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Simultaneous Determination of Six Components in Wanjinxiang Shushuang Ointment by QAMS

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Abstract [**Objectives**] To regulate the quality of Wanjinxiang Shushuang Ointment through simultaneously quantifying menthol, camphor, 1,8-cineole, linalool, borneol and caryophyllene oxide by QAMS (quantitative analysis of multi-components by single-marker). [**Methods**] The method was performed using an Agilent DB-WAX (30 m × 0.32 mm, 0.25 μm) polyethylene glycol chromatographic column; with nitrogen employed as a carrier gas. The constant pressure was 4.73 psi; and the injection temperature was 240 ℃, with a shunt ratio : of 10 : 1; The hydrogen flame ion detector with a detector temperature of 240 ℃; the injection volume was 0.3 μL. To verify the accuracy and applicability of QAMS, the results were compared with those obtained using the internal standard method (naphthalene). [**Results**] In Wanjinxiang Shushuang Ointment, menthol, camphor, 1,8-cineole, linalool, borneol, caryophyllene oxide and naphthalene were well separated by the same chromatography with good linearity in their respective ranges ($R \geqslant 0.9992$). The average recoveries were 99.66%, 101.03%, 98.07%, 98.24%, 101.39%, and 103.39% with *RSD*s of 0.69%, 1.52%, 1.25%, 1.94%, 1.44%, and 2.74%, respectively. The QAMS is similar to the internal standard method. [**Conclusions**] This simple, accurate method with high precision, separation and reproducibility can serve as a reference for the quality control of Wanjinxiang Shushuang Ointment.

Key words Wanjinxiang Shushuang Ointment, *Blumea balsamifera* (L.) DC., Chemical component, Quantitative analysis of mult-components by single-marker(QAMS), Quality control

1 Introduction

The Wanjinxiang Shushuang Ointment contains *Blumea balsamifera* (L.) DC., 1,8-cineole, and menthol as its main components. This ointment has disinfectant and anti-inflammatory properties, which can help reduce swelling, pain, and prevent mosquito bites. *B. balsamifera* (L.) DC., also called Niuer wormwood and Dafeng wormwood, is a herbaceous perennial plant from the asteraceae family's genus Blumea. It is a popular medicinal plant in ethnic minority areas of China, like Li, Miao, and Zhuang, as well as in Southeast Asian countries due to its effects of clearing heat, reducing itching, inhibiting inflammation, and providing antioxidant properties[4–6]. Several studies have demonstrated that *B. balsamifera* (L.) DC. contains several active components like borneol, camphor, linalool, among others. These components promote osmosis, antimicrobial, and other essential pharmacological activities[7–12]. Additionally, research has indicated that the ointment's menthol is present in topical pain-relieving, itch-reducing, antiseptic and cooling products[13–15], 1,8-cineole exhibits insect repellent properties[16–17]. It is consistent with the primary therapeutic function of this formula and can serve as an indicator for quality control purposes.

Currently, there is a lack of comprehensive quality control standards for Wanjinxiang Shushuang Ointment, and only a limited number of studies have addressed its quality standards. Chinese medicine has a complex component, an unclear target point, and mechanism of action, making it challenging to fully evaluate its quality based solely on the analysis of a single component's content and it is only through the joint control of multiple index components to ensure the quality control of the required herbs that the quality of the medicines can be more comprehensively and fully controlled[18–19]. In this study, we simultaneously determined the content of six active components using QAMS, a simple, reliable, and accurate method that provides scientific reference for the quality control of Wanjinxiang Shushuang Ointment.

2 Instruments and materials

Agilent 7890, Agilent 6890 Gas Chromatographs, DB-WAX, HP-INNOWax, DB-1701 polyethylene glycol chromatographic column, produced by Agilent USA; XS105DU electronic analytical balance produced by Mettler Toledo, Switzerland.

1,8-cineole, caryophyllene oxide, camphor, linalool reference substance (purity: ≥98%; batch No.: wkq22011706, wkq21092408, wkq21112403, wkq21052711), purchased from Sichuan Weikeqi Biological Technology CO., LTD; menthol, borneol (purity: ≥98%; batch No.: 110728-201707, 110881-200706), purchased from Sinopharm Chemical Reagent Co. Ltd.; naphthalene (purity: ≥99.7%; batch No.: 20080515), purchased from Tianjin Zhiyuan Chemical Reagent Co.; methanol (analytically pure, batch No.: 20210906) purchased from Tianjin

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Fuyu Fine Chemical Co., Ltd.; Wanjinxiang Shushuang Ointment (batch No.: 20210219, 20210415, 20210620) from Guizhou Hongyu Pharmaceutical Co., Ltd.

