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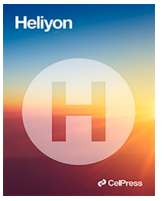

Research article

GC-MS method for simultaneous determination and pharmacokinetic investigation of five volatile components in rat plasma after oral administration of the essential oil extract of *Pogostemon cablin*

Yameng Zhu [a,c,1], Huizi Ouyang [a,b,1], Wenhan Lin [c], Wenwen Li [c], Xiunan Cao [c], Yanxu Chang [c], Jun He [c,*]

[a] First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, 300193, Tianjin, China
[b] National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, 300193, Tianjin, China
[c] State Key Laboratory of Component-based Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, China

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ABSTRACT

Pogostemon cablin (PC) is a traditional Chinese medicine (TCM) and food as well as an important essential oil plant in China. PC essential oil exerts pharmacological effects such as anti-inflammatory, anti-oxidant, anti-platelet, anti-thrombotic, and anti-depressant. This study established a reliable and sensitive gas chromatography-mass spectrometry (GC-MS) method for the simultaneous determination of the pharmacokinetics of patchouli alcohol, β-elemene, β-caryophyllene, caryophyllene oxide, and farnesol in the plasma of rats after oral administration of PC essential oil extract. Using ethyl acetate to prepare the plasma samples, and p-menthone was used as the internal standard (IS). An HP5-MS column (0.25 μm × 0.25 mm × 30 m) was used for chromatographic separation, and detection was performed in selected ion monitoring (SIM) mode. The accuracies of intra-day and inter-day for all analytes displayed a range of −6.7 %–9.2 %, with precision below 12.5 %. Extraction recoveries for analytes ranged from 74.0 to 106.4 % and matrix effects ranged from 92.4 to 106.9 %. Stability results have demonstrated that the relative standard deviations (RSD) of analytes were below 12.1 %. Therefore, the developed GC-MS method successfully evaluated the pharmacokinetics of five volatile components in PC essential oil extract administered orally to rats.

1. Introduction

Pogostemon cablin (PC) is the aerial part of dried *Pogostemon cablin* (Blanco) Benth. (Pogostemun, Lamiaceae), and native to Southeast Asia. It is now widely cultivated in China, Indonesia, the Philippines, and Thailand [1]. In Traditional Chinese Medicine (TCM), PC was used to improve indigestion, and relieve vomiting and diarrhea [2]. Research in recent years has revealed that PC has abundant pharmacological activities, including immune regulation, anti-bacterial, anti-oxidant, anti-tumor, and antiviral due to it containing various active components such as monoterpenes, triterpenes, sesquiterpenes, phytosterols, flavonoids, glycosides,

* Corresponding author.
E-mail address: hejun673@tjutcm.edu.cn (J. He).
[1] Contributed equally to this work.

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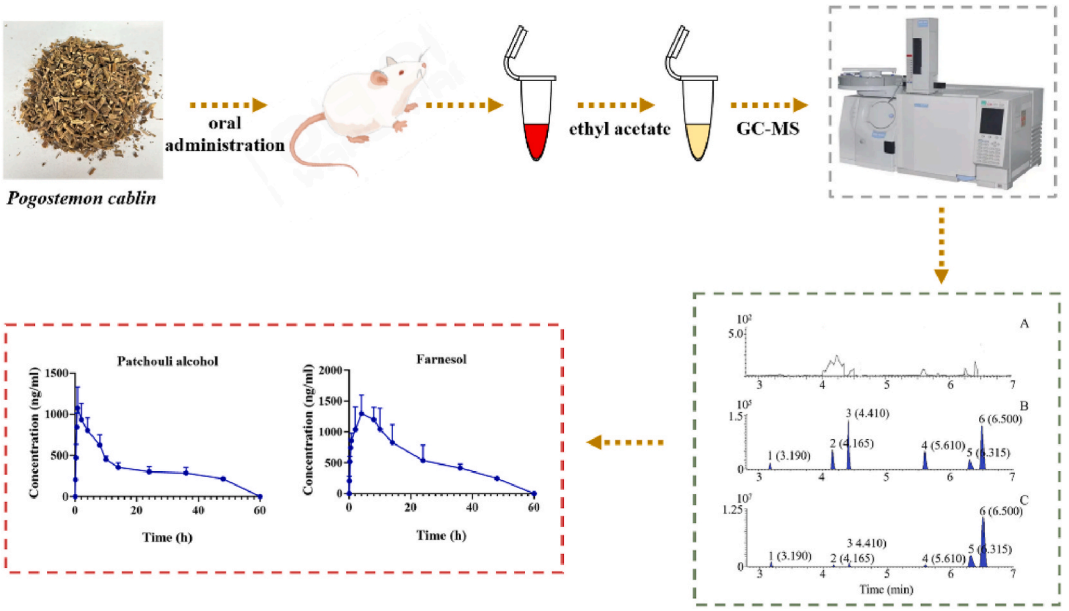

