Simultaneous Determination of Active Ingredients in Ethnomedicine *Gaultheria leucocarpa var. yunnanensis* and its Medicinal Preparation by Capillary Electrophoresis with Electrochemical Detection

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Abstract

A simple and rapid capillary electrophoresis (CE) with electrcochemical detection (ED) method has been established for the simultaneous determination of seven active ingredients in the stems and roots of *Gaultheria leucocarpa var. yunnanensis* and its medicinal preparation, including (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid. The effects of working potential, pH, and concentration of running buffer, separation voltage, and injection time on CE–ED are systematically investigated. Under the optimum conditions, the seven analytes could be completely separated within 23 min in a borax running buffer (pH 8.7). A good linear relationship is obtained over three orders of magnitude with detection limits (signal-to-noise ratio = 3) ranging from 5×10^{-8} g/mL to 3×10^{-7} g/mL for the analytes. The proposed method is successfully used in the analysis of real samples after a relatively simple extraction procedure, and the assay results are satisfactory.

Introduction

Gaultheria leucocarpa var. yunnanensis, commonly known as Tou guxiang, belongs to the family of Ericaceae and is widely distributed in the southern regions of the Changjiang River in china, especially in Yunnan and Guizhou provinces (1). It is used in both traditional Chinese medicine and in the minority folklore medicine for the cure of rheumatic arthritis, swelling pain, trauma, chronictracheitis, cold and vertigo, and acute and chronic prostatitis (1–3). All the different parts of *Gaultheria leucocarpa var. yunnanensis* have different medical functions. The dry roots of *Gaultheria leucocarpa var. yunnanensis* is claimed to possess the functions of eliminating dampness, promoting blood circulation, regulating breathing, and relieving pains, and the stems and leaves are mainly used to treat tetter (1). Its preparation can be used in series of remedies for rheumatic arthritis and lumbar hyperosteogeny (4). In recent years, many investigations have been made on the chemical constituents and biological activities of *Gaultheria leucocarpa var.yunnanensis*, which contains some important compounds including flavonoids, diterpenoids, triterpenoids, organic acids, coumarins, and sterols (2,5–7). Some of these have attracted further attention

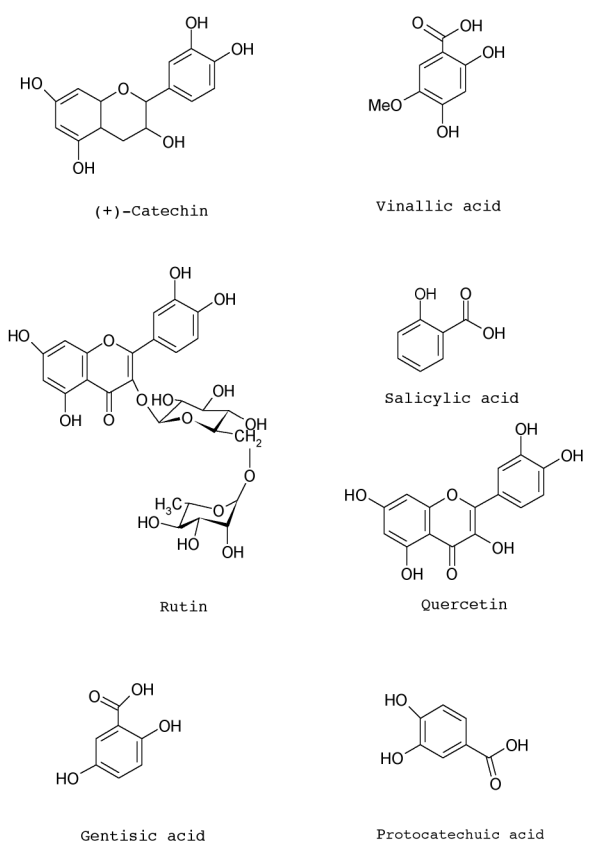

Figure 1. Molecular structures of the analytes: (+)-catechin, rutin, gentisic acid, vanillic acid, salicylic acid, quercetin, and protocatechuic acid.