3 Methods

3.1 Gas chromatographic conditions Column: Agilent DB-WAX (30 m×0.32 mm, 0.25 μm); carrier gas: high-purity nitrogen (purity ≥99.999%); Injection temperature: 240 ℃; shunt ratio: 10∶1; hydrogen flame ion detector: 240 ℃; the injection volume: 0.3 μL. Initial temperature: 40 ℃ (retention for 2 min), rising to 230 ℃ at 10 ℃/min (retention for 10 min).

3.2 Solution preparation

3.2.1 Preparation of internal standard solution. Precise quantities of naphthalene were placed in a 25 mL volumetric flask, which was then diluted and adjusted with methanol to be scaled up and prepared into an internal standard solution with a concentration of 0.446 0 mg/mL.

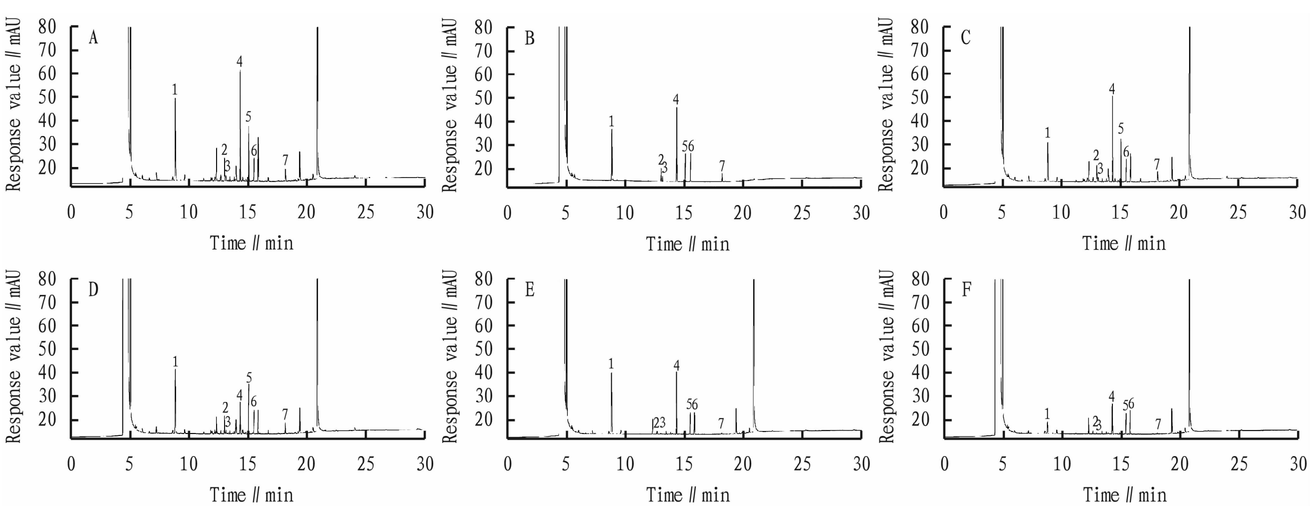
3.2.2 Preparation of tested sample solution. A weight of 2.0 g of Wanjinxiang Shushuang Ointment was carefully measured and placed into a 10 mL volumetric flask. Then, 0.5 mL of internal standard solution was added, the mixture was diluted, and fixed with methanol to the necessary scale.

3.2.3 Preparation of deficiency sample solution. According to the prescription and production process of Wanjinxiang Shushuang Ointment make 1, 8-cineole deficiency sample, menthol deficiency sample and *Blumea balsamifera* (L.) DC. deficiency sample.

3.2.4 Preparation of reference solution. Precise amounts of menthol, camphor, 1, 8-cineole, linalool, borneol and caryophyllene oxide were accurately measured in methanol to create a mixture of reference solution containing 4.650 mg/mL menthol, 0.220 mg/mL camphor, 1.690 mg/mL cineole, 0.270 mg/mL linalool, 0.800 mg/mL borneol, and 0.690 mg/mL caryophyllene oxide.