Fig. 1. A reliable and sensitive GC-MS method for determination of after oral administration of PC essential oil extract in rat plasma and pharmacokinetic study.

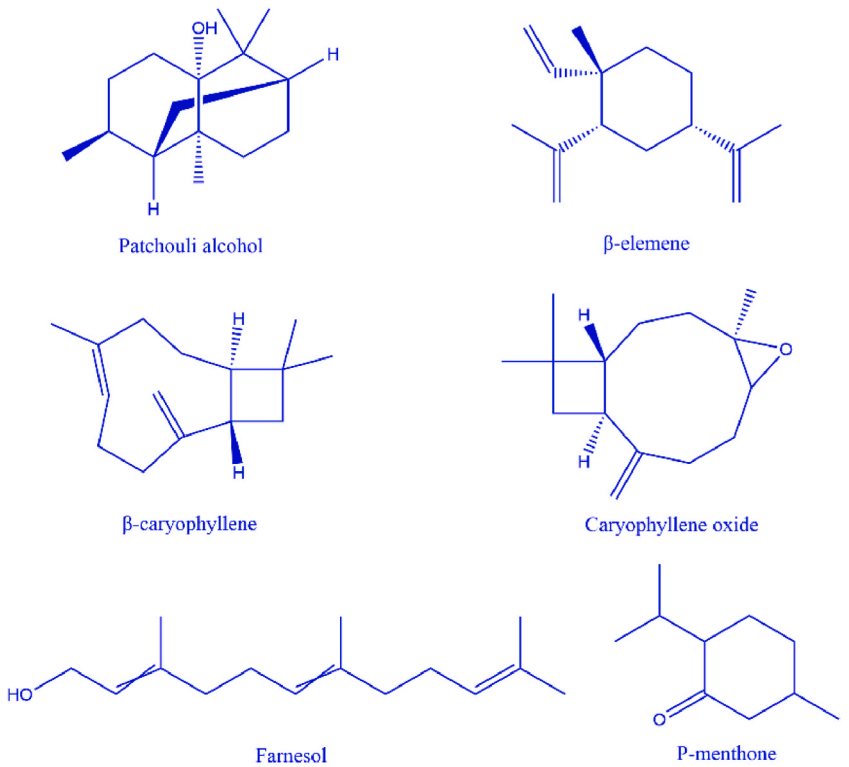

Fig. 2. The five analytes and IS chemical structures.

alcohols, and aldehydes [3–5]. PC is also an important commercial plant widely used in perfume, beverage, agar, soap, and food industries [6].

PC, one of the top 20 essential oil producers, has tremendous economic potential [7]. The leaves and stems of PC are rich in volatile components. Currently, analytical studies on its chemical composition focus on the volatile oil, patchouli oil. Patchouli oil is vital to the

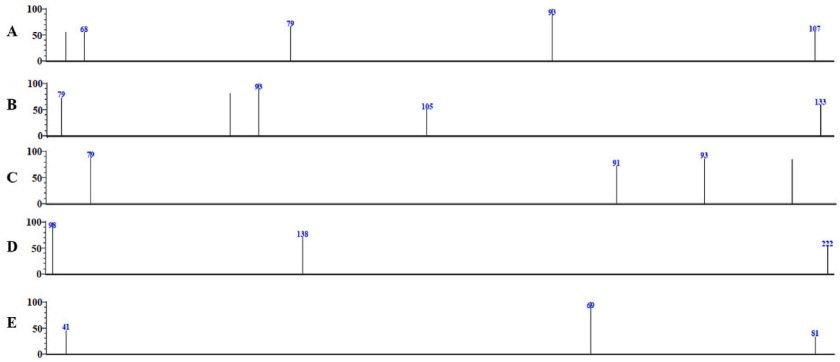

Fig. 3. The secondary mass spectrogram of β-elemene (A), β-caryophyllene (B), caryophyllene oxide (C), patchouli alcohol (D), and farnesol (E).

