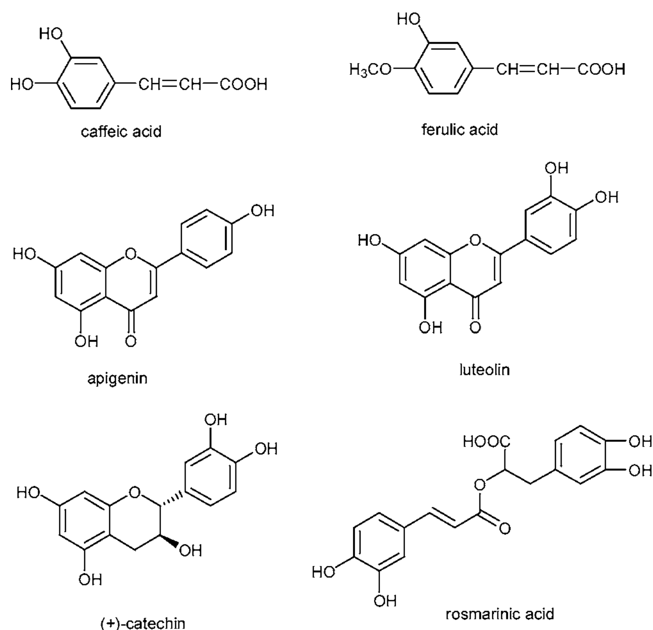

Figure 1. Molecular structures of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid.

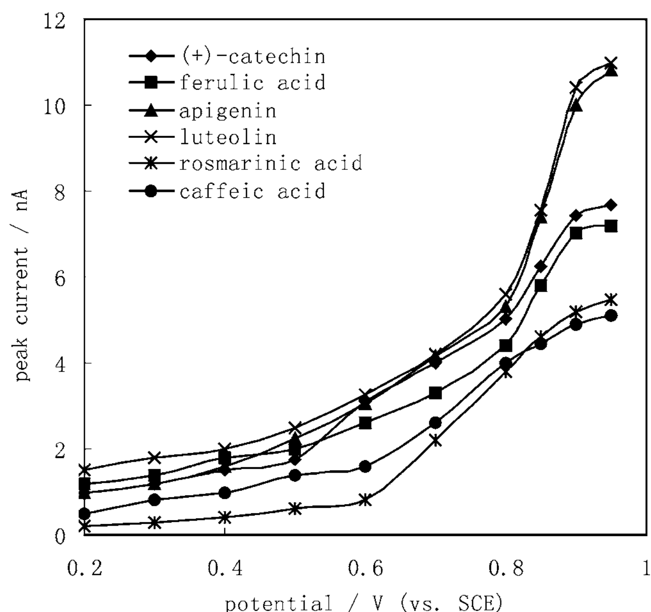

Figure 2. Hydrodynamic voltammograms (HDVs) for (+)-catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid in CE. Working conditions: fused-silica capillary, 25 μm i.d. × 75 cm; working electrode, 300 μm diameter carbon disk electrode; running buffer, 100 mmol/L borate buffer (pH 8.7); separation voltage, 18 kV; electrokinetic injection, 8 s (18 kV); concentrations, 2.0×10^{-5} g/mL for (+)-catechin, ferulic acid, apigenin, and luteolin and 5.0×10^{-5} g/mL for rosmarinic acid and caffeic acid.

pounds and partially charged organics such as phenolic compounds at a high pH. It provides faster analysis time and better separation efficiency than HPLC and consumes only small amounts of aqueous solvents. CE has environmental and economic advantages; however, HPLC is better in terms of accuracy, sensitivity, and precision. Therefore, CE has been proposed as a complementary technique to HPLC for the separation of phenolic compounds present in herbs such as phenolic acids and flavones. In combination with electrochemical detection (ED), CE-ED offers high sensitivity and good selectivity for electroactive species.

In 2000, the U.S. Food and Drug Administration (FDA) published a draft of the *Guidance for Industry Botanical Drug Products*. Before a plant drug becomes legally marketed, its spectroscopic or chromatographic fingerprints and chemical assay of characteristic markers are required. CE should find more applications in this area. In this work, CE-ED was proposed for the determination of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid (**Figure 1**) in the leaves and seeds of *P. frutescens* L. The leaves grow quickly in June, July, and August and are usually collected from the beginning of July until the end of August; the seeds are usually collected from the beginning of September to late October. Samples were collected at different growth stages to monitor the change of the contents of the active components. The optimization, detailed characterization, and advantages of CE-ED for the simultaneous determination of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid in *P. frutescens* L. are reported in the following sections.