3.3 Methodology examination

3.3.1 Exclusivity Test. In accordance with Section **2.2**, separately tested the tested sample solution, deficiency sample solution and reference solution. The peaks of menthol, camphor, 1, 8-cineole, linalool, borneol and caryophyllene oxide and naphthalene were separated well, and the peak positions of the tested sample substances and the reference substance have the same retention time, and the peak positions of the components to be tested show no corresponding peaks in the deficiency sample solution. The results are illustrated in Fig. 1.



Note: A. sample solution; B. mixed standard solution; C. 1, 8-cineole deficiency sample; D. menthol deficiency sample; E. *Blumea balsamifera* (L.) DC. deficiency sample; F. cineole, menthol, *Blumea balsamifera* (L.) DC. deficiency sample; 1. 1, 8-cineole; 2. camphor; 3. linalool; 4. menthol; 5. borneol; 6. naphthalene; 7. caryophyllene oxide.

Fig. 1 Gas chromatograms of various constituents

3.3.2 Linear relationship examination. Precisely measured 0.30, 0.40, 0.50, 1.00, and 2.00 mL of mixed reference solution in a 5 mL volumetric flask. Then, added 0.5 mL of the internal standard solution of naphthalene and dilute with a solution of methanol to reach the mark. Five different concentrations of the standard mixed solution were derived in this manner. A standard curve was plotted with calculated by the concentration of the reference substance and the ratio of the peak area of each component to the peak area of the internal standard as the abscissa and ordinate coordinates, the results showed that there was a strong linear correlation among the contents of 6 components. The results are shown in Table 1.

Table 1 Linear relationship of various constituents

Component	Linear equation	r	Linear range mg/mL
Menthol	$Y=2\,075.5X+3.611\,2$	1.000 0	0.027 9 – 0.186 0
Camphor	$Y=1\,851.9X+0.295\,2$	0.999 9	0.001 3 – 0.008 8
1, 8-cineole	$Y=930.13X+0.750\,9$	0.999 7	0.010 1 – 0.067 6
Linalool	$Y=628.94X+0.313\,5$	0.999 8	0.001 6 – 0.010 8
Borneol	$Y=1\,497.7X+0.541\,2$	1.000 0	0.004 8 – 0.032 0
Caryophyllene oxide	$Y=183.36X+1.469\,5$	0.999 2	0.004 1 – 0.027 6

3.3.3 Precision test. The working mixed reference solution injected for six consecutive times under chromatographic conditions, the peak area of each component was recorded and their *RSD*s were calculated. The *RSD* of menthol, camphor, 1, 8-cineole, linalool, borneol and caryophyllene oxide was 0.94%, 1.75%, 2.25%, 1.65%, 1.93% and 1.76%, respectively, which indicated that the developed method had a good precision.

3.3.4 Repeatability test. Stability was investigated by analyzing the tested sample solution at 0, 3, 6, 12, and 24 h at room temperature and recording peak area of each component. The results in the showed that the *RSD* value of peak area of menthol, camphor, 1, 8-cineole, linalool, borneol and caryophyllene oxide was 1.69%, 2.87%, 2.51%, 1.87%, 2.39% and 1.06%, respectiviesly, indicating that the tested sample solutions were stable within 24 h.

3.3.5 Sample recovery test. Accurately weighed 2.0 g of the Wanjinxiang Shushuang Ointment (batch No.: 20210219), 9 portions, added the mixed reference solution according to 80%, 100%, 120% of each component, diluted with methanol to the scale line, and inject the sample for detection. The average recoveries of 6 samples are shown in Table 2. The results show that the method is accurate.

4 Results and analysis

4.1 Relative correction factors The mixed control solution was aspirated and injected into the sample for detection. In this experiment, 1, 8-cineole was used as an internal reference standard, and the correction factor of the remaining five components to be tested were calculated through the peak areas of the internal reference standard 1, 8-cineole and the peak areas of the remaining five components to be tested. (According to the formula: $f = \frac{A_i/c_i}{A_s/c_s}$, where A_i denotes the peak area of the internal reference standard, c_i denotes to the mass concentration of the internal reference standard, A_s denotes the peak area of the component to be measured, c_s denotes the mass concentration of the component to be measured[20].) The relative correction factor of the remaining components to be measured with the internal reference standard 1, 8-cineole were calculated. ($R_f = f_s/f_{1,\ 8\text{-cineole}}$, f_s is the correction factor for the other components.). The *RSD* value reflects the stability of the calculated results [21]. The results are shown in Table 3.

