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Study of polyethylene glycol as a green solvent in the microwave-assisted extraction of flavone and coumarin compounds from medicinal plants

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ABSTRACT

In this paper, the application of polyethylene glycol (PEG) aqueous solution as a green solvent in microwave-assisted extraction (MAE) was firstly developed for the extraction of flavone and coumarin compounds from medicinal plants. The PEG solutions were optimized by a mono-factor test, and the other conditions of MAE including the size of sample, liquid/solid ratio, extraction temperature and extraction time were optimized by means of an orthogonal design $L_9(3^4)$. Subsequently, PEG-MAE, organic solvent-MAE, and conventional heating reflux extraction (HRE) were evaluated with nevadensin extraction from *Lysionotus pauciflorus*, aesculin and aesculetin extraction from *Cortex fraxini*. Furthermore, the mechanism of PEG-MAE was investigated, including microwave-absorptive property and viscosity of PEG solutions, the kinetic mechanism of PEG-MAE and different microstructures of those samples before and after extraction. Under optimized conditions, the extraction yields of nevadensin from *L. pauciflorus*, aesculin and aesculetin from *C. fraxini* were 98.7%, 97.7% and 95.9% in a one-step extraction, respectively. The recoveries of nevadensin, aesculin and aesculetin were in the range of 92.0–103% with relative standard derivation lower than 3.6% by the proposed procedure. Compared with organic solvent-MAE and conventional extraction procedures, the proposed methods were effective and alternative for the extraction of flavone and coumarin compounds from medicinal plants. On the basis of the results, PEG solution as a green solvent in the MAE of active compounds from medicinal plants showed a great promising prospect.

1. Introduction

MAE is a rapid and effective extraction technique compared with traditional extraction techniques and has been applied to extract biological active compounds from different matrices [1–3]. Comparing with other modern extraction techniques such as supercritical fluid extraction and pressurized liquid extraction, MAE is easy to use and the systems are cheaper. However, abundant organic solvents used are problematic in the extraction/separation of biological active compounds from the herb because of their toxicity, volatility and flammability. So the design of safe and environmentally benign extraction solvents and processes has played an increasingly important role in the development of sample pretreatment and separation technologies. There are many potential advantages of replacing organic solvents with water or various types of aqueous solutions, such as low cost, reduced flammability, reduced toxicity, and reduced environmental risk. Leading organic solvent alternatives including supercritical fluids [4], ionic liquids [5], and solventless conditions, represent an increasingly significant choice for the replacement of traditional organic solvents. However, supercritical fluids as solvents have some obvious disadvantages such as elevated pressures required, relative high costs of investment and unusual operating conditions. Ionic liquids as solvents also suffer from the disadvantages of tedious preparation, high price, as well as limited knowledge of their toxicity.

Recently, polyethylene glycol (PEG) solutions have attracted increasing interest as novel solvents due to their excellent properties and potential application in analytical chemistry [6]. PEGs have a number of benign characteristics. Owing to their good biocompatibility and low immunogenicity, PEGs are on the Food and Administration's (FDA's) GRAS (Compounds Generally Recognized as Safe) list, and have been approved by the FDA for internal consumption. Unlike organic solvents, low molecular weight liquid PEGs are nonvolatile. PEGs also have low flammability and are biodegradable. On the other hand, PEGs have been found to be stable to acid, base, high temperature, high oxidation systems, and reduction systems. Moreover, PEGs have good miscibility with water and organic solvents, as well as good solubility for various organic compounds. Compared with ionic liquids, PEGs have other advantages, such as much lower cost, completely non-halogenated

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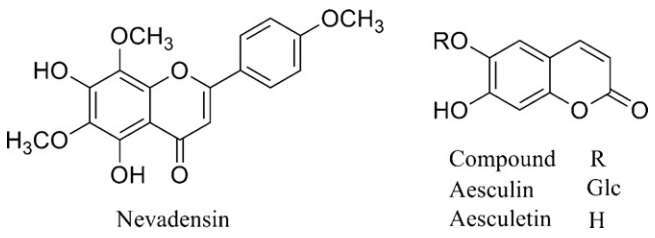

Fig. 1. Chemical structure of the target compounds.

and well known low toxicity. Therefore, PEGs are used as environmentally friendly solvents in sample preparation especially in separating analytes such as aqueous two-phase systems extraction [7,8] and cloud point extraction [9,10]. Moreover, PEGs are polar molecules and are suitable for energy dissipation with microwaves. Therefore, PEGs as solvents in the MAE of organic compounds would be a preference. However, there are no reports on MAE of bioactive substances from herb samples using PEGs as solvents.