perfume and cosmetics industry due to it has fixative properties that make other perfumes last longer [8,9]. There is a wide range of uses for patchouli oil in aromatherapy, including relieving depression and stress, calming nerves, and controlling appetite. Patchouli oil also has characteristics of anti-bacterial, anti-inflammatory, anti-oxidant, and anti-depressant [10–14]. Various compounds have been reported in patchouli oil, such as patchouli alcohol, β-caryophyllene, β-patchoulene, β-elemene, β-pinene, and α-selinene [15,16]. As a primary bioactive component of PC, patchouli alcohol accounts for between 20 and 26 % of the total weight of patchouli oil and is used as a phytochemical indicator to determine the quality of PC and patchouli oil [2]. As an important component in PC, patchouli alcohol has been extensively studied for its anti-inflammatory, antioxidant, anti-tumor, analgesic, vasodilatory, and other pharmacological effects [17–19].

Pharmacokinetics is one of the key steps in evaluating the absorption characteristics of different active components in TCM. Understanding pharmacokinetics is important for elucidating the mechanism of action. Moreover, it helps to reduce toxic side effects, optimize dosing regimens, and guide the clinical application of drugs [20]. Studies on the pharmacokinetics of patchouli alcohol have been reported, but this is not sufficiently representative of the pharmacokinetics of the PC *in vivo* [21,22].

This study established a reliable and sensitive gas chromatography-mass spectrometry (GC-MS) method to simultaneously determine the contents of five components (patchouli alcohol, β-elemene, β-caryophyllene, caryophyllene oxide, and farnesol) in the plasma of rats after oral administration of PC essential oil extract (Fig. 1). Successful application of GC-MS method in pharmacokinetic studies in rats, which provided an important reference for further development and clinical rational use of PC.

2. Materials and methods

2.1. Chemicals and reagents

The standards of patchouli alcohol, β-elemene, β-caryophyllene, caryophyllene oxide, and p-menthone (internal standard [IS], purity ≥98 %) were received from Chengdu Desite Bio-Technology Co., Ltd. (Chengdu, China). The Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China) provided farnesol. The chemical structures of the five components and IS are displayed in Fig. 2. Ethyl acetate and *n*-hexane were provided by Concord Technology (Tianjin) Co., Ltd. (Tianjin, China).

PC (*Pogostemon cablin*), also known as "Guanghuoxiang", was obtained from Guangdong province, China. The crude drugs were authenticated as *Pogostemon cablin* by Professor Jun He of Tianjin University of Traditional Chinese Medicine. PC is stored at the State Key Laboratory of Component-based Chinese Medicine, Tianjin University of Traditional Chinese Medicine, China.

2.2. Instruments and conditions

The HP5-MS column (0.25 μm × 0.25 mm × 30 m) was used for chromatographic separation, with an AOC-20i autosampler. A 99.99 % high-purity helium carrier gas was utilized at a flow rate of 1.8 mL/min. The column temperature was programmed as follows: starting at 120 °C, increasing to 180 °C at a rate of 20 °C/min, and then to 210 °C at a rate of 10 °C/min. The injection volume was set at 1 μL, the split ratio at 20:1, and the injection temperature at 250 °C.

Mass spectrometric data were collected using electron impact mode and quantified using selected ion monitoring (SIM) mode. Temperatures were set at 240 °C for the transmission line and 230 °C for the ionization source. The total run time was 7.0 min and the characteristic ions (*m*/*z*) of the β-elemene, β-caryophyllene, caryophyllene oxide, patchouli alcohol, and farnesol were *m*/*z* 93/79/107, *m*/*z* 93/79/133, *m*/*z* 79/93/91, *m*/*z* 98/138/222 and *m*/*z* 69/41/81, respectively. The secondary mass spectrogram of β-elemene, β-caryophyllene, caryophyllene oxide, patchouli alcohol, and farnesol are shown in Fig. 3.

