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Electromigration Profiles of *Cynomorium songaricum* Based on Capillary Electrophoresis with Amperometric Detection

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A high-performance capillary electrophoresis with amperometric detection (CE-AD) method has been developed for the simultaneous determination of the pharmacologically active ingredients in *Cynomorium songaricum* in this work. Under the optimum conditions, phloridzin, epicatechin, catechin, naringenin, rutin, luteolin, quercetin, gallic acid, and protocatechuic acid can be well separated or nearly baseline separated (epicatechin and catechin peaks) within 31 min at the separation voltage of 14 kV in a 50 mmol L^{-1} Borax running buffer (pH 9.0). Detection limits (S/N=3) ranged from 5.7×10^{-8} to 8.5×10^{-9} g mL^{-1} for all nine analytes. This procedure was successfully used for the analysis and comparison of the content difference of *C. songaricum* samples collected from different places based on their electrophorograms or "electromigration profiles".

KEYWORDS: ***Cynomorium songaricum*; electromigration profiles; capillary electrophoresis; amperometric detection**

INTRODUCTION

Cynomorium songaricum, the stem of *C. songaricum* Rupr. (Suo-yang, family Cynomoriaceae), forms the basis of various rare Chinese herbal medicines (*1*). It has been used as a medicinal herb in folk medicine for reinforcing kidneys, invigorating yang, benefiting the anima, and moisturizing the intestine. Therefore, *C. songaricum*, as a kind of traditional Mongolian medicine, has been titled as "elixir" and "desert ginseng". Modern research from the fields of chemistry, pharmacology, and clinic has shown that *C. songaricum* extract has many bioactivities, especially in immunity, resisting consenescence, regulating endorrinosity, improving sexual function, and relaxing the bowels, and it is an ideal medicine for treating senile diseases without side effects (*2*−*6*). *C. songaricum* has increasingly attracted considerable attention of researchers from Asia, Europe, and North America, especially East Asia, and is gradually becoming one of the most popular medicinal herbs, of which phytopharmaceuticals are easily accessible throughout the world and have no side effects. Furthermore, because of its wide adaptation and resistance to drought and sand, *C. songaricum* Rupr. offers great promise for arid areas (*7*). Therefore, it is necessary to develop a sensitive, selective, dependable, and relative simple assay method to determine the active ingredients in *C. songaricum*.

So far there are a series of compounds isolated from the polar fraction of *C. songaricum* elucidated structurally by chemical evidence and spectral methods (*8*−*11*). Pharmacological studies reveal that the stem of *C. songaricum* mainly contains organic acids, flavonoids, steroids and triterpenes, etc. Flavonoids are also an important kind of plant estrogen (phytoestrogen). Because of the association of many phytoestrogens and other members of the polyphenol family with specific diseases or toxicity-related issues, phytoestrogens have drawn rising interests from analytical chemists in the past decade. Modern animal research has revealed that flavonoids (*12*−*15*) and some phenolic acids (*16*, *17*) show speeding cruor, antioxidant, radioprotective, antimutagenic, and anticarcinogenic effects, respectively. As phytoestrogens are present in many plants and food products, it is interesting work to find some new plant species for extracting bioactive ingredients.

However, only a few reports can be found for the analysis of one or some bioactive ingredients in *C. songaricum*, including the amino acid analytical instrument (*18*, *19*), UV spectrophotometry approaches (*20*, *21*), TLC (*22*), and HPLC (*23*, *24*). Proline is taken as the only compound for differentiating *C. songaricum* by TLC, and no content index is recorded in the Chinese pharmacopoeia (*25*). Capillary electrophoresis (CE) is becoming increasingly recognized as an important analytical separation technique due to its speed, efficiency, reproducibility, ultra-small sample volume, and ease of clearing up the contaminants. In combination with amperometric detection (AD), CE-AD offers high sensitivity and good selectivity for electroactive species (*26*, *27*). In comparison with HPLC, CE is a more efficient separation method without complicated operation and high cost. However, to our knowledge, this technique has not been fully explored yet, and its application for the analysis of *C. songaricum* has not been conducted.