4.2 Robustness of RCFs In order to investigate the robustness of RCFs in different chromatographic conditions, Agilent 7890, Agilent 6890 of gas chromatographs and DB-WAX (30 m×0.32 mm, 0.25 μm), HP-INNOWax (30 m×0.32 mm, 0.25 μm), DB-1701 column (30 m×0.25 mm, 0.25 μm) three different models of chromatographic columns were examined, the mixed standard solution was injected and analyzed. The results showed that the RCFs of each component were not significantly different under different types of instruments and columns. The results are shown in Table 4.

4.3 Relative retention time Calculation of the content of each component through the relative correction factor needs to confirm the location of the peaks of menthol, camphor, linalool, borneol and caryophyllene oxide to achieve the purpose of one measurement and multiple evaluations. In this study, the relative retention time method was used to calculate the relative retention

Table 2 Determination results of recovery test

Compound	Original mg	Added amount mg	Detected amount mg	Recovery %	Average recovery %	*RSD* %
Menthol	0.242 3	0.188 8	0.430 1	99.49	99.66	0.69
	0.241 8	0.188 8	0.429 7	99.54		
	0.239 1	0.188 8	0.426 2	99.12		
	0.243 1	0.232 3	0.475 0	99.82		
	0.239 4	0.232 3	0.473 9	100.95		
	0.240 1	0.232 3	0.471 3	99.52		
	0.250 6	0.290 4	0.542 4	100.48		
	0.246 6	0.290 4	0.533 1	98.66		
	0.243 1	0.290 4	0.531 7	99.38		
Camphor	0.011 5	0.009 0	0.020 7	102.56	101.03	1.52
	0.011 9	0.009 0	0.020 9	1.003 3		
	0.011 7	0.009 0	0.020 6	0.992 2		
	0.011 5	0.011 0	0.022 7	1.014 5		
	0.012 0	0.011 0	0.023 2	1.014 5		
	0.011 8	0.011 0	0.023 1	1.027 7		
	0.011 8	0.013 8	0.025 5	0.993 2		
	0.011 6	0.013 8	0.025 3	0.992 8		
	0.011 7	0.013 8	0.025 9	1.029 0		
1, 8-cineole	0.085 6	0.066 3	0.152 1	1.003 7	98.07	1.25
	0.088 3	0.066 3	0.153 3	0.981 0		
	0.091 0	0.066 3	0.156 5	0.988 6		
	0.085 8	0.081 6	0.166 3	0.986 9		
	0.086 0	0.081 6	0.165 7	0.976 9		
	0.088 5	0.081 6	0.167 7	0.970 5		
	0.086 1	0.102 0	0.184 0	0.959 8		
	0.088 9	0.102 0	0.188 5	0.976 9		
	0.089 0	0.102 0	0.189 2	0.982 1		
Linalool	0.013 9	0.010 9	0.024 5	0.970 9	98.24	1.94
	0.015 1	0.010 9	0.025 9	0.990 8		
	0.015 5	0.010 9	0.026 3	0.986 3		
	0.014 0	0.013 5	0.027 4	0.989 5		
	0.015 5	0.013 5	0.028 9	0.991 5		
	0.014 9	0.013 5	0.028 2	0.981 5		
	0.014 8	0.016 9	0.031 6	0.996 8		
	0.014 3	0.016 9	0.031 1	0.997 5		
	0.015 1	0.016 9	0.030 9	0.936 6		
Borneol	0.041 5	0.032 4	0.073 5	0.989 3	101.39	1.44
	0.041 5	0.032 4	0.074 8	1.028 8		
	0.041 1	0.032 4	0.074 4	1.030 1		
	0.041 0	0.039 8	0.081 0	1.004 9		
	0.040 7	0.039 8	0.081 4	1.022 2		
	0.041 2	0.039 8	0.082 1	1.025 7		
	0.041 1	0.049 8	0.090 9	0.999 8		
	0.041 7	0.049 8	0.091 7	1.004 7		
	0.041 4	0.049 8	0.092 2	1.019 7		
Caryophyllene oxide	0.008 5	0.006 6	0.015 3	1.019 8	103.64	2.74
	0.008 3	0.006 6	0.015 0	1.010 6		
	0.008 1	0.006 6	0.014 9	1.025 6		
	0.008 0	0.008 2	0.016 5	1.041 7		
	0.008 6	0.008 2	0.016 9	1.017 2		
	0.007 7	0.008 2	0.016 4	1.066 2		
	0.008 0	0.010 2	0.018 9	1.068 6		
	0.008 4	0.010 2	0.018 6	1.000 0		
	0.008 1	0.010 2	0.019 1	1.078 4		