2.3. Preparation of the essential oil extract of PC

The PC samples (250.0 g) were extracted through a volatile oil extractor under 2.5 L of water reflux for 10 h. The extracted essential

oils were collected and dehydrated with anhydrous sodium sulfate for 24 h to obtain 3 mL of volatile oils, which were then stored at 4 °C until analyzed. The contents of β-elemene, β-caryophyllene, caryophyllene oxide, patchouli alcohol, and farnesol in PC determined by the developed GC-MS method were 5950.48, 2949.28, 6214.95, 15649.49, and 13362.33 μg/mL, respectively.

2.4. Preparation of standard and quality control (QC) samples

β-elemene, β-caryophyllene, caryophyllene oxide, patchouli alcohol, farnesol, and p-menthone (IS) were weighed separately and each was diluted using ethyl acetate to create standard stock solutions with a concentration of 1 mg/mL. Various working solutions were then prepared by further diluting these standard stock solutions with ethyl acetate.

Add an appropriate amount of mixed working solution and 10 μL IS to prepare the calibration solution into 100 μL blank rat plasma to obtain the concentration: 133, 266, 665, 1330, 2660, 6650, and 13300 ng/mL for β-elemene; 60, 120, 300, 600, 1200, 3000, and 6000 ng/mL for β-caryophyllene; 400, 800, 2000, 4000, 8000, 20000, and 40000 ng/mL for caryophyllene oxide; 200, 400, 1000, 2000, 4000, 10000, and 20000 ng/mL for patchouli alcohol; 100, 200, 500, 1000, 2000, 5000, and 10000 ng/mL for farnesol. Quality control (QC) samples were prepared in the same manner for three concentrations (low, medium, and high). Until analyzed, all solutions were maintained at 4 °C.

2.5. Plasma sample preparation

A 100 μL plasma sample was combined with 10 μL ethyl acetate, then followed by adding 10 μL IS (10 μg/mL). After vortexing the mixture for 1 min, 130 μL of methyl acetate was added and vortexed for an additional 5 min to ensure thorough mixing.

2.6. Method validation

2.6.1. Specificity

To determine the specificity, the chromatograms of different samples were compared. This encompassed evaluating the chromatograms of blank plasma samples, spiked plasma samples containing analytes, as well as plasma samples collected subsequent to the oral administration of PC essential oil extract. By analyzing these chromatograms, the specificity of the PC essential oil extract was determined.

2.6.2. Linearity and LLOQ

Calibration curves were created through plotting the ratios of peak areas for each analyte and IS against the concentration of the respective analyte. To determine the relationship between the variables, a linear regression equation with a weighting factor of $1/x$ was applied. The lower limit of quantification (LLOQ) was determined by considering the baseline noise, which exhibited a signal-to-noise ratio of around 10.

2.6.3. Precision and accuracy

Six QC samples at three concentrations were used to assess precision and accuracy of analytes (133, 1330, and 13300 ng/mL for β-elemene; 60, 600, and 6000 ng/mL for β-caryophyllene; 400, 4000, and 40000 ng/mL for caryophyllene oxide; 200, 2000, and 20000 ng/mL for patchouli alcohol; 100, 1000, and 10000 ng/mL for farnesol) within one day or on three consecutive days. The RSD was used to determine the intra-day and inter-day precisions, and the accuracy was evaluated by RE.

2.6.4. Extraction recovery and matrix effect

The extraction recovery rates were calculated by comparing the peak areas of QC samples and post-extracted samples at three concentrations. Matrix effects were assessed by comparing peak areas of the post-extraction QC samples with those obtained from non-extracted.

2.6.5. Stability

Plasma samples were stabilized for 12 h in an autosampler, 4 h at room temperature, three freeze-thaw cycles, and 7 days at −80 °C to assess the stability of analytes.

2.7. Pharmacokinetic study

Six male rats weighing 220 ± 10 g were utilized in this study. Before the experiment, the rats were fasted for 12 h without restriction of water intake. PC essential oil extract was dissolved in a 0.5 % CMC-Na aqueous solution and administered orally to the rats at a dosage of 1.0 mL/kg (1.1 g/kg). Before and following oral administration, Blood samples (300 μL) were collected from the rats' fundus venous plexus (0, 0.083, 0.25, 0.5, 0.75, 2, 4, 8, 10, 14, 24, 36, 48, and 60 h). A clean tube was then used to collect the blood samples, which were immediately frozen at −80 °C until further analysis could be performed.

