

基于 UHPLC-Q-TOF-MS 技术分析暗紫贝母花的化学成分

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摘要:目的 为充分利用川贝母资源,本研究通过超高效液相色谱-四极杆飞行时间质谱联用技术(UHPLC-Q-TOF-MS)鉴定暗紫贝母花中化学成分。方法 采用 Thermo Hypersil GOLD aQ(2.1 mm × 150 mm, 1.9 μm) 色谱柱, 0.1% 甲酸水溶液-甲醇梯度洗脱, 流速 0.3 mL · min⁻¹, 柱温 35 °C, 采用电喷雾电离源(ESI), 正、负离子模式分别扫描。结果 通过对照品比对、一级质谱精确测定相对分子质量、二级质谱碎片信息, 结合文献报道, 从暗紫贝母花中鉴定了 71 个化学成分, 包括生物碱 24 个, 黄酮 12 个, 氨基酸 9 个等, 并鉴定出贝母素甲、贝母素乙等有效成分。结论 本研究运用 UHPLC-Q-TOF-MS 技术对其化学组成进行了详尽解析, 结果表明暗紫贝母花存在潜在的药用价值, 研究结论为其资源的合理开发与可持续利用提供了科学依据。

关键词:暗紫贝母花;超高效液相色谱-四极杆飞行时间质谱联用;成分鉴定;裂解规律

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Analysis of Chemical Constituents in Flower of *Fritillaria unibracteata* P. K. Hsiao et K. C. Hsia by UHPLC-Q-TOF-MS

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ABSTRACT:OBJECTIVE To identify the chemical constituents in flower of *Fritillaria unibracteata* P. K. Hsiao et K. C. Hsia (F-F, Chinese: Anzibeimu Hua) by employing ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry(UHPLC-Q-TOF-MS) for the optimal utilization of *Fritillaria cirrhosa* D. Don (Chinese: Chuanbeimu) resources. **METHODS** The separation was conducted on Thermo Hypersil GOLD aQ column (2.1 mm × 150 mm, 1.9 μm) eluted with methanol-0.1% formic acid in a gradient mode at a flow rate of 0.3 mL · min⁻¹, the column temperature was maintained at 35 °C, and positive and negative electrospray ionization(ESI) was adopted for determine the chromatographic effluents. **RESULTS** Through comparison with reference standards, precise molecular weight determination from primary mass spectrometry, and analysis of fragmentation patterns from secondary mass spectrometry, combined with existing literature, 71 chemical constituents were identified in F-F. These identified compounds encompass 24 alkaloids, 12 flavonoids, 9 amino acids, and additional substances, including the notable detection of peimine and peiminine within F-F. **CONCLUSION** This study employs UHPLC-Q-TOF-MS technology to conduct an exhaustive analysis of the chemical composition of F-F. From the results, it can be seen that the F-F possess potential medicinal value. This study provides scientific basis for the rational development and sustainable utilization of its resources.

KEY WORDS: *Fritillaria unibracteata* flower; UHPLC-Q-TOF-MS; chemical constituent identification; cleavage pattern

川贝母是四川道地药材,被收载于《中国药典》2020 年版,共有 6 种基原植物:包括川贝母(*Fritillaria unibracteata* P. K. Hsiao et K. C. Hsia)、甘肃贝母(*Fritillaria przewalskii* Maxim.)、暗紫贝母(*Fritillaria unibracteata* Hsiao et K. C. Hsia)、甘肅贝母(*Fritillaria przew-*

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alskii Maxim.)、梭砂贝母 (*Fritillaria delavayi* Franch.)、太白贝母 (*Fritillaria taipaiensis* P. Y. Li) 或瓦布贝母 [*Fritillaria unibracteata* Hsiao et K. C. Hsia var. *wabuensis* (S. Y. Tang et S. C. Yue) Z. D. Liu, S. Wang et S. C. Chen]。川贝母以其鳞茎入药,具有清热润肺、化痰止咳的功效^[1],其疗效在医疗实践中获得了广泛认可^[2-3]。川贝母中含有氨基酸类、有机酸类、黄酮类、生物碱类、皂苷类、香豆素类化学成分等,其中生物碱类是川贝母中重要的化学成分,在《中国药典》2020年版中也以生物碱作为含量控制指标^[1]。

川贝母根据其不同的形态特征,常被分为“松贝”“青贝”“炉贝”与“栽培品”等商品规格^[1],其中“松贝”因其优良的品质而历来被认为是川贝母中最优品,而暗紫贝母即是“松贝”的主要来源^[4]。基于其良好的临床效果,近年来川贝母的需求量持续攀升^[5],野生川贝母自然繁殖率低且由于人为过度采挖^[6],导致川贝母资源长期供不应求,人工种植虽有所突破,但产量十分有限,因此市场价格持续高企。