MATERIALS AND METHODS

Apparatus. In this work, a capillary zone electrophoresis with amperometric detection system was laboratory-built and was similar to that described previously (*25*). A ±30 kV high-voltage DC power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided a voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential, and the outlet end of the capillary was maintained at ground. The separations were undertaken in a 75 cm length, 25 μm i.d., and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ). To protect the operator from the high voltage and ensure the safety of the CE-ED system, the entire capillary, the buffer reservoirs for CE, and all electrodes were enclosed in a Plexiglas box with a safety switch wired to turn off the power supply whenever the box was opened. The whole system was assembled in a 10 m^2 Faraday room that was air-conditioned at 20 °C to minimize the effects of external noise sources.

A three-electrode cell system consisting of a 300 μm diameter carbon disk working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode was used in combination with a BAS LC-3D amperometric detector (Bioanalytical Systems, West Lafayette, IN). Before use, the carbon disk electrode was polished with emery paper, then sonicated in doubly distilled water, and finally positioned carefully opposite the outlet of the capillary and arranged in a wall-jet configuration (*26*). The distance between the tip of the working electrode and the capillary outlet was adjusted to ~25 μm by comparison with the bore (25 μm) in the capillary while being viewed under a microscope. The electropherograms were recorded using a chart record (XWTD-164, Shanghai Dahua Instrument Factory, China). CE was performed in a 100 mmol/L borate buffer (pH 8.7) used as the running buffer at a separation voltage of 18 kV. The potential applied to the working electrode was 0.90 V (versus SCE). Before each run in CE experiments, the capillary was sequentially rinsed with 0.1 mol/L hydrochloric acid, doubly distilled water, and 0.1 mol/L sodium hydroxide, 3 min for each, and then with running buffer until the current inside the capillary was stable. This was important to get a reproducible electroosmotic flow (EOF). Samples were injected electrokinetically at 18 kV for 8 s.

Reagents and Solutions. Catechin, apigenin, luteolin, rosmarinic acid, and caffeic acid were purchased from Sigma (St. Louis, MO); ferulic acid was obtained from Shanghai Reagent Factory (Shanghai, China). Stock solutions of the analytes (1.0×10^{-3} g/mL each) were prepared in anhydrous ethanol (A.R. grade) and were diluted to the appropriate concentration with running buffer (100 mmol/L, $Na_2B_4O_7$–H_3BO_3, pH 8.7) for the construction of calibration curves. Before use, all solutions were filtered through 0.22 μm nylon filters.

Sample Preparation. The leaves and seeds of *P. frutescens* L. were collected from the campus of the Medical Center of Quanzhou Normal University (Fujian, China) and were kindly identified by Professor J.

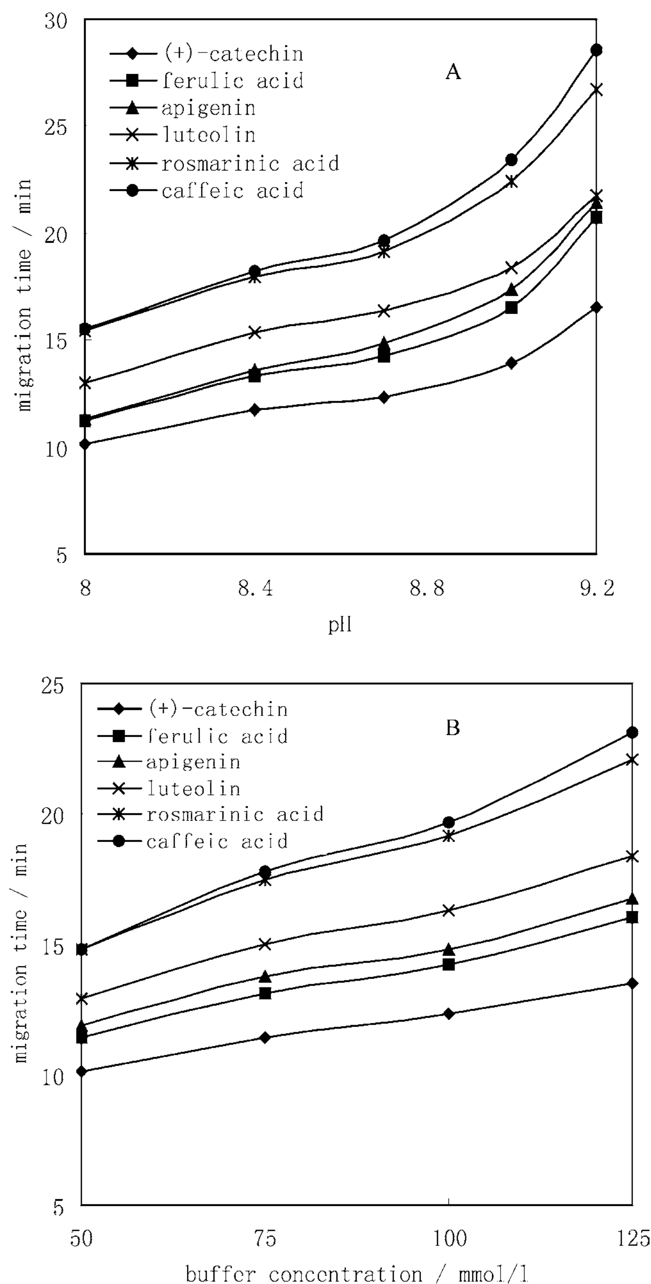

Figure 3. Effect of buffer pH (**A**) and concentration (**B**) on the migration time of (+)-catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid. Working potential: 0.90 V (vs SCE); other conditions were as in **Figure 2**.