The rats were administered a single oral dose of essential oil extract equivalent to the human clinical equivalent dose, but only a few compounds were detected. In the present study, after giving different doses of PC essential oil extract orally to rats, it was found that a single oral dose of 1.0 mL/kg of PC essential oil extract was able to detect more components and obtain complete concentration-time curves for two components, and no abnormal variations were found in the fur, skin, eyes, and behavioral pattern of the rats, with no

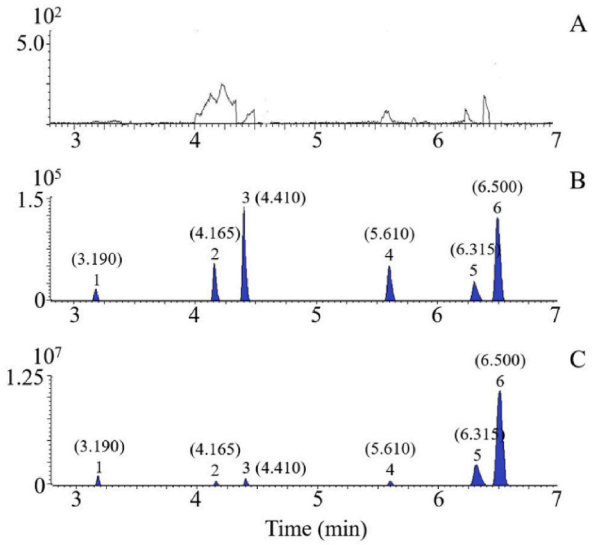

Fig. 4. The chromatograms of various samples: a blank plasma sample (A), a blank plasma sample combined with both the five analytes and IS (B), and plasma samples obtained after oral intake 2 h of the PC essential oil extract (C) (1. p-menthone (IS), 2. β-elemene, 3. β-caryophyllene, 4. caryophyllene oxide, 5. patchouli alcohol, 6. farnesol).

signs of coma, sleep, lethargy, tremor, diarrhea, and salivation. Therefore, 1.0 mL/kg of PC essential oil extract was administered to rats in this study.

2.8. Data analysis

The mean ± standard deviation was used to represent the data. MassHunter Workstation software (Agilent, USA, version B.09.00) was used for quantitative computation of plasma analyte levels. DAS Software (version 3.0; Medical College of Wannan, China) was employed for the determination of pharmacokinetic parameters.

3. Result and discussion

3.1. GC-MS conditions optimization

SIM mode was selected for the determination of the five analytes. Moreover, initial (50, 80, 100, and 120 °C) and final (200, 240, 260, and 280 °C) column temperature, injector temperature (210, 250, and 270 °C), and split ratio (5:1, 10:1, and 20:1) were optimized in the present study for better area response and symmetrical peaks.

3.2. Selection of extraction method

The selection of extraction methods liquid-liquid extraction (LLE) and protein precipitation were common sample preparation methods, both of which were tested during method development. LLE used organic extraction solvents, including ethyl acetate and *n*-hexane. In addition, the protein precipitation method was examined using methanol and acetonitrile, respectively. The results showed that both *n*-hexane and ethyl acetate LLE could be used for repeated extraction of five analytes with good recovery. In this study, ethyl acetate was chosen as the extraction solvent to avoid interference from endogenous plasma substances on analyte determination post-treatment with *n*-hexane.

3.3. Method validation

3.3.1. Specificity

The selectivity of the method was evaluated by examining the chromatograms of different samples: a blank plasma sample (A), a blank plasma sample combined with both the five analytes and IS (B), and plasma samples obtained after oral intake of the PC essential oil extract (C). The findings revealed that there were no interference peaks detected at the retention times of the five analytes and IS (Fig. 4 A-C).

3.3.2. Linearity and LLOQ

The correlation coefficients of analytes were greater than 0.999. The results suggested that the calibration profiles of the five

Table 1
Standard curve regression equation, linear range, and LLOQ of five components.

