DOI: 10.19600/j.cnki.issn2152 - 3924.2025.01.003

Analysis of Chemical Components of *Cocculus laurifolius* DC. Based on Liquid Chromatography-Mass Spectrometry

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Abstract [**Objectives**] To analyze the main chemical components in *Cocculus laurifolius* DC. by ultra-high performance liquid chromatography-quaternary rod/electrostatic field orbital hydrazine high resolution mass spectrometry. [**Methods**] Using Welch AQ-C_{18} chromatographic column (150 mm × 2.1 mm, 1.8 μm), gradient elution was performed with 0.1% formic acid aqueous solution (A)-methanol (B) as the mobile phase, and electrospray ESI ionization source and simultaneous mass spectrometry scanning mode of positive and negative ions were used. [**Results**] 26 kinds of chemical component were identified or inferred, including 3 organic acids, 5 flavonoids, 4 alkaloids, 1 coumarin and 13 others. [**Conclusions**] The UPLC-Q-Exactive HRMS technique is simple, which lays a foundation for the drug-efficacy material basis and medicinal quality evaluation of *C. laurifolius* DC.

Key words *Cocculus laurifolius* DC., UPLC-Q-Exactive HRMS, Chemical component, Alkaloids, Flavonoids

1 Introduction

Cocculus laurifolius DC. is a plant of the genus *Cocculus* of the Menispermaceae family, and is also known as Mufangji, Shanlizhi, Xiaoshishu. It is mainly distributed in Guangxi, Guangdong, Yunnan, Hainan and other regions. It is a traditional medicinal plant and is used as a medicine for promoting the body's blood circulation and nervous system stability and regulating the body fluid. It has the effects of dispelling wind, relieving pain, dispersing blood stasis and reducing swelling. It is often used to treat rheumatism, low back pain, hypertension, edema and other diseases in Zhuang medicine[1]. Modern research shows that *C. laurifolius* DC. contains a variety of alkaloids chemical components and pharmacological effects. For example, Tsakadze *et al.*[2], scholars from the Socialist Republic of Georgia, isolated 10 alkaloid components from the dried leaves of *C. laurifolius* DC., including coculine ($C_{17}H_{21}NO_2$), coculi-dine ($C_{18}H_{23}NO_2$), coclafinin ($C_{16}H_{19}NO_2$), cocclafin hy-drochloride ($C_{17}H_{21}NO_2HCl$), coclaurune ($C_{17}H_{19}NO_3$), isoboldine ($C_{19}H_{21}NO_4$), norisoboldine ($C_{18}H_{19}NO_4$). Zhang and Yue[3] isolated two new bisbenzylisoquinoline alkaloids, α, α'-dioxo-7'-o-demethylstebisimine and 7'-o-demethylstebisimine, and 14 known alkaloids from the roots of *C. laurifolius* DC. Pakistani scholar Ajaib *et al.*[4] used methanol to extract concentrated extracts from the leaves and bark of *C. laurifolius* DC., and measured the antibacterial activity of 4 strains of bacteria and 2 strains of fungi. The results showed that both leaf and bark extracts had significant control effects on bacteria and fungi, and bark chloroform extract had significant DPPH free radical scavenging activity. Pakistani scholar Maqbool *et al.*[5] evaluated the anticonvulsant and neuroprotective effect of ethanol extract of *C. laurifolius* DC. leaves on strychnine-induced convulsions in rats. The results showed that ethanol extract (200 and 400 mg/kg) had obvious anticonvulsant activity, which was manifested as delaying seizure and death time after strychnine-induced convulsions. At the same time, the extract had neuroprotective activity, which may be related to the antioxidant properties of the extract, and the acute toxicity experiment of the extract did not show any toxicity or neurotoxicity side effects. In addition, there are literature reports on the genetic information and phylogenetic position of chloroplast genome of this plant[6]. However, the overall analysis method of complex chemical composition of *C. laurifolius* DC. has not been reported at present. Therefore, this study used ultra-high performance liquid chromatography-quaternary rod/electrostatic field orbital trap high-resolution mass spectrometry to screen and systematically analyze the active ingredients in the Zhuang medicine—*C. laurifolius* DC., aiming to provide a reference for basic research on the pharmacodynamic substances of *C. laurifolius* DC.