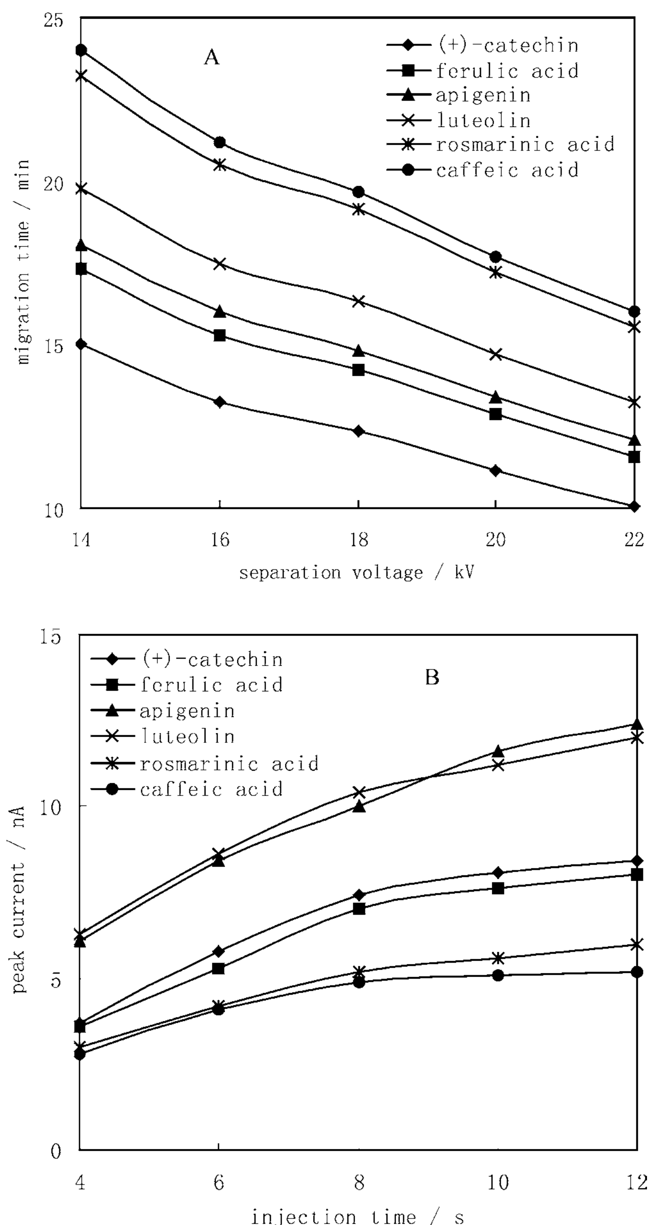

Figure 4. Effect of separation voltage on the migration time of (+)-catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid (**A**) and effect of injection time on the analytes' peak current (**B**). Working potential: 0.90 V (vs SCE); other conditions were as in **Figure 2**.

Lu (Department of Pharmacology, Medicinal Center of Quanzhou Normal University, Fujian, China).

Three batches of leaves and seeds of *P. frutescens* L. were collected from the same plants and dried in air. The batch numbers (040715, 040805, and 040825 for leaves; 040905, 040925, and 041015 for seeds) were based on the collection dates. All samples were dried at 60 °C for 2 h and then ground into powder in a mortar. An accurate weight amount of the powders (2.0000 ± 0.0005 g) was extracted with 10 mL of 80% ethanol for 2 h in an ultrasonic bath at ambient temperature. Next, each of the samples was filtered through filter paper first and then through a 0.22 μm nylon filter. Sample solutions were stored at 4 °C in the dark. The sample solutions were diluted using the running buffer just prior to CE analysis.

RESULTS AND DISCUSSION

Hydrodynamic Voltammograms (HDVs). In amperometric detection the potential applied to the working electrode directly affects the sensitivity, detection limit, and stability. Therefore, the effect of the working electrode potential on the peak current (calculated by measuring the peak height) of the analytes was investigated by ploting peak currents versus detection potential to obtain optimum detection. As shown in **Figure 2**, all of the analytes display similar profiles, with rapid increase of the response starting at 0.60V (versus SCE). When the applied potential passes 0.90V (versus SCE), however, the peak currents of the analytes increase much more slowly. Although an applied potential of >0.90 V (versus SCE) results in higher peak currents, both the baseline noise and the background current increase substantially. The high background current leads to an unstable baseline, which is a disadvantage for sensitive and stable detection. The potential applied to the working electrode was, therefore, maintained at 0.90 V (versus SCE), where the background current is not too high and the signal-to-noise (S/N) ratio is the highest.