清初名医刘潜江在《本经疏证》(中品)草十五味中就曾提到:“是皆误于以贝母为其根,而遗却其附根以生,连累相著,且有分解一层矣”,意为不要仅仅关注于贝母鳞茎部分,需要对其他部分也适当考虑,提示人们在药物功效与药用部位上应更加细致与全面,在药用部位细分上,不应该仅仅局限于鳞茎上。

贝母属植物的花部通常被直接丢弃,然而根据前期调研,部分川贝母种植基地中,有种植工人提到暗紫贝母花(Flower of *Fritillaria unibracteata*, F-F)采摘晒干后可泡水后饮用。此外,其他贝母属植物的花部,如浙贝母花^[7-8],平贝母花^[9]与湖北贝母花^[10],也被发现含有与鳞茎相似的生物碱等活性成分,表明其可能有一定的药用价值。然而,关于川贝母花的相关研究尚属空白。鉴于当前川贝母资源紧缺且价格昂贵,扩大川贝母药用部位可能为缓解资源压力问题提供一种解决方案。

超高效液相色谱-四极杆飞行时间质谱联用(UHPLC-Q-TOF-MS)技术因其高分辨率、稳定性和准确性,已成为中医药物质基础研究中的重要分析工具^[11-12]。因此,本研究选择已基本实现人工种植的暗紫贝母的花部为研究对象,采用UHPLC-Q-TOF-MS技术,对其化学成分进行系统分析,为川贝母资源的进一步合理开发和综合利用提供科学的依据。

1 材料与方法

1.1 药物与试剂

F-F为采集于四川阿坝藏族羌族自治州阿坝县的暗紫贝母的干燥花,由四川省药品检验研究院(四川省医疗器械检测中心)副主任中药师齐景梁鉴定。

对照品:贝母素甲(批号110750-200612)、贝母素乙(批号110751-201712)、腺苷(批号110879-202204)、原儿茶酸(批号110809-201906)、咖啡酸(批号110885-201703)、阿魏酸(批号110773-201614)、脯氨酸(批号140677-201808)、水仙苷(批号111977-201501)、秦皮乙素(批号110741-202109)、精氨酸(批号140685-201707)、尿嘧啶(批号100469-201302)、亮氨酸(批号110876-200204)、槲皮素(批号100081-201509)、烟酸(批号100434-201102)、木犀草素(批号111520-202006)、西贝母碱(批号110767-201710)、贝母辛(批号111892-201402)、西贝母碱苷(批号111917-201202)(中国食品药品检定研究院)。甲醇、甲酸铵与甲酸(质谱级,美国Fisher公司),其他试剂为分析纯。

1.2 仪器

Agilent 1290-6540 UHPLC-Q-TOF-MS(美国Agilent公司);Millipore Q超纯水系统(美国Millipore公司)。

1.3 样品与对照品制备

取F-F粉末(过三号筛)1g,精密称定,置具塞锥形瓶中,精密加入体积分数80%甲醇25mL,称定质量,超声处理30min,再称定质量,用体积分数80%甲醇补足减失的质量,摇匀,滤过,取续滤液,即得F-F供试品溶液。

取各对照品适量,精密称定,分别加甲醇溶解并定容至10mL,制成单一对照品储备液,于4℃冰箱中避光保存,备用。临用前取各对照品储备液适量并混合,用甲醇稀释至各对照品浓度约为 $10\mu\text{g}\cdot\text{mL}^{-1}$ 的混合对照品溶液。

1.4 色谱条件

色谱柱 Thermo Hypersil GOLD aQ (2.1 mm × 150 mm, 1.9 μm),以0.1%甲酸溶液为流动相A,以甲醇为流动相B,梯度洗脱:0~15.5 min,95%~50% A;15.5~25 min,50%~45% A;25~42 min,45%~5% A;42~47 min,5% A;47~47.1 min,5%~95% A;47.1~50.1 min,95% A。流速 $0.3\text{ mL}\cdot\text{min}^{-1}$;柱温 $35\text{ }^{\circ}\text{C}$;进样量 $1\mu\text{L}$ 。

1.5 质谱条件

带鞘流气的电喷雾离子源(AJS-ESI+/-);气体温度: $325\text{ }^{\circ}\text{C}$;气体流速: $8\text{ L}\cdot\text{min}^{-1}$;喷雾器压力:

0.24 MPa;鞘气温度:350 ℃;鞘气流速:11 L·min⁻¹;碎裂电压:110 V;喷雾电压:Pos 4 000 V/neg 3 500 V;喷嘴电压:Pos 0 V/neg 1 000 V。Agilent MassHunter 数据采集软件(Ver. B. 08. 00);MassHunter Workstation 定量分析软件(Ver. B. 07. 00)。