2 Materials and methods

2.1 Materials

2.1.1 Medicinal materials. The herbal medicine of *C. laurifolius* was collected from Yaoshi Mountain in Guangxi University of Chinese Medicine in December 2022. It was identified by Professor Wen Haicheng from College of Zhuang Medicine of Guangxi University of Chinese Medicine as a dried ground part of *C. laurifolius* DC., a plant of the genus *Cocculus*, and it was crushed into

Received: December 13, 2024 Accepted: January 8, 2025

Supported by Scientific Research Project of China Medical Association of Minorities(2022M2038-310401); Guangxi First-class Discipline Project for Traditional Chinese Medicine (GuiJiaoKeYan 202201); Scientific Research and Training Project for College Students of Guangxi University of Chinese Medicine (2023DXS14); Funding Project for High-level Innovation Team and Outstanding Scholars in Guangxi Universities (GuiJiaoRen 201407); NATCM's Project of High-level Construction of Key TCM Disciplines/Medicine for Ethnic Minorities (Zhuang Medicine) (ZYYZDXK-2023164); Guangxi Higher Education Key Laboratory for the Research of Toxic Diseases in Zhuang Medicine (GuiJiaoKeYan 202210).

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coarse powder for later use.

Methanol was analytically pure, formic acid was chromatographically pure, and water was ultrapure.

2.1.2 Instruments. Direct-Q® 5 pure water/ultrapure water all-in-one system (German Merck Millipore); D3024R micro-refrigerated centrifuge (Beijing Dragon Laboratory Instruments Limited); MX-F vortex oscillator (Wuhan ServiceBio Co., Ltd.); JP-040S ultrasonic cleaner (Skymen Cleaning Equipment Shenzhen Co., Ltd.); UltiMate 3000 RS ultra-high performance chromatograph (Thermo Fisher Scientific, Inc.); Q-Exactive quaternary rod/electrostatic field orbital trap high-resolution mass spectrometry (Thermo Fisher Scientific China Co., Ltd.

2.2 Methods

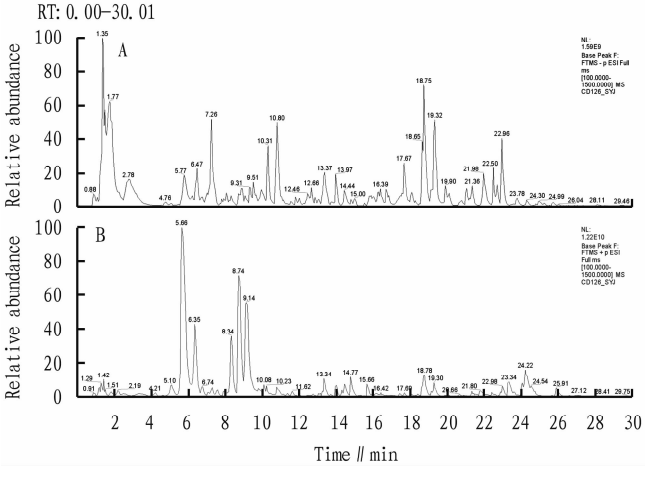
2.2.1 Preparation of test solution. The coarse powder of this product was passed through a No. 1 sieve, then 0.5 g of the powder was weighed accurately, and placed in a 50 mL beaker flask. 10.00 mL of 70% methanol solution was added and the flask was weighed. It was extracted ultrasonically (power 250 W, frequency 40 kHz) for 40 min and cooled. 70% methanol was used to make up for the lost weight, and it was shaken well, and filtered. The filtrate was passed through a 0.22 μm microporous membrane to obtain the test solution.