In this work we developed a sensitive and reliable method for the determination of nine active ingredients in *C. songaricum* and its pharmaceuticals by CE-AD, which have similar molecular structures as shown in **Figure 1**. Further, this procedure was successfully used for the analysis of the content difference of *C. songaricum* samples collected from different places based

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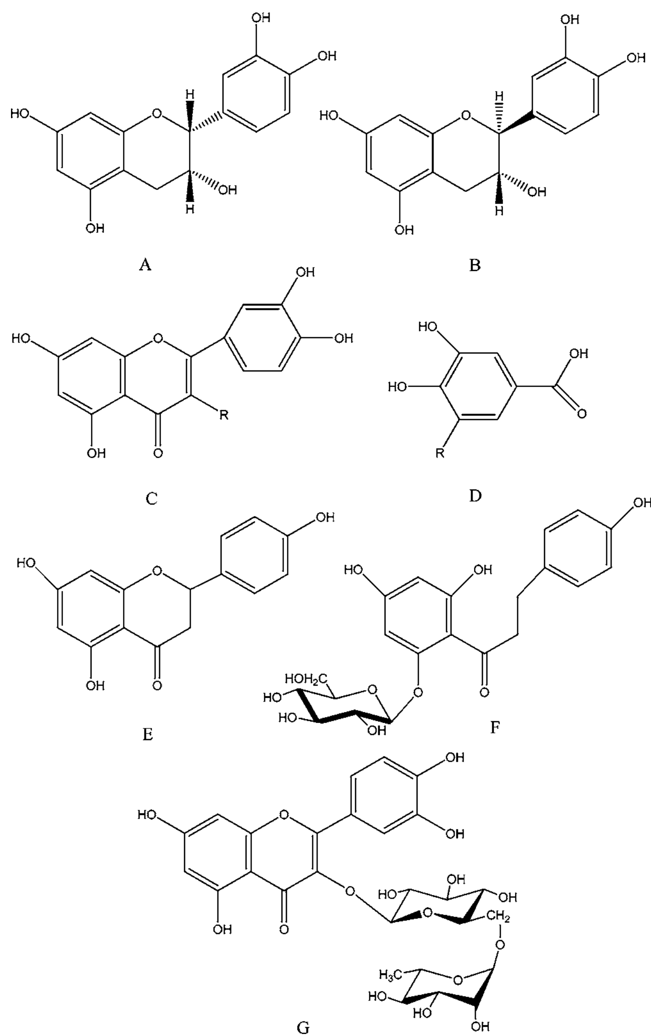

Figure 1. Molecular structures of analytes. (**A**) epicatechin; (**B**) catechin; (**C**) luteolin (R = H), quercetin (R = OH); (**D**) gallic acid (R = OH), protocatechuic acid (R = H); (**E**) naringenin; (**F**) phloridzin; (**G**) rutin.

on their electromigration profiles or "electrochemical characteristic chromatograms".

MATERIALS AND METHODS

Apparatus. The laboratory-built CE-AD system has been constructed in this work and is similar to that described previously (*26*). A ±30 kV high-voltage power supply (Shanghai Institute of Nuclear Research, China) provided separation voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential, and the outlet end was maintained at ground. A 75 cm length of 25 μm i.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ) was used for the separation. Samples were all injected electrokinetically, applying 14 kV for 6 s.

The design of the CE-AD detector was based on the end-column approach in which the working electrode is simply placed at the outlet of the separation capillary and detection is carried out in the same solution reservoir that contains the grounding electrode for CE instrument. A carbon-disk electrode with 300 μm diameter was employed as the working electrode. Before use, the surface of the carbon-disk electrode was polished with emery sandpaper, sonicated in deionized water, and then positioned carefully opposite the capillary outlet with the aid of an Oriel Corp. (Stratford, CT) Model 14901 micropositioner. A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode, and a SCE (saturated calomel electrode) reference electrode were used in combination with a BAS LC-4C amperometric detector (Biochemical System, West Lafayette, IN). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument factory, China). All experiments were performed at room temperature.

