基于UPLC-Q-TOF-MS/MS代谢组学技术筛选不同基原圆柏品种鉴别的化学标志物

赵紫薇[1], 彭　芳[2], 张　琨[1], 罗玉婷[2], 曾宇骄[2], 武鑫玥[2], 唐　策[1*], 范　刚[1*]

(1. 成都中医药大学民族医药学院, 四川 成都 611137;　2. 成都中医药大学药学院, 四川 成都 611137)

摘要: 本研究采用超高效液相色谱-四极杆-飞行时间质谱 (UPLC-Q-TOF-MS/MS) 非靶向代谢组学技术, 分析鉴定圆柏药材的整体化学成分, 并结合主成分分析和偏最小二乘判别分析筛选不同基原圆柏品种鉴别的化学标志物。结果共检测出58个化学成分, 鉴定了其中46个化学成分, 包括26个黄酮类、8个有机酸类及其衍生物、4个苯丙素类、3个萜类、5个其他类成分, 其中methylsyringin和ekersenin为首次在圆柏中鉴定的成分。在正离子模式下筛选出12个品种鉴别的化学标志物, 在负离子模式下筛选出13个品种鉴别的化学标志物。结果表明, UPLC-Q-TOF-MS/MS代谢组学技术结合化学计量学方法, 可有效揭示不同基原圆柏药材的化学成分差异, 为其品种鉴别和质量控制提供参考。

关键词: 圆柏; UPLC-Q-TOF-MS/MS; 代谢组学; 品种鉴定

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Selection of chemical markers for identification of different species of Juniperri Caulis et Folium based on UPLC-Q-TOF-MS/MS metabonomics technology

ZHAO Zi-wei[1], PENG Fang[2], ZHANG Kun[1], LUO Yu-ting[2], ZENG Yu-jiao[2], WU Xin-yue[2], TANG Ce[1*], FAN Gang[1*]

(*1. School of Ethnic Medicine of Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China; 2. School of Medicine of Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China*)

Abstract: In this study, untargeted metabolomics technology based on ultra-high-performance liquid chromatography-quadrupole/time of flight mass spectrometry (UPLC-Q-TOF-MS/MS) was used to analyze and identify the overall chemical components of Juniperri Caulis et Folium. Chemical markers for the identification of different Juniperri Caulis et Folium species were screened by integrated principal component analysis and partial least squares discriminant analysis. A total of 58 chemical components were detected and 46 of them were identified, including 26 flavonoids, 8 organic acids and their derivatives, 4 phenylpropanoids, 3 terpenoids, and 5 other components. Among them, methylsyringin and ekersenin were identified for the first time. In the positive ion mode, 12 markers were screened, and in the negative ion mode, 13 markers were screened for species identification. In summary, UPLC-Q-TOF-MS/MS metabonomics technology combined with chemometrics method can effectively reveal the chemical composition differences of different Juniperri Caulis et Folium species, and provide reference for its species identification and quality control.

Key words: Juniperri Caulis et Folium; UPLC-Q-TOF-MS/MS; Metabonomics; Species identification

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*通讯作者 E-mail: fangang1111@163.com; tangce@cdutcm.edu.cn

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圆柏为常用藏药材, 味苦性凉, 能清热、消炎、干黄水, 常用于治疗肾病, 尿涩, 膀胱病, 关节炎, 月经不调等[1]。圆柏是典型的多基原药材, 同属多种植物均能入药。课题组前期对西藏、青海、四川、云南等藏区藏医院、藏药制药企业及药材市场的圆柏药材使用情况进行了调查, 发现使用的品种主要为滇藏方枝柏*Juniperus indica*、香柏*J. pingii* var. wilsonii、高山柏*J. squamata*、大果圆柏*J. tibetica*和祁连圆柏*J. przewalskii*[2]。圆柏的基原多样性和混淆使用不利于市场监管, 也影响其质量稳定性和可控性。因此, 采用现代科学技术筛选可鉴别圆柏不同基原品种的化学标志物, 对于其质量控制具有重要的意义。