time of menthol, camphor, linalool, borneol and caryophyllene oxide through the retention time of 1, 8-cineole, the mixed standard solution was injected and analyzed. ($\Delta t_{Rs/R1,\ 8\text{-cineole}} = t_{Rs} - t_{R1,\ 8\text{-cineole}}$, t_{Rs} is the retention time of the sample component, $t_{R1,\ 8\text{-cineole}}$is the retention time of internal reference standard.) The results are shown in Table 5 and none of them exhibit a significant difference.

4.4 Determination of sample content Precise weighing of a sample of Wanjinxiang Shushuang Ointment (batch No.: 20210219, 20210415, 20210620). Three batches of samples were prepared as tested sample solution, and three portions of each batch were prepared in parallel. To evaluate the feasibility of the QAMS method, the contents of menthol, camphor, 1, 8-cineole, linalool, borneol and caryophyllene oxide were determined and calculated by QAMS method and internal standard method respectively, and the results of the above two kinds of results were analyzed by t-test[15]. The results demonstrate that there is no significant statistical difference between the internal standard method and the QAMS method. Hence, it proves that QAMS is a feasible option for quality controlling the active components in Wanjinxiang Shushuang Ointment. The results are shown in Table 6.

Table 3 Relative correction factors for various constituents

No.	Relative correction factor				
	Camphor	Linalool	Menthol	Borneol	Caryophyllene oxide
1	0.474 5	1.233 2	0.443 0	0.606 7	0.421 9
2	0.473 5	1.278 5	0.447 5	0.608 7	0.428 8
3	0.464 7	1.254 7	0.434 8	0.583 3	0.432 8
4	0.466 7	1.241 2	0.439 8	0.580 6	0.416 8
5	0.470 6	1.270 6	0.439 0	0.598 8	0.438 3
6	0.471 5	1.270 6	0.440 3	0.600 1	0.426 1
Mean	0.471 9	1.258 1	0.440 7	0.596 4	0.427 5
RSD//%	1.28	1.44	0.97	1.98	1.800

Table 4 Effect of relative correction factors of different instruments and columns

Instrument	Chromatographic columns	Relative correction factor				
		Camphor	Linalool	Menthol	Borneol	Caryophyllene oxide
Agilent 7890B	DB-WAX	0.475 8	1.274 1	0.442 1	0.595 4	0.430 7
	HP-INNOWax	0.469 3	1.273 4	0.438 3	0.604 9	0.445 2
	DB-1701	0.466 2	1.279 3	0.439 7	0.610 7	0.427 2
Agilent 6890B	DB-WAX	0.473 3	1.233 4	0.447 3	0.589 6	0.432 9
	HP-INNOWax	0.470 1	1.267 9	0.445 1	0.613 3	0.436 4
	DB-1701	0.475 9	1.287 2	0.443 9	0.619 3	0.438 8
	Mean	0.473 4	1.264 7	0.442 7	0.605 5	0.435 2
	RSD//%	0.65	1.62	0.76	1.86	1.47

Table 5 Relative retention time of various constituents min

Instrument	Chromatographic columns	Relative correction factor				
		Camphor	Linalool	Menthol	Borneol	Caryophyllene oxide
Agilent 7890	Agilent DB-WAX	1.470 1	1.484 4	1.620 1	1.701 9	2.053 7
	Agilent HP-INNOWax	1.469 5	1.469 5	1.610 2	1.690 5	2.114 8
	Agilent DB-1701	1.448 4	1.448 4	1.611 1	1.674 8	2.018 3
Agilent 6890	Agilent DB-WAX	1.471 7	1.485 5	1.621 3	1.703 2	2.055 9
	Agilent HP-INNOWax	1.469 7	1.469 7	1.610 4	1.688 7	2.111 0
	Agilent DB-1701	1.450 2	1.450 2	1.591 2	1.669 6	2.013 5
	Mean	1.463 3	1.468 0	1.610 7	1.688 1	2.061 2
	RSD//%	0.74	1.09	0.67	0.81	2.12