Compound	Calibration curve	Correlation coefficients (r)	Linear range (ng/mL)	LLOQ (ng/mL)
β-Elemene	$Y = 2.6804E^{-003}X - 1.5053$	0.999	133–13300	133
β-Caryophyllene	$Y = 6.2735E^{-003}X - 0.1691$	0.999	60–6000	60
Caryophyllene oxide	$Y = 1.4918E^{-003}X - 8.6818E^{-002}$	0.999	400–40000	400
Patchouli alcohol	$Y = 1.2982E^{-003}X + 4.9309E^{-004}$	0.999	200–20000	200
Farnesol	$Y = 4.9882E^{-003}X - 0.1154$	0.999	100–10000	100

Table 2
Accuracy and precision of five components in rat plasma (n = 6).

Compound	Spiked concentration (ng/mL)	Intra-day			Inter-day		
		Measured (ng/mL)	RE (%)	RSD (%)	Measured (ng/mL)	RE (%)	RSD (%)
β-Elemene	133	137.7 ± 6.9	5.0	3.5	137.3 ± 5.9	4.3	3.2
	1330	1306.9 ± 77.0	5.9	−1.7	1393.1 ± 51.8	3.7	4.7
	13300	12760.4 ± 292.1	2.3	−4.1	12793.0 ± 187.3	1.5	−3.8
β-Caryophyllene	60	62.2 ± 1.3	2.1	3.6	62.9 ± 1.1	1.8	4.8
	600	614.5 ± 23.2	3.8	2.4	591.8 ± 14.4	2.4	−1.4
	6000	5930.2 ± 141.2	2.4	−1.2	5847.5 ± 106.7	1.8	−2.5
Caryophyllene oxide	400	418.2 ± 21.1	5.0	4.6	424.7 ± 19.0	4.5	6.2
	4000	4006.8 ± 264.2	6.6	0.2	4029.6 ± 240.2	6.0	0.7
	40000	39631.7 ± 332.6	0.8	−0.9	38826.4 ± 862.8	2.2	−2.9
Patchouli alcohol	200	208.1 ± 5.69	2.7	4.1	218.5 ± 8.2	3.7	9.2
	2000	2106.8 ± 107.1	5.1	5.3	1927.4 ± 127.4	6.6	−3.6
	20000	19461.7 ± 1415.9	7.3	−2.7	21298.4 ± 1028.6	4.8	6.5
Farnesol	100	102.4 ± 12.8	12.5	2.4	103.5 ± 5.6	5.4	3.5
	1000	1025.7 ± 55.8	5.4	2.6	1062.5 ± 75.0	7.1	6.3
	10000	9331.4 ± 721.7	7.7	−6.7	9760.4 ± 974.4	10.0	−2.4

Table 3
Matrix effect and extraction recovery of five components in rat plasma (n = 6).

Compound	Spiked concentration (ng/mL)	Extraction recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
β-Elemene	133	96.5 ± 7.7	7.9	98.8 ± 9.4	9.5
	1330	93.4 ± 8.2	8.8	103.9 ± 3.6	3.4
	13300	74.9 ± 3.9	5.2	94.1 ± 5.4	5.7
β-Caryophyllene	60	82.3 ± 9.0	10.9	92.4 ± 7.3	7.9
	600	95.8 ± 3.4	3.6	106.9 ± 9.1	8.5
	6000	74.0 ± 1.9	2.6	95.1 ± 4.8	5.0
Caryophyllene oxide	400	97.6 ± 5.1	5.2	93.1 ± 6.7	7.2
	4000	97.5 ± 6.0	6.1	100.0 ± 3.9	3.9
	40000	85.8 ± 2.3	2.6	101.7 ± 2.56	2.5
Patchouli alcohol	200	93.6 ± 9.0	9.6	97.4 ± 7.9	8.1
	2000	104.5 ± 9.9	9.4	106.6 ± 9.1	8.5
	20000	102.0 ± 8.8	8.6	96.5 ± 8.7	9.0
Farnesol	100	101.8 ± 5.8	5.7	101.2 ± 9.8	9.7
	1000	106.4 ± 12.2	11.5	96.4 ± 5.9	6.1
	10000	98.6 ± 8.8	9.0	102.8 ± 3.0	2.9

analytes had good linear relationships within their respective concentration ranges (Table 1).

3.3.3. Precision and accuracy

The accuracy and intra and inter-day precision of QC samples under three concentrations are listed in Table 2. Both intra and inter-day RSDs were below 12.5 %, and RE ranged from −6.7 % to 9.2 %. The results revealed that the method has good precision and accuracy.