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because of their antiviral, anti-inflammatory (8) and antioxidant activities for scavenging radicals (9). In this study, (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid (the molecular structures of these compounds are shown in Figure 1) in *Gaultheria leucocarpa var. yunnanensis* were of particular interest because of their various pharmacological activities. For example, rutin has a anti-inflammatory effect and exerted a partial inhibitory effect on degranulation of fMet-Leu-Phe/CB-stimulated neutrophils (10). Salicylic acid is used as a topical keratolytic and as an external antiseptic and antifungal (11). Catechin, as flavonoids, treat vascular, viral, gastrointestinal, microbial, and inflammatory illnesses (12). Quercetin has important medical functions upon breast cancer by inhibiting PI and PIP kinases (13).

Simple, economical, and efficient methods for the simultaneous analysis and quantitative measurement of multiple constitutes in *Gaultheria leucocarpa var. yunnanensis* are necessary in order to establish a quality standard for the drug. In 2000, the U.S. Food and Drug Administration (FDA) published a draft of 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 (14). However, because of the composition diversity, analysis of the active ingredients of *Gaultheria leucocarpa var.yunnanensis* is a challenging task. Work published in recent years has shown that high-performance liquid chromatography has been applied as a method of choice in the analysis of salicylic acid and gaulthersides present in *Gaultheria leucocarpa var. yunnanensis* (15,16), but there has been no comprehensive study dealing with the simultaneous determination of seven active ingredients. Therefore, a simple and rapid method for the separation and determination of the investigated compounds has been developed. Capillary electrophoresis (CE) with electrochemical detection (ED) is a powerful technique that affords rapid and high-resolution separations (10^4 to 10^6 theoretical plates) while requiring only a few microliters of the sample.

In this work, the focus is on CE with ED for the determination of seven ingredients: (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid in the roots and stems of *Gaultheria leucocarpa var. yunnanensis* and its preparation.

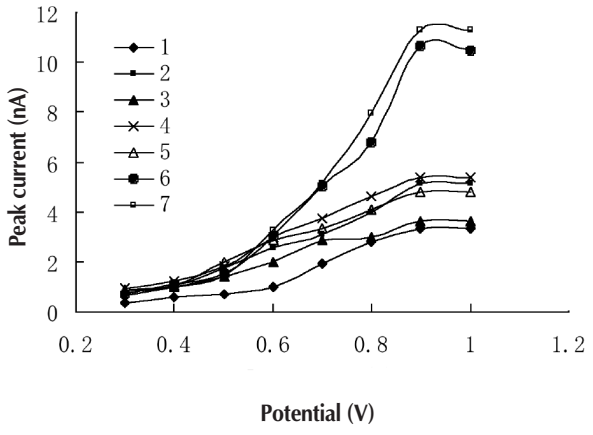

Figure 2. Hydrodynamic voltammograms for (+)-catechin, 1; rutin, 2; gentisic acidl, 3; vanillic acid, 4; salicylic acid, 5; quercetin, 6; and protocatechuic acid, 7. Experimental conditions: Fused-silica capillary, 25-µm i.d. × 70-cm; working electrode, 300-µm diameter carbon disk electrode; running buffer, 60 mmol/L borate buffer (pH 8.7); separation voltage, 16 kV; electrokinetic injection, 8 s (16 kV); concentration, 2.0×10^{-5} g/mL for all the analytes.

Experimental

Apparatus

The laboratory-built CE–ED system was constructed and is similar to that described previously (17,18). A high-voltage (± 30 kV) power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided voltage between the ends of the capillary. The separation was undertaken in a 70 cm × 25-µm i.d. and 360-µm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ). A three-electrode electrochemical cell consisted of a 300-µm diameter carbon disc working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. An electrochemical Analyzer CHI 830B (CH Instruments, Austin, Texas) was used as the amperometric detector, which was connected to a high-performance PC installed with the Windows XP operating system. Before use, the carbon disc electrode was polished with emery paper and sonicated in doubly distilled water, and carefully positioned opposite the outlet of the capillary. The distance between the tip of the working electrode and the capillary outlet was as close as possible so that the CE effluent directly impinged upon the electrode surface. The capillary was rinsed with 0.1 mol/L NaOH 30 min before use. The injector electrode was kept at high positive voltage, and the electrochemical cell for detection was kept at ground. All electrodes were enclosed in a plexiglass box with a safety switch wired to turn off the power supply whenever the box was opened (19).