近年来, 代谢组学技术已广泛运用于植物药材的整体成分分析、品种鉴别和质量评价等研究中[3]。超高效液相色谱-四极杆-飞行时间串联质谱法 (ultra-high-performance liquid chromatography-quadrupole/time of flight mass spectrometry, UPLC-Q-TOF-MS/MS) 技术是代谢组学研究的主要工具[4], 具有高灵敏度、高分辨率、高质量精度及扫描范围广等优点[5,6]。目前, 张娇等[7]、马明芳等[8]对圆柏单一基原品种进行了化学成分研究, 但不同基原圆柏药材之间的化学成分是否有差异仍然未知。本研究采用基于高分辨质谱的植物代谢组学技术对圆柏药材中的小分子代谢物进行全面分析表征, 同时结合主成分分析 (principal components analysis, PCA)、偏最小二乘法判别分析 (partial least squares discriminant analysis, PLS-DA)等多元统计学方法对不同品种间的代谢物进行比较分析, 以期找出化学标志物, 为圆柏的品种鉴别和质量评价提供新方法。

材料与方法

仪器 Waters Acquity UPLC^{TM}超高效液相色谱仪 (美国Waters公司), 配有四元高压泵, 自动进样器, PDA检测器, Empower色谱工作站, 连接Q-TOF analyzer in a SYNAPT G2 HDMS system高分辨质谱仪, 配备电喷雾离子源和MassLynx v4.1质谱工作站; Sorvall Legend Micro 17高速离心机 (美国Thermo Fisher Scientific公司); BT125D十万分之一电子天平 (德国Sartorius公司); SB-4200DTD超声波清洗器 (宁波新芝生物科技股份有限公司)。

试剂 海波拉亭-7-*O*-*β*-*D*-吡喃葡萄糖苷 (批号: DST202019-053)、methylsyringin (批号: DST202020-044)、槲皮苷 (批号: DST202007-001)、海波拉亭-7-*O*-*β*-*D*-吡喃木糖苷 (批号: DST202019-029)、ekersenin (批号: DST202004-009)、柏木双黄酮 (批号: DST202010-002)、穗花衫双黄酮 (批号: DTS201025-046)、扁柏双黄酮 (批号: DST200619-009) 和罗汉松双黄酮A (批号: DST200919-053) 均购于成都乐美天医药公司, 纯度 > 98%。色谱级乙腈, 色谱级甲酸 (美国Sigma公司)、亮氨酸脑啡肽 (美国Waters公司), 蒸馏水 (广州屈臣氏食品饮料有限公司)。甲醇 (成都市科隆化学品有限公司) 等试剂均为分析纯。

样品 本研究共收集66批圆柏药材, 经重庆市中药研究院秦松云副研究员鉴定为滇藏方枝柏*J. indica*、香柏*J. pingii* var. *wilsonii*、高山柏*J. squamata*、大果圆柏*J. tibetica*和祁连圆柏*J. przewalskii*, 样品信息详见表1。

色谱条件 色谱柱为Acquity UPLC® BEH C18 (100 mm × 2.1 mm, 1.7 μm); 流动相为0.1%甲酸水 (A)−0.1%甲酸乙腈 (B), 梯度洗脱程序为: 0～3 min, 4%～5% B; 3～7 min, 5%～7% B; 7～9 min, 7%～9% B; 9～13 min, 9%～10% B; 13～24 min, 10%～18% B; 24～30 min, 18%～28% B; 30～35 min, 28%～30% B; 35～55 min, 30%～50% B; 55～58 min, 50%～52% B; 58～60 min, 52%～65% B; 60～65 min, 65%～65% B。流速为0.4 mL·min^{-1}, 柱温为40℃, 进样量为1 μL。

质谱条件 采用Waters SYNAPT G2 HDMS系统。以氮气作为雾化、锥孔气, 源温度: 150 ℃, 反向锥孔气流: 50 L·h^{-1}, 脱溶剂气温度: 450 ℃, 脱溶剂气流: 800 L·h^{-1},

Table 1 Samples information of Juniperri Caulis et Folium. SC: SiChuan Province; Tibet Aut. Pre.: Tibetan Autonomous Prefecture; QH: QingHai Province; YN: YunNan Province; TCM: Traditional Chinese Medicine; Tibet Aut. Reg.: Tibet Autonomous Region

No.	Origin	Location	Collection time
QL-01	*J. przewalskii*	Shuangqiaogou, Dawei Township, Xiaojin County, Aba Tibetan and Qiang Autonomous Prefecture, SC	2019.07.03
QL-02	*J. przewalskii*	Zeku County, Huangnan Tibet Aut. Pre., QH	2020.07.17
QL-03	*J. Przewalskii*	Xiongrang Village, Zeku County, Huangnan Tibet Aut. Pre., QH	2020.07.17
QL-04	*J. przewalskii*	Tibetan Medical Hospital of Golog Tibet Aut. Pre., QH	2020.07.31
QL-05	*J. przewalskii*	Lajia Town, Maqin County, Golog Tibet Aut. Pre., QH	2020.07.30
QL-06	*J. przewalskii*	Xiangda Town, Nangqian County, Yushu Tibet Aut. Pre., QH	2020.08.03
QL-07	*J. przewalskii*	Maixiu Town, Zeku County, Huangnan Tibet Aut. Pre., QH	2020.08.22
QL-08	*J. przewalskii*	Maixiu Town Longcanggou, Zeku County, Huangnan Tibet Aut. Pre., QH	2020.08.22