1.6 数据处理

为了便于对 F-F 中成分进行系统性的分析,本研究收集贝母属已鉴定化学成分的对照品进行质谱分析,总结出其色谱保留行为、裂解规律以及特征碎片离子等。再通过 PubChem、SciFinder、Chem-spider 及 CNKI 等数据库和相关文献的检索对有关贝母属植物的化学成分信息进行收集汇总,包括名称、分子式、结构式等。然后,采用 MassHunter 分析软件对一级质谱提供的精确准分子离子进行分析,计算未知化合物的元素组成和可能的分子式,再选择目标化合物的二级质谱碎片离子信息,与对照品、数据库及文献报道提供的保留时间、裂解规律等信息进行比对,以确认化学成分及结构。

2 结果

2.1 化合物鉴定结果

按“1.6”项下方法对 F-F 化合物色谱峰进行提取匹配和分析鉴定,结果共鉴定了 71 个化合物,其中有 18 个化合物通过对照品确认。正、负离子模式下的总离子流图(TIC)见图 1,所鉴定化学成分的具体信息见表 1。

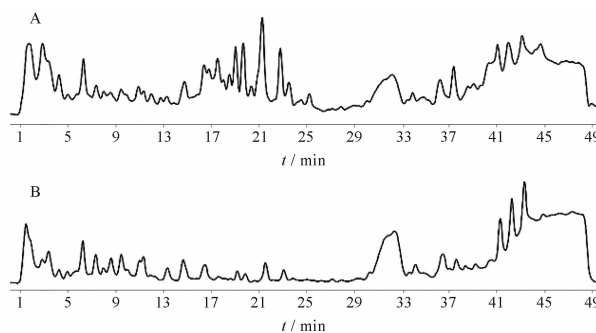


图 1 暗紫贝母花(F-F)正(A)负(B)离子模式的总离子流图
Fig. 1 TIC plots of positive mode(A) negative mode(B) of F-F

表 1 F-F 超高效液相色谱-四极杆飞行时间质谱联用(UHPLC-Q-TOF-MS)分析结果

Tab. 1 Results of UHPLC-Q-TOF-MS analyses of F-F

No.	Name	Formula	<i>t_R</i> /min	ESI +/-	<i>m/z</i> (MS)	<i>m/z</i> (MS/MS)	Error/ × 10 ⁻⁶	Structure class
1	Arginine ^[1]	C ₆ H ₁₄ N ₄ O ₂	1.21	[M + H] ⁺	175.119 9	158.090 0, 116.064 8	-5.40	Amino acid
2	Anthranilic acid ^[13]	C ₇ H ₇ NO ₂	1.31	[M + H] ⁺	138.055 5	94.066 2, 93.056 1, 92.050 4	-3.98	Organic acid
3	Proline ^[1]	C ₅ H ₉ NO ₂	1.39	[M + H] ⁺	116.069 9	70.065 8	6.13	Amino acid
4	L-Valine ^[14]	C ₅ H ₁₁ NO ₂	1.62	[M + H] ⁺	118.085 3	72.081 8, 55.055 0	8.16	Amino acid
5	Adenine ^[13]	C ₅ H ₅ N ₅	1.69	[M + H] ⁺	136.062 4	119.036 3, 92.025 3, 67.030 1	-4.56	Amino acid
6	Citric acid or isomer ^[15]	C ₆ H ₈ O ₇	1.69	[M - H] ⁻	191.021 2	387.009 8, 129.020 0, 111.010 2	-7.68	Organic acid
7	Nicotinic acid ^[1]	C ₆ H ₅ NO ₂	1.74	[M + H] ⁺	124.040 0	78.034 9, 53.039 2	-5.65	Vitamin
8	Citric acid or isomer ^[15]	C ₆ H ₈ O ₇	1.75	[M - H] ⁻	191.021 1	387.009 0, 129.020 5, 111.011 5	-7.47	Organic acid
9	Uracil ^[1]	C ₄ H ₄ N ₂ O ₂	2.17	[M + H] ⁺	113.034 7	96.008 2, 95.023 5	-1.3	Nucleoside
10	Uridine ^[16]	C ₉ H ₁₂ N ₂ O ₆	2.25	[M + H] ⁺	245.078 1	113.035 4, 96.009 1, 70.029 2	-5.27	Nucleoside
11	L-Tyrosine ^[14]	C ₉ H ₁₁ NO ₃	2.4	[M + H] ⁺	182.082 5	165.055 1, 147.044 7, 136.076 6, 123.045 1, 91.055 1, 77.039 2	-7.35	Amino acid
12	L-isoleucine ^[14]	C ₆ H ₁₃ NO ₂	2.62	[M + H] ⁺	132.102 9	86.097 7, 69.070 9, 57.058 0	-7.59	Amino acid
13	L-leucine ^[1]	C ₆ H ₁₃ NO ₂	2.80	[M + H] ⁺	132.102 8	86.097 5, 69.071 4, 57.058 3	-6.83	Amino acid
14	Adenosine ^[1]	C ₁₀ H ₁₃ N ₅ O ₄	2.87	[M + H] ⁺	268.105 6	136.062 4, 57.034 3	-5.88	Nucleoside
15	Pyridoxine ^[14]	C ₈ H ₁₁ NO ₃	2.94	[M + H] ⁺	170.081 8	152.071 6, 134.061 2, 124.077 0, 106.066 6, 77.039 7, 65.038 7	-3.73	Vitamin
16	Guanosine ^[16]	C ₁₀ H ₁₃ N ₅ O ₅	3.06	[M + H] ⁺	284.100 3	152.057 2, 135.031 0, 98.060 8	-4.79	Nucleoside
17	5'-Deoxyadenosine ^[13]	C ₁₀ H ₁₃ N ₅ O ₃	3.08	[M + H] ⁺	252.110 4	136.062 2, 117.054 7	-5.11	Nucleoside
18	Phenylalanine ^[13]	C ₉ H ₁₁ NO ₂	4.10	[M + H] ⁺	166.087 0	120.081 9, 103.055 2, 93.070 7, 77.039 6	-4.51	Amino acid
19	Protocatechuic acid ^[1]	C ₇ H ₆ O ₄	5.05	[M - H] ⁻	153.020 3	109.030 8, 108.022 9, 65.003 7, 53.030 9,	-6.28	Organic acid
20	6-O-caffeoyl-α-D-fructofuranosyl-(2→1)-α-D-glucopyranoside ^[17]	C ₂₁ H ₂₈ O ₁₄	5.76	[M - H] ⁻	503.143 5	485.134 2, 341.111 6, 323.079 9, 281.073 5, 221.047 6, 179.034 9, 161.024 6	-5.69	Phenylpropanoids
21	5'-Deoxy-5'-methylthio adenosine ^[18]	C ₁₁ H ₁₅ N ₅ O ₃ S	6.58	[M + H] ⁺	298.098 1	136.062 6, 127.021 1, 119.106 4, 97.029 5	-4.25	Nucleoside
22	Aesculetin ^[1]	C ₉ H ₆ O ₄	8.35	[M + H] ⁺	179.034 5	151.039 2, 123.044 9, 105.034 6, 77.039 5	-3.45	Coumarin
23	Caffeic acid ^[1]	C ₉ H ₈ O ₄	8.87	[M - H] ⁻	179.034 8	135.046 3, 107.051 3	1.01	Organic acid
24	Acetoside ^[17]	C ₂₁ H ₂₈ O ₁₃	9.59	[M - H] ⁻	487.148 2	307.080 6, 163.040 5, 145.030 3	-5.09	Sucrose ester