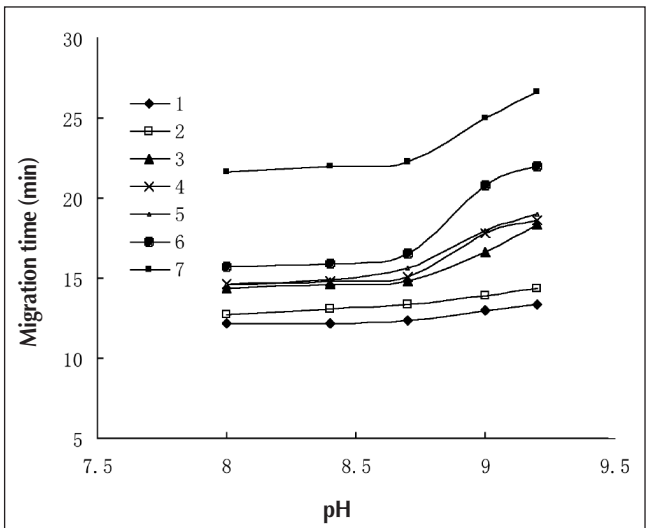

Figure 3. Effect of buffer pH on the migration time. Working potential, + 0.90 V (vs. SCE); other conditions and compounds same as in Figure 2.

Reagents

Reagents were of analytical grade and solvents were of chromatographic purity. (+)-Catechin were purchased from Sigma (St. Louis, MO). Rutin and gentisic acid were purchased from Aldrich (Milwaukee, WI); vanillic acid, salicylic acid, quercetin, and protocatechuic acid were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The roots and stems of the herb *Gaultheria leucocarpa var. yunnanensis* were purchased from a drug store in Loudi (Hunan, China). *Gaultheria leucocarpa var. yunnanensis* syrup was obtained from Guizhou Tongji Tang Pharmacal Company (Guizhou, China). Stock solutions of (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid (2.0×10^{-3} g/mL, each) were prepared in anhydrous ethanol, stored in the dark at 4°C, and diluted to the desired concentrations with the running buffer (60 mmol/L borate buffer, pH = 8.7). Before use, all solutions were filtered through 0.22-µm nylon filters.

Sample preparation

Approximately 3 g of roots or stems of each sample were ground into powder in a mortar and accurately weighed. Each weighed sample was extracted with 8 mL 95% ethanol and maintained in the dark at 4°C for 18 h, before being extracted by ultrasonication for 1 h. The samples were filtered through filter paper and a 0.22-µm nylon membrane syringe filter in turn. Next, a total of extracted solutions was diluted with 95% ethanol to 10 mL. Then, 0.1 mL root and 0.5 mL stem of each sample solution were again diluted with the running buffer to 1 mL. *Gaultheria leucocarpa var. yunnanensis* syrup was directly filtered through a 0.22-µm syringe filter, and then a 0.1-mL portion of filtrate was diluted with the running buffer to 1 mL. After being filtered through a 0.22-µm syringe filter, all solutions could be injected directly to the CE–ED system with high voltage electric injection for analysis. Before use, all sample solutions were stored in the dark at 4°C.