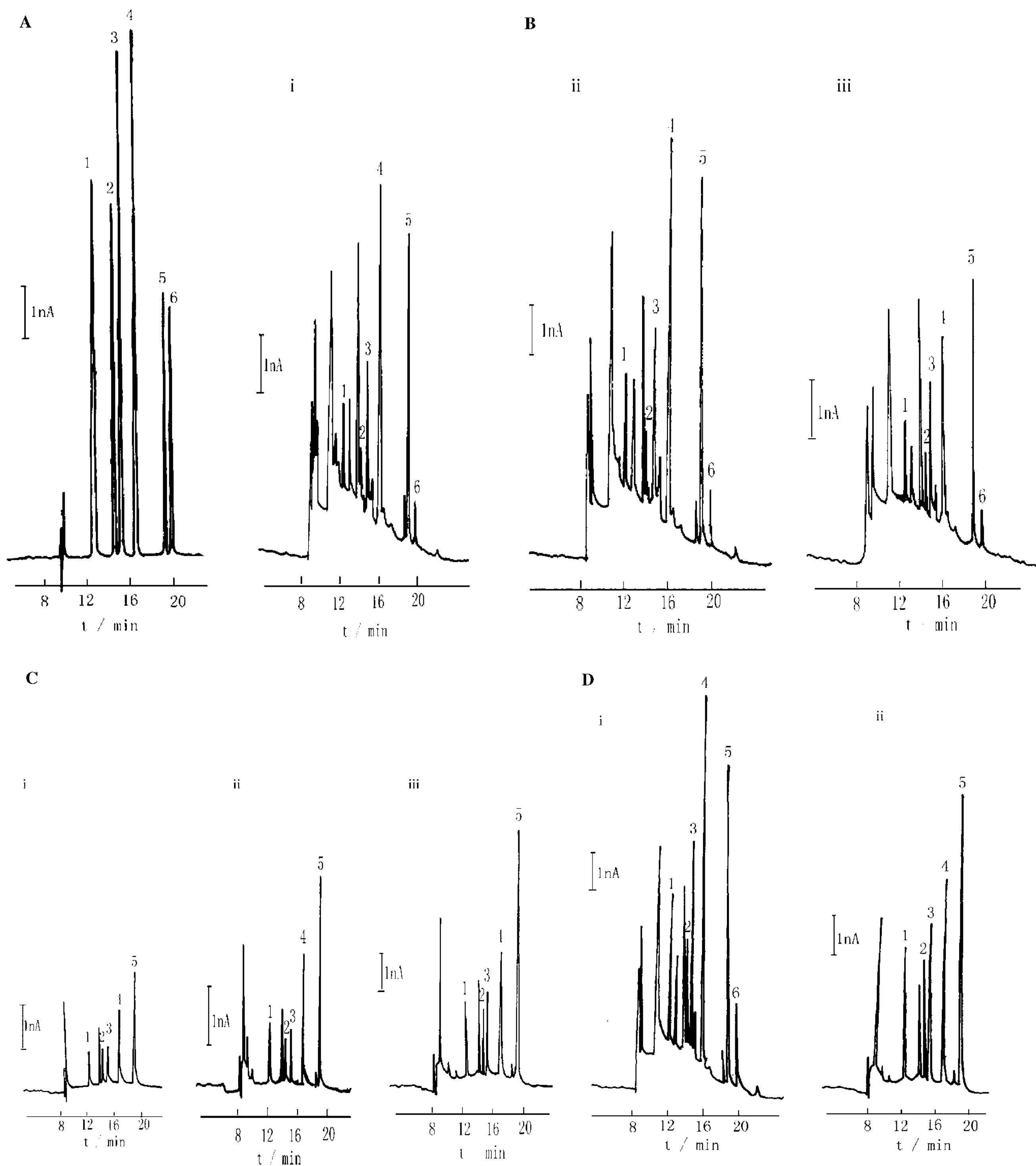

Figure 5. Electropherogram of a standard mixture solution [2.0×10^{-5} g/mL for (+)-catechin, ferulic acid, apigenin, and luteolin, 5.0×10^{-5} g/mL for rosmarinic acid and caffeic acid] (**A**); typical electropherograms of *P. frutescens* L. leaves (**B**) (i, 040715; ii, 040805; iii, 040825); typical electropherograms of *P. frutescens* L. seeds (**C**) (i, 040905; ii, 040925; iii, 041015); and electropherograms of the spiked extracts (**D**) (i, 040805; ii, 041015; for catechin, ferulic acid, apigenin, and luteolin, the concentration added is 4.0×10^{-6} g/mL; for rosmarinic acid and caffeic acid, the concentration added is 1.0×10^{-5} g/mL). Dilution for all samples: 1:25. Peak identification: (1) (+)-catechin; (2) ferulic acid; (3) apigenin; (4) luteolin; (5) rosmarinic acid; (6) caffeic acid. Working potential: 0.90 V (vs SCE); other conditions were as in **Figure 2**.

