Int. J. Electrochem. Sci., 15 **(2020)** 5002 – 5017, doi: 10.20964/2020.06.33

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Simultaneous Electrochemiluminescence Determination of Sinomenine, Cepharanthine and Tetrahydropalmatine in *Stephania epigaea* by Capillary Electrophoresis Coupled with Ultrasonic-Assisted Aqueous Two-Phase Extraction

Shuangjiao Sun[1,2], Yanfen Wei[2], Hao Wang[2], Lifu Tang[2] and Biyang Deng[2,]

[1] School of pharmacy, Shaoyang University, Shaoyang 422000, China
[2] State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmaceutical Sciences, Guangxi Normal University, Guilin 541004, China
[*]E-mail: dengby16@163.com

Received: 8 January 2020 / *Accepted:* 18 March 2020 / *Published:* 10 May 2020

Three alkaloids in *Stephania epigaea* were extracted using ultrasonic-assisted extraction in an aqueous two-phase system. Based on separation by CE with acetonitrile as an additive, this work first established a simple and sensitive CE-ECL method for simultaneous determination of sinomenine, cepharanthine and tetrahydropalmatine in *Stephania epigaea*. The experimental conditions were optimized. The three alkaloids had the highest extraction rates in the aqueous two-phase system compared with the other extraction solutions studied in this paper. Under the optimized experimental conditions, the three alkaloids were optimally separated within 10 min and displayed linear concentration ranges for sinomenine, cepharanthine and tetrahydropalmatine of 0.08-4.0 μg/mL, 0.08-4.0 μg/mL and 0.04-6.0 μg/mL, respectively. The detection limits of sinomenine, cepharanthine and tetrahydropalmatine (S/N=3) were 0.027, 0.024 and 0.013 μg/mL, respectively. This method was successfully applied for the determination of sinomenine, cepharanthine and tetrahydropalmatine in *Stephania epigaea* extracts with recoveries of 98.4%, 102.4% and 101.1%, respectively.

Keywords: Capillary electrophoresis; Electrochemiluminescence; Aqueous two-phase; Ultrasonic extraction; *Stephania epigaea*

1. INTRODUCTION

Stephania epigaea (*S. epigaea*) is produced in Guangdong, Guangxi, Yunnan and other places of China and is the medicinal plant of Menispermaceae and grows mainly in limestone hills. It is a kind of natural drug that contains abundant amounts of alkaloids, such as tetrahydropalmatine, cepharanthine,

crebanine, sinoacutine, and palmatine, and is widely used in Chinese ethnic minority medicine [1]. Cepharanthine (Figure S1a) can treat inflammation, septic shock, and various cancers [2] and inhibit the replication of HIV-1, suppress the growth of primary lymphoma and induce apoptosis of cancer cells [3-5]. The combination of cepharanthine and chemotherapy drugs can significantly increase the accumulation of anti-cancer drugs in cells, reduce the resistance of cancer cells and improve the effect of chemotherapy [6]. Tetrahydropalmatine (Figure S1b) is a widely used traditional painkiller that has good effects in treating pain caused by gastrointestinal or hepatobiliary disease. Studies have also shown that tetrahydropalmatine could treat high blood pressure and protect against liver injury [7-8]. Sinomenine (Figure S1c) can resist inflammation and relieve pain, suppress immunity, release histamine, reduce blood pressure and treat rheumatic diseases [9-14]. At the same time, sinomenine could inhibit the growth of tumour cells in the lymphatic system, curb the spread of breast tumours [15-17], and help alleviate ischaemia induced by cerebral injuries [18]. High-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) [19], HPLC with photodiode array detection (HPLC-DAD) [20], and liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) [21] have been used to analyse cepharanthine. HPLC [22,23] and reverse-phase HPLC (RP-HPLC) [24,25] were employed to determine tetrahydropalmatine. HPLC [26] and voltammetry [27] could be used in the analysis of sinomenine. NMR spectroscopy could simultaneously determine sinomenine and dicentrine in *S. epigaea* [28]. However, there have been no reports on the simultaneous determination of the three alkaloids until now. Compared with other reported methods, electrochemiluminescence detection has undergone vibrant development [29,30], and electrochemiluminescence detection coupled with capillary electrophoresis (CE-ECL) has been widely used in pharmaceutical analysis since it has the advantages of efficient separation, no environmental pollution, rapid analysis, low consumption of reagents, simplicity of operation, high sensitivity and wide linearity [31-36]. The determination of sinomenine by CE-ECL has been reported [35], but the other two alkaloids have not been determined by this method. In this paper, a CE-ECL method for the simultaneous determination of cepharanthine, tetrahydropalmatine and sinomenine in *S. epigaea* was developed. Ultrasonic extraction is a simple and efficient extraction method [37,38]. Aqueous two-phase extraction technology as an efficient separation technology has gained increasing attention. It has unique characteristics of short separation times, low interfacial tension, small mass transfer resistance, low environmental pollution, easy scaling and continuous operation [39,40] and has been widely used in the separation and purification of bioactive macromolecule materials [41]. An aqueous two-phase system composed of a lower alcohol with salt has characteristics of low viscosity, fast hierarchical speed, easy recovery, and low environmental pollution and has been increasingly used for the extraction and separation of constituents from medicinal plants [42,43]. However, there have been few reports about the use of this technology for alkaloid extraction of cepharanthine, tetrahydropalmatine and sinomenine [44,45]. Since the three alkaloids have good medicinal value, simultaneous determination of them in *S. epigaea* is of great practical importance to the development and production of *S. epigaea* products.

In this paper, an ultrasonic-assisted aqueous two-phase extraction system was used to obtain alkaloids from *S. epigaea*, and the integrated extraction and separation of the alkaloids by using a simple home-made device was achieved. Based on separation by CE and using acetonitrile as an additive, a

simple and sensitive method for simultaneous ECL determination of sinomenine, cepharanthine and tetrahydropalmatine in *S. epigaea* was developed.

2. EXPERIMENTAL

2.1. Instruments and reagents

A CE–ECL testing system (MPI-B) produced by Xi'an Remex Electronic Science-Tech Co., Ltd. (Xi'an, China) was used and it included a high-voltage CE power supply, an autosampler, a multifunction chemiluminescence detector, a multichannel data collection analyser and a digital readout. The end-column ECL cell was a three-electrode system that included a Pt-disk working electrode, a Ag/AgCl (saturated KCl) reference electrode and a Pt-wire auxiliary electrode. An uncoated fused-silica capillary (75 μm × 50 cm) was purchased from Yongnian Optical Conductive Fibre Plant (Hebei, China). An SK3200H ultrasonic cleaner (Shanghai Kudos Ultrasonic Instrument Co., Ltd., Shanghai, China), an HSJ-4A model pH meter (Shanghai Precision and Scientific Instrument Corporation, Shanghai, China), a TG16-W high-speed, refrigerated centrifuge (Hunan Xiangyi Centrifuge Instrument Co., Ltd., Changsha, China) and a DZF-300 vacuum drying oven (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd, Zhengzhou, China) were also used in the experiment.