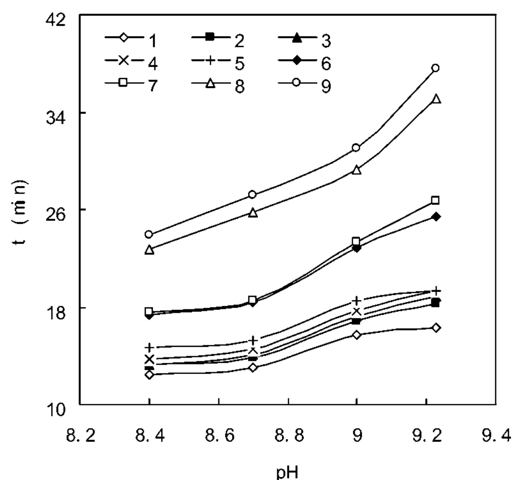

Figure 2. Effects of the running buffer pH on the migration time of the analytes. Fused-silica capillary: 25 μm i.d. × 75 cm; working electrode: 300 μm diameter carbon disk electrode; working electrode potential: +950 mV (vs SCE); concentration of running buffer: 50 mmol L^{-1}; separation voltage: 14 kV; injection time: 6 s /14 kV; peak identification: (1) phloridzin, (2) epicatechin, (3) catechin, (4) naringenin, (5) rutin, (6) luteolin, (7) quercetin, (8) gallic acid, and (9) protocatechuic acid; concentration of analytes: 1.0×10^{-5} g mL^{-1} each.

Chemical and Reagents. Phloridzin, epicatechin, catechin, naringenin, and luteolin were purchased from Sigma (St. Louis, MO), rutin was purchased from Aldrich (Milwaukee, WI), and quercetin, gallic acid, and protocatechuic acid were obtained from Shanghai Reagent Factory (Shanghai, China); they were all used as received. *C. songaricum* samples were collected from Gansu province, Qinghai province, and Xinjiang, Neimenggu, and Ningxia municipalities (China).

Stock solutions of nine analytes (1.00×10^{-3} g mL^{-1}, each) were prepared in anhydrous ethanol (A.R. grade), stored in the dark and at 4 °C, and were diluted to the desired concentration with the running buffer ($H_3BO_3-Na_2B_4O_7$ buffer with pH value from 8.4 to 9.2). Before use, all solutions were filtered through 0.22 μm nylon filters.

Sample Preparation. About 2 g of *C. songaricum* samples collected from different places or its pharmaceuticals were ground into powder in a mortar and then accurately weighed. Each weighed sample was extracted with 10 mL anhydrous ethanol (A. R. grade) and water (4:1) for 30 min in an ultrasonic bath. Then each of the samples was filtered through filter paper first and then through a 0.22 μm syringe filter. After filtration, the solutions were injected directly to the CE-AD system for analysis. Before use, all sample solutions were stored in the dark.

RESULTS AND DISCUSSION

Separation Conditions in CE. The acidity of the running buffer affects the ζ-potential, the electroosmotic flow (EOF), as well as the overall charge of the analytes, which determine the migration time and the separation of the analytes. The effect of the running buffer pH on the migration time of the analytes was investigated in the pH range of 8.4−9.2. As shown in **Figure 2**, when pH is lower than 8.7, epicatechin and catechin cannot be separated; luteolin and quercetin have the same separation problem. Since the analytes migrate counter-electroosmotic, the apparent migration time of analyte usually increases with increasing pH value, as well as the resolution improved for all analytes. When pH is higher than 9.2, naringenin cannot be separated from rutin. Meanwhile, the peak current is low and the peak shape becomes poor. At pH 9.0, the nine analytes can be well separated or nearly baseline separated (epicatechin and catechin peaks) within a relatively short time.

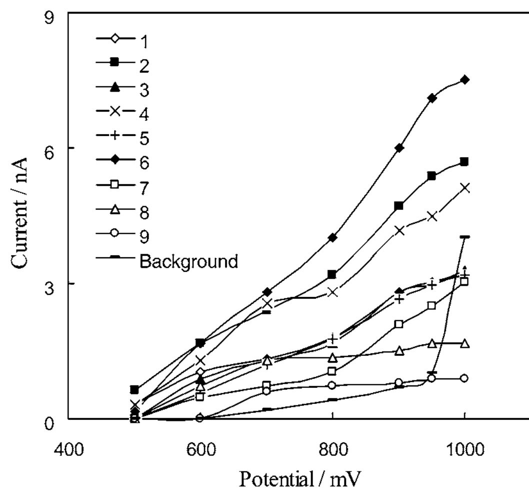

Figure 3. Hydrodynamic voltammograms (HDVs) in CE-AD: phloridzin (1), epicatechin (2), catechin (3), naringenin (4), rutin (5), luteolin (6), quercetin (7), gallic acid (8) and protocatechuic acid (9). The pH value of running buffer is 9.0, and other experiment conditions are the same as in **Figure 2**.