Results and Discussion

Effect of the potential applied to the working electrode

The potential applied to the working electrode directly affects the sensitivity, detection limit, and stability of this method. Therefore, hydrodynamic voltammetry was investigated to obtain optimum detection conditions. As shown in Figure 2, when the applied potential exceeded + 0.60 V [versus the satu-

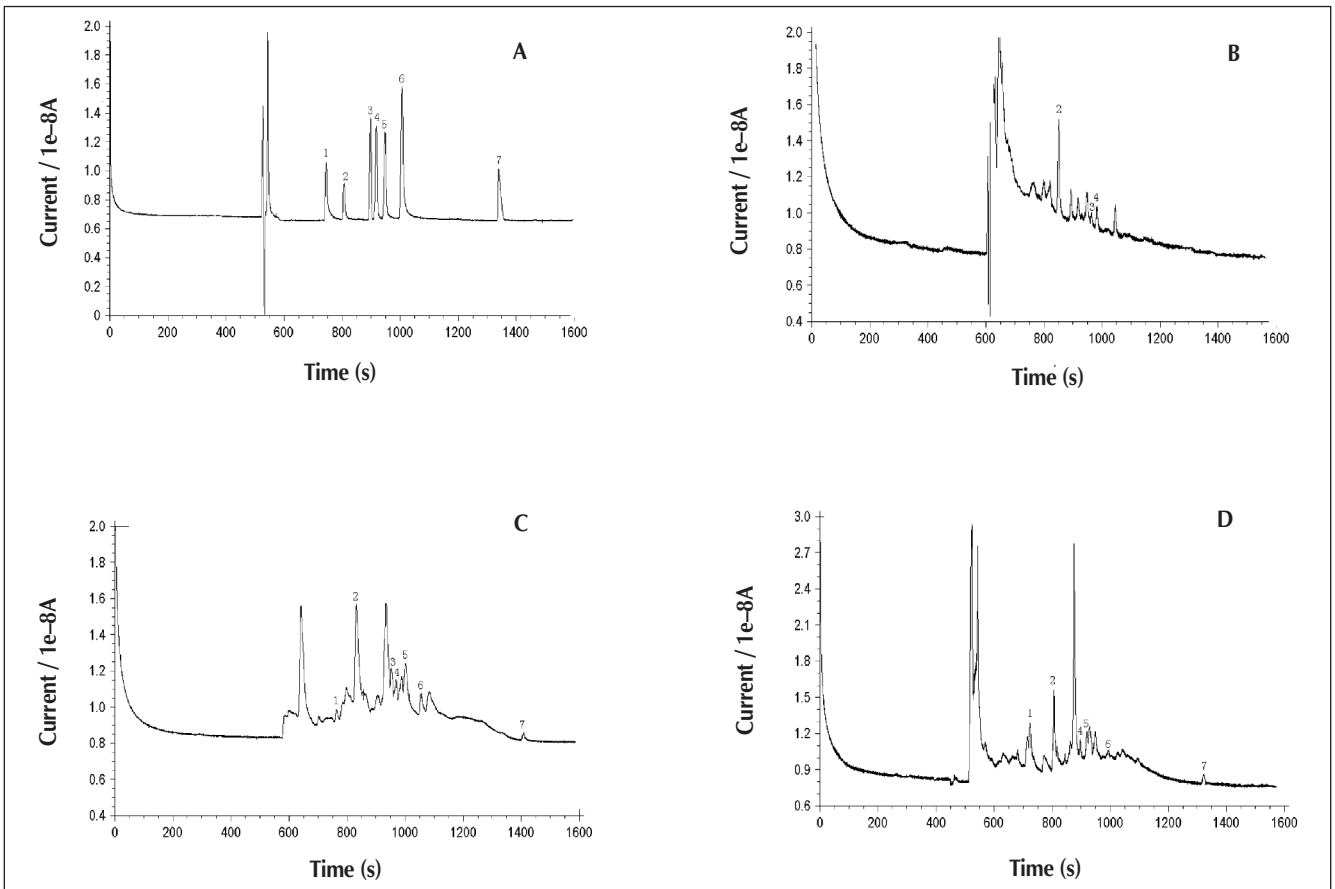

Figure 4. Electropherograms of standard solution (10.8 µg/mL (+)-catechin, 17.2 µg/mL rutin, 21.5 µg/mL gentisic acid, 10.8 µg/mL vanillic acid, 10.8 µg/mL salicylic acid, 17.2 µg/mL quercetin, and 34.4 µg/mL protocatechuic acid) (A); a sample solution of dried Gaultheria leucocarpa var. yunnanensis stem (B); a sample solution of dried *Gaultheria leucocarpa var. yunnanensis* root (C); and a sample solution of Gaultheria leucocarpa var. yunnanensis syrup (D). Working electrode potential is + 900 mV (versus SCE); other conditions are the same as in Figure 2. Peak identification: (+)-catechin, 1; rutin, 2; gentisic acid, 3; vanillic acid, 4; salicylic acid, 5; quercetin, 6; and protocatechuic acid, 7.

rated calomel electrode (SCE)], all analytes could generate oxidation current at the working electrode. When the applied potential was between 0.60 and 0.90 V (versus SCE), the peak current of each analyte increased with an increasing applied potential; however, when the applied potential exceeded 0.90 V (versus SCE), both the baseline noise and the background current increased strongly, resulting in an unstable baseline that was not conducive to sensitive and stable detection. Therefore, the applied potential to the working electrode was maintained at + 0.90V (vs. SCE), where the background current was not too high and the signal-to-noise (S/N) ratio was the highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

Effects of the pH value and the buffer concentration

Three buffers, namely phosphate, borate, and a phosphate–borate buffer, were tested. The experimental results showed that under the same conditions, the peak current of analytes in the borate system was much higher and more stable than that in phosphate and phosphate–borate systems. So the borate buffer was employed as the running buffer in this work. The pH dependence of the migration time was investigated in the pH range of 8.0–9.2. As shown in Figure 3, the migration time of all analytes increased with an increasing pH value, and separation of the analytes could be achieved between pH 8.7 and 9.2. When the pH was lower than 