Lysionotus pauciflorus (Shidiaolan in Chinese) is a small shrub widespread in southern China, and its whole plant is used as a Chinese traditional medicinal herb for the treatment of lymph node tuberculosis, cough with tachypnoea and rheumatic pains [11,12]. One of the most important bioactive compounds in sample is nevadensin (5,7-dihydroxy-6,8,4′,-trimethoxyflavone, Fig. 1), which is a flavonoid compound with multiple pharmacological effects such as antibacterial, anti-inflammatory, antihypertensive and free radical-scavenging activities [13,14]. *Cortex fraxini*, a kind of commonly used Chinese herbal drug, is officially listed in the Chinese Pharmacopoeia [15]. *C. fraxini* could inhibit the growth of dysentery bacillus. Furthermore, it has also been shown to possess expectorant, antitussive and antiasthmatic effects [16]. Aesculin and aesculetin which are the main effective constituents of *C. fraxini* have coumarin as their parent structure (as shown in Fig. 1). Traditionally, abundant organic solvents, including methanol and ethanol, were utilized to extract nevadensin from the Chinese herb by maceration at room temperature [13,14], heating reflux extraction [17] and ultrasonic-assisted extraction [18]. The conventional extraction methods for aesculin and aesculetin from *C. fraxini* not only use toxic organic solvents but also need a long time for the extraction [19–22]. Some simple and environmental-friendly method should be established.

In this work, the potentiality of PEG as an alternative solvent in MAE was investigated. PEGs of different molecular weights and different concentrations were used to extract flavone and coumarin compounds from medicinal plants by MAE, respectively, and various extraction factors were considered systematically. The extraction yields and the extraction conditions of these compounds in PEG-MAE were compared with those in organic solvent-MAE, conventional HRE and maceration extraction (ME). In addition, the properties of PEG solutions and kinetic mechanism of PEG-MAE were also studied. The microstructures of *L. pauciflorus* and *C. fraxini* before and after extractions were investigated by scanning electron microscopy.

2. Experimental

2.1. Reagents and samples

Dried *L. pauciflorus* and *C. fraxini* were purchased from the Laoyitang medicinal material emporium in Anhui and Qingping medicinal material emporium in Guangzhou, respectively. The materials were triturated, passed through stainless steel sieves with 0.90, 0.45, 0.30 and 0.15 mm mesh sizes and stored in closed desiccators. The same batch of sample was used here in the experiments.

HPLC grade acetonitrile used for mobile phase was purchased from Merck (Germany). Standards of nevadensin, aesculin and aesculetin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Different molecular weights of chemical pure PEG solutions including PEG-200, PEG-400, PEG-600, PEG-1000, PEG-2000, PEG-4000, and PEG-10000 were purchased from Guangdong Guanghua Chemical Factory (Guangzhou, China). Other reagents were analytical grade and purchased from Guangzhou chemical reagent factory (Guangzhou, China). Distilled water was used throughout the study.

2.2. Apparatus

MAE experiments were performed with an MAS-II microwave oven (Sineo Microwave Chemistry Technology Company, Shanghai, China) with a frequency of 2450 MHz and a maximum delivered power of 1000 W. The heating reflux extractor composed of a 100 mL round-bottom flask, a condenser tube and a boiling regulator.

HPLC analysis was carried out on a HPLC integrated system Shimadzu LC-2010C_{HT} (Shimadzu, Japan) which consists of an SCL-10Avp system controller, two LC-10ATvp liquid chromatography pumps, an SPD-10Avp UV–vis detector and a Model 7725 injection valve furnished with a 20 μL loop. The chromatographic data were recorded and processed with the Class-VP Workstation software (Shimadzu, Japan).

Viscosity measurement was carried out on a NDJ-1 rotational viscometer (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). A NS-100 single piston reciprocating peristaltic pump was used in the study of kinetic mechanism. A XL-30 scanning electron microscope (Philips, Eindhoven, Netherlands) was used to obtain microstructure pictures of dried sample before and after extraction.

2.3. Extraction procedures

For PEG-MAE, a 1.0 g amount of sample was put into a round-bottom flask with a certain volume of PEG solution. The microwave extraction was performed at a certain temperature for some time. The PEG solution was optimized by a mono-factor test and the influential factors such as particle size, extraction temperature, extraction time and ratio of liquid to solid were optimized by means of an orthogonal design L_9 (3^4).

For organic solvent-MAE of nevadensin from *L. pauciflorus*, 1.0 g sample of 0.30–0.15 mm size was put into a round-bottom flask with 20 mL methanol. The microwave extraction was performed at 65 °C for 10 min. The optimum organic solvent-MAE conditions for aesculin and aesculetin in *C. fraxini* were the same as the organic solvent-MAE conditions for nevadensin of *L. pauciflorus* except that the volume of methanol was 10 mL.

For HRE, 1.0 g sample of 0.30–0.15 mm size was put into a round-bottom flask with 40 mL PEG solution. The extraction temperature was controlled by heating in a water bath at 80 °C for 40 min.

For maceration extraction, 1.0 g sample of 0.30–0.15 mm size was put into a round-bottom flask with 40 mL PEG solution. The extraction was performed at room temperature for 12 h.