续表 1 (continued)

No.	Name	Formula	t_R /min	ESI ⁺ / ⁻	m/z (MS)	m/z (MS/MS)	Error/ $\times 10^{-6}$	Structure class
25	Sipeimine-3 β -D-glucoside ^[1]	C ₃₃ H ₅₃ NO ₈	11.57	[M+H] ⁺	592.387 6	574.367 8	-5.42	Alkaloid
26	1-O-Glycerol ferulate ^[19]	C ₁₃ H ₁₆ O ₆	12.07	[M-H] ⁻	267.087 0	252.065 5, 175.040 2, 149.060 7, 133.030 8	1.54	Phenylpropanoid
27	Ferulic acid ^[1]	C ₁₀ H ₁₀ O ₄	12.18	[M-H] ⁻	193.051 8	178.028 3, 134.038 4, 133.030 2	-6.02	Organic acid
28	(2S)-3-Acetoxy-2-(hexopyranosyloxy)propyl 3-(4-hydroxyphenyl)acrylate ^[19]	C ₂₀ H ₂₆ O ₁₁	12.24	[M-H] ⁻	441.142 7	163.040 8, 145.030 8	-5.57	Phenylpropanoid
29	Sipeimine ^[1]	C ₂₇ H ₄₃ NO ₃	12.46	[M+H] ⁺	430.334 3	412.318 2, 138.125 0	-6.36	Alkaloid
30	Edpetiline or isomer ^[20]	C ₃₃ H ₅₃ NO ₈	12.86	[M+H] ⁺	592.387 4	574.370 9, 287.056 1	-5.08	Alkaloid
31	Peimine ^[1]	C ₂₇ H ₄₃ NO ₃	13.99	[M+H] ⁺	430.333 8	412.323 4	-5.19	Alkaloid
32	Peimine ^[1]	C ₂₇ H ₄₅ NO ₃	14.28	[M+H] ⁺	432.349 7	414.339 0, 398.305 3, 176.143 3, 138.111 7, 124.112 0	-5.75	Alkaloid
33	Hyperoside ^[14]	C ₂₁ H ₂₀ O ₁₂	14.57	[M-H] ⁻	463.089 8	300.028 9, 271.026 5, 243.029 8, 151.004 6	-3.45	Flavonoid
34	Rutin and isomer ^[14]	C ₂₇ H ₃₀ O ₁₆	14.71	[M-H] ⁻	609.147 4	463.146 5, 301.035 7, 300.028 9	-2.12	Vitamin
35	Rutin and isomer ^[14]	C ₂₇ H ₃₀ O ₁₆	14.93	[M-H] ⁻	609.147 6	463.146 9, 301.035 2, 300.029 5	-2.44	Vitamin
36	Edpetiline or isomer ^[20]	C ₃₃ H ₅₃ NO ₈	14.94	[M+H] ⁺	592.388 1	574.375 7	-6.27	Alkaloid
37	Yibeinoside A or isomer ^[21]	C ₃₃ H ₅₃ NO ₇	15.13	[M+H] ⁺	576.392 3	414.334 0, 396.328 9, 98.098 1	-4.90	Alkaloid
38	Peimisine ^[1]	C ₂₇ H ₄₁ NO ₃	15.40	[M+H] ⁺	428.318 2	410.300 5, 393.274 5, 337.213 4	-5.33	Alkaloid
39	Yibeinoside A or isomer ^[21]	C ₃₃ H ₅₃ NO ₇	15.58	[M+H] ⁺	576.392 3	414.335 9, 396.325 4, 98.096 7	-4.90	Alkaloid
40	3'-O-Methylquercetin 3-galactoside ^[14]	C ₂₂ H ₂₂ O ₁₂	15.75	[M-H] ⁻	477.104 8	449.108 2, 357.062 9, 314.044 4, 243.031 8	-1.99	Flavonoid
41	Astragaln ^[15]	C ₂₁ H ₂₀ O ₁₁	15.76	[M-H] ⁻	447.094 7	285.039 8, 284.035 5, 255.028 0, 227.037 6	-3.16	Flavonoid
42	Litseagermacrane ^[15]	C ₁₅ H ₂₄ O ₂	15.82	[M+H] ⁺	237.186 4	219.175 2, 167.106 9, 151.112 5, 135.118 3, 107.086 5	-6.32	Terpenoid