Table 6 Sample content determination results (n = 3)

Compound	20210219			20210415			20210620		
	ISM//mg/g	QAMS//mg/g	*RSD*//%	ISM//mg/g	QAMS//mg/g	*RSD*//%	ISM//mg/g	QAMS//mg/g	*RSD*//%
1, 8-cineole	0.525 6	–	–	0.536 5	–	–	0.531	–	–
Camphor	0.099 4	0.099 8	0.24	0.096 9	0.097 4	0.27	0.104 3	0.104 9	0.26
Linalool	0.087 4	0.086 2	0.71	0.087 4	0.086 3	0.68	0.093 9	0.092 9	0.55
Menthol	1.462 0	1.466 5	0.15	1.469 0	1.474 3	0.18	1.543 0	1.548 5	0.18
Borneol	0.391 0	0.392 5	0.20	0.403 9	0.405 4	0.19	0.416 3	0.418 0	0.20
Caryophyllene oxide	0.083 9	0.084 7	0.47	0.085 8	0.084 3	0.88	0.086 9	0.087 5	0.34

5 Discussion

5.1 Selection of internal standards Chinese medicines have complex components. In this study, the internal standard method can be utilized to eliminate errors due to changes in operating conditions. This makes the measurement and calculation of the relative peak areas of the internal standard and the components to be measured more precise. To reduce experimental error, it is advisable to choose an internal standard with a peak position that is either close to the measured component's peak position or falls between the multiple chromatographic peaks of the measured component. This experiment evaluated diethyl malonate, m-xylene, n-propanol, n-butanol, and naphthalene as internal standards. The use of naphthalene as an internal standard revealed that it was positioned between borneol and caryophyllene oxide peaks, the duration of its peak was more optimal. Subsequently, the components intended to be measured could be accurately differentiated. This approach could effectively prevent the occurrence of systematic errors.

5.2 Chromatographic conditions It was found that under constant temperature, the separation effect was poor due to the long interval between the peaks of the active components and the inability of the separation degree to meet the standard, and the separation effect was poor. Compared with the constant temperature determination, the temperature programmed can accelerate the peak time of high boiling point substances, reduce the diffusion and shorten the time, so this study adopts the temperature programmed method. The effect on the chromatographic peak separation was also investigated from several aspects, such as: injection volume, shunt ratio, initial temperature, temperature increase rate, *etc*. After experimental verification, the initial temperature was determined to be 40 ℃, the shunt ratio was 10 : 1, and the injection volume was 0.3 μL. Examination of three different models of columns, including DB-WAX, HP-INNOWax and DB-1701, showed that the DB-WAX column performed better than the other models in terms of the response value, the peak shape and the degree of separation. The DB-WAX column was found to be better than the other columns in terms of response value, peak shape and separation, which was more suitable for the analysis of the active components of Wanjinxiang Shushuang Ointment.

5.3 Selection internal reference standard The initial study reveals that the 1, 8-cineole extract content in the samples was high and low-cost. It also had a short peak time, and there were fewer interfering peaks in the vicinity of 1, 8-cineole. As an internal reference standard adhering to the QAMS choice principle, it is stable, inexpensive and readily available.

6 Conclusions

Focusing on Wanjinxiang Shushuang Ointment, we established a reliable and efficient QAMS method to simultaneously determine the concentration levels of menthol, camphor, cineole, linalool, borneol, and caryophyllene oxide. Through a comparison of the QAMS and internal standard methods, the results indicated no significant differences, which is consistent with the modernized quality control of traditional Chinese medicine. This suggests that the QAMS method is a feasible option for the quality control of Wanjinxiang Shushuang Ointment and provides insight into the comprehensive quality control of this preparation.

References

[1] WANG YH, ZHANG YR. Variations in components and antioxidant activities of essential oils from leaves of Luodian *Blumea balsamifera* from different harvest times in China[J]. PLoS One, 2020, 15(6): e0234661.

[2] YANG H, GAO Y, LONG L, *et al*. Antibacterial effect of *Blumea balsamifera* (L.) DC. essential oil against *Staphylococcus aureus*[J]. Archives of microbiology, 2021, 203(7): 3981–3988.