The extraction yield of target compound was defined as follows:

$$\text{Yield}\ (\%) = \frac{\text{Mass of target compound in one-step extraction solution}}{\text{Sum of the mass of target compound in sample}} \times 100$$

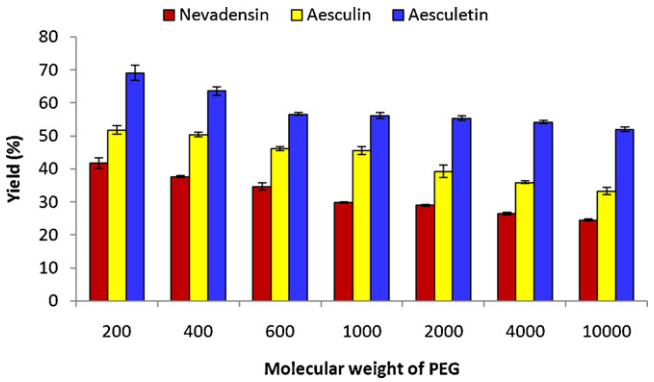

Fig. 2. Effect of the molecular weight of PEG on extracting the target compounds from samples ($n = 3$). The MAE conditions were as follows: the size of sample was 0.90–0.45 mm, the extraction temperature was 40 °C, the extraction time was 2 min, and the ratio of liquid/solid was 20:1.

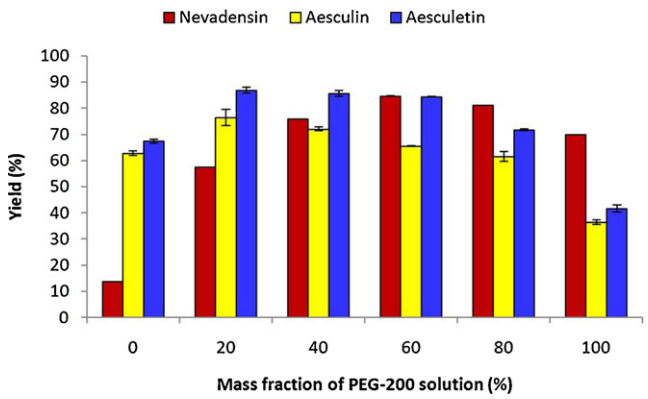

Fig. 3. Effect of the concentration of PEG-200 on extracting the target compounds from samples ($n = 3$). The MAE conditions were as follows: the size of sample was 0.90–0.45 mm, the extraction temperature was 40 °C, the extraction time was 2 min, and the ratio of liquid/solid was 20:1.

The mass of target compound in extraction solution (one-step extraction) was analyzed by RP-HPLC. The sum mass of target compound in sample was determined by analysis of the total extraction solutions by extracting continuously for three times with PEG solution under the optimum MAE conditions. In this work, the experimental results showed that the mean of total mass of nevadensin in *L. pauciflorus*, aesculin and aesculetin in *C. fraxini* was 2.04 mg/g, 19.56 and 6.05 mg/g, respectively.

2.4. Analysis

The extracts obtained were filtrated and the solvent was added until a final volume of 100 mL. The extraction solution was filtrated through a 0.45 μm microporous membrane for subsequent HPLC analysis. Chromatographic separations of nevadensin in *L. pauciflorus*, aesculin and aesculetin in *C. fraxini* were performed on a Diamonsil C_{18} column (250 mm × 4.6 mm i.d., 5 μm, Dikma, China) and a Diamonsil C_{18} column (200 mm × 4.6 mm i.d., 5 μm, Dikma, China), respectively, both equipped with an Easy Guard C_{18} guard column (10 mm × 4.6 mm i.d., Dikma, China) at 25 °C.

The conditions of HPLC analysis for nevadensin of *L. pauciflorus* were as follows. The mobile phase was composed of acetonitrile–0.5% acetic acid aqueous solution (60:40, v/v). The flow rate was 1.0 mL/min, the injection volume was 10 μL and the detection wavelength was set at 284 nm.

The conditions of HPLC analysis for aesculin and aesculetin in *C. fraxini* were as follows. The mobile phase was composed of acetonitrile–0.5% acetic acid aqueous solution (12:88, v/v). The flow rate was 1.0 mL/min, the injection volume was 10 μL and the detection wavelength was set at 340 nm.

3. Results and discussion

3.1. Study of PEG-MAE conditions

3.1.1. Optimization of PEG solutions by a mono-factor test

PEG solutions of different molecular weights were studied and the extraction efficiencies were compared, which are shown in Fig. 2. The extraction yields of nevadensin, aesculin and aesculetin declined from 41.8% to 24.5%, from 51.8% to 33.2% and from 69.1% to 51.9%, respectively, as the molecular weight of PEG increased from 200 to 10000. The results showed that the molecular weight of PEG had significant effects on the extraction of nevadensin from *L. pauciflorus*, aesculin and aesculetin from *C. fraxini*. The reasons were that the less molecular weight of PEG, the less viscosity PEG solution would have, which was beneficial for the mass transfer during extraction, and the polarity of the PEG solutions increased as the molecular weight of PEG decreased. Therefore, PEG-200 was chosen for further application.