2.2.2 Chromatographic conditions. Chromatographic column Welch AQ-C_{18} (150 mm × 2.1 mm, 1.8 μm); flow rate: 0.30 mL/min; mobile phase: 0.1% formic acid aqueous solution (A)-methanol (B), gradient elution (0 – 1 min, 2% B; 1 – 5 min, 2% – 20% B; 5 – 10 min, 20% – 50% B; 10 – 15 min, 50% – 80% B; 15 – 20 min, 80% – 95% B; 20 – 27 min, 95% B; 27 – 28 min, 95% – 2% B; 28 – 30 min, 2%); column temperature: 35 ℃; autosampler temperature: 10.0 ℃; injection volume: 5.00 μL.

2.2.3 Mass spectrometry conditions. UPLC-Q-Exactive HRMS system; ESI electrospray ion source, electrospray voltage: 3.2 kV (positive, negative), capillary temperature: 300 ℃; scanning mode: full mass/dd-MS^2 positive and negative ion scanning, resolution: 1 7 500 – 70 000 FWHM, scan range: 100.0 – 1 500.0 *m/z*, auxiliary gas: nitrogen (purity ≥ 99.999%), 15 Arb, 350 ℃.

3 Results and analysis

3.1 UPLC-Q-Exactive HRMS analysis The chemical composition of traditional Chinese medicine is complex, and the detailed chemical composition cannot be identified by a single mode. Therefore, the positive and negative ion mode was used to determine the test solution in this experiment. The total ion current diagram is shown in Fig. 1.



NOTE A. negative ions; B. positive ions.

Fig. 1 Total ion current diagram of *Cocculus laurifolius* DC.

3.2 Structural identification The data on *C. laurifolius* DC. compounds collected by high-resolution liquid chromatography-mass spectrometry were qualitatively analyzed by Compound Discoverer 3.3 database, and mass spectrometry information such as compound retention time (t_R) and accurate relative molecular mass were obtained. Through the primary ion peak, combined with the database and the characteristic cleavage rules of the compound, each chromatographic peak was speculated, and the compound structure of each chromatographic peak was finally determined. Finally, 26 chemical component were identified, including 3 organic acids, 5 flavonoids, 4 alkaloids, 1 coumarin and 13 others. The results are shown in Table 1.

Table 1 Structural identification results of *Cocculus laurifolius* DC.

Peak No.	T_R min	Compound	Ionic mode	Molecular formula	Theoretical value	Actual value	Secondary fragments // *m/z*	Category
1	1.426	Trigonelline	$[M+H]^+$	$C_7H_7NO_2$	137.136	137.047	121.065, 110.060, 94.065, 92.050, 79.042, 65.039	Alkaloids
2	2.258	Nicotinamide	$[M+H]^+$	$C_6H_6N_2O$	122.125	122.048	112.039, 106.029, 96.044, 80.050, 68.050, 53.039	Others
3	2.793	Citric acid	$[M-H]^-$	$C_6H_8O_7$	192.124	192.026	129.018, 112.010, 111.007, 87.007, 85.028, 67.017, 57.033	Organic acids
4	3.808	Salsolinol	$[M+H]^+$	$C_{10}H_{13}NO_2$	179.220	179.094	163.075, 151.075, 137.059, 117.070, 85.029	Alkaloids
5	5.673	Isorhapontigenin	$[M+H-H_2O]^+$	$C_{15}H_{14}O_4$	258.269	258.089	241.085, 231.101, 227.069, 213.090, 181.064, 153.069	Others
6	8.322	Syringic acid	$[M+H]^+$	$C_9H_{10}O_5$	198.173	198.052	181.085, 155.070, 140.046, 123.044, 95.049	Organic acids

(To be continued)

(Continued)