Besides the pH value, the running buffer concentration is also an important parameter. The effect of the running buffer concentration on migration time was also studied ranging from 20 to 100 mmol L^{-1}, and the optimum running buffer concentration is 50 mmol L^{-1} (pH 9.0).

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of EOF and the migration velocity of the analytes, which in turn determines the migration time of the analytes. As expected, higher separation voltage gives shorter migration time for all analytes. However, when the separation voltage exceeds 16 kV, baseline noise becomes larger. Therefore the optimum separation voltage selected is 14 kV, at which good separation can be obtained for all analytes within 31 min.

The injection time determining the amount of sampling affects both peak current and peak shape. The effect of injection time on peak current was studied by varying injection time from 2 to 10 s at 14 kV. It was found that the peak current increases with increasing sampling time. When the injection time is longer than 6 s, peak current nearly levels off and peak broadening becomes more severe. In this experiment, 6 s (14 kV) is selected as the optimum injection time.

Conditions of AD. In amperometric detection 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 results. As shown in **Figure 3**, when the applied potential exceeds +600 mV (vs SCE), all analytes can generate oxidation current at the working electrode, and the oxidation currents of catechin, rutin, and quercetin increase rapidly. When the applied potential is greater than +1000 mV (vs SCE), both the baseline noise and the background current increase very strongly, resulting in an unstable baseline, which is a disadvantage for sensitive and stable detection. Therefore the applied potential to the working electrode was maintained at +950 mV (vs SCE) where the background current is not too high and the *S*/*N* ratio is the highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

Through the experiments above, the optimum conditions for determining phloridzin, epicatechin, catechin, naringenin, rutin, luteolin, quercetin, gallic acid, and protocatechuic acid have been decided. The applied potential to the working electrode was selected at +950 mV (vs SCE), and the injection time was 6 s (14 kV). They can be well separated or nearly baseline separated (epicatechin and catechin peaks) within 31 min at the separation voltage of 14 kV in a 50 mmol L^{-1} Borax running buffer (pH 9.0). The typical electropherogram for a standard solution of the nine analytes was shown in **Figure 4A**.

Reproducibility, Linearity, Detection Limits, and Recovery. The reproducibility of the peak current is estimated by making repetitive injections of a standard mixture solution (1.0×10^{-5} g mL^{-1} for each analyte) under the selected optimum conditions ($n = 7$). The relative standard derivations (RSDs) of the peak current are 2.5%, 1.7%, 2.4%, 1.9%, 2.2%,

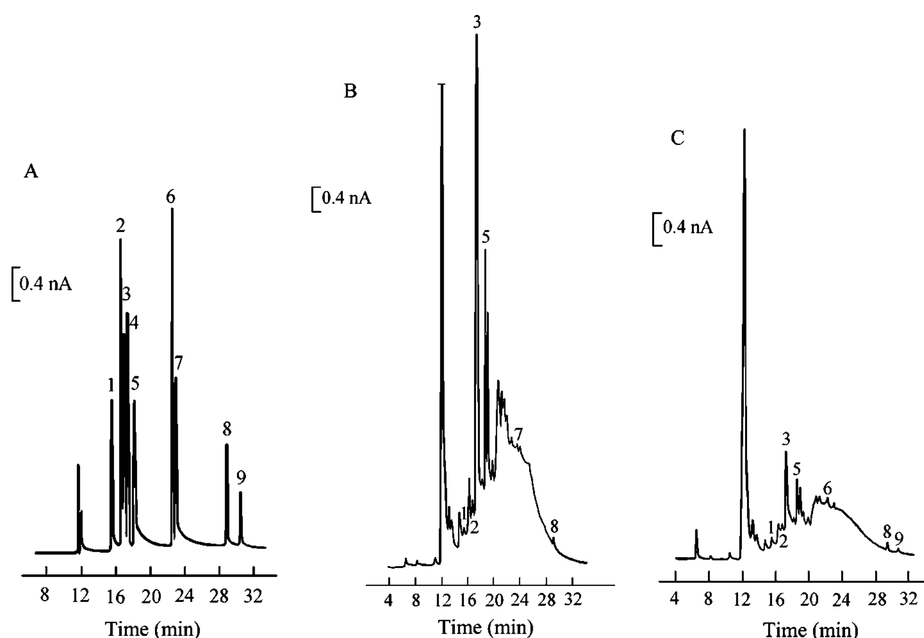

Figure 4. Electropherograms of a standard mixture solution (1.0×10^{-5} g mL^{-1} each) (**A**) and of sample solutions of *C. songaricum* from Gansu (**B**) and Xinjiang (**C**). The pH value of running buffer is 9.0, and the working electrode potential is +950 mV. Other experiment conditions and peak identifications are the same as in **Figure 2**.