Effects of pH and Concentration of the Buffer. The acidity and concentration of the running buffer play an important role in CE for its effect on zeta potential (ζ) and electroosmotic flow (EOF), as well as the overall charge of all the analytes, which affect the migration time and the separation of the analytes. Therefore, it is important to study their influences on CE to obtain optimum separations. The effect of the running buffer pH on the migration time of the investigated analytes is shown in **Figure 3A**. The running buffer was 100 mmol/L borate buffer at five different pH values (8.0, 8.4, 8.7, 9.0, and 9.2). As shown in **Figure 3A**, the resolution of apigenin and ferulic acid is poor at pH 8.0. When the running buffer pH increases, the resolution

of all compounds is improved, with migration time increased. At pH 8.7 the analytes can be well separated; it is also found that the peak current is low, and the peak shape becomes poor when the pH value exceeds 8.7. The analytes were migrated in the dissociated forms; the electrostatic force was unchanged with pH but the electroosmotic flow was decreased as the pH was increased, which results in the gradual increase of retention time with increasing pH values for all of the analytes. When the pH is >8.7, both the carboxyl and phenolic hydroxyl groups of the analytes are dissociated to form the carboxylate−phenolate divalent anions, which were more strongly pulled back by electrostatic force. pH is not an isolated parameter; it also influences the ionic strength of the solution, and the velocity of EOF was decreased with increasing concentration of electrolyte. Therefore, 100 mmol/L borate buffer at pH 8.7 was chosen as the running buffer in consideration of the peak current, resolution, and analytical time.

Increasing the running buffer concentrations (ionic strength) generally decreases the EOF, thus lengthening the migration times. The reduced EOF with the increasing buffer concentrations is due to the decrease of the thickness of the diffusion of the double layer at the inner capillary wall. Thus, the resolution is also increased with the increasing ionic strength. Results show that higher buffer concentrations led to longer migration times and decreasing electrophoretic mobility. However, higher buffer concentrations (>100 mmol/L) also have a negative effect on the detection limits because the peak currents of all analytes decrease and the effect of Joule heat becomes more pronounced, which is in accordance with the report by Tsuda et al. (*27*) describing that the velocity of the electroosmotic flow was decreased with increasing concentration of electrolyte, therefore resulting in the decrease of peak currents. At 100 mmol/L, all compounds were well separated with the total migration time of 20 min and the migration order of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid, respectively (**Figure 3B**). Consequently, 100 mmol/L borate buffer (pH 8.7) was selected as a compromise among resolution, efficiency, and analysis time and employed for subsequent optimization.

Effects of Separation Voltage and Injection Time. The separation voltage affects the electric field strength, which in turn affects the EOF and the migration velocity of charged particles, which determine the migration time of the analytes. Moreover, higher separation voltage may result in higher Joule heating. The effect of separation voltage on the migration time of the analytes is shown in **Figure 4A**: increasing the voltage gives shorter migration times but also increases the background noise, resulting in a higher detection limit. Although the resolution of analytes can be improved to some extent, too low a separation voltage will increase the analytical time considerably, which in turn causes severe peak broadening. On the basis of experiments, 18 kV was chosen as the optimum voltage to accomplish a good compromise.

The effect of injection time on CE separation was investigated by varying the sampling time (4, 6, 8, 10, and 12 s at a voltage of 18 kV, as shown in **Figure 4B**). It was found that both the peak current and the peak width increase with increasing sampling time. When injection time is >8 s, the peak current levels off and peak broadening becomes severe. In this experiment, 8 s (18 kV) was selected as the optimum injection time.

Through the experiments above, the optimum conditions for the determination of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid were decided. The typical electropherogram for a standard solution of the analytes is shown in **Figure 5A**, as we can see baseline separation could be achieved within 20 min.

Table 1. Injection, Intraday, and Interday Precision of the Analytes in the Standard Solution[a]

	precision (%RSD)					
	peak height			migration time		
compound	injection ($n = 7$)	intraday ($n = 10$)	interday ($n = 6$)	injection ($n = 7$)	intraday ($n = 10$)	interday ($n = 6$)
(+)-catechin	2.8	3.0	3.5	0.8	1.0	1.2
ferulic acid	2.5	2.4	3.8	0.6	0.8	1.3
apigenin	2.0	3.5	3.2	1.2	1.3	1.8
luteolin	3.2	3.0	2.8	1.0	0.8	1.5
rosmarinic acid	2.6	2.8	4.5	0.6	0.7	0.9
caffeic acid	1.8	2.5	4.8	0.8	0.9	1.6

[a] Working potential is 0.90 V (vs SCE). Other conditions were as in **Figure 2**.