The effects of different concentrations (mass fraction) of PEG-200 on the extraction yields are shown in Fig. 3. The extraction yield of nevadensin increased with the increasing concentration of PEG-200 at early stage, and slightly fell at late stage. The results showed that PEG solutions obtained much higher extraction yields compared with water. There might be two reasons. Firstly, PEG increased the solubility of nevadensin due to its good dissolving capacity. Secondly, PEG changed the dissipation factor of solution and the transfer of energy from microwaves to sample, which affected extraction efficiency and speed of energy transferring. At late stage, the increasing concentration of PEG-200 resulted in the high viscosity of the solution, which slowed the mass transfer during extraction. Therefore, 60% PEG-200 solution was selected for the extraction of nevadensin from *L. pauciflorus*. Similar results occurred in the extraction of aesculin and aesculetin from *C. fraxini*. However, 20% PEG-200 solution obtained the highest extraction yields of the two compounds. That is because aesculin and aesculetin are more polar than nevadensin, and mass fraction of PEG-200 solutions larger than 20% has higher viscosity which is not good for the mass transfer during extraction.

3.1.2. Optimization of MAE conditions by an orthogonal design

The optimization of MAE parameters was investigated with an orthogonal design L_9 (3^4). The factors were the size of sample (A), extraction temperature (B), extraction time (C) and liquid/solid ratio (D). The factors and the corresponding levels used in the orthogonal design are shown in Table 1. Nine experimental trials were performed according to the orthogonal design and the results are also shown in Table 1. The K and R values were calculated and listed in Table 2.

In Table 2, all of those factors had an influence on the extraction yields of the three target compounds. Comparing the R values, the influence of factors on the mean extraction yield of nevadensin decreased in the order: extraction temperature, the size of sample, extraction time and ratio of liquid to solid. The influence of factors on the mean extraction yields of aesculin and aesculetin decreased in the order: the size of sample, extraction temperature, extraction time and ratio of liquid to solid. The analysis of variance of the extraction yields also indicated that the extraction temperature and the size of sample in MAE had obvious influence on extraction yields of the three target compounds. The reasons were that PEG solutions were viscous liquids which had worse diffusion ability, and high extraction temperature could decrease the viscosity

Table 1
Experimental conditions and extraction yields[a] extracted with the orthogonal design L_9 (3^4) (n = 3).

No.	Factor				Yield (mean ± SD, %)		
	A Particle size (mm)	*B* Temperature (°C)	*C* Time (min)	*D* Liquid/solid ratio (mL/g)	Nevadensin	Aesculin	Aesculetin
1	0.90–0.45	40	5	10:1	51.6 ± 1.3	72.8 ± 1.9	55.5 ± 0.8
2	0.90–0.45	60	10	20:1	76.7 ± 1.1	80.3 ± 2.5	73.4 ± 0.3
3	0.90–0.45	80	15	30:1	95.7 ± 1.5	84.8 ± 2.3	78.5 ± 2.4
4	0.45–0.30	40	10	30:1	70.0 ± 1.1	75.0 ± 0.5	78.7 ± 1.1
5	0.45–0.30	60	15	10:1	86.0 ± 0.3	80.4 ± 1.4	85.8 ± 1.0
6	0.45–0.30	80	5	20:1	92.4 ± 0.9	80.4 ± 2.4	83.3 ± 2.7
7	0.30–0.15	40	15	20:1	91.4 ± 0.6	88.1 ± 1.6	87.0 ± 2.8
8	0.30–0.15	60	5	30:1	95.4 ± 0.6	90.8 ± 2.2	89.8 ± 3.1
9	0.30–0.15	80	10	10:1	98.2 ± 0.7	97.7 ± 1.4	95.9 ± 1.3

[a] Each extraction yield is the mean of three independent experiments.

Table 2
Analysis of L_9 (3^4) test results.

Sample	Compounds	Factor[a]	K_1[b]	K_2	K_3	R[c]
L. pauciflorus	Nevadensin	*A*	74.7	82.8	95.0	20.3
		B	71.0	86.0	95.4	24.4
		C	79.8	81.6	91.0	11.2
		D	78.6	86.8	87.0	8.4
C. fraxini	Aesculin	*A*	79.3	78.6	92.2	13.6
		B	78.6	83.8	87.6	9.0
		C	81.3	84.3	84.4	3.1
		D	83.6	82.9	83.5	0.7
	Aesculetin	*A*	69.1	82.6	90.9	21.8
		B	73.7	83.0	85.9	12.2
		C	76.2	82.7	83.8	7.6
		D	79.1	81.2	82.3	3.3

[a] For key to factors, see Table 1.
[b] $K_i^F = (1/3)$ the extraction yield of target compounds at F_i.
[c] $R_i^F = \max\{K_i^F\} - \min\{K_i^F\}$, here F and i means extraction factor and setting level, respectively.

of PEG solutions. Moreover, it was difficult for PEG solutions to permeate through the larger size of sample, so that target compounds in sample could not be transferred into the solutions quickly or completely. According to the largest donating rule, the largest value which affects the extraction yields should be the selected value. In Table 2, K_1–K_3 were the average yields under every level of an investigating variable, respectively. Therefore, considering time and solvent saving, the optimum experimental conditions for nevadensin of *L. pauciflorus* were as follows: the size of sample was 0.30–0.15 mm, the extraction temperature was 80 °C, the extraction time was 10 min, and the ratio of liquid/solid was 20:1. The optimum experimental conditions for aesculin and aesculetin in *C. fraxini* were the same as the conditions for nevadensin of *L. pauciflorus* except that the ratio of liquid/solid was 10:1. Under the optimum MAE conditions, the extraction yields of nevadensin, aesculin and aesculetin were 98.7%, 97.7% and 95.9%, respectively. This indicated that the extraction yield could be enhanced using a combination of those factors at different levels in the preparation process.