Cepharanthine and tetrahydropalmatine standards were obtained from Chengdu Institute of Biology of Chinese Academy of Sciences (Chengdu, China). A sinomenine standard was obtained from Shanghai Yuanye Biology Science & Technology Co., Ltd. (Shanghai China). Tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate was purchased from Alfa Aesar (Johnson Matthey, Ward Hill, MA, USA). Na_2HPO_4, Na_3PO_4, NaH_2PO_4, NaOH, Tween 80 (TW-80), anhydrous alcohol and propyl alcohol were obtained from Xilong Chemical Co., Ltd. (Guangdong China). Sodium dodecyl sulfate (SDS), polyvinylpyrrolidone (PVP) and cyclodextrin (β-CD) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetonitrile was purchased from Guangdong Guanghua Science & Technology Co., Ltd. (Guangdong, China). The three alkaloid stock solutions (0.1 mg/mL) were prepared using anhydrous ethanol and stored at 4 °C. *S. epigaea* was collected from the Guangxi Institute of Botany (Guilin, Guangxi, China). All chemicals used in the experiment were of analytical reagent grade, and the water used was double-distilled water (DDW). Before CE analysis, all solutions were filtered through 0.45 μm membrane filters (Shanghai Xinya Purification Material Factory, Shanghai, China).

2.2. Procedure

Before beginning analysis, a new capillary was activated by filling it with NaOH (0.1 mol/L) for 12 h, and then 0.1 mol/L NaOH, DDW, and a corresponding running buffer (RB) were used to flush the activated capillary for 10 min. The working electrode surface was polished with 0.3 μm alumina powder and cleaned with DDW in an ultrasonic cleaner before use. To maintain good reproducibility of the method, the $Ru(bpy)_3^{2+}$–phosphate solution was replaced every 2 h during the experiment. Phosphate (12 mmol/L) in 30% acetonitrile (pH 7.5) was used as an RB, and samples were introduced into the

capillary with electrokinetic injection at 12 kV for 12 s and separated at 14 kV in all experiments. The detection potential was fixed at 1.22 V, and the potential of the photomultiplier tube (PMT) was operated at 800 V with a magnification of 3 in the experiments.

2.3. Sample preparation

An ultrasonic-assisted aqueous two-phase extraction system was used to obtain alkaloids from *S. epigaea*. *S. epigaea* samples were dried to a constant weight at 70 °C in a ventilated space after washing the fresh tubers of *S. epigaea* and cutting them into slices. The dried samples were powdered and sifted through a 100 mesh sieve, and then a 0.1000 g powder sample was added to a 9 mL aqueous two-phase system composed of ethanol and ammonium sulfate and extracted in an ultrasonic bath for 10 min at 200 W. After the extraction, the sample was placed on a home-made simple cover for separation (as shown in Figure 1, the separation cover consisted of a centrifuge cover, glass tube, transparent plastic hose and clamp). The transparent plastic hose was clamped, the centrifuge tube was turned upside down, and the clip was opened to separate the solution after solvent partitioning. removing the upper solution, the lower solution was extracted twice, and then the upper solutions were merged and diluted with anhydrous ethanol to 25 mL.

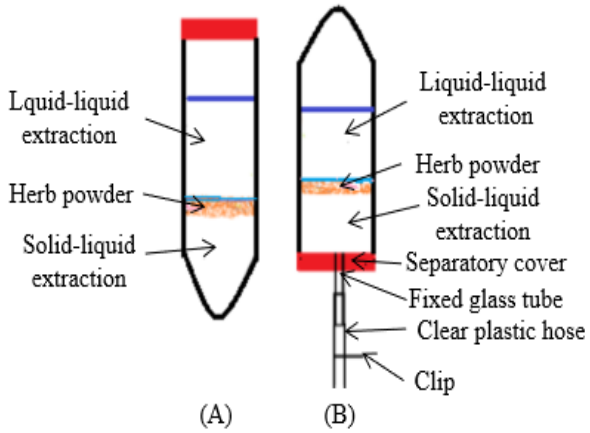

Figure 1. Schematic diagram of alkaloid extraction and extract liquid separation; a. schematic diagram of alkaloid extraction; b. schematic diagram of extract liquid separation

3. RESULTS AND DISCUSSION

3.1. Electrochemical action of three alkaloids with $Ru(bpy)_3^{2+}$

Electrochemiluminescence reactions consist of electrochemical reactions and chemiluminescence reactions. According to the electrochemiluminescence mechanism of $Ru(bpy)_3^{2+}$[46], the reaction between $Ru(bpy)_3^{2+}$ and the analyte is the key factor affecting the ECL intensity. ECL signals generated by cepharanthine, tetrahydropalmatine and sinomenine with $Ru(bpy)_3^{2+}$ in PBS were studied (Figure S2). When only $Ru(bpy)_3^{2+}$ and PBS were used, the ECL intensity was weak (Figure S2a) and

increased when sinomenine (Figure S2b), cepharanthine (Figure S2c) or tetrahydropalmatine (Figure S2d) was added to $Ru(bpy)_3^{2+}$-PBS. These results showed that the three alkaloids could be analysed with ECL detection.

3.2. Choice of additives

When the RB solution did not contain additives, the electrophoretic peaks of the three alkaloids completely overlapped. The effects of SDS, PVP, TW-80 and β-CD with mass concentrations of 1%, 2%, and 3% on the RB solution for separation were tested. The effects of propyl alcohol and acetonitrile with volume concentrations of 10%, 20%, 30%, and 40% were also examined. The results showed that different concentrations of SDS, PVP, TW-80, β-CD and propyl alcohol could not separate the analytes. The ECL peaks of the three alkaloids overlapped in the RB (Figure 2a). There were three peaks, but they overlapped in RB-10% acetonitrile (Figure 2b). The separation of the three alkaloids was improved but still failed to be entirely separated in RB-20% acetonitrile (Figure 2c). The three alkaloids were separated completely and had the strongest ECL intensity in RB-30% acetonitrile (Figure 2d). The separation efficiency was further improved in RB-40% acetonitrile, but the sensitivity was lowered, and the analysis time was extended (Figure 2e). Based on this, 30% acetonitrile was chosen as the RB additive. Compared with the CE-ECL electropherograms of individual standard alkaloids under the same experimental conditions, it could be concluded that peaks 1, 2 and 3 were sinomenine, cepharanthine and tetrahydropalmatine, respectively.