Peak No.	T_R min	Compound	Ionic mode	Molecular formula	Theoretical value	Actual value	Secondary fragments // m/z	Category
7	9.011	4 '-hydroxy-3'-methoxyacetophenone	$[M+H]^+$	$C_9H_{10}O_3$	166.174	166.063	152.046, 123.044, 106.041, 95.049, 78.047	Others
8	10.266	Citral	$[M+H]^+$	$C_{10}H_{16}O$	152.233	152.120	135.116, 125.059, 111.044, 107.049, 93.070, 81.070, 71.049	Others
9	10.642	2-indolone	$[M+H]^+$	C_8H_7NO	133.147	133.053	106.065, 105.033, 79.054,	Others
10	11.335	Sinomenine	$[M+H]^+$	$C_{19}H_{23}NO_4$	329.390	329.162	298.146, 284.103, 281.117, 269.080, 241.085, 216.101	Alkaloids
11	11.351	Jasmonic acid	$[M+H]^+$	$C_{12}H_{18}O_3$	210.270	210.125	193.122, 151.111, 133.101, 91.054, 81.070, 69.070	Organic acids
12	13.154	7-hydroxycoumarin	$[M+H]^+$	$C_9H_6O_3$	162.142	162.031	135.043, 133.028, 103.054, 95.049, 79.054	Coumarins
13	14.988	Luteolin	$[M-H]^-$	$C_{15}H_{10}O_6$	286.236	286.047	199.039, 175.039, 151.002, 133.028, 107.012	Flavonoids
14	15.588	Apigenin	$[M+H]^+$	$C_{15}H_{10}O_5$	270.237	270.052	153.018, 119.049, 91.054, 69.070	Flavonoids
15	15.688	Diosmetin	$[M+H]^+$	$C_{16}H_{12}O_6$	300.263	300.063	286.046, 258.051, 229.048, 95.085	Flavonoids
16	15.872	Corchorifatty acid F	$[M-H]^-$	$C_{18}H_{32}O_5$	328.443	328.224	291.197, 229.144, 211.133, 171.101, 97.064, 85.028	Others
17	16.54	Caryophylline	$[M+H]^+$	$C_{15}H_{24}O$	220.350	220.182	203.179, 185.132, 179.143, 161.132, 119.085, 93.070, 81.070	Others
18	16.944	Glycitein	$[M+H]^+$	$C_{16}H_{12}O_5$	284.263	284.068	270.051, 242.056, 225.184, 153.017, 95.085, 81.070	Others
19	16.997	Fumarine	$[M+H]^+$	$C_{20}H_{19}NO_5$	353.369	353.125	339.109, 324.085, 295.083, 278.080	Alkaloids
20	17.707	Naltrexone	$[M+H]^+$	$C_{20}H_{23}NO_4$	341.400	341.162	324.158, 297.111, 265.085, 198.054, 163.075	Others
21	18.118	Ar-turmerone	$[M+H]^+$	$C_{15}H_{20}O$	216.319	216.151	119.085, 117.070, 91.054	Flavonoids
22	18.548	Genistein	$[M-H]^-$	$C_{15}H_{10}O_5$	270.237	270.052	241.050, 225.055, 197.060, 181.064	Flavonoids
23	21.348	Palmitoylethanolamide	$[M+H]^+$	$C_{18}H_{37}NO_2$	299.492	299.282	283.262, 272.294, 109.101, 95.085, 85.101, 62.060	Others
24	21.663	Oleoylethanolamide	$[M+H]^+$	$C_{20}H_{39}NO_2$	325.529	325.297	311.114, 202.086, 174.091, 95.086, 83.086, 62.060	Others
25	22.496	Stearylamide	$[M+H]^+$	$C_{18}H_{37}NO$	283.492	283.287	116.107, 102.091, 72.045, 57.070	Others
26	23.195	Ergosterol peroxide	$[M+H]^+$	$C_{28}H_{44}O_3$	428.647	428.328	411.324, 383.328, 231.210, 199.132, 121.101, 95.086	Others

4 Discussion

It is reported in the literature that alkaloids are the main components of *C. laurifolius* DC.[7]. From the experimental identification results, *C. laurifolius* DC. contains many alkaloids; a small quantity of coumarin compounds, such as 7-hydroxycoumarin, also contain a large number of flavonoids, and flavonoids such as arturmerone and genistein were first found in *C. laurifolius* DC.

UPLC-Q-Exactive HRMS technology can be used to systematically and qualitatively analyze the chemical components of Chinese herbal medicines, comprehensively clarify their chemical substance basis, and enrich the information of chemical components of Chinese herbal medicines[8]. This experiment laid a foundation for the evaluation of the quality of *C. laurifolius* DC. and the research of compound preparations containing *C. laurifolius* DC.

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