3.2. Method validation

The three target compounds were identified by their chromatograms and retention times in comparison with those of the authentic standard compounds. As shown in Figs. 4 and 5, the PEG in extracts had no effect on the retention of target compounds. Compared with the extraction solution of methanol (Fig. 4C), the extraction solution of PEG (Fig. 4B) had more impurity, which was owed to the extensive solubility of PEG.

The calibration curves of nevadensin, aesculin and aesculetin, which related the concentrations to the peak areas, showed good linearity over the range of 0.5–68.5 mg/L, 0.6–272.5 mg/L and 0.5–242.5 mg/L, respectively. The limit of detections of the three target compounds, which were evaluated on the basis of a signal-to-noise ratio of 3, were 0.06, 0.07 and 0.09 mg/L, respectively.

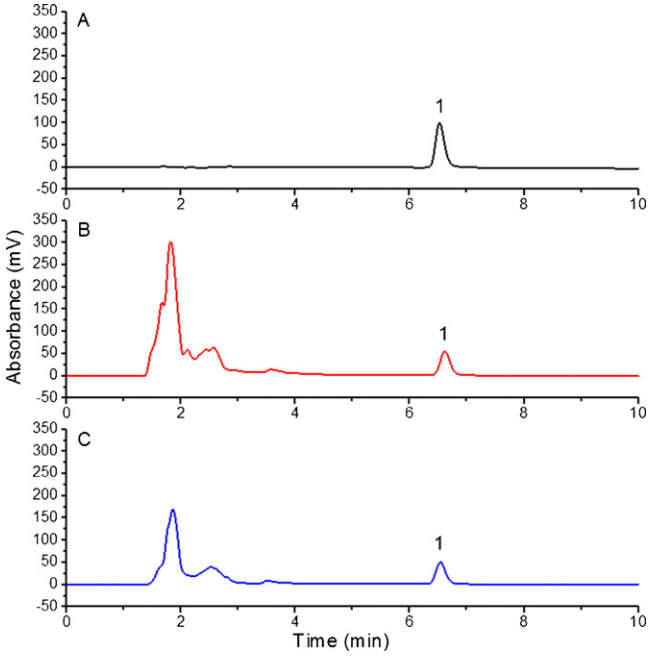

Fig. 4. Chromatograms of standard solution of 34.2 mg/L nevadensin (A), extract of *L. pauciflorus* by PEG solution (B), and extract of *L. pauciflorus* by methanol (C). Peak 1, nevadensin.

The reproducibility was estimated by five repetitive samples extracted by MAE at the optimum conditions. The relative standard

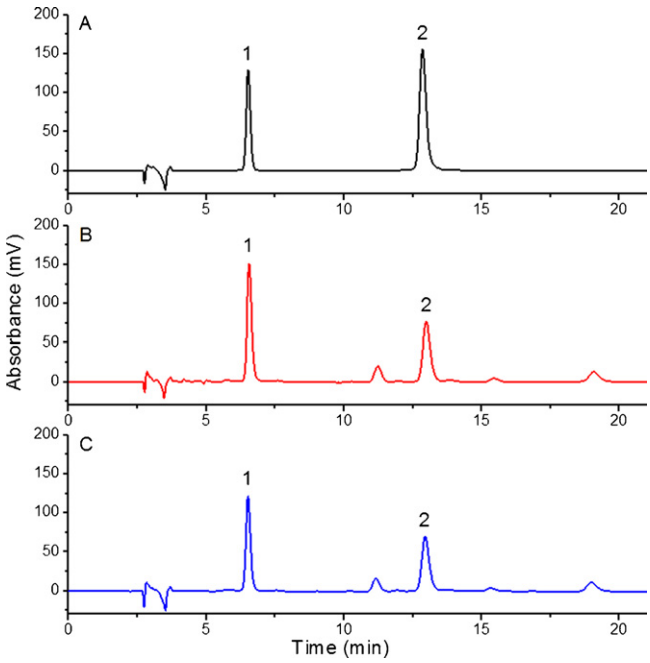

Fig. 5. Chromatograms of standard solution of aesculin (98.1 mg/L) and aesculetin (87.3 mg/L) (A), extract of *C. fraxini* by PEG solution (B) and extract of *C. fraxini* by methanol (C). Peak 1, aesculin; peak 2, aesculetin.

derivations (RSDs) of nevadensin, aesculin and aesculetin ranged from 1.1% to 2.1%. The results indicated that the repeatability of the methods was good.

The recovery experiments of target compounds at the optimum conditions were evaluated by standard-addition method at different concentration levels. The results are shown in Table 3. The recoveries of nevadensin, aesculin and aesculetin were in the range of 92.4–103% with RSDs lower than 3.6%. The reproducibility and recovery proved that the method had good precision and accuracy.