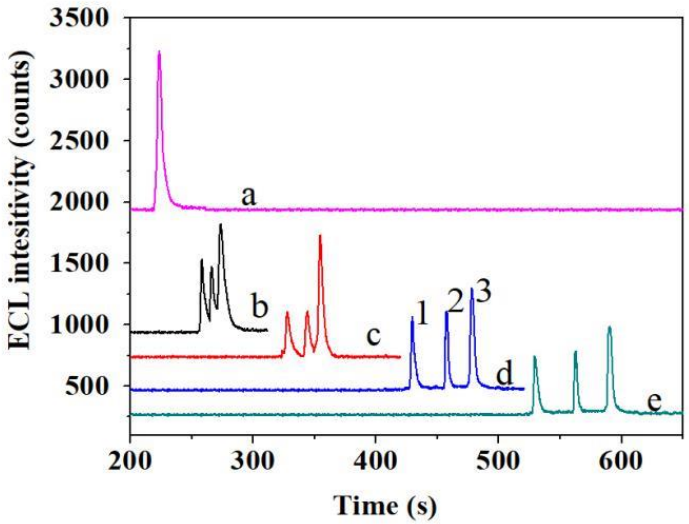

Figure 2. Effect of different acetonitrile contents on the separation and ECL intensity. Detection conditions were as follows: electrokinetic injection, 10 kV ×10 s; separation voltage, 12 kV; detection potential, 1.25 V; running buffer, 10 mmol/L PBS at pH 7.5; detection solution, 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L PBS at pH 7.5; standard solution, 1.00 μg/mL sinomenine, 1.00 μg/mL cepharanthine and 1.00 μg/mL tetrahydropalmatine; a. 0% acetonitrile; b. 10% acetonitrile; c. 20% acetonitrile; d. 30% acetonitrile; and e. 40% acetonitrile. Peak 1, sinomenine; peak 2, cepharanthine; and peak 3, tetrahydropalmatine

3.3. Optimization of detection potential

The ECL emission of the reaction between $Ru(bpy)_3^{2+}$ and analyte is mainly dependent on the formation of oxidized $Ru(bpy)_3^{3+}$ [47-49]. The detection potential on the working electrode thus greatly influences the ECL intensity. ECL intensity was measured to optimize the detection potentials of sinomenine, cepharanthine and tetrahydropalmatine. As shown in Figure 3, the ECL signal intensities of the alkaloids were relatively weak when the detection potential was set to 1.10 V and 1.15 V and then reached a maximum at 1.22 V, so the detection potential of 1.22 V was chosen.

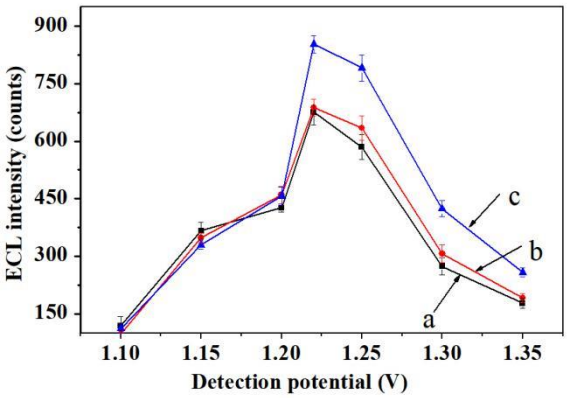

Figure 3. Effect of detection potential on ECL intensity. Detection conditions were as follows: electrokinetic injection, 10 kV ×10 s; separation voltage, 12 kV; running buffer, 10 mmol/L PBS, pH 7.5 and 30% acetonitrile; detection solution, 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L PBS at pH 7.5; standard solution, 1.00 μg/mL sinomenine, 1.00 μg/mL cepharanthine and 1.00 μg/mL tetrahydropalmatine. a. sinomenine; b. cepharanthine; c. tetrahydropalmatine

3.4. Effect of RB concentration and pH in the detection cell

The RB concentrations in the detection cell remarkably influenced the ECL intensity. According to reference [50], 50 mmol/L buffer containing 5 mmol/L $Ru(bpy)_3^{2+}$ in the detection cell was chosen. The pH has an effect on ECL intensity since it is the major factor influencing the reaction between $Ru(bpy)_3^{2+}$ and an analyte. The effects of pH on ECL intensities in the detection cell were studied from pH values of 5.5 to 9.5. As shown in Figure S3, the ECL intensities of sinomenine, cepharanthine and tetrahydropalmatine reached maximum values when the pH was 8.0, so a pH value of 8.0 in the detection cell was selected for further experiments.

3.5. Effect of the concentration and pH of the running buffer solution

The pH of the running buffer solution would influence the zeta potential, electroosmotic flow and charges of analytes in the inner wall of the capillary, which would affect not only the ECL intensity but also the migration time and resolution of the analytes [51,52]. The effects of running buffer (containing 30% acetonitrile) pH on ECL intensity were investigated. As shown in Figure S4, when the

concentration was kept at 10 mmol/L, the ECL intensities of the three alkaloids had maximum values, and their resolution was greater than 2.0 at pH 7.5. As shown in Figure S5, the running buffer pH was maintained at 7.5, and the concentration of the running buffer was changed from 6.0 to 18 mmol/L. The three analytes had the highest ECL signals using 12 mmol/L buffer solution. In the experiment, 12 mmol/L phosphate buffer (containing 30% acetonitrile) at pH 7.5 was selected.

3.6. Optimization of separation voltage

Both electrophoretic and electroosmotic velocities were proportional to the field strength. The current would increase, and the analysis time would shorten with increasing separation voltage. However, too high of a separation voltage would result in Joule heat, which affects the resolution of the analytes. At the same time, the separation voltage would affect the ECL intensities since the microenvironment of the capillary outlet would be affected by the effluent from the capillary when the separation voltage was changed [53]. The separation voltages from 8 kV to 20 kV were investigated to choose the optimal separation voltages. As shown in Figure 4, sinomenine, cepharanthine and tetrahydropalmatine all had maximum ECL intensities at 14 kV. The influence of separation voltage on the separation was also studied, and the adjacent alkaloid resolution decreased with increasing separation voltage. The resolutions of both the adjacent alkaloids exceeded 1.5 at a voltage of 14 kV. In subsequent experiments, the separation voltage was set to 14 kV.