[3] PANG Y, ZHANG Y, HUANG L, *et al*. Effects and mechanisms of total flavonoids from *Blumea balsamifera* (L.) DC. on skin wound in rats[J]. International Journal of Molecular Sciences, 2017, 18(12): 2766.

[4] UTHAISAKEE. Antimicrobial activity of *Blumea balsamifera* (L.) DC. extracts and essential oil[J]. Natural Product Research, 2011, 25(19): 1849–1856.

[5] PANG YX, WANG D, FAN ZW, *et al*. *Blumea balsamifera*: A phytochemical and pharmacological review[J]. Molecules (Basel, Switzerland), 2014, 19(7): 9453–9477.

[6] FU WJ, WANG D, PANG YX, *et al*. Effect of *Blumea balsamifera* oil on percutaneous absorption of salbutamol sulfate tradit[J]. Chinese Journal of Experimental Traditional Medical Formulae, 2013, 19(14): 174–177. (in Chinese).

[7] LIU SY, YU L, YU S, *et al*. Borneol in cardio-cerebrovascular diseases: pharmacological actions, mechanisms, and therapeutics[J]. Pharmacological research, 2021(169): 105627.

[8] LU Y, GENG X, WANG J, *et al*. Current status of preclinical safety evaluation of borneol[J]. Chinese Journal of New Drugs, 2016, 25(6): 645–649, 658. (in Chinese).

[9] HE R, ZHANG Z, XU L, *et al*. Antibacterial mechanism of linalool emulsion against *Pseudomonas aeruginosa* and its application to cold fresh beef[J]. World Journal of Microbiology & Biotechnology, 2022, 38(4): 56.

[10] HERMAN, ANDRZEJ, TAMBOR, *et al*. Linalool affects the antimicrobial efficacy of essential oils[J]. Current microbiology. 2016, 72(2): 165–172.

[11] WEIBAO K, HUANRAN H, YAN G, *et al*. Antifungal activity of camphor against four phytopathogens of *Fusarium*[J]. South African Journal of Botany, 2022(148): 437–45.

[12] LUYAO G, HAIMING C, WENXUE C, *et al*. Linalool against *Hafnia alvei*, its antibacterial mechanism revealed by metabolomic analyses[J]. Food Bioscience, 2023(51): 102316.

[13] CHENG H, AN X. Cold stimuli, hot topic: An updated review on the biological activity of menthol in relation to inflammation[J]. Front Immunol, 2022(13): 1023746.

[14] LIU B, FAN L, BALAKRISHNA S, *et al*. TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain[J]. Pain, 2013, 154(10): 2169–2177.

[15] M DO, NICOLA A, D JB, *et al*. Skin application of menthol enhances maximal isometric lifting performance[J]. Journal of Strength and Conditioning Research, 2023, 37(3): 564–573.

[16] JUSTINO N, NETO G, SILVA ID, *et al*. *Rosmarinus officinalis* L. essential oil and its majority compound 1, 8-cineole at sublethal amounts induce no direct and cross protection in Staphylococcus aureus ATCC 6538[J]. Foodborne Pathogens and Disease, 2012, 9(12): 1071–1076.

[17] JUERGENS UR. Anti-inflammatory properties of the monoterpene 1.8-cineole: Current evidence for co-medication in inflammatory airway diseases[J]. Drug research, 2014, 64(12): 638–646.

[18] ZHAO CJ, CHENG JB, LI CY, *et al*. Quality evaluation of *Acanthopanax senticosus* via quantitative analysis of multiple components by single marker and multivariate data analysis[J]. Journal of Pharmaceutical and Biomedical Analysis, 2021(201): 114090.

[19] PENG Y, DONG M, ZOU J, *et al*. Analysis of the HPLC fingerprint and QAMS for Sanhuang Gypsum Soup[J]. Journal of Analytical Methods in Chemistry, 2018(2018): 5890973.

[20] WANG S, GAN Y, KAN H, *et al*. Exploitation of HPLC analytical method for simultaneous determination of six principal unsaturated fatty acids in oviductus ranae based on quantitative analysis of multi-components by single-marker (QAMS)[J]. Molecules (Basel, Switzerland), 2021, 26(2): 479.

[21] SU YT, PENG Y, REN J, *et al*. Quantitative analysis of berberidis cortex via quantitative analysis of multicomponents by single marker (QAMS) combined with fingerprint and chemometrics methods[J]. Journal of Analytical Methods in Chemistry, 2022(2022): 8042631.