The stability was investigated by determining the varieties of target compounds in PEG extraction solutions on 7 separate days. All the RSDs of intra-day and inter-day were less than 2.5% and 3.2%, respectively. The results showed that target compounds were stable in the PEG extraction solutions.

3.3. Comparison of different extraction procedures

In order to compare the extraction efficiency of the PEG solution with organic solvent, methanol was used to extract target compounds from the medicinal plants. PEG-MAE was performed at the optimum conditions in Section 3.1, and methanol-MAE was performed at the optimum conditions in Section 2.3. The chromatograms of methanol extraction solutions are shown in Figs. 4 and 5. The results showed that PEG solution and methanol had different extraction selectivity on substances in sample. The extraction yields of nevadensin, aesculin and aesculetin by using methanol were 90.4%, 88.3% and 88.8%, respectively, which were slightly lower than those by using PEG-200. Additionally, methanol is volatile and flammable, and it is harmful to environment. Therefore, PEG solution was a possible alternative solvent in the MAE of flavone and coumarin compounds from medicinal plants.

HRE and ME were also carried out in comparison study. The HRE conditions were liquid/solid ratio 40:1, extraction time 40 min and extraction temperature 80 °C. The extraction yields of nevadensin, aesculin and aesculetin by HRE were 92.2%, 89.8% and 97.6%, respectively, with the recoveries between 94.6% and 102%. The ME conditions were liquid/solid ratio 40:1, extraction time 12 h and extraction temperature 25 °C. The extraction yields of nevadensin, aesculin and aesculetin by ME were 84.9%, 85.3% and 96.9%, respectively, with the recoveries between 89.0% and 101%. Compared with HRE and ME, MAE could obtain higher extraction yields by using less solvent at shorter extraction time, which is due to its unique extraction mechanism. During MAE procedure, microwaves directly heat solvent and sample. Therefore, the direct interaction of microwaves with PEG solutions and free water molecules present in the cells resulted in the subsequent rupture of the cells and the release of intracellular products into the solvent. The results shown above indicated that MAE was a more rapid and effective sample preparation technique.

3.4. Analysis of real samples

The present method was applied for the determination of target compounds in real samples from different regions. *L. pauciflorus* from Jiangxi, Anhui, Yunnan and Guizhou provinces, *C. fraxini* from Gansu, Jilin, Guangxi and Liaoning provinces were studied. The results showed that nevadensin in *L. pauciflorus* from different regions ranged from 2.01 to 3.12 mg/g with the corresponding RSDs less than 2.5%. The recoveries of nevadensin were in the range of 92.0% and 106.0% and the corresponding RSDs were less than 5.3%. Aesculin and aesculetin in *C. fraxini* from different regions ranged from 7.36 to 21.45 mg/g and 2.35 to 6.05 mg/g with the corresponding RSDs less than 2.7%, respectively. The recoveries of aesculin and aesculetin were in the range of 98.0% and 104.3% with the corresponding RSDs less than 2.1%. The results indicated that the PEG-MAE technique was feasible for extracting nevadensin from *L. pauciflorus* and for extracting aesculin and aesculetin from *C. fraxini*.

3.5. Mechanism of PEG-MAE

3.5.1. The investigation of PEG solution properties

As an alternative solvent used in MAE, the microwave-absorptive property of the solvent is very important. It is difficult for a solvent which is unsuitable for energy dissipation with microwaves to be widely used in MAE procedure. Besides, PEG solutions are viscous fluids, and the viscosity of the extraction solvent would significantly influence the heating conduction and the mass transfer. So, both the microwave-absorptive property and the viscosity of PEG solutions were studied.

The microwave-absorptive properties of different solutions including water, methanol, ethanol, PEG-200, PEG-400, PEG-600, 60% PEG-200, 60% PEG-400 and 60% PEG-600 were investigated by comparing the heating rates. The time of different solutions used in achieving different temperatures is recorded in Table 4. The heating rates were the slopes of the calibration curve constructed by plotting the temperatures achieved vs the time used. Comparing the heating rates of different solutions under microwave radiation in Table 4, the heating rate of PEG-200 was 1.6 times higher than that of water and was 1.4 times higher than that of methanol, which demonstrated the good microwave-absorptive property of PEGs. And the heating rates of PEGs decreased from PEG-200 to PEG-600, the probable reasons were that the polarity of PEG solutions decreased as the molecular weight of PEG increased and the viscosity of PEG solutions increased at the same time, which decelerated the heat exchange. Different molecular weights of 60% PEG solutions have no significant difference in the heating rates.

The viscosities of different mass fractions of PEG-200 solutions were studied by detecting the viscosities of the solutions at 30 °C and 70 °C. The results are shown in Fig. 6A. The viscosity of PEG-200 solution increased as the mass fraction increased, especially after the mass fraction higher than 60%. The results can explain why the extraction yield of nevadensin slightly fell after the mass fraction reached 60% in Section 3.1.2. The viscosity of PEG-200 solution increased more rapidly at 30 °C than that at 70 °C, which means that

Table 3
Recovery of the target compounds from samples ($n = 3$).