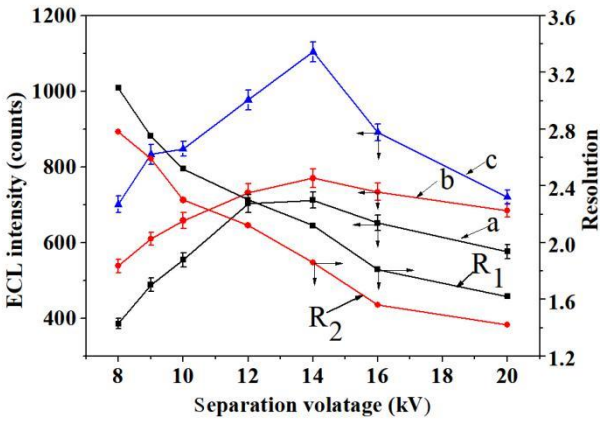

Figure 4. Effect of separation voltage on ECL and R. Detection conditions were as follows: running buffer, 12 mmol/L; other conditions are outlined in Figure S5. a, sinomenine; b, cepharanthine; and c, tetrahydropalmatine. R_1 is the resolution between sinomenine and cepharanthine, and R_2 is the resolution between cepharanthine and tetrahydropalmatine

3.7. Optimization of injection voltage and injection time

Fixing the injection time, the influence of the injection voltage (from 6 to 18 kV) on ECL intensities was studied. The ECL intensities of sinomenine, cepharanthine and tetrahydropalmatine increased with increasing injection voltage from 6 to 12 kV. The ECL intensities of the three analytes

reached their maximum (Figure 5) and exhibited resolutions between two adjacent peaks greater than 1.5 when the injection voltage was 12 V. In consideration of the resolutions and sensitivities of the three alkaloids, the injection voltage was set at 12 kV. The influence of injection time on resolution and ECL intensities was also investigated. The ECL intensities of sinomenine, cepharanthine and tetrahydropalmatine strengthened with increasing injection times from 6 s to 10 s (Figure S6). The ECL intensities of sinomenine and cepharanthine did not change much, but they significantly increased for tetrahydropalmatine, and the resolutions between the two components exceeded 1.5 when the injection time was 12 s. Considering the resolutions and sensitivities, 12 s was chosen as the optimum injection time.

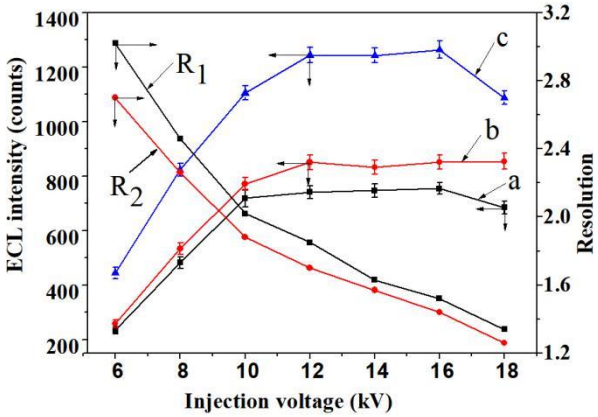

Figure 5. Effect of injection voltage on ECL and R. Detection conditions were as follows: separation voltage, 12 kV; the other conditions are listed in Figure 4; a. sinomenine; b. cepharanthine; c. tetrahydropalmatine; R_1. resolution between sinomenine and cepharanthine; R_2. resolution between cepharanthine and tetrahydropalmatine

3.8. The choice of an aqueous two-phase system

In this study, alkaloids in *S. epigaea* were extracted through aqueous two-phase extraction, which was formed by organic matter and salt. $(NH_4)_2SO_4$, NaCl, Na_2CO_3, Na_2SO_4 or Na_3PO_4 with concentrations from 0.5 g to 2.0 g were added into the mixed solution of 5 mL ethanol and 5 mL water to study the formation of an aqueous two-phase system. The experimental results showed that the ethanol-water mixture became an aqueous two-phase system with the addition of $(NH_4)_2SO_4$, Na_2CO_3 and Na_2SO_4 but not with the addition of Na_3PO_4 and NaCl. The aqueous two-phase system had the characteristics of fast layering and a clear interface in the presence of $(NH_4)_2SO_4$, and the aqueous two-phase system formed by Na_2CO_3 or Na_2SO_4 addition easily produced a precipitant with the characteristics of a narrow aqueous two-phase range and an unclear interface. In this paper, $(NH_4)_2SO_4$ was chosen to form an aqueous two-phase system with an ethanol-water mixed solution.

3.9. Effect of $(NH_4)_2SO_4$ level

The effect of the $(NH_4)_2SO_4$ level on the formation of the aqueous two-phase system with the mixed solution of 5 mL ethanol and 5 mL water was investigated, and the results showed that two phases

would not appear when the $(NH_4)_2SO_4$ level was less than 0.5 g, and $(NH_4)_2SO_4$ would precipitate out when the $(NH_4)_2SO_4$ level was more than 2.0 g. The aqueous two-phase system was stable when the level of $(NH_4)_2SO_4$ was in the range of 0.6 g to 1.8 g. *S. epigaea* (0.1 g) was extracted by the aqueous two-phase system that contained $(NH_4)_2SO_4$ from 0.6 g to 1.8 g for 15 min, and the extraction was diluted to 25 mL. The effect of the $(NH_4)_2SO_4$ level on the ECL intensities of the three alkaloids was investigated under the optimized experimental conditions. As shown in Figure 6, the ECL intensities of sinomenine, cepharanthine and tetrahydropalmatine were strongest when the $(NH_4)_2SO_4$ level was 1.2 g, so 1.2 g $(NH_4)_2SO_4$ was selected as the optimum salt level in subsequent experiments.

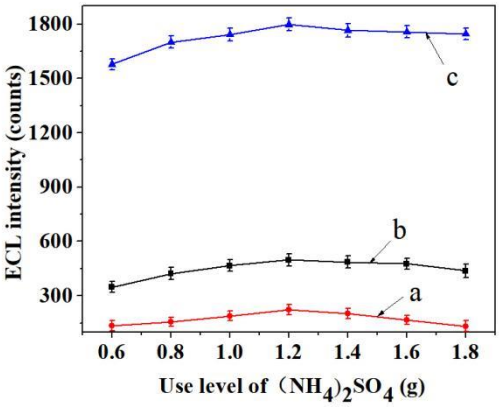

Figure 6. Effect of $(NH_4)_2SO_4$ use level on ECL. Detection conditions included an injection time of 12 s, and the other conditions are listed in Figure 6S; a. cepharanthine; b. sinomenine; and c. tetrahydropalmatine

3.10. Effect of ethanol volume

When the water volume was kept at 5 mL and the $(NH_4)_2SO_4$ mass was 1.2 g in the aqueous two-phase system, the effect of the ethanol level in the aqueous two-phase system on the extraction efficiency was investigated according to the ECL intensities of the three alkaloids, which were extracted from 0.1 g *S. epigaea* for 15 min. As shown in Figure S7, sinomenine, cepharanthine and tetrahydropalmatine had the strongest ECL intensities when the ethanol volume was 4.0 mL, so 4.0 mL ethanol and 5 mL water were selected as the optimum volumes in the aqueous two-phase system.

3.11. Effect of ultrasonication time

Setting the ultrasonic power at 200 W, the effect of ultrasonication time on the extraction of *S. epigaea* was studied. As shown in Figure S8, the ECL intensities of sinomenine, cepharanthine and tetrahydropalmatine were weak when the ultrasonication time was 5 min, became stronger when the ultrasonication time was 10 min, and then hardly changed with increasing ultrasonication time. In this paper, a *S. epigaea* sample was extracted for 10 min at 200 W.