Continued

No.	Origin	Location	Collection time
XB-01	*J. pingii* var. *wilsonii*	Dege County, Ganzi Tibet Aut. Pre., SC	2019.07.24
XB-02	*J. pingii* var. *wilsonii*	Jiaduo Village, Ganzi County, Ganzi Tibet Aut. Pre., SC	2019.06.27
XB-03	*J. pingii* var. *wilsonii*	China Tibet Hospital, Derong County, Ganzi Tibet Aut. Pre., SC	2019.06.27
XB-04	*J. pingii* var. *wilsonii*	Tibetan Hospital of Ganzi Tibet Aut. Pre., SC	2019.07.22
XB-05	*J. pingii* var. *wilsonii*	TCM Market of Chengdu International Trade City, SC (No. 6-1-1158)	2020.06.25
XB-06	*J. pingii* var. *wilsonii*	TCM Market of Chengdu International Trade City, SC (No. 6-1-0725)	2020.07.22
XB-07	*J. pingii* var. *wilsonii*	TCM Market of Chengdu International Trade City, SC (No. 6-1-1158)	2020.07.22
XB-08	*J. pingii* var. *wilsonii*	Lhasa Agricultural Product Quality Supervision, Inspection and Testing Center, Tibet Aut. Reg.	2020.07.12
XB-09	*J. pingii* var. *wilsonii*	Affiliated Hospital of Tibet Medical University, Tibet Aut. Reg.	2020.07.22
XB-10	*J. pingii* var. *wilsonii*	International Tibetan TCM Trading City, Tibet Aut. Reg.	2020.07.22
XB-11	*J. pingii* var. *wilsonii*	Tibetan Medical Hospital of Golog Tibet Aut. Pre., QH	2020.07.31
XB-12	*J. pingii* var. *wilsonii*	Tibetan Hospital of Chengduo County, Yushu Tibet Aut. Pre., QH	2020.08.02
XB-13	*J. pingii* var. *wilsonii*	Kangding Airport, Ganzi Tibet Aut. Pre., SC	2020.08.08
XB-14	*J. pingii* var. *wilsonii*	Kangding Airport, Ganzi Tibet Aut. Pre., SC	2020.08.08
XB-15	*J. pingii* var. *wilsonii*	Baima Snow Mountain, Deqin County, YN	2020.08.13
XB-16	*J. pingii* var. *wilsonii*	The Second Pass of Baima Snow Mountain, Deqin County, YN	2020.08.14
XB-17	*J. pingii* var. *wilsonii*	Sangri County, Lashannan City, Tibet Aut. Reg.	2020.08.15
XB-18	*J. pingii* var. *wilsonii*	Near Gabila, Kangding City, Ganzi Tibet Aut. Pre., SC	2020.08.18
XB-19	*J. pingii* var. *wilsonii*	Mangkang County, Changdu, Tibet Aut. Reg.	2020.08.16
DZ-01	*J. indica*	Dege County, Ganzi Tibet Aut. Pre., SC	2019.07.23
DZ-02	*J. indica*	Tu'er Mountain Foot, Litang County, Ganzi Tibet Aut. Pre., SC	2019.09.28
DZ-03	*J. indica*	International Tibetan TCM Trading City, Tibet Aut. Reg.	2019.08.01
DZ-04	*J. indica*	International Tibetan TCM Trading City, Tibet Aut. Reg.	2020.06.21
DZ-05	*J. indica*	Northern Grassland Medicinal Materials Development Co., Ltd of Tibet Aut. Reg.	2020.06.26
DZ-06	*J. indica*	Rui Junfang Biotechnology Development Co., Ltd of Tibet Aut. Reg.	2020.06.25
DZ-07	*J. indica*	Tibetan Hospital of Lingzhi City, Tibet Aut. Reg.	2020.07.16