3.3.4. Extraction recovery and matrix effect

The extraction recoveries ranged from 74.0 to 106.4 %, and the matrix effect ranged from 92.4 to 106.9 % (Table 3). This method showed accurate and acceptable extraction recoveries and matrix effects.

3.3.5. Stability

The presentation of the QC sample stability under different conditions were presented in Table 4. The RSDs of the tested analytes were all below 12.1 %. This revealed that the GC-MS method developed could reliably determine the presence of the five analytes.

Table 4
Stability of five components in rat plasma (n = 6).

Compound	Spiked concentration (ng/mL)	4 h at room temperature		12 h in an autosampler		Three freeze-thaw cycles		7 days at −80 °C	
		Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)
β-Elemene	133	136.2 ± 2.8	2.0	139.5 ± 4.2	3.0	140.8 ± 4.0	2.9	137.7 ± 4.2	3.0
	1330	1358.0 ± 21.6	1.6	1360.8 ± 35.4	2.6	1317.1 ± 41.4	3.1	1370.2 ± 51.1	3.7
	13300	12823.0 ± 166.4	1.3	12691.3 ± 299.4	2.4	13067.0 ± 203.7	1.6	12580.6 ± 233.7	1.9
β-Caryophyllene	60	67.3 ± 5.1	7.6	62.3 ± 1.7	2.7	66.2 ± 3.8	5.7	64.0 ± 6.2	9.7
	600	598.8 ± 29.6	4.9	601.9 ± 25.7	4.3	595.6 ± 12.6	2.1	570.9 ± 30.5	5.3
	6000	5982.1 ± 90.2	1.5	5690.6 ± 145.5	2.6	5746.1 ± 326.8	5.7	5853.5 ± 139.6	2.4
Caryophyllene oxide	400	431.8 ± 11.5	2.7	441.6 ± 27.0	6.1	432.0 ± 11.0	2.5	441.6 ± 22.7	5.1
	4000	4070.0 ± 48.8	1.2	4153.9 ± 98.6	2.4	4153.6 ± 20.8	0.5	4119.9 ± 129.5	3.1
	40000	39546.8 ± 512.6	1.3	37952.9 ± 1677.6	4.4	40438.0 ± 699.0	1.7	39280.3 ± 855.2	2.2
Patchouli alcohol	200	226.0 ± 8.2	3.6	201.6 ± 8.4	4.2	213.8 ± 10.3	4.8	209.6 ± 15.4	7.3
	2000	1877.6 ± 32.5	1.7	1878.1 ± 78.1	4.2	1979.2 ± 53.6	2.7	1920.8 ± 78.0	4.1
	20000	19166.6 ± 468.9	2.4	19339.0 ± 563.8	2.9	19913.5 ± 675.1	3.4	19008.9 ± 507.2	2.7
Farnesol	100	101.3 ± 8.1	8.0	103.3 ± 10.8	10.4	102.0 ± 5.5	5.3	108.1 ± 11.0	10.1
	1000	1084.8 ± 83.4	7.7	1054.6 ± 88.1	8.4	1108.5 ± 68.0	6.1	1073.1 ± 59.8	5.6
	10000	8990.5 ± 1085.2	12.1	9976.4 ± 894.1	9.0	9805.1 ± 636.4	6.5	9805.1 ± 636.4	6.5