3.12. Choice of extractant

The aqueous two-phase system (the mixture of 4 mL ethanol, 5 mL water and 1.2 g $(NH_4)_2SO_4$), an ethanol-water solution (the mixture of 4 mL ethanol and 5 mL water), and 9 mL ethanol were used to extract sinomenine, cepharanthine and tetrahydropalmatine from 0.1000 g *S. epigaea* powder using an ultrasonic bath at 140 W for 10 min.

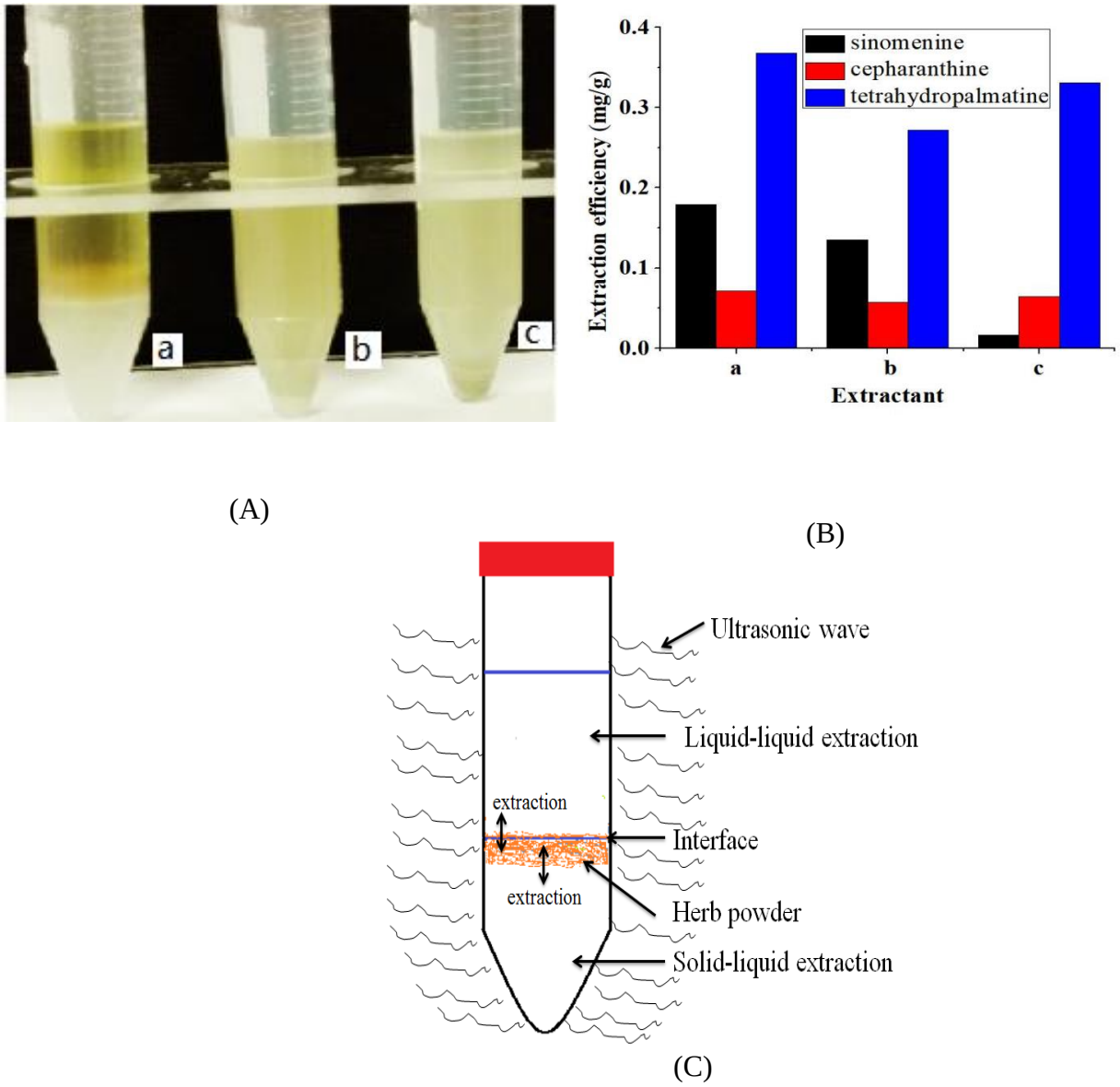

Figure 7. The effects of different extractants on the extraction efficiency (A); the extraction efficiency of different extractants (B); the schematic diagram of aqueous two-phase extraction assisted by ultrasonication (C), a. aqueous two-phase extraction; b. ethanol-water extraction; c. anhydrous ethanol extraction

The sample powder was extracted twice since the third extract solution had very weak ECL intensities for sinomenine, cepharanthine and tetrahydropalmatine. The extracting solutions were combined and detected under the optimal conditions. The extractants of centrifuge tubes a, b and c were the aqueous two-phase system, ethanol-water solution and ethanol, respectively (Figure 7A). As shown

in Figure 7A, centrifuge tube a had obvious two-phase separation, and the *S. epigaea* powder was located at the interface of the two phases. The schematic diagram of the extraction with the aqueous two-phase system is shown in Figure 7C. Under the optimized experimental conditions, the contents of the three alkaloids were determined by CE-ECL. As shown in Figure 7B, the contents of sinomenine, cepharanthine and tetrahydropalmatine in *S. epigaea* were 0.179, 0.071 and 0.368 mg/g for the aqueous two-phase system, 0.135, 0.057 and 0.272 mg/g for the ethanol-water solution, and 0.0162, 0.064 and 0.331 mg/g for ethanol, respectively. Thus, it can be seen that the aqueous two-phase system had the highest extraction efficiency compared with that of the ethanol-water solution and ethanol.

3.13. Sample analysis

Under the optimized conditions, blank samples (Figure 8a), *S. epigaea* samples (Figure 8b), *S. epigaea* samples spiked with sinomenine, cepharanthine and tetrahydropalmatine (Figure 8c) and standards (Figure 8d) were analysed by the developed method. As shown in Figure 8a, the blank sample had no obvious ECL peaks. In addition to the three sample peaks, there were some unknown ECL signal peaks in Figure 8b, and they may have been generated by other alkaloids present in the *S. epigaea* sample. The determination of sinomenine, cepharanthine and tetrahydropalmatine in *S. epigaea* was not inhibited by other alkaloids. The ECL intensities are shown in Figure 8b indicates that the contents of sinomenine, cepharanthine and tetrahydropalmatine in the *S. epigaea* sample were 0.179 mg/g, 0.071 mg/g and 0.368 mg/g, respectively. The recovery experiments of the three alkaloids were also investigated. The recoveries and RSDs are shown in Table 1. The recoveries of sinomenine, cepharanthine and tetrahydropalmatine were 98.4%, 102.4% and 101.1%, respectively.