Table 1. Regression Equations and Detection Limits[a]

compd	regression eq[b]	correlation coeff	linear range (g mL^{-1})	detection limit (10^{-8} g mL^{-1})
phloridzin	$y = 1.45 \times 10^5 x + 0.05$	0.9995	1×10^{-7}–1×10^{-4}	1.7
epicatechin	$y = 2.55 \times 10^5 x + 0.03$	0.9993	1×10^{-7}–1×10^{-4}	1.2
catechin	$y = 1.88 \times 10^5 x + 0.05$	0.9992	1×10^{-7}–5×10^{-5}	1.5
naringenin	$y = 2.28 \times 10^5 x - 0.10$	0.9991	1×10^{-7}–1×10^{-4}	1.3
rutin	$y = 1.42 \times 10^5 x - 0.01$	0.9994	1×10^{-7}–1×10^{-4}	1.7
luteolin	$y = 3.11 \times 10^5 x + 0.08$	0.9996	1×10^{-7}–1×10^{-4}	0.8
quercetin	$y = 1.41 \times 10^5 x - 0.14$	0.9991	5×10^{-7}–1×10^{-4}	5.5
gallic acid	$y = 1.02 \times 10^5 x - 0.04$	0.9996	1×10^{-7}–5×10^{-5}	3.8
protocatechuic acid	$y = 3.83 \times 10^4 x + 0.03$	0.9993	2×10^{-7}–1×10^{-4}	5.7

[a] CE-AD conditions are the same as in **Figure 4**. [b] In the regression equation, the *x* value is the concentration of analytes (g mL^{-1}), and the *y* value is the peak current (nA).

Table 2. Determination Results of Recovery in This Method with *C. songaricum* Sample (Qinghai) ($n = 3$)[a]

ingredient	original amount (g mL^{-1})	added amount (g mL^{-1})	found (g mL^{-1})	recovery (%)	RSD (%)
phloridzin	0.8×10^{-6}	1.0×10^{-5}	1.06×10^{-5}	98.1	2.7
epicatechin	0.2×10^{-6}	1.0×10^{-5}	1.00×10^{-5}	98.0	3.9
catechin	6.1×10^{-6}	1.0×10^{-5}	1.63×10^{-5}	101.2	2.3
naringenin	NF	1.0×10^{-5}	1.02×10^{-5}	102.0	3.4
rutin	0.2×10^{-6}	1.0×10^{-5}	1.06×10^{-5}	103.9	3.5
luteolin	3.9×10^{-6}	1.0×10^{-5}	1.37×10^{-5}	98.6	2.6
quercetin	0.3×10^{-6}	1.0×10^{-5}	1.06×10^{-5}	102.9	4.2
gallic acid	1.3×10^{-6}	1.0×10^{-5}	1.15×10^{-5}	101.8	3.1
protocatechuic acid	0.7×10^{-6}	1.0×10^{-5}	1.04×10^{-5}	97.2	4.6

[a] CE-AD conditions are the same as in **Figure 4**.

Table 3. Assay Results for *C. songaricum* Samples ($n = 3$)[a]

ingredients	*C. songaricum* samples (μg/g)						
	Gansu[b]	Qinghai[b]	Xinjiang[b]	Neimenggu[b]	Ningxia[b]	granules[c]	capsules[c]
phloridzin	35.4[d] (5.1)[e]	38.1 (5.0)	36.8 (5.1)	36.2 (4.7)	11 (6.9)	48.4 (3.9)	24.3 (4.0)
epicatechin	49 (4.3)	12.2 (5.7)	12.3 (6.0)	74.2 (3.8)	12.2 (5.3)	88.6 (2.1)	121.2 (2.2)
catechin	2319 (1.4)	318 (3.4)	251 (2.5)	832 (2.5)	419 (5.0)	92.9 (3.5)	291.2 (2.1)
naringenin	NF	NF	NF	NF	NF	NF	NF
rutin	1416 (2.6)	197 (3.9)	179 (4.3)	752 (2.0)	268 (3.7)	41.3 (4.0)	223.6 (2.6)
luteolin	NF	12 (5.3)	5.45 (5.7)	11 (4.4)	9.78 (5.6)	4.4 (5.2)	5.6 (5.5)
quercetin	14.7 (5.8)	66.7 (4.3)	NF	23.7 (6.0)	11.8 (5.8)	NF	NF
gallic acid	49.6 (6.1)	73 (4.9)	26.8 (5.3)	127 (3.6)	56.9 (4.3)	604.6 (1.4)	813.2 (1.3)
protocatechuic acid	NF	35.4 (5.4)	35.3 (5.6)	35.4 (5.4)	34.2 (5.1)	57.3 (5.0)	70.6 (4.9)