Sample	Compounds	Original amount (mg/g)	Added amount (mg/g)	Found amount (mg/g)	Recovery (%)	RSD (%)
L. pauciflorus	Nevadensin	2.04	0.93	2.92	94.1	1.6
			1.86	3.90	99.8	0.9
			2.78	4.91	103.1	1.0
C. fraxini	Aesculin	19.56	4.00	23.26	92.5	1.3
			8.00	27.47	98.8	1.1
			16.00	34.68	94.5	1.2
	Aesculetin	6.05	1.00	6.97	92.4	3.1
			2.00	8.09	102.2	0.9
			5.00	10.81	95.2	3.6

Table 4
Comparison of heating rates of different solutions under microwave radiation ($n = 3$).[a]

Temperature (°C)	Time (s)								
	Water	Methanol	Ethanol	PEG-200	PEG-400	PEG-600	60% PEG-200	60% PEG-400	60% PEG-600
20	0	0	0	0	0	0	0	0	0
30	21	15	15	15	15	15	18	17	18
40	32	22	22	22	23	24	26	25	26
50	42	29	28	28	30	30	33	32	33
60	52	42	35	34	36	38	39	39	39
70	62	–	45	40	44	46	45	45	45
80	71	–	–	47	52	56	51	51	51
90	85	–	–	54	62	66	–	–	–
100	–	–	–	62	72	79	–	–	–
K[b]	0.876	0.998	1.174	1.384	1.186	1.082	1.210	1.216	1.218

[a] Each time is the mean of three independent experiments. The standard deviation was less than 1.4 s.
[b] K, the heating rate of solution.

temperature has significant influence on the viscosity of PEG-200 solutions.

The influence of temperature on the viscosity of PEG-200 and 60% PEG-200 solutions was studied by detecting the viscosities at different temperatures. The results are shown in Fig. 6B. As the temperature increased, the viscosity of PEG-200 and 60% PEG-200 solutions decreased rapidly, especially that of pure PEG-200 solution. The less viscosity PEG solution has, the more diffusivity PEG solution would have, which was beneficial for the heating conduction and mass transfer during extraction. Therefore, the concentration of PEG solution and the temperature achieved by microwave radiation would greatly influence the extraction ability of PEG solution during the extraction of bioactive compounds from natural products.

3.5.2. Study of kinetic mechanism

To investigate the changes of the extraction yields during MAE process, the PEG-MAE kinetic mechanism was studied compared with the methanol-MAE in this study. The results of kinetic curves for the PEG-MAE and methanol-MAE of nevadensin from *L. pauciflorus*, aesculin and aesculetin from *C. fraxini*, according to time, are shown in Fig. 7, respectively.

For nevadensin from *L. pauciflorus*, the extraction yield increased rapidly at early stage and then reached an equilibrium concentration. For methanol-MAE, the extraction yield of nevadensin reached the corresponding maximum value (about 87%) before 8 min. And for PEG-MAE, the extraction yield of nevadensin arrived to the maximum value (about 96%) after 10 min. The above results indicated that methanol-MAE could reach an equilibrium concentration a little faster than PEG-MAE in the extraction of nevadensin from *L. pauciflorus*. That is probably because the viscosity of methanol is much less than 60% PEG aqueous solution, which is beneficial for the diffusion and mass transfer during extraction. However, PEG-MAE could obtain a higher maximum extraction yield than methanol-MAE, the probable reason was that the extraction temperature of PEG-MAE was higher than that of methanol-MAE. The results of the MAE kinetic mechanism indicated that about 10 min of MAE time was enough to obtain a high extraction yield of

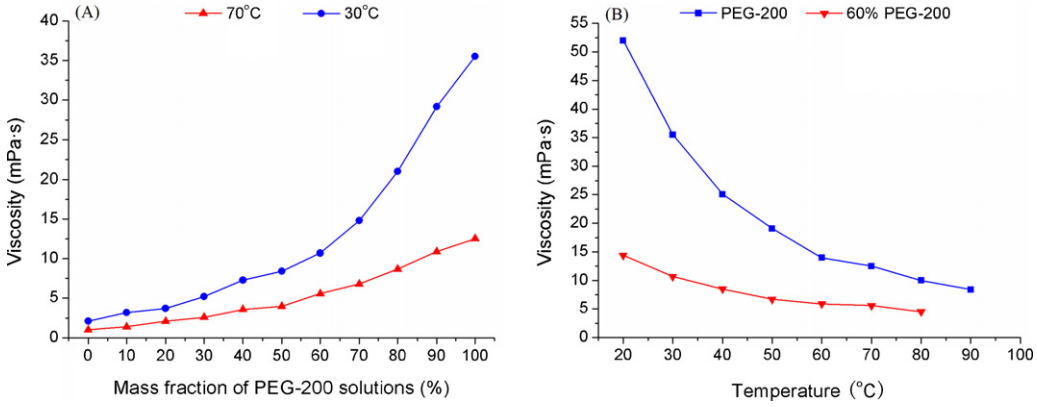

Fig. 6. (A) The viscosity of different mass fractions of PEG-200 solutions ($n = 3$). (B) The viscosity of PEG-200 solutions at different temperatures ($n = 3$).