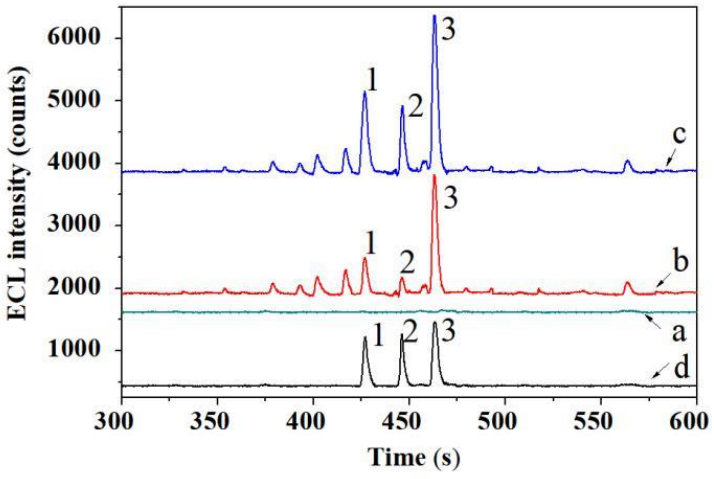

Figure 8. CE-ECL electropherograms of a blank sample, *S. epigaea* sample, a spiked *S. epigaea* sample and standards. (a) blank sample; (b) *S. epigaea* sample; (c) *S. epigaea* sample spiked with 1.0 μg/mL sinomenine, 1.0 μg/mL cepharanthine and 1.0 μg/mL tetrahydropalmatine; (d) standard solution of 1.0 μg/mL sinomenine, 1.0 μg/mL cepharanthine and 0.8 μg/mL tetrahydropalmatine. Peak 1 corresponds to sinomenine; peak 2, cepharanthine; peak 3, tetrahydropalmatine

Table 1. Analytical results and recoveries of three alkaloids in *S. epigaea* (n = 6)

Alkaloids	Content (mg/g)	Added (mg/g)	Founded (mg/g)	Recovery (%)	RSD (%)
Sinomenine	0.179	0.250	0.425	98.4	2.1
Cepharanthine	0.071	0.125	0.199	102.4	3.3
Tetrahydropalmatine	0.368	0.375	0.747	101.1	1.4

4. CONCLUSION

A simple and sensitive method was developed with acetonitrile as a separation additive for the simultaneous detection of sinomenine, cepharanthine and tetrahydropalmatine in *S. epigaea* extracts using CE-ECL with aqueous two-phase and ultrasonic-assisted extraction. The method had the advantages of a short analysis time, efficient separation, easy operation, low sample consumption, high sensitivity and a wide linear range.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (21765004), the Hunan Science Foundation of China (2018JJ2363), the Guangxi Science Foundation of China (2019GXNSFAA245076), and the Innovation Project of Guangxi Graduate Education (YCSZ2013039). The research fund of State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources (Guangxi Normal University) (CMEMR2018-C18) is gratefully acknowledged.

SUPPORTING INFORMATION

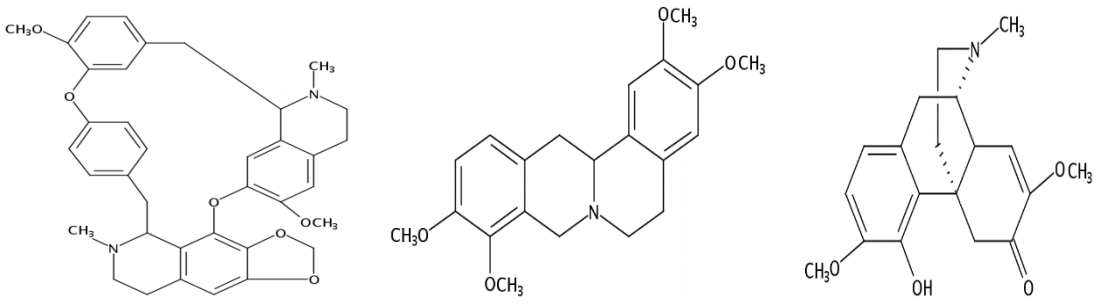

Figure S1. The molecular structures of cepharanthine, tetrahydropalmatine and sinomenine

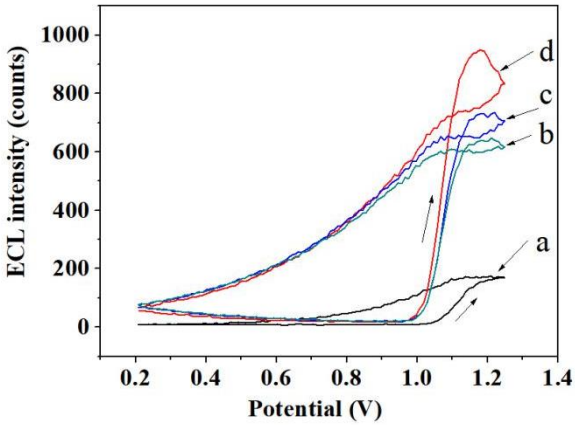

Figure S2. The effect of potential on ECL intensity
Detection conditions: scan rate, 100 mV/s; a. 5 mmol/L $Ru(bpy)_3^{2+}$+50 mmol/L phosphate buffer (pH 7.5); b. a + 1.0 μg/mL sinomenine; c. a + 1.0 μg/mL cepharanthine; d. a + 1.0 μg/mL tetrahydropalmatine.

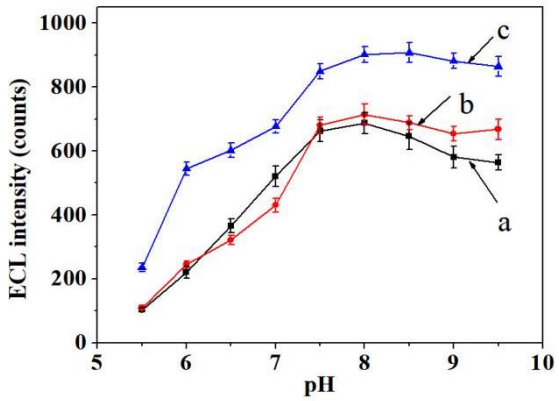

Figure S3. Effect of the detection buffer pH on ECL intensity
Detection conditions: detection potential, 1.22 V; other conditions are outline in Figure 3;
a. sinomenine: b. cepharanthine; c. tetrahydropalmatine.

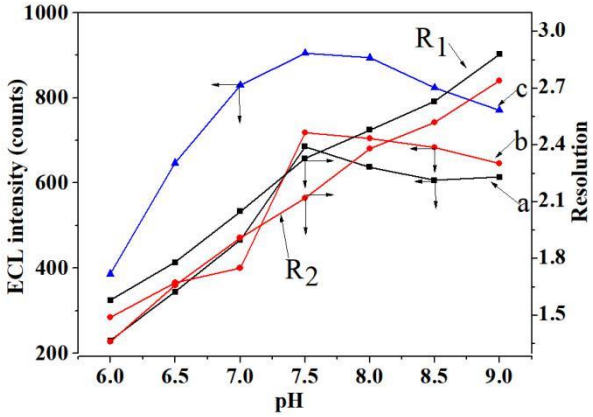

Figure S4. Effect of running buffer pH on ECL intensity and R
Detection conditions: solution in detection cell, pH 8.0; other conditions are outlined in Figure S3.
a. sinomenine; b. cepharanthine; c. tetrahydropalmatine; R_1: resolution between sinomenine and cepharanthine; R_2: resolution between cepharanthine and tetrahydropalmatine.