8.7, syringic acid could not be separated from vanillic acid. Moreover, a higher pH value resulted in a long analysis time and easy oxidation of the analytes. Therefore, pH 8.7 was selected as the optimum pH value. Along with the pH value, the running buffer concentration is also an important parameter, as it affects peak height and theoretical plate number. The effect of the running buffer concentration on migration time was studied, and the optimum running buffer concentration was 60 mmol/L.

Effects of separation voltage and injection time

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of electroosmotic flow and the migration velocity of the analytes, which in turn determines the migration time of the analytes. A higher separation voltage gives a shorter migration time for the majority of these ingredients. However, when the separation voltage exceeded 16 kV, baseline noise became larger. Therefore the optimum separation voltage selected was 16 kV, at which good separation could be obtained for all analytes within 23 min. The injection time determining the amount of sampling affects both peak current (oxide electric current of the ingredients at work electric potential 0.90 V versus SCE) and peak shape. The effect of injection time on peak current was studied by varying injection time from 2 to 10 s at 16 kV. When the injection time was longer than 8 s, the peak current nearly leveled off and peak broadening became more severe. In this experiment, 8 s (16 kV) was selected as the optimum injection time.

According to the above results, the optimum conditions for (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid were decided. A 60-mmol/L borate buffer (pH 8.7) was used as the running buffer at a separation voltage of 16 kV. The potential applied to the working electrode was + 0.90 V (versus SCE). Samples were injected electrokinetically at 16 kV for 8 s.

The typical electrophoregram for a standard solution of the seven analytes is shown in Figure 4A, and it can be seen that good separation can be achieved within 23 min.

Method validation

Stability of the solutions

The stability of standard and sample solutions were determined by monitoring the peak area of standard mixture solutions and sample solutions over a period of one day. The results showed that the peak area and migration time of each analyte were almost unchanged [relative standard deviation (RSD) % < 3.5] and that no significant degradation was observed within the given period, indicating the solutions were stable for at least 24 h.