Precision, Linearity, and Detection Limits. The precision of the method was determined by measuring the repeatability of injection ($n = 7$), intraday ($n = 10$), and interday ($n = 6$) analyses in the standard mixture solution of 2.0×10^{-5} g/mL (+)-catechin, ferulic acid, apigenin, and luteolin and 5.0×10^{-5} g/mL rosmarinic acid and caffeic acid under the optimum conditions in this experiment. The results are listed in **Table 1**. The relative standard deviations (RSDs) of peak current varied from 1.8 to 4.8%, and the migration time varied from 0.6 to 1.8% for the analytes.

A series of standard solutions of (+)-catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid ranging from 1.0×10^{-7} to 1.0×10^{-3} g/mL in concentration were tested to determine the linearity of the determination. Results from regression analysis of calibration curves are listed in **Table 2**. Sensitivity was evaluated by determination of the limit of detection (LOD) and the limit of quantification (LOQ) (see **Table 2**). The LOD was determined as the concentration with a S/N ratio of 3, whereas the LOQ was the concentration with a S/N ratio of 10. The LOD and LOQ for rosmarinic acid (*28*) in HPLC are 2.5 and 5.5 μg/mL, respectively, whereas in CE-ED they are 1 and 3 μg/mL, respectivelyy. The LODs for luteolin and apigenin are 0.25 and 0.3 μg/mL, respectively, and the LOQs for luteolin and apigenin in HPLC are both 0.35 μg/mL (*29*). As we can see from **Table 2**, the sensitivity of CE-ED on similar flavonoids is comparable to that of HPLC.

Sample Analysis and Recovery. Under optimum conditions, the determination of (+)-catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid in real samples was carried out according to the procedures described earlier. Typical electropherograms of the leaves and the seeds of *P. frutescens* L. are shown in panels **B** and **C**, respectively, of **Figure 5**. The migration times in CZE in fused-silica capillary tubes can be varied ∼1−2% due to the nonrepeatable EOF, which is caused by the unstable surface condition of the inner wall of the tubes and the change in the effective electric field strength (*30*). The components in natural plants are complicated, and the matrix of real samples is different from that of standard solutions. Additionally, migration times are highly sensitive to slight changes in buffer pH and ionic strength. It is observed in the experiment that the migration time of the phenolic compounds in different samples (**Figure 5B**,**C**) shifted from those of the standard solutions (**Figure 5A**), which made the identification by migration time unreliable; further identification of the peaks is confirmed by spiking experiments, and typical electropherograms of spiked extracts are shown in **Figure 5D**. The assay results are listed in **Table 3**. The assay results in this work agree with those obtained by HPLC (*22*, *31*), in which the amount of

Table 2. Results of Regression Analysis on Calibration and Detection Limits[a]

compound	regression equation $y = a + bx$[b]	correlation coefficient	linear range (μg/mL)	LOD[c] (g/mL)	LOQ[d] (g/mL)
(+)-catechin	$Y = 372286x - 0.03$	$R = 0.9997$	1–1000	5×10^{-7}	1×10^{-6}
ferulic acid	$Y = 354286x - 0.02$	$R = 0.9997$	1–1000	5×10^{-7}	1×10^{-6}
apigenin	$Y = 501714x - 0.08$	$R = 0.9995$	1–1000	2×10^{-7}	5×10^{-7}
luteolin	$Y = 524000x - 0.12$	$R = 0.9996$	1–1000	2×10^{-7}	4×10^{-7}
rosmarinic acid	$Y = 104571x - 0.05$	$R = 0.9996$	5–1000	1×10^{-6}	3×10^{-6}
caffeic acid	$Y = 96057x + 0.095$	$R = 0.9996$	5–1000	1×10^{-6}	5×10^{-6}

[a] Working potential is 0.90 V (vs SCE). Other conditions were as in **Figure 2**. [b] Where *y* and *x* are the peak current (nA) and concentration of the analytes (g/mL), respectively. [c] Limit of detection, corresponding to concentrations giving signal-to-noise ratio of 3. [d] Limit of quantification, corresponding to concentrations giving signal-to-noise ratio of 10.

Table 3. Assay Results of the Analytes in *P. frutescens* L. Leaves and Seeds ($n = 3$, 10^{-5} g/g)[a]

	P. frutescens L. leaves			*P. frutescens* L. seeds		
compound	040715	040805	040825	040905	040925	041015
(+)-catechin	10.9	18.8	6.5	5.3	9.3	13.4
ferulic acid	4.8	9.3	6.9	5.7	6.6	12.9
apigenin	14.4	17.6	11.9	4.0	5.9	11.6
luteolin	31.5	37.8	15.7	8.1	14.3	16.0
rosmarinic acid	143.5	179.5	114.5	63.7	116.1	162.5
caffeic acid	22.1	30.8	15.7	NF[b]	NF	NF

[a] Working potential is 0.90 V (vs SCE). Other conditions were as in **Figure 2**. [b] Not found.

rosmarinic acid from the leaves of *P. frutescens* L. ranged from 0.99 to 7.6 mg/g. Most research work emphasizes particularly the analysis of the rosmarinic acid in *P. frutescens* L. In fact, synergistic effects between various constituents may exist. This is the first time that the contents of the analytes in *P. frutescens* L. are determined simultaneously.