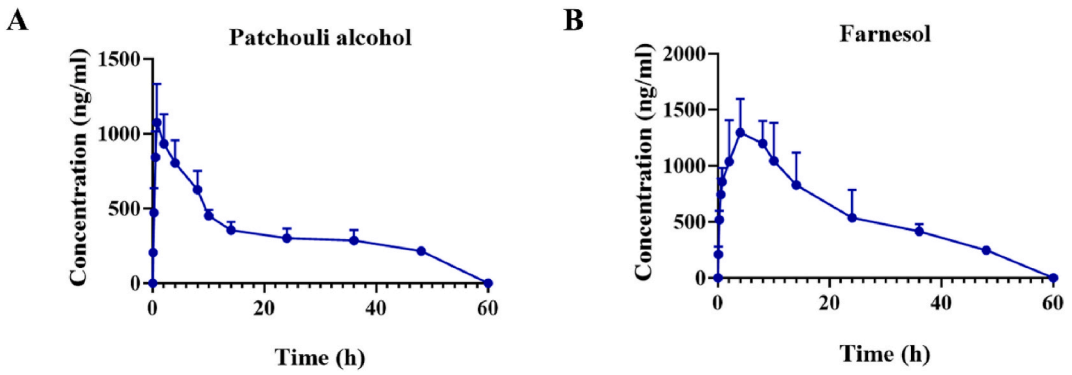

Fig. 5. Mean plasma concentration-time profiles of patchouli alcohol (A) and farnesol (B) after oral administration of the PC essential oil extract.

Table 5
Main pharmacokinetic parameters of patchouli alcohol and farnesol in rats (mean ± SD, n = 6).

Parameters	Patchouli alcohol	Farnesol
C_{max} (ng/mL)	1108.5 ± 221.0	1413.7 ± 255.2
T_{max} (h)	1.3 ± 0.7	6.0 ± 2.6
$T_{1/2\alpha}$ (h)	2.1 ± 0.3	4.5 ± 4.4
$AUC_{(0\text{-}tn)}$ (μg/L*h)	20319.3 ± 1591.3	32149.6 ± 6469.1
$AUC_{(0\text{-}\infty)}$ (μg/L*h)	20319.3 ± 1591.3	32534.1 ± 6949.2

3.4. Pharmacokinetic study

Utilizing the validated method to analyze the pharmacokinetics of the five compounds following the administration of the essential oil extract from PC. The presence of β-elemene, β-caryophyllene, and caryophyllene oxide in rat plasma was solely observed at the initial plasma sampling instances, rendering the attainment of a comprehensive pharmacokinetic profile arduous. Consequently, these analytes were omitted from the study findings. The mean plasma concentration-time profiles of the patchouli alcohol and farnesol are displayed in Fig. 5. Additionally, as seen from the results of the main pharmacokinetic parameters (Table 5), the concentration maximum (C_{max}) of the patchouli alcohol and farnesol were 1108.5 ± 221.0 and 1413.7 ± 255.2 ng/mL, respectively, indicating that both compounds had high blood concentrations and complete absorption. The concentration maximum (T_{max}) and plasma half-life ($T_{1/}$

$_2$) of patchouli alcohol and farnesol were 1.3 ± 0.7 and 6.0 ± 2.6 h, 2.1 ± 0.3 and 4.5 ± 4.4 h respectively, demonstrating that patchouli alcohol was rapidly absorbed into the circulatory system and its absorption rate was faster than farnesol.

At present, there is no method for concurrently determining multiple components in rat plasma in rat plasma subsequent to the oral administration of PC essential oil extract. However, there are some reports on the determination of plasma samples after oral or intravenous administration of patchouli alcohol [21,22]. The pharmacokinetic profile of orally administered PC essential oil extract in the present study was slightly different from that observed in the pure form, which may be due to the complexity of interactions between multiple TCM compositions *in vivo* [23].

4. Conclusion

In this study, a rapid and sensitive GC-MS method was established and validated to simultaneously determine the five components (β-elemene, β-caryophyllene, caryophyllene oxide, patchouli alcohol, and farnesol) in rat plasma of rats after oral administration of PC essential oil extract. The results revealed that the blood concentrations of patchouli alcohol and farnesol were high, their absorptions were complete, and the absorption of patchouli alcohol was faster than farnesol. This method was successfully utilized to investigate the pharmacokinetics of these compounds after oral administration of PC, providing a valuable reference for the clinical application and additional exploration into the pharmacology of PC.

Data availability statement

Data associated with the study has not been deposited into a publicly available repository. Data are available from the corresponding author upon reasonable request.

Ethics statement

This study was approved by The Animal Ethic Review Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2023059). Informed consent was not required for this study as it involved basic animal experiments, and no clinical trials were conducted.

CRediT authorship contribution statement

Yameng Zhu: Writing – original draft, Investigation. **Huizi Ouyang:** Investigation. **Wenhan Lin:** Investigation, Formal analysis. **Wenwen Li:** Data curation. **Xiunan Cao:** Validation. **Yanxu Chang:** Supervision. **Jun He:** Writing – review & editing, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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