Linearity

The linear relationships between the concentrations of the analytes and the corresponding peak-area ratios were assessed by analyzing a series of concentrations of the analytes. A series of mixed standard solutions from 1.0×10^{-8} g/mL to 2.0×10^{-4} g/mL were tested. The results of regression analysis on calibration curves and detection limits are presented in Table I.

Table I. Results of Regression Analysis on Calibration and the Detection Limits*

Compound	Regression equation $Y = a + bX$†	Correlation coefficient	Linear range (µg/mL)	Detection limit‡ (µg/mL)
(+)-catechin	$Y = 6082.9X + 0.7763$	0.9996	0.1–150	0.05
rutin	$Y = 2435.6X + 0.0065$	0.9993	0.4–100	0.15
gentisic acid	$Y = 3885.9X + 0.9534$	0.9991	0.1–200	0.10
vanillic acid	$Y = 10428X + 0.0724$	0.9998	0.2–100	0.10
salicylic acid	$Y = 4360.5X + 0.8908$	0.9993	0.5–100	0.20
quercetin	$Y = 9994.7X + 0.1333$	0.9994	0.3–200	0.10
protocatechuic acid	$Y = 2150.9X + 0.9753$	0.9993	0.2–150	0.10

* CE–ED conditions are as given in the legend to Figure 2.
† Where Y and X are the peak area (nQ) and concentration (mg/mL) of the analytes, respectively.
‡ The detection limits corresponding to concentrations giving signal-to-noise ratio of 3.

Reproducibility

The reproducibility of the peak area and migration time was estimated by making repetitive injections of a standard mixture solution (2.0×10^{-5} g/mL for each analyte) under the optimum conditions ($n = 6$). The relative RSDs of the peak area and migration time were 2.0% and 1.6% for (+)-catechin, 2.3% and 2.1% for rutin, 2.6% and 2.2% for gentisic acid, 1.9% and 2.2% for vanillic acid, 1.8% and 2.4% for salicylic acid, 2.7% and 2.5% for quercetin, and 2.9% and 3.2% for protocatechuic acid.

Limit of detection and limit of quantitation

The limits of detection (LOD) were evaluated on the basis of a signal-to-noise ratio of 3. The calibration curves exhibited excellent linear behavior over a concentration range of about three orders of magnitude with the detection limits ranging from 3.0 $\times 10^{-8}$ g/mL to 1.0 $\times 10^{-7}$ g/mL for all the analytes.

The limit of quantitation (LOQ) is defined as the level at or above which the measurement precision is satisfactory for quantitative analysis. In this case, LOQ was evaluated on the basis of a signal-to-noise ratio of 10. The LOQ were 1.3 $\times 10^{-7}$ g/mL, 4.5 $\times 10^{-7}$ g/mL, 1.0 $\times 10^{-7}$ g/mL, 2.2 $\times 10^{-7}$ g/mL, 5.0 $\times 10^{-7}$ g/mL, 3.1 $\times 10^{-7}$ g mL, and 2.0 $\times 10^{-7}$ g/mL for (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid, respectively.

Table II. Assay* Results for *Gaultheria leucocarpa var. yunnanensis* Stem, Root, and its preparation, *Gaultheria leucocarpa var. yunnanensis* Syrup (*n* = 3)

Ingredient	Found	RSD (%)
Dried Gaultheria leucocarpa var. yunnanensis root		
(+)-catechin	27.8 µg/g	3.1
rutin	395 µg/g	2.9
gentisic acid	17.2 µg/g	3.0
Vanillic acid	12.5 µg/g	2.5
salicylic acid	90.7 µg/g	3.2
quercetin	41.2 µg/g	2.9
protocatechuic acid	41.0 µg/g	2.7
Dried Gaultheria leucocarpa var. yunnanensis stem		
(+)-catechin	N.F.†	—
rutin	141 µg/g	—
gentisic acid	13.0 µg/g	3.8
vanillic acid	13.0 µg/g	3.1
salicylic acid	N.F.	—
quercetin	N.F.	—
protocatechuic acid	N.F.	—
Gaultheria leucocarpa var. yunnanensis syrup		
(+)-catechin	51.9 µg/mL	2.7
rutin	127 µg/mL	3.0
gentisic acid	62.6 µg/mL	2.9
vanillic acid	6.00 µg/mL	3.3
salicylic acid	29.5 µg/mL	3.2
quercetin	6.26 µg/mL	2.8
protocatechuic acid	13.4 µg/mL	2.9

* CE–ED conditions are as given in the legend to Figure 2.
† N.F. means not found.