43	Kaempferol ^[14]	C ₁₅ H ₁₀ O ₆	16.04	[M-H] ⁻	285.041 9	151.004 2, 135.043 8, 133.030 2, 83.014 7, 65.725 8	-5.03	Flavonoid
44	Isorhamnetin-3-glucoside ^[14]	C ₂₂ H ₂₂ O ₁₂	16.29	[M-H] ⁻	477.104 8	315.049 9, 314.042 3, 299.021 8, 243.028 6, 151.003 1	-1.99	Flavonoid
45	Narcissoside ^[1]	C ₂₈ H ₃₂ O ₁₆	16.44	[M-H] ⁻	623.163 8	315.052 3, 271.026 5, 151.004 6	-5.04	Flavonoid
46	Ebeedinone ^[21]	C ₂₇ H ₄₃ NO ₂	16.79	[M+H] ⁺	414.338 3	396.327 9, 119.085 5, 98.096 4, 95.085 3	-3.98	Alkaloid
47	Puqienine E ^[20]	C ₂₈ H ₄₃ NO ₃	16.82	[M+H] ⁺	442.334 0	424.319 3	-5.50	Alkaloid
48	Quercitrin ^[15]	C ₂₁ H ₂₀ O ₁₁	17.15	[M-H] ⁻	447.094 7	314.042 5, 300.024 4, 271.019 5, 243.030 6	-3.16	Flavonoid
49	Hupeheninoside ^[21]	C ₃₃ H ₅₅ NO ₇	17.18	[M+H] ⁺	578.407 9	398.341 2, 98.097 5, 85.0291, 69.033 9	-4.80	Alkaloid
50	β -Solanine or isomer ^[22]	C ₃₉ H ₆₃ NO ₁₁	17.62	[M+H] ⁺	722.451 3	560.394 4, 398.344 2, 380.332 0, 98.097 6	-5.42	Alkaloid
51	Spirosol-5-en-3-ol 4-O- α -L-rhamnopyranosyl-(1-6)- β -D-glucopyranoside or isomer ^[19]	C ₃₉ H ₆₃ NO ₁₁	17.73	[M+COOH] ⁻	766.440 0	720.434 9, 179.056 3, 161.046 1	-2.43	Alkaloid
52	Quercetin ^[1]	C ₁₅ H ₁₀ O ₇	18.23	[M+H] ⁺	303.051 8	257.042 2, 229.048 3, 201.050 8, 153.018 8	-6.19	Flavonoid
53	β -Solanine or isomer ^[22]	C ₃₉ H ₆₃ NO ₁₁	18.41	[M+H] ⁺	722.451 3	560.397 1, 398.342 1, 380.333 4, 98.097 2	-5.42	Alkaloid
54	Formononetin-7-O-glucoside ^[19]	C ₂₂ H ₂₂ O ₉	19.01	[M-H] ⁻	429.121 6	253.073 7, 193.051 0, 161.024 9	-5.80	Flavonoid
55	Demissidine-3-O- β -D-glucopyranosyl (1 \rightarrow 4) glucopyranoside or isomer	C ₃₉ H ₆₅ NO ₁₁	19.24	[M+COOH] ⁻	768.457 6	722.450 8, 161.046 8, 101.025 5	-5.02	Alkaloid
56	Luteolin ^[1]	C ₁₅ H ₁₀ O ₆	19.29	[M-H] ⁻	285.041 3	133.029 9	-6.81	Flavonoid
57	α -Solamarine or isomer ^[22]	C ₄₅ H ₇₃ NO ₁₆	21.18	[M+H] ⁺	884.504 5	738.443 7, 414.337 5	-4.85	Alkaloid
58	Puqiedinone ^[21]	C ₂₇ H ₄₃ NO ₂	21.26	[M+H] ⁺	414.338 3	396.327 1, 119.084 5, 105.070 3, 95.084 4, 81.070 6	-3.98	Alkaloid
59	Podophyllotoxin ^[19]	C ₂₂ H ₂₂ O ₈	21.55	[M-H] ⁻	413.126 9	219.070 5, 193.053 5, 175.039 3, 134.030 1	-6.54	Lignan