DZ-08	*J. indica*	Zheduoshan, Kangding City, Ganzi Tibet Aut. Pre., SC	2020.07.01
DZ-09	*J. indica*	Sejila Mountain, Linzhi City, Tibet Aut. Reg.	2020.07.01
DZ-10	*J. indica*	Shannan City, Tibet Aut. Reg.	2020.08.05
DZ-11	*J. indica*	Kongse Township, Daofu County, Ganzi Tibet Aut. Pre., SC	2020.08.06
DZ-12	*J. indica*	Yangxi Road, Heqing County, Dali City, YN	2020.08.06
DZ-13	*J. indica*	Derong Sino Tibetan Hospital, Ganzi Tibet Aut. Pre., SC	2020.08.07
DZ-14	*J. indica*	Baima Snow Mountain, Deqin County, Diqing Tibet Aut. Pre., YN	2020.08.11
DZ-15	*J. indica*	Dalanggu Village, Xiangcheng County, Ganzi Tibet Aut. Pre., SC	2020.08.15
DZ-16	*J. indica*	Tiegohe, Litang County, Ganzi Tibet Aut. Pre., SC	2020.08.13
DZ-17	*J. indica*	Shengping Town, Deqin County, Diqing Tibet Aut. Pre., YN	2020.08.17
DZ-18	*J. indica*	Rui Junfang Biotechnology Development Co., Ltd of Tibet Aut. Reg.	2020.08.16
DZ-19	*J. indica*	Lulang Town, Linzhi County, Linzhi City, Tibet Aut. Reg.	2020.08.25
GS-01	*J. squamata*	Guinan County, Hainan Tibet Aut. Pre., QH	2019.09.28
GS-02	*J. squamata*	Tibetan Hospital, Dege County, Ganzi Tibet Aut. Pre., SC	2019.07.24
GS-03	*J. squamata*	Huzhu Tu Autonomous County, Haidong City, QH	2019.09.28
GS-04	*J. squamata*	Tibetan Hospital, Huangzhong District, Xining City, QH	2019.09.27
GS-05	*J. squamata*	TCM Market of Chengdu International Trade City, SC (No. 6-1-1158)	2020.07.16
GS-06	*J. squamata*	Tibetan Hospital, Qinghai Province	2020.07.17
GS-07	*J. squamata*	Tibetan Hospital of Hainan Tibet Aut. Pre., QH	2020.07.17
GS-08	*J. squamata*	Tibetan Hospital of Zeku County, Qinghai Province	2020.07.17
GS-09	*J. squamata*	Medicinal Materials Market of Qinghai Sanzhi Trading Co., Ltd, QH	2020.07.17
GS-10	*J. squamata*	Zheduoshan, Kangding City, SC	2020.07.16
GS-11	*J. squamata*	Shannan City, Tibet Aut. Reg.	2020.08.05
GS-12	*J. squamata*	Tibetan Hospital of Nangqian County, Yushu Tibet Aut. Pre., QH	2020.08.03
GS-13	*J. squamata*	Xiazha Town, Shiqu County, Ganzi Tibet Aut. Pre., SC	2020.08.06
GS-14	*J. squamata*	Baima Snow Mountain, Deqin County, YN	2020.08.13
GS-15	*J. squamata*	Litang County, Ganzi Tibet Aut. Pre., SC	2020.08.17
DG-01	*J. tibetica*	Tibetan Medicine Factory of Naqu City, Tibet Aut. Reg.	2019.08.01
DG-02	*J. tibetica*	TCM Market of Chengdu International Trade City, SC (No. 6-1-0725)	2020.06.27
DG-03	*J. tibetica*	Tibetan Hospital of Chengduo County, Yushu Tibet Aut. Pre., QH	2020.08.02
DG-04	*J. tibetica*	Shannan Xueyuan Medicinal Materials Co., Ltd, Tibet Aut. Reg.	2020.08.05
DG-05	*J. tibetica*	Tibetan Medicine Co., Ltd. of Tibet Tibetan Medical College, Tibet Aut. Reg.	2020.08.17