LOD and LOQ

Under optimum conditions, the determination of (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid, as well as the medicinal preparation for *Gaultheria leucocarpa var. yunnanensis*, was carried out according to the procedures previously described. Typical electrophoregrams obtained from the herb *Gaultheria leucocarpa var. yunnanensis* stems (B), *Gaultheria leucocarpa var. yunnanensis* roots (C), and *Gaultheria leucocarpa var. yunnanensis* syrup (D) are shown in Figure 4. The assay results are listed in Table II.

Under the optimum conditions, the recovery and reproducibility experiments were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by the standard addition method. The average recoveries and RSDs for the analytes are listed in Table III (*n* = 3).

Table III. The Determination Results of Recovery for this Method* using *Gaultheria leucocarpa var. yunnanensis* Root from Hunan Province (*n* = 3)

Ingredient	Original amount (µg/mL)	Added amount (µg/mL)	Found amount (µg/mL)	Recovery (%)	RSD (%)
Dried Gaultheria leucocarpa var. yunnanensis root					
(+)-Catechin	2.78.	5.00	7.89	102.2	3.0
Rutin	39.5	10.00	49.0	95.0	2.7
Gentisic acid	1.72	2.00	3.81	95.5	2.9
Vanillic acid	1.25	2.00	3.17	96.0	3.4
Salicylic acid	9.07	10.00	19.33	102.6	1.8
Quercetin	4.12	5.00	9.27	103.0	2.9
Protocatechuic acid	4.10	5.00	8.90	96.0	2.2

* CE–ED conditions are as given in the legend to Figure 2.

Conclusion

Under the optimum conditions, the proposed procedure was applied to the determination of active ingredients of the root and stem of the herb *Gaultheria leucocarpa var. yunnanensis* and its preparation. Through the external standard method, the linearity relationship was found between peak area and sample concentration, and from the migration time of analytes compared with the electropherogram of standard mixture solution, the active ingredients (+)-catechin, rutin, gentisic acid, vallinic acid, salicylic acid, quercetin, and protocatechuic acid in the herb samples were identified and determined. The assay results show that *Gaultheria leucocarpa var. yunnanensis* contains a relatively high level of rutin, and from the electropherographs of different parts of herb samples, there are significant differences in the kind and content of active ingredients in the stem and root of *Gaultheria leucocarpa var. yunnanensis*; for example, (+)-catechin, salicylic acid, quercetin, and protocatechuic acid have been determined in roots of *Gaultheria leucocarpa var. yunnanensis*, but not in terms of it.

The work presents the first application of CE–ED for the simultaneous assay of seven active ingredients in stems and roots of *Gaultheria leucocarpa var. yunnanensis* and its medicinal preparation, *Gaultheria leucocarpa var. yunnanensis* syrup. The previously described assay results indicate that this method

is accurate, sensitive, and reproducible. It is a useful quantitative technique for the analysis and quality control of the herb *Gaultheria leucocarpa var. yunnanensis*. In particular, the assay results demonstrate the kind and content diversity of different parts of this herb. Furthermore, the different parts of this herb can be used as an alternative source material of pharmacy.