续表 1 (continued)

No.	Name	Formula	t_R /min	ESI ⁺ / ⁻	m/z (MS)	m/z (MS/MS)	Error/ $\times 10^{-6}$	Structure class
60	Puqienine A ^[21]	C ₂₈ H ₄₇ NO ₃	21.74	[M + H] ⁺	446.364 8	428.352 5, 253.196 0, 145.100 0, 107.087 2, 105.069 7, 98.095 6, 95.085 6, 81.071 0	-4.33	Alkaloid
61	Imperialine N-oxide ^[20]	C ₂₇ H ₄₃ NO ₄	21.77	[M + H] ⁺	446.329 0	428.315 1	-5.65	Alkaloid
62	Ebeienine ^[21]	C ₂₇ H ₄₃ NO ₂	22.26	[M + H] ⁺	414.338 3	396.327 3, 98.085 5, 81.071 1, 67.053 8	-3.98	Alkaloid
63	Spirosol-5-en-3-ol 4-O- α -L-rhamnopyranosyl-(1-6)- β -D-glucopyranoside or isomer ^[19]	C ₃₉ H ₆₃ NO ₁₁	23.13	[M + COOH] ⁻	766.440 0	720.435 9, 179.056 2, 161.047 4	-2.43	Alkaloid
64	α -Solamarine or isomer ^[22]	C ₄₅ H ₇₃ NO ₁₆	23.36	[M + H] ⁺	884.504 5	738.444 3, 414.339 4	-4.85	Alkaloid
65	Demissidine-3-O- β -D-glucopyranosyl-(1 \rightarrow 4) glucopyranoside or isomer ^[19]	C ₃₉ H ₆₅ NO ₁₁	24.95	[M + COOH] ⁻	768.457 6	722.451 9, 161.046 8, 101.025 2	-5.02	Alkaloid
66	Pinellie acid ^[19]	C ₁₈ H ₃₄ O ₅	28.07	[M - H] ⁻	329.235 4	229.145 9, 211.136 3, 171.103 9	-6.21	Organic acid
67	2 β , 3 β , 14-Trihydroxy-cholest-7-en-6-one 3-O- α -L-rhamnopyranosyl-(1-3)-[β -D-glucopyranosyl-(1-2)]- β -D-glucopyranoside ^[19]	C ₅₁ H ₈₄ O ₂₃	31.01	[M + COOH] ⁻	1109.543 9	917.475 5, 901.492 1, 755.419 6	-5.03	Saponin
68	(2 α , 3 β , 5 α , 25R)-2-Hydroxy-yspirostan-3-O- α -L-rhamnopyranosyl-(1-2)- β -D-glucopyranosyl-(1-2)- β -D-glucopyranoside ^[19]	C ₄₅ H ₇₄ O ₁₈	31.89	[M + COOH] ⁻	947.489 6	901.484 5, 755.421 6	-4.30	Saponin
69	α -Linolenic acid or isomer ^[13]	C ₁₈ H ₃₀ O ₂	37.26	[M + H] ⁺	279.234 0	149.024 3, 81.070 7, 67.054 9	-7.70	Organic acid
70	α -Linolenic acid or isomer ^[13]	C ₁₈ H ₃₀ O ₂	37.44	[M + H] ⁺	279.234 0	149.025 0, 81.070 3, 67.054 4	-7.70	Organic acid
71	9-Hydroxy-10(E), 12(Z)-octadecadienoic acid ^[19]	C ₁₈ H ₃₂ O ₃	38.67	[M - H] ⁻	295.230 3	277.220 4, 195.141 0, 183.012 8	-8.21	Organic acid

注:¹⁾为对照品确认。

Note:¹⁾a confirmation of the reference standards.