隔: 0.02 s, 质荷比: m/z 100～1 500 Da, 锁定质量数 (亮氨酸脑啡肽): 正离子模式: m/z 556.277 1 $[M+H]^+$, 负离子模式 m/z 554.261 5 $[M-H]^-$。

供试品溶液制备 取圆柏药材粉末 (过三号筛) 约1 g, 精密称定, 置具塞锥形瓶中, 精密加入甲醇 30 mL, 称定重量, 超声处理 (功率 250 W, 频率 40 kHz) 30 min, 放冷, 再次称定重量, 用甲醇补足减少的重量, 摇匀, 滤过, 取滤液, 13 000 $r \cdot min^{-1}$ 离心 15 min, 取上清液过 0.22 μm 的微孔滤膜, 即得。

数据处理与统计分析 查阅国内外圆柏及其同属柏科植物化学成分研究相关文献, 收集圆柏中各类化学成分, 建立圆柏质谱数据库, 将数据库导入 UNIFI 1.8 (Waters, 美国) 在线软件进行化学成分鉴定。通过人工校验和核实精确分子量、出峰时间、裂解碎片、加合离子峰, 筛选质量误差在±5 ppm内, 确认和指证化合物。将UPLC-Q-TOF-MS/MS获得的原始数据导入Progenesis QI软件进行峰提取、峰匹配及归一化前处理后, 筛选并导出鉴别化合物数据。利用SIMCA-P 14.1软件对预处理后的数据进行PCA、PLS-DA分析, 根据变量重要性投影值 (variable importance in projection, VIP) > 3、$P < 0.05$ 筛选潜在差异代谢物。潜在差异代谢物采用Metaboanalyst 5.0进行热图分析。

结果

1 圆柏化学成分的质谱鉴定

对不同基原的66批圆柏样品进行UPLC-Q-TOF-MS/MS分析, 结果发现各基原圆柏样品的总离子流图基本相似。结合UNIFI自动匹配化合物与人工校验化合物, 共检测出58个化学成分, 鉴定了其中46个化学成分, 其中在正离子模式下鉴定了37个成分, 在负离子模式下鉴定了42个成分, 包括26个黄酮类成分、8个有机酸类成分及其衍生物、4个苯丙素类成分、3个萜类成分、5个其他类成分, 以及12个未知成分。总离子流图见图1, 各化合物的质谱信息见表2[9-38]。

1.1 黄酮类成分 本研究从圆柏药材中鉴定了26个黄酮类化合物。黄酮类成分易发生典型的逆狄尔斯–阿德尔反应 (Retro Diels-Alder reaction, RDA), 易失去 H_2O、CO和 C_2H_2O 等。其中双黄酮类的裂解方式可根据C环断裂位置的不同分为三种类型: 穗花衫双黄酮裂解类型 (在Ⅱ环1/3、0/4位更易发生裂解)、罗波斯塔双黄酮类型 (在Ⅰ环1/3、1/4位更易发生裂解)、扁柏双黄酮类型 (C-O键易发生裂解)[34]。因此, 根据不同的

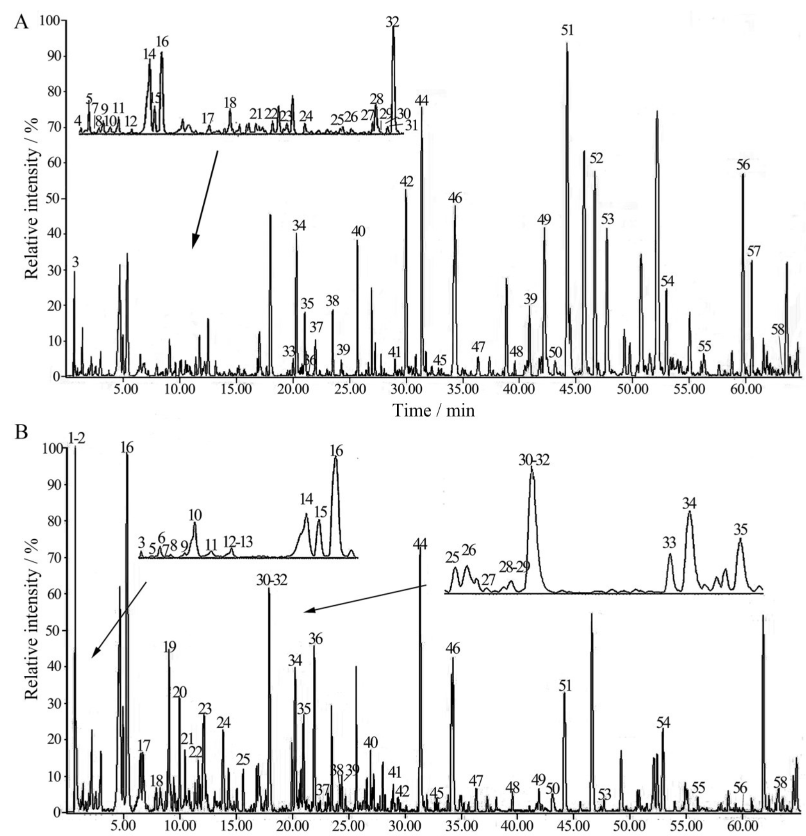

Figure 1 The base peak ion (BPI) current of Juniperri Caulis et Folium by UPLC-Q-TOF-MS/MS at positive (A) and negative (B) ion modes. Figure 1 corresponds to the peak labels in Table 2

Table 2 MS/MS information of the major constituents in Juniperri Caulis et Folium. *Represents the compound identified by comparison of reference substances

No.	t_R /min	Molecular formula	Theoretical mass (*m*/*z*)	Measured mass $[M+H]^+$/ $[M+Na]^+$ (ppm)	Measured mass $[M-H]^-$ (ppm)	MS/MS (*m*/*z*)	Compound	Ref.
1	0.62	$C_7H_{12}O_6$	192.063 4	/	191.055 4 (−1.0)	173.044 9 $[M-H_2O]^-$ (−0.6); 127.039 5 $[M-H_2O-CO_2]^-$ (1.6)	Auinic acid	
2	0.68	$C_6H_{12}O_6$	180.063 4	/	179.055 4 (−1.1)	161.044 6 $[M-H-H_2O]^-$ (−2.5)	Fructose	
3	0.93	$C_9H_{11}NO_3$	181.073 9	182.081 7 (0.5)	180.066 1 (−3.3)	163.044 6 $[M-NO_3]^-$ (−2.5); 136.076 3 $[M+H-H_2O-CO]^+$ (0.7)	*L*-Tyrosine	
4	0.95	$C_{10}H_{13}N_5O_4$	267.096 8	268.104 9 (1.1)	/	136.072 2 $[M+H-C_5H_8O_4]^+$ (0.0); 119.049 7 $[M+H-NH_3-C_5H_8O_4]^+$ (0.8); 94.040 7 $[M+H-C_5H_8O_4-NH_2CN]^+$ (2.1)	Adenosine	[9]
5	1.21	$C_7H_6O_5$	170.021 5	171.029 6 (1.8)	169.013 2 (−3.0)	125.023 6 $[M-COOH]^-$ (−2.4); 127.039 7 $[M+H-COO]^+$ (1.6); 79.018 0 $[C_5H_3O]^-$ (2.5)	Gallic acid	
6	1.31	$C_{12}H_{22}O_{11}$	342.116 2	/	341.108 4 (−3.6)	179.049 7 $[M-H-glc]^-$ (−1.1); 161.044 6 $[M-H-glc-H_2O]^-$ (−2.5); 119.034 4 $[C_4H_7O_4]^-$ (−1.7)	Sucrose	
7	1.36	$C_{11}H_{16}O_8$	276.084 5	299.074 8 (1.7)	275.076 8 (0.4)	/	Unknown	
8	1.51	$C_7H_6O_3$	138.031 7	/	137.023 6 (−2.2)	93.033 7 $[M-H-CO_2]^-$ (−3.2)	Salicylic acid	
9	1.93	$C_{30}H_{26}O_{13}$	594.137 3	595.145 7 (0.8)	593.128 4 (−1.9)	441.080 8 $[M-glc]^-$ (0.5); 423.070 8 $[M-glc-H_2O]^-$ (0.7); 305.065 3 $[C_{15}H_{13}O_7]^-$ (−0.3); 283.024 3 $[C_{15}H_7O_6]^-$ (0.7); 255.028 7 $[C_{14}H_7O_5]^-$ (1.2)	Gallocatechin-(4*α*→8)-catechin or catechin-(4*α*→8)-gallocatechin	[10]
10	2.33	$C_7H_6O_4$	154.026 0	/	153.018 9 (0.7)	109.028 7 $[M-COOH]^-$ (−2.8)	Protocatechuic acid	[11]
11	2.48	$C_{16}H_{18}O_9$	354.095 1	377.084 9 (2.2)	353.087 9 (0.6)	191.055 8 $[M-H-C_9H_6O_3]^-$ (1.0); 173.044 9 $[M-H-C_9H_6O_3-H_2O]^-$ (−0.6); 161.023 2 $[M-H-C_7H_{12}O_6]^-$ (−4.3); 135.044 4 $[M-H-C_7H_{10}O_6-CO]^-$ (−1.5); 109.028 5 $[M-H-C_7H_{10}O_6-CO-C_2H_2]^-$ (−4.6)	Chlorogenic acid	[12]
12	2.52	$C_{30}H_{26}O_{13}$	594.137 3	595.145 1 (−0.2)	593.130 2 (1.2)	441.082 4 $[M-H-glc]^-$ (0.5); 423.071 9 $[M-glc-H_2O]^-$ (0.7); 305.066 0 $[C_{15}H_{13}O_7]^-$ (−0.3); 283.024 5 $[C_{15}H_7O_6]^-$ (0.7); 255.029 6 $[C_{14}H_7O_5]^-$ (1.2)	Gallocatechin-(4*α*→8)-catechin or catechin-(4*α*→8)-gallocatechin	
13	2.97	$C_{15}H_{14}O_6$	290.079 0	291.087 5 (2.1)	289.070 8 (−1.4)	579.149 8 $[2M+H]^+$ (−0.9); 577.133 7 $[2M-H]^-$ (−1.6); 245.080 7 $[M-H-CO_2]^-$ (−2.9); 229.050 1 $[M-H-H_2O-C_2H_2O]^-$ (3.4); 203.070 0 $[M-H-H_2O-C_3O_2]^-$ (−3.9); 187.038 8 $[M-H-CO_2-C_3H_6O]^-$ (−3.7); 179.033 8 $[M-H-C_6H_6O_2]^-$ (−3.4); 161.023 1 $[M-H-CO_2-2C_2H_2O]^-$ (5.0); 137.024 0 $[M-H-C_8H_8O_3]^-$ (−1.4); 123.044 6 $[M-H-C_9H_8O_3]^-$ (0.0)	Epicatechin	[13]
14	4.63	$C_{30}H_{26}O_{12}$	578.520 0	579.151 4 (1.9)	577.134 4 (−0.3)	427.103 7 $[M+H-C_8H_7O_3]^+$ (1.9); 409.093 0 $[M+H-C_8H_7O_3-H_2O]^+$ (1.7); 407.076 4 $[M-H-C_8H_7O_3-H_2O]^-$ (−0.7); 291.087 1 $[M+H-C_8H_7O_3-C_7H_4O_3]^+$ (0.7); 289.072 0 $[M+H-C_{15}H_{14}O_6]^+$ (2.8)	Proanthocyanidin B1	[14]
15	4.93	$C_9H_8O_3$	164.047 3	/	163.038 9 (−3.7)	147.044 1 $[M-H-H_2O]^-$ (−3.4); 119.049 7 $[M-H-CH_2O_2]^-$ (0.0)	*p*-Hydroxycinnamic acid	

Continued

No.	t_R /min	Molecular formula	Theoretical mass (m/z)	Measured mass $[M+H]^+$/ $[M+Na]^+$ (ppm)	Measured mass $[M-H]^-$ (ppm)	MS/MS (m/z)	Compound	Ref.
16	5.31	$C_{15}H_{14}O_6$	290.079 0	291.087 6 (2.1)	289.071 0 (−0.7)	245.044 8 $[M-H_2O-C_2H_2]^-$ (−0.8); 227.070 9 $[M-H-CO_2-H_2O]^-$ (0.4); 203.070 8 $[M-H-C_3H_2O_3]^-$ (0.0); 205.049 9 $[M-H-C_4H_4O_2]^-$ (−1.0); 179.034 2 $[M-H-C_6H_6O_2]^-$ (−1.1); 151.039 3 $[C_8H_7O_3]^+$ (−1.3); 139.039 3 $[C_7H_7O_3]^+$ (−1.4); 137.032 6 $[C_7H_5O_3]^-$ (−2.2); 125.023 7 $[C_6H_5O_3]^-$ (−1.6); 109.028 7 $[C_6H_5O_2]^-$ (−2.8)	Catechin	[15]
17	6.55	$C_{30}H_{26}O_{12}$	578.520 0	579.159 1 (−0.3)	577.134 2 (−0.7)	427.1029 $[M+H-C_8H_7O_3]^+$ (0.5); 409.091 9 $[M+H-C_8H_7O_3-H_2O]^+$ (−1.0); 407.1414 $[M-H-C_8H_7O_3-H_2O]^-$ (0.7); 291.087 1 $[M+H-C_8H_7O_3-C_7H_4O_3]^+$ (0.7); 289.071 4 $[M+H-C_{15}H_{14}O_6]^+$ (0.7)	Proanthocyanidin B	[16]
18	8.44	$C_{21}H_{22}O_{11}$	450.116 2	451.124 4 (0.9)	449.108 5 (0.2)	287.054 6 $[M-H-glc]^-$ (−3.5); 289.071 1 $[M+H-glc]^+$ (−0.3); 259.059 7 $[M-H-glc-H_2O]^-$ (−3.5); 151.002 7 $[M-H-glc-C_8H_8O_2]^-$ (−2.6); 107.013 3 $[M-H-glc-C_8H_7O_2-CO-OH]^-$ (1.9); 125.023 7 $[M-H-glc-C_7H_4O_4]^-$ (−1.6)	Eriodictyol-7-*O*-glucoside	[17]
19	9.75	$C_{20}H_{18}O_{11}$	434.084 9	/	433.077 1 (−1.4)	867.161 8 $[2M-H]^-$ (−0.2); 301.034 8 $[C_{15}H_9O_7]^-$ (0.0)	Quercetin-3-*O*-*β*-*D*-glucopyranoside	[18]
20	10.07	$C_9H_8O_4$	180.042 6	/	179.034 7 (1.7)	161.023 8 $[M-H-H_2O]^-$ (−0.6)	Caffeic acid	
21	10.11	$C_9H_6O_3$	162.031 7	163.039 7 (1.2)	161.023 4 (−3.1)	/	Unknown	
22	11.6	$C_{16}H_{26}O_8$	346.162 8	369.152 9 (1.1)	345.154 8 (−0.3)	/	Unknown	
23	12.13	$C_{24}H_{32}O_{11}$	496.194 5	519.184 0 (−0.4)	495.184 2 (−3.6)	/	Unknown	
24	13.83	$C_{25}H_{34}O_{12}$	526.205 0	549.194 5 (−0.5)	525.198 2 (1.9)	/	Unknown	
25	15.61	$C_{25}H_{32}O_{10}$	492.199 5	515.188 5 (−1.6)	491.192 5 (1.6)	/	Unknown	
26	15.78	$C_{21}H_{20}O_{12}$	464.095 5	465.104 2 (1.9)	463.087 4 (−0.6)	303.050 5 $[M+H-glc]^+$ (−4.0); 301.034 8 $[M-H-glc]^-$ (3.2); 271.023 6 $[C_{14}H_7O_6]^-$ (−2.6); 257.094 6 $[M+H-glc-CH_2O_2]^+$ (1.6); 255.065 1 $[M+H-glc-CH_2O_2]^-$ (−2.4)	Hyperoside	
27	16.98	$C_{21}H_{20}O_{12}$	464.095 5	465.104 3 (2.2)	463.087 6 (−0.2)	929.194 6 $[2M+H]^+$ (0.0); 927.183 2 $[2M-H]^-$ (0.1); 301.034 3 $[M-H-glc]^-$ (1.7); 303.050 7 $[M+H-glc]^-$ (0.7); 151.003 0 $[C_7H_3O_4]^+$ (3.3)	Hypolaetin-7-*O*-*β*-*D*-glucopyranoside*	
28	17.05	$C_{27}H_{30}O_{16}$	610.517 5	611.162 0 (1.2)	609.145 3 (−0.5)	465.104 1 $[M+H-C_6H_{10}O_4]^+$ (1.7); 463.087 8 $[M-H-C_6H_{10}O_4]^-$ (0.2); 301.034 1 $[M-H-rutinose]^-$ (−2.3); 303.051 2 $[M+H-rutinose]^+$ (2.3); 271.024 3 $[M-H-rutinose-CO]^{-}$ (0.0); 257.044 9 $[M+H-rutinose-CO_2]^+$ (−0.4); 255.029 0 $[M-H-rutinose-CO_2]^-$ (−1.2); 151.039 2 $[M-H-rutinose-C_8H_4O_5-CO]^-$ (−2.0)	Rutinum	[19]
29	17.10	$C_{20}H_{18}O_{12}$	450.079 8	451.087 9 (0.4)	449.072 5 (1.1)	/	Unknown	
30	17.67	$C_{26}H_{34}O_{11}$	522.210 1	545.199 6 (−0.6)	521.202 8 (1.0)	545.199 6 $[M+Na]^+$ (−0.6); 521.202 8 $[M-H]^-$ (1.0)	Isolarisiresinol-2*α*-*O*-*β*-*D*-glucoside or isolarisiresinol-3*α*-*O*-*β*-*D*-glucoside, isomer 1	[20]

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No.	t_R /min	Molecular formula	Theoretical mass (*m/z*)	Measured mass $[M+H]^+$/ $[M+Na]^+$ (ppm)	Measured mass $[M-H]^-$ (ppm)	MS/MS (*m/z*)	Compound	Ref.
31	17