Acknowledgments

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References

1. M. Xiaojun, Z. Junhua, and C. Xinzi. Studies on resources of ethnomedicine *Gaultheria leucocarpa var. yunnanensis*. *Chin. Materia Medica* **26:** 85–89 (2001).
2. M. Xiaojun, Z. Ling, D. Chengfang, S. Bingyu, Z. Junjua, and C. Xinzi. Advances in studies on *Gaultheria leucocarpa var. yunnanensis* and medicinal plants of Gaultheria L. *Chin. Trad. and Herbal Drugs* **32:** 945–49 (2001).
3. Editor Committee of Jiangsu Chinese Medical College. *Traditional Chinese Medicine Dictionary*. Shanghai Science and Technology Publishing House, Shanghai, 1977, pp. 1879–1980.
4. C. Changxin. 45 instances of the treatment of hyperosteogeny with Tougu Xiangtang. *Chin. Med.* **7:** 36 (1995).
5. Z. Zhizhen, G. Dean, and L. Changlin. Studies on the chemical constituents of *Gaulthheria yunnanesis*. *Chin. Trad. and Herbal Drugs* **8:** 508-511 (1998).
6. Z. Zhizhen, G. Dean, and L. Changlin. Studies on the chemical constituents of *Gaulthheria yunnanesis*. *Chin. Trad. and Herbal Drugs* **3:** 113-116 (1999).
7. Z. Zhizhen, W. Hong, Z. Yuguo, L. Changlin, G. Dean, and Z. Junhua. Studies on chemical constituents and biological activities of Gaultheria leucocarpa var. yunnanensis. *J. Beijing Med. Coll.* **1:** 77 (1998).
8. Z. Bin, H. Xiaoli, D. Yi, and D. Guanhua. Gaultherin. A natural salicylate derivative from Gaultheria yunnanensis: towards a better non-steroidal anti-inflammatory drug. *Eur. J. Pharmacol*. **530:** 166–71(2006).
9. K. Briviba and H. Sies. *Natural Antioxidants in Human Health and Disease*, B. Frei, Ed. Academic Press, NY, 1994, pp. 107–12.
10. L. Selloum, H. Bouriche, C. Tigrine, and C. Boudoukha. Anti-inflammatory effect of rutin on rat paw oedema, and on neutrophils chemotaxis and degranulation. *Exp Toxic Pathol*. **54:** 313–18 (2003).
11. J.D. Goss. Improved liquid chromatography of salicylic acid and some related compounds on a phenyl column. *J. Chromatogr. A* **828:** 267–71 (1998).
12. Y. Yusuf. Catechins in foods. *Trends Food Sci. Technol*. **17:** 64–71 (2006).
13. Y.A. Yeh, M. Herenyiova, and G. Weber. Quercetin: synergistic action with carboxyamidotriazole in human breast carcinoma cell. *Life Sci.* **57:** 1285–92 (1995).
14. P. Youyuan, L. Fanghui, and Y. Jienong. Quantitative and qualitative analysis of flavonoid markers in Frucus aurantii of different geographical origin by capillary electrophoresis with electrochemical detection. *J. Chromatogr. B* **830:** 224–30 (2006).
15. Z. Zhizhen, G. Dean, and L. Changlin. Quantitative analysis of Gaultherin in *Gaultheria leucocarpa var. yunnanensis* by HPLC. *Chin. Materia Medica* **24:** 1642–51 (1999).
16. M. Xiaojun, J. Zhaohui, and L. Xiansheng. Quantitative analysis of salicylic acid in *Gaultheria leucocarpa var. yunnanensis* by HPLC. *Guizhou Med. J.* **29:** 72–73 (2005).
17. F.Yuzhi, G. Fangyu, F. Xiaoming, and Y. Jiannong. Determination of priority phenols in wastewater by capillary electrophoresis with electrochemical detection. *Chin. J. Anal. Chem.* **26:** 694–97 (1998).
18. F. Changguo, S. Lining, and F. Yuzhi. Determination of adenosine phosphate by co-electroosmotic capillary electrophoresis with amperometric detection. *Anal. Chim. Acta* **399:** 259–63 (1999).
19. F. Xiaoming, Y. Jiannong, and F. Yuzhi. Determination of polyhydroxy antibiotics by capillary zone electrophoresis with amperometric detection at a nickel electrode. *Anal. Chim. Acta* **329:** 49–55 (1996).

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