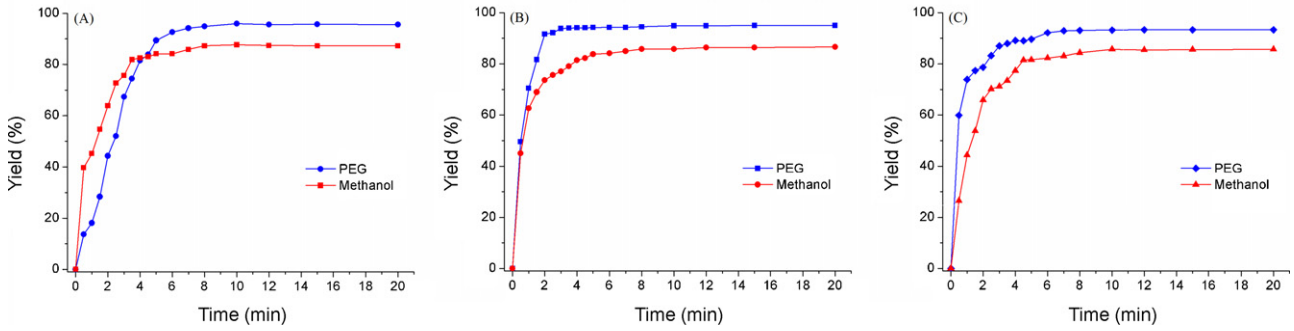

Fig. 7. Kinetic curves of nevadensin (A), aesculin (B) and aesculetin (C) extracted from samples using PEG-200 solution and methanol, respectively.

nevadensin from *L. pauciflorus* when PEG solution was used as a solvent.

For aesculin and aesculetin from *C. fraxini*, the extraction yields also increased rapidly at early stage and then reached an equilibrium concentration. However, there is much difference between the flavone compound and coumarin compound in their MAE kinetic mechanism. The extraction yields of aesculin and aesculetin reached the corresponding maximum values (about 95.0% and 93.0%) after 3 min and 6 min in PEG-MAE, while 8 min and 10 min were taken in methanol-MAE to reach the corresponding maximum values (about 86.0% and 85.5%) for aesculin and aesculetin, respectively. The above results indicated that PEG-MAE could reach an equilibrium concentration much faster than methanol-MAE in the extraction of aesculin and aesculetin from *C. fraxini*. That is probably because the good dissolving capacity of PEG solution for coumarin compounds and the higher extraction temperature could result in the fast rupture of the cells. Furthermore, PEG-MAE could obtain higher maximum extraction yields than methanol-MAE. The results of the MAE kinetic mechanism indicated that there were advantages both in the extraction rates and maximum extraction yields for the extraction of coumarin compounds from *C. fraxini* when PEG solution was used as a solvent.

3.5.3. Structural changes after extraction

In order to further elucidate the extraction mechanism, the identification of microstructures of samples over variant conditions was carried out by SEM. Compared samples before extraction (Figs. S1A and S2A, see Supplementary Data) with samples after PEG-MAE (Figs. S1B and S2B), the microstructures of *L. pauciflorus* and *C. fraxini* changed obviously after PEG-MAE. The surface of *L. pauciflorus* and *C. fraxini* was greatly destroyed and the structure of cells walls was ruptured after PEG-MAE, which resulted in exposing the target compounds to PEG solution. The results suggested that the mechanism of PEG-MAE was based on an explosion at the cell level, which was in accord with the hypothesis investigated by Paré et al. [23]. The structures of *L. pauciflorus* and *C. fraxini* obtained by methanol-MAE were also severely destroyed (Figs. S1C and S2C), there were some ruptures of cell wall structures observed in *L. pauciflorus*, but no significant ruptures of cell wall structures were observed in *C. fraxini*. The difference between PEG-MAE and methanol-MAE may be caused by the different microwave-absorptive properties of extraction solutions. The better microwave-absorptive property of PEG solution caused the higher heating rate, which resulted in more destruction to the cell walls. However, the microstructures of *L. pauciflorus* and *C. fraxini* were not considerably changed and ruptured after HRE (Figs. S1D and S2D). The results indicated that the solvent transferred into the matrix and extracted the compounds by solubilization, therefore little destruction of the microstructure of sample occurred and more solvent and long extraction time were used in HRE process.

4. Conclusion

In this work, PEG aqueous solution was proved to be a possible alternative green solvent in the MAE of flavone and coumarin compounds from medicinal plants. Compared with conventional extraction procedures, the proposed method could provide higher extraction yields with considerable reduction in extraction time and solvent consumption. What is more, PEG solution has nice microwave-absorptive property, which has significant advantage in MAE procedure. The PEG-MAE kinetic mechanism indicated that about 10 min was enough to obtain high extraction yields of the target compounds. And according to the SEM results, the enhanced extraction was mainly based on the destruction of sample microstructures in PEG-MAE process. The proposed technique was a green, simple, rapid and effective extraction method for separation of flavone and coumarin compounds from the Chinese herb. And with the development of green sample preparation techniques, PEG solution as a green solvent in the MAE of useful substances in natural sources showed a great promising prospect.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.04.031.

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