[a] CE-AD conditions are the same as in **Figure 4**. [b] The place name is the origin of the *C. songaricum* samples. [c] The pharmaceuticals of *C. songaricum.* [d] The data is the content of analytes in *C. songaricum* samples. [e] The data in brackets is RSD%.

1.5%, 3.4%, 2.7%, and 3.0% for phloridzin, epicatechin, catechin, naringenin, rutin, luteolin, quercetin, gallic acid, and protocatechuic acid, respectively. The reproducibility exhibited in the present study shows that it is feasible to determine the above analytes based on CE-AD.

To determine the linearity of phloridzin, epicatechin, catechin, naringenin, rutin, luteolin, quercetin, gallic acid, and protocatechuic acid, a series of standard solutions from 1.0×10^{-7} to 1.0×10^{-4} g mL^{-1} were tested. The peak current and concentration of each analyte were subjected to regression analysis to calculate the calibration equations and correlation coefficients. The results of regression analysis on calibration curves were summarized in **Table 1**. The results show that within the concentration range indicated in **Table 1** there was an excellent correlation between peak current and concentration of each analyte. The limit of detection (LOD) was established based on a signal-to-noise ratio of 3. The LOD of nine analytes ranged from 5.7×10^{-8} to 8.5×10^{-9} g mL^{-1}; detailed data were shown in **Table 1**.

To evaluate the precision and accuracy of the method, the recovery experiments under the optimum conditions were also conducted with *C. songaricum* sample (Xinjiang) ($n = 3$). Recovery was determined by the standard addition method, and the results are listed in **Table 2**. The results indicate the method is accurate enough for the simultaneous determination of the above analytes.

Sample Analysis and Discussion. Under the optimum conditions, the proposed procedure was applied for the determination of bioactive ingredients in *C. songaricum* samples and its pharmaceuticals based on CE-AD. Typical electropherograms of *C. songaricum* samples are shown in **Figure 4B,C**. By a standard addition method and the migration time of analytes compared with the electropherogram of the standard mixture solution (**Figure 4A**), the active ingredients—namely phloridzin (1), epicatechin (2), catechin (3), naringenin (4), rutin (5), luteolin (6), quercetin (7), gallic acid (8), and protocatechuic acid (9)—in different *C. songaricum* samples can be determined. The results are listed in **Table 3**. The assay results showed that

C. songaricum samples contain an abundance of polyphenols and flavonoids—this is particularly true for catechin and rutin—and the bioactivities of these compounds are in agreement with the pharmacological functions of *C. songaricum*. Therefore, we considered that catechin and rutin could be recommended as index constituents for quality control of *C. songaricum* materials (*28*).

As we can see from **Figure 4**, significant differences among these sample electropherograms can be found; in other words, the peak structure including peak number and peak height of these electropherograms are noticeably different. For example, peak 3 in **Figure 4B** (Gansu) is about 9.2 times higher than that of **Figure 4C** (Xinjiang). Therefore, these electromigration profiles or "electrochemical chromatograms" can be effectively used for the comparison of component diversity of medicinal herbs planted in different places. Besides, the above assay results show that the overall amount of nine active constituents in the Gansu sample (1 g) is about 7.1 times as that of Xinjiang sample (1 g) as shown in **Table 3**. So, it is necessary to establish quality herb gardens of *C. songaricum* to guarantee its pharmaceutical effect.

In this paper, a developed CE-AD procedure was successfully used for the analysis and comparison of the content difference of *C. songaricum* samples collected from different places based on their electrophorograms or "electromigration profiles". The above assay results indicate that CE-AD is accurate, sensitive, and reproducible, providing a useful quantitative method for the analysis of *C. songaricum*. Furthermore, it suggests that it is valuable to exploit the phytopharmaceuticals of *C. songaricum*, and it will be a new source of functional foods and medicines.

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