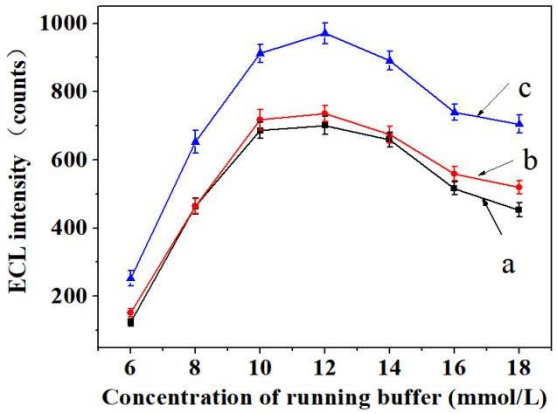

Figure S5. Effect of the running buffer concentration on ECL intensity
Detection conditions: the running buffer pH was 7.5, and the other conditions are shown in Figure S4. a. sinomenine, b. cepharanthine, c. tetrahydropalmatine; R_1: resolution between sinomenine and cepharanthine; R_2: resolution between cepharanthine and tetrahydropalmatine.

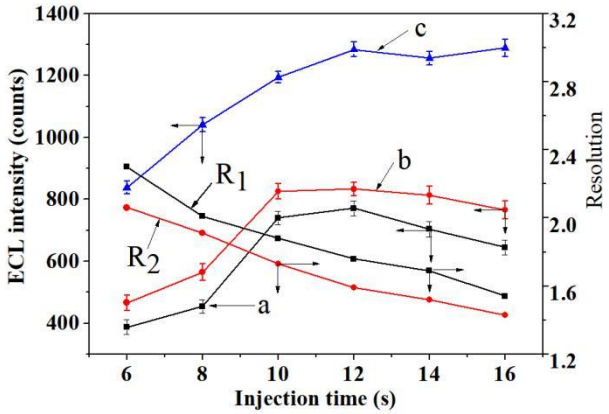

Figure S6. Effect of injection time on ECL intensity and resolution
Detection conditions: 12 kV injection voltage; other conditions are outlined in Figure 5. a. sinomenine, b. cepharanthine, c. tetrahydropalmatine; R_1: resolution between sinomenine and cepharanthine; R_2: resolution between cepharanthine and tetrahydropalmatine.

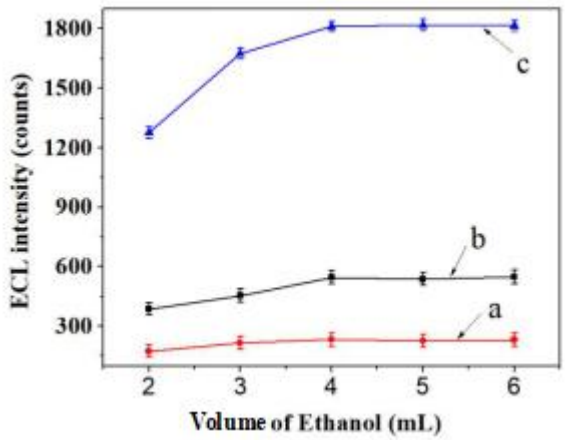

Figure S7. Effect of ethanol volume on ECL intensity
Detection condition: 1.2 g $(NH_4)_2SO_4$; other detection conditions are outlined in Figure 6; a. cepharanthine; b. sinomenine; c. tetrahydropalmatine

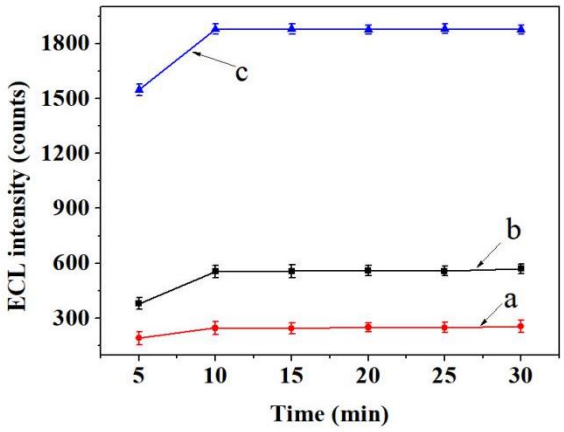

Figure S8. Effect of ultrasonication time on ECL intensity
Detection condition: 4 mL ethanol; other detection conditions are shown in Figure S7;
a. cepharanthine; b. sinomenine; c. tetrahydropalmatine.

References

1. J. Dong, L. Cai, Y. S. Fang, H. Xiao, Z. J. Li, Z. T. Ding, *Fitoterapia*,104 (2015) 102-107.
2. V. Payon, C. Kongsaden, W. Ketchart, A. Mutirangu, P. Wonganan, *Planta Med*., 85 (2019) 41-47.
3. M. Okamoto, M. Ono, M. Baba, *Aids Res. Hum. Retro.*, 14 (1998) 1239-1245.
4. N. Takahashimakise, S. Suzu, M. Hiyoshi, T. Ohsugi, H. Katano, K. Umezawa, *Int. J. Cancer*, 125 (2009) 1464–1472.
5. S. Gao, X. Li, X. Ding, W. Qi, Q. Yang, *Cell. Physiol. Biochem*., 41 (2017) 1633-1648.
6. A. Rattanawong, V. Payon, W. Limpanasittikul, C. Boonkrai, A. Mutirangura, P. Wonganan, *Oncol. Rep.*, 39 (2018) 227-238.
7. Z. Zhou, W. Zhao, W. Shi, Y. Xiao, Z. Ma, J. Xue, J. Tang, *Front. Pharmacol.*, 10 (2019) 336-347.
8. N. T. Tung, C. S. Tran, H. A. Nguyen, T. L. Nguyen, S. C. Chi, D. D. Nguyen, *Int. J. pharm.*, 537 (2018) 19-21.
9. X. Qian, Z. Zhao, W. Shang, Z. Xu, B. Zhang, H. Cai, *Mol. Med. Rep.*, 18 (2018) 49-58.
10. S. Li, J. Han, D. S. Wang, Q. Yang, B. Feng, W. Kang, M. Zhao, *Metab. Brain. Dis.*, 32 (2017) 211-219.
11. M. C. Lagerstr, *Scand. J. Pain*, 7 (2015) 15-16.
12. T. Gao, J. Hao, Z. Wiesenfeldhallin, D. Wang, X. Xu, *Eur. J. Pharm*., 721 (2013) 5-11.
13. Q. Wang, X. Li, *Int. Immunopharmacol.*, 11 (2011) 373-376.
14. M. Xu, L. Liu, B. Deng, X. Cai, *Planta Med.*, 74 (2008) 1423-142.
15. B. Zhao, L. Liu, J. Mao, K. Liu, W. Fan, J. Liu, *Biomed. Pharmacother.*, 96 (2017) 1036-1044.
16. H. Zhang, Y. Ren, X. Tang, K. Wang, Y. Liu, Z. Li, X. Li, P. Liu, C. Zhang, J. He, *Sci. Rep.*, 5 (2015) 1-10.
17. L. Song, D. Liu, Y. Zhao, J. He, H. Kang, Z. Dai, X. Wang, S. Zhang, Y. Zan, *Biochem. Biophys. Res. Commun.*, 464 (2015) 705-710.
18. W. Wu, P. Wu, X. Chen, Z. Zhang, J. Gu, Y. Yang, Q. Xiong, L. Ni, F. Wang, J. Chen, *Brit. J. Pharmacol.*, 164 (2011) 1445-1459.
19. Y. Deng, W. Wu, S. Ye, *Pharm. Boil.*, 55 (2017) 1775-1779.
20. S. Bory, S. S. Bun, B. Baghdikian, F. Mabrouki, S. K .Cheng, R. Elias, H. Bune, E. Olliver, *Nat. Prod. Commun.*, 5 (2010) 877-882.
21. R. Dong, Z. Fang, H. Gao, G. Hao, G. Liu, T. Shan, Z. Liu, *Chromatographia*, 73 (2011) 75-81.
22. Z. Hong, G. Cai, W. Ma, J. Wen, Y. Chai, G. Fan, *Biomed. Chromatogr.*, 26 (2012) 749-753.