2.2 代表化合物结构解析过程

2.2.1 生物碱类化学成分 贝母中的贝母素甲与贝母素乙等生物碱类化学成分被认为是镇咳祛痰的有效成分^[23],具有毒性低、不良反应少、不易复发等优点^[24]。本节以贝母素甲阐述生物碱类化学成分的裂解过程。

化合物 32 在正离子模式下,其准分子离子峰为 m/z 432.349 7 [M + H]⁺,主要二级质谱离子碎片有 m/z 414.339 0、398.305 3、176.143 3、138.111 7、124.112 0 等。其中碎片 m/z 414.339 0 由母离子 m/z 432.349 7 脱去 1 分子 H₂O 形成,紧接着脱去 1 分子 CH₄形成碎片 m/z 398.305 3。根据加合物不同攻击位点,存在 3 个特征离子碎片:碎片 m/z 176.143 3、 m/z 138.111 7、 m/z 124.112 0,分别由母离子脱去 C₁₅H₂₈O₃、C₁₈H₃₀O₃ 与 C₁₉H₃₂O₃ 形成。根据以上裂解规律,结合对照品,鉴定该化合物为贝母素甲(C₂₇H₄₅NO₃)(图 2)。

2.2.2 黄酮类化学成分 黄酮类化学成分常与糖结合形成苷或以游离苷元的形式存在,其裂解过程容易发生逆-狄尔斯-阿尔德(Retro Diels-Alder, RDA)裂解,脱去糖基等。本节以水仙苷阐述黄酮类化学成分的裂解过程。

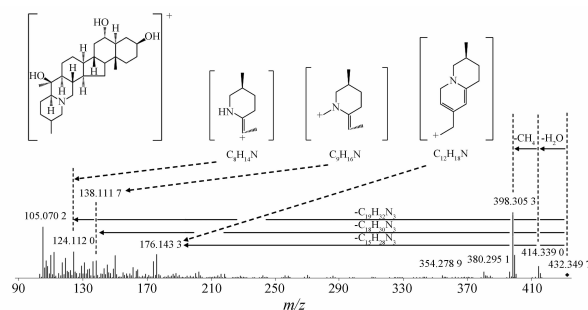


图 2 32 号化合物贝母素甲

Fig. 2 NO. 32 compound peimine

化合物 45 在负离子模式下,其准分子离子峰为 m/z 623.163 8 [M - H]⁻,主要二级质谱离子碎片有 m/z 315.052 3、271.026 5、151.004 6 等。其中碎片 m/z 315.052 3 由母离子 m/z 623.163 8 连续脱去 C₆H₁₀O₄ 与 C₆H₁₀O₅ 基团形成,再脱去 1 分子 CO₂ 形成 m/z 271.026 5,离子碎片 m/z 151.004 6 是由水仙苷发生 RDA 裂解,脱去 C₂₁H₂₈O₁₂ 而形成。结合对照品和文献^[25],鉴定该化合物为水仙苷(C₂₈H₃₂O₁₆)(图 3)。

2.2.3 有机酸类化学成分 有机酸是一类具有羧基、磺酸基、亚磺酸基与硫酸基等酸性基团的有机

化合物,具有抗菌、抗肿瘤等活性^[26]。本节以咖啡酸阐述有机酸类化学成分的裂解过程。

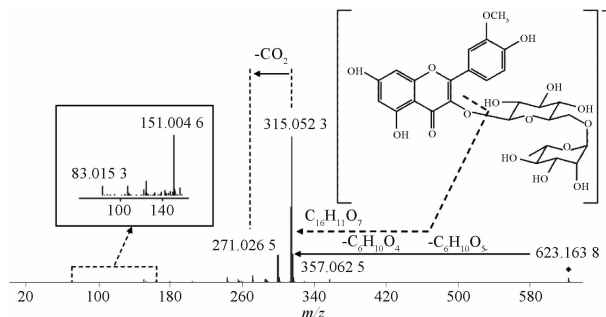


图3 45号化合物水仙苷
Fig.3 NO.45 compound narcissoside

化合物23在负离子模式下,其准分子离子峰为 m/z 179.0348 $[M-H]^-$,主要二级质谱离子碎片有 m/z 135.0463、107.0513等。其中碎片 m/z 135.0463由母离子 m/z 179.0348脱去1个 CO_2 基团形成,接着脱去1个 CO 基团形成 m/z 107.0513,结合对照品和文献^[26],鉴定该化合物为咖啡酸($C_9H_8O_4$) (图4)。