By comparing the electropherograms of the standard solution (**Figure 5A**) and spiking experiments, it was found that all samples has identical profiles (except for caffeic acid, which is not found in the seeds) on the basis of relative peak heights and migration times. The active ingredients, namely, catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid, were found in the leaves of *P. frutescens* L., so all six constituents can be defined as common peaks in the fingerprint of *P. frutescens* L. leaves under the selected conditions. Caffeic acid is not found in the seeds of *P. frutescens* L., and the electropherograms are relatively simple compared with those of the leaves. However, the content of individual analytes varied greatly in different samples, indicating that growth stages and the parts of plant had great impact on the contents of the constituents investigated. As we can see from **Table 3**, the content of the flavonoids became lower when the leaves became older, whereas in seeds the content of flavonoids increased with ripeness. Proper harvest time should be the time when the content of the flavonoids in the samples is relatively high. The proposed method provided a simple, reliable way not only to determinte the active compounds in the *P. frutescens* L. but also to select the proper time to harvest the medicinal plants.

The recovery and reproducibility experiments under the optimum conditions were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by standard addition method: accurate amounts of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid were added to the diluted extracts of the samples, and the results obtained from *P. frutescens* L. leaves (batch 040805) are listed in **Table 4**. The recovery for all samples varied from 92 to 106%. The above assay results indicate that this method is accurate, sensitive, and reproducible, providing a useful quantitative method for the analyses of active ingredients in *P. frutescens* L.

Table 4. Results of the Recovery of This Method ($n = 3$, 10^{-5} g/mL)[a]

compound	original amount	added amount	found amount	recovery (%)	RSD (%)
(+)-catechin	18.8	10.0	28.4	96	3.5
ferulic acid	9.3	10.0	18.5	92	2.8
apigenin	17.6	10.0	27.1	95	3.0
luteolin	37.8	30.0	66.0	94	3.6
rosmarinic acid	179.5	100	277.5	98	3.8
caffeic acid	30.8	30	62.0	104	3.2

[a] Working potential is 0.90 V (vs SCE). Other conditions were as in **Figure 2**.

Conclusion. This work presents the first application of CE-ED for the determination of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid in *P. frutescens* L. The realization of such analysis is more economical in comparison to HPLC because the consumption of electrolytes is negligible, and the use of organic solvents is practically avoided, and the capillary is much easier to wash. The reproducibility of quantitative analysis is satisfactory. ED coupled with CE enables selective and sensitive detection of the electroactive constituents in the crude drug, and simplification of the electropherograms for only electroactive constituents could be detected. Samples do not need derivatization before determination because the analytes could be directly detected on the working electrode. It is concluded that CE-ED is a powerful technique for the constituents and fingerprint study of natural plants and has become an alternative, competitive, and supplementary method for HPLC, because of its special attributes.

LITERATURE CITED

(1) Shetty, K. Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochem.* **2004**, *39*, 789−803.

(2) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481−504.

(3) Pitchersky, E.; Gang, D. R. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends Plant Sci.* **2000**, *5*, 459−445.

(4) Douglas, C. J. Phenylpropanoid metabolism and lignin biosynthesis: from weeds to trees. *Trends Plant Sci.* **1996**, *1*, 171−178.

(5) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(6) Lemanska, K.; Szymusiak, H.; Tyrakowsla, B.; Zielinski, R.; Soffers, A. E. M.; Rietens, I. M. C. M. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biol. Med.* **2001**, *31*, 869−881.

(7) Henderson, B. E.; Ross, R. K.; Pike, M. C. Toward the primary prevention of cancer. *Science* **1991**, *254*, 1131−1138.

(8) Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

(9) Smith, M. A.; Perry, G.; Richey, P. L.; Sayre, L. M.; Anderson, V. E.; Beal, M. F.; Kowall, N. Oxidative damage in Alzheimer's. *Nature* **1996**, *382*, 120−121.

(10) Peterson, J.; Dwyer, J. Flavonoids: dietary occurrence and biochemical activity. *Nutr. Res.* **1998**, *18*, 1995−2018.

(11) Materska, M.; Perucka, I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *J. Agric. Food Chem.* **2005**, *53*, 1750−1756.

(12) Saito, M.; Hosoyama, H.; Ariga, T. Antiulcer activity of grape seed extract and procyanidins. *J. Agric. Food Chem.* **1998**, *46*, 1460−1464.

(13) Sökmen, M.; Serkedjieva, J.; Daferera, D.; Gulluce, M.; Polissiou, M.; Tepe, B.; Akpulat, H. A.; Sahin, F.; Sokmen, A. In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. *J. Agric. Food Chem.* **2004**, *52*, 3309−3312.