23. Y. Yuan, X. Li, Z. Liu, L. Shi, L. Li, Y. Li, *Acta Pharm. Sin.*, 31 (1996) 286-290.
24. J. Ou, L. Kong, C. Pan, X. Su, X. Lei, H. Zou, *J. Chromatogr. A*, 1117 (2006) 163-169.
25. Y. Yuan, Z. Liu, X. Li, *Biomed. Chromatogr.*, 10 (1996) 11-14.
26. Y. Zhou, C. Guo, H. Chen, Y. Zhang, X. Peng, P. Zhu, *J. Anal. Methods Chem.*,1 (2015) 1-5.
27. J. Guan, Z. Wang, C. Wang, Q. Qu, G. Yang, X. Hu, *Int. J. Electrochem. Sci.*, 2 (2007) 572-582.
28. J.W. Dong, X.J. Li, L. Cai, J.Y. Shi, Y.F. Li, C. Yang, Z.J. Li, *J. Pharmaceut. Biomed. Anal.*, 160 (2018) 330-335.
29. F. Yang, Z. Peng, C. Gu, B. Liu, K. Zhou, *Int. J. Electrochem. Sci.*, 14 (2019) 6292-6302.
30. G. Mo, X. He, C. Zhou, D. Ya, J. Feng, C. Yu, B. Deng, *Biosens. Bioelectron.*, 126 (2019) 558-564.
31. Z. Liu, W. Qi, G. Xu, *Chem. Soc. Rev.*, 44 (2015) 3117-3142.
32. Y. Liu, Y. Liu, M. Zhou, K. Huang, J. Cao, H. Wang, Y. Chen, *J. Chromatogr. A, 1340* (2014) 128-133.
33. S. Sun, Y. Wei, H. Wang, Y. Cao, B. Deng, *Talanta*, 179 (2018) 213-220.
34. F. Yang, K. Zhou, Y. Lu, H. Yoshida, H. Yang, *Int. J. Electrochem. Sci.*, 14 (2019) 9159-9169.
35. Zhou, Y. Ma, X. Ren, X. Zhou, L. Li, H. Chen, *Anal. Chim. Acta*, 587 (2007) 104-109.
36. Y. M. Liu, W. Tian, Y. X. Jia, H. Y. Yue, *Biomed. Chromatogr.*, 23 (2009)1138-1144.
37. M. I. S. Melecchi, V. F. Péres, C. Dariva, C. A. Zini, F. C. Abad, M. M. Martinez, E. B. Caramao. *Ultrason. Sonochem*., 13 (2006) 242-250.
38. J. Y. Wu, L. D. Lin, F. T. Chau , *Ultrason Sonochem.*, 8 (2001) 347-352.
39. M. Iqbal, Y. Tao, S. Xie, Y. Zhu, D. Chen, X. Wang, H. I. Hussain, *Biol. Proced. Online* , 18 (2016) 18-34.
40. P. A. J. Rosa, A. M. Azevedo, S. Sommerfeld, W. Backer. M.R. Aires-Barros. *Biotechnol. Adv.*, 29 (2011) 559-567.
41. R. R. Soares, A. M. Azevedo, J. M. Van Alstine, M. R. Aires-Barros, *Biotechnol. J.*, 10 (2015) 1158-1169.
42. Y. Guo, J. Han, D. Zhang, L. Wang, L. Zhou, *Ultrason. Sonochem.*, 20 (2013) 125-132.
43. L. Li, X. Li, J. Ding, Y. Liu, Q. Wu, X. Wang, M. Li, Y. Jin, *Chem. J. Chinese U.*, 37 (2016) 454-459.
44. W. Zhang, D. Zhu, H. Fan, X. Liu, Q. Wan, X. Wu, P. Liu, J. Tang, *Sep. Purif. Technol.*, 141 (2011) 13-123.
45. Q. Cao, S. Li, C. He, K. Li, F. Liu, *Anal. Chim. Acta*, 590 (2007) 187-194.
46. J. K. Leland, M. J. Powel, *J. Electrochem. Soc.*, 137(1990) 3127-3131.
47. Z. Chen, Y. Zu, *J. Electroanal. Chem.*, 612 (2008) 151-155.
48. H. Zheng, Y. Zu, *J. Phys. Chem. B*, 109 (2005) 12049-12053.
49. H. Qiu, J. Yan, X. Sun, J. Liu, W. Cao, X. Yang, E. Wang, *Anal. Chem.*, 75 (2003) 5435-5440.
50. B. Deng, C. Su, Y. Kang, *Anal. Bioanal. Chem.*, 385 (2006) 1336-1341.
51. Y. Tao, X. Zhang, J. Wang, X. Wang, N. Yang, *J. Electroanal. Chem.*, 674 (2012) 65-70.
52. H. Duan, J. Cao, J. Yang, H. Wang, Y. Liu, *Talanta*, 154 (2016) 341-345.
53. J. Guo, M. Wang, H. Guo, R. Chang, H. Yu, G. Zhang, A. Chen, *Biomed. Chromatogr.*, 33(2019) 4646-4654.