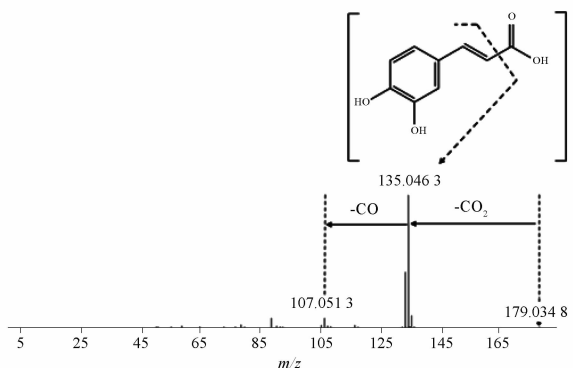


图4 23号化合物咖啡酸
Fig.4 NO.23 compound caffeic acid

2.2.4 氨基酸类化学成分 氨基酸,是含有碱性氨基和酸性羧基的有机化合物,本节以精氨酸阐述有机酸类化学成分的裂解过程。

化合物1在正离子模式下,其准分子离子峰为 m/z 175.1199 $[M+H]^+$,主要二级质谱离子碎片有 m/z 158.0900、116.0684等。其中碎片 m/z 158.0900由母离子 m/z 175.1199脱去1个 NH_3 基团形成,接着脱1个 CH_2N_2 基团形成 m/z 116.0684,结合对照品和文献^[26],鉴定该化合物为精氨酸($C_6N_{14}H_4O_2$) (图5)。

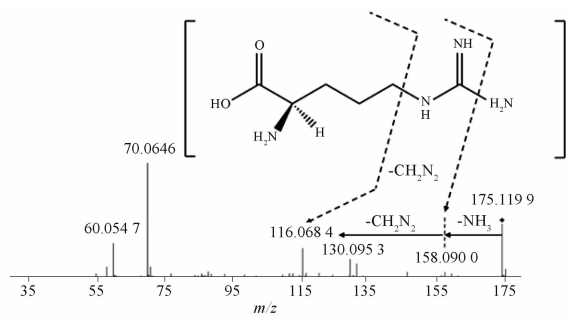


图5 1号化合物精氨酸
Fig.5 NO.1 compound arginine

2.2.5 香豆素类化学成分 香豆素类化学成分具有抗菌抗炎、止咳、化痰、平喘等功效^[27],与贝母中生物碱类化学成分药效重合,可能存在药物协同作用,本节以秦皮乙素阐述香豆素类化学成分的裂解过程。

化合物22在正离子模式下,其准分子离子峰为 m/z 179.0345 $[M+H]^+$,主要二级质谱离子碎片有 m/z 151.0392、123.0449、105.0346与77.0395等。其中碎片 m/z 151.0392与123.0449由母离子 m/z 179.0345逐步脱去1个 CO 基团形成,接着脱去1个 H_2O 基团形成 m/z 105.0346,随后再次脱去1个 CO 基团形成 m/z 77.0395,结合对照品,鉴定该化合物为秦皮乙素($C_9H_6O_4$) (图6)。

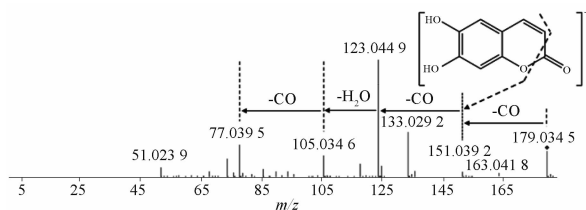


图6 22号化合物秦皮乙素
Fig.6 NO.22 compound aesculetin

3 讨论

本研究对分析条件进行了多重优化,在考察流动相时,水相分别比较体积分数0.1%甲酸溶液、10 mmol·L⁻¹甲酸铵溶液、10 mmol·L⁻¹甲酸铵溶液(含体积分数0.1%甲酸),有机相分别比较了甲醇和乙腈,在考察供试品制备时分别考察了甲醇、80%甲醇和50%甲醇等溶剂,以质谱响应值较好且峰数量最多作为评判标准,最终分别确定了体积分数0.1%甲酸溶液-甲醇的流动相洗脱体系和体积分数80%甲醇供试品制备溶剂。

川贝母,作为四川的传统道地药材,承载着悠久

的历史与丰富的文化内涵,在当前其市场供需矛盾不断加剧的背景下,也促使人们思考如何更加高效与创新地利用这一珍贵资源。川贝母的花部,尽管在某些地区已有零星的使用,其潜在的药用价值却尚未得到系统的科学研究和开发。在此前提下,本研究首次聚焦于暗紫贝母的花部,运用前沿的UH-PLC-Q-TOF-MS技术对其化学组成进行了详尽解析,共鉴定出71个化学成分,特别是24个传统上被认为与川贝母鳞茎的化痰止咳等功效密切相关的生物碱成分,如贝母素甲、贝母素乙和西贝母碱等,还鉴定了12个黄酮类、10个有机酸类、9个氨基酸类、6个核苷类、4个维生素类、3个苯丙素类以及少量皂苷类、香豆素类、蔗糖酯类、木脂素类等成分。这一结果不仅揭示了F-F潜在的药用价值,更为其资源的合理开发与可持续利用开辟了新途径。

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