(14) Mukherjee, S. K. A revision of Labiateae of the Indian empire. *Records Bot. Surv., India* **1940**, *14*, 186.

(15) Liu, J. H.; Steigel, A.; Reininger, E.; Bauer, R. Two new prenylated 3-benzoxepin derivatives ascyclooxygenase inhibitors from *Perilla frutescens* var. *acuta*. *J. Nat. Prod.* **2000**, *63*, 403−405.

(16) Nakamura, Y.; Ohto, Y.; Murakami, A.; Ohigashi, H. Superoxide scavenging activity of rosmarinic acid from *Perilla frutescens* Britton Var. *acuta* f. *viridis*. *J. Agric. Food Chem.* **1998**, *46*, 4545−4550.

(17) Nakazawa, T.; Ohsawa, K. Metabolites of orally administered *Perilla frutescens* extract in rats and humans. *Biol. Pharm. Bull.* **2000**, *23*, 122−127.

(18) Ishikura, N. Anthocyanins and flavones in leaves and seeds of *Perilla* plant. *Agric. Biol. Chem.* **1981**, *45*, 1855−1860.

(19) Aritomi, M.; Kumori, T.; Kawasaki, T. Cyanogenic glycosides in leaves of *Perilla frutescens* var. *acuta*. *Phytochemistry* **1985**, *24*, 2438−2439.

(20) Masahiro, T.; Risa, M.; Harutaka, Y. Novel antioxidants isolated from *Perilla frutescens* Britt. var. *crispa* (Thunb) Hand.-Mazz. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 1093.

(21) Osakabe, N.; Yasuda, A.; Natsume, M.; Sanbongi, C.; Kato, Y.; Osawa, T.; Yoshikawa, T. Rosmarinic acid, a major polyphenolic component of *Perilla frutescens*, reduces lipopolysaccharide (lps)-induced liver injury in D-galactosamine (D-galn) sensitized mice. *Free Radical Biol. Med.* **2002**, *33*, 798−806.

(22) Makino, T.; Furuta, Y.; Wakushima, H.; Fujii, H.; Saito, K.; Kano, Y. Anti-allergic effect of *Perilla* frutescens and its active constituents. *Phytother. Res.* **2003**, *17*, 240−243.

(23) Miliauskas, G.; van Beek, T. A.; de Waard, P.; Venskutonis, R. P.; Sudholter, E. J. R. Identification of radical scavenging compounds in *Rhaponticum carthamoides* by means of LC-DAD-SPE-NMR. *J. Nat. Prod.* **2005**, *68*, 168−172.

(24) Proestos, C.; Chorlanopoulos, N.; Nychas, G. J. E., Komaitis, M. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem.* **2005**, *53*, 1190−1195.

(25) Fang, Y. Z.; Fang, X. M.; Ye, J. N. Determination of polyhydroxy antibiotics by capillary electrophoresis with amperometric detection at a copper electrode. *Chem. J. Chin. Univ.* **1995**, *16*, 1514−1518.

(26) Ye, J. N.; Baldwin, R. P. Determination of amino acids and peptides by capillary electrophoresis and electrochemical detection at a copper electrode. *Anal. Chem.* **1994**, *66*, 2669−2674.

(27) Tusda, T.; Nomura, K.; Nakagawa G. Open-tubular microcapillary liquid-chromatography with elctroosmosis flow using a UV detector. *J. Chromatogr.* **1982**, *248*, 241−247.

(28) Parejo, I.; Viladomat, F.; Bastida, J.; Codina, C. Development and validation of a high-performance liquid chromatographic method for the analysis of antioxidative phenolic compounds in fennel using a narrow bore reversed phase C18 column. *Anal. Chim. Acta* **2004**, *512*, 271−280.

(29) Torre-Carbot, K. D. L.; Jauregui, O.; Gimeno, E.; Castellote, A. I.; Lamuela-Raventoas, R. M.; Loapez-Sabater, M. C. Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. *J. Agric. Food Chem.* **2005**, *53*, 4331−4340.

(30) Liang, H. R.; Siren, H.; Riekkola, M. L.; Vuorela, P.; Vuorela, H.; Hiltunen, R. Optimized separation of pharmacologically active flavonoids from *Epimedium* species by capillary electrophoresis. *J. Chromatogr. A* **1996**, *746*, 123−129.

(31) Nakamura, Y.; Ohto, Y.; Murakami, A.; Ohigashi, H. Superoxide scavenging activity of rosmarinic acid from *Perilla frutescens* Britton Var. *acuta* f. *viridis. J. Agric. Food Chem.* **1998**, *46*, 4545−4550.

Received for review June 9, 2005. Revised manuscript received August 14, 2005. Accepted August 17, 2005. We are grateful for financial support provided by the National Science Foundation of China (Grants 20375013, 20335040, and 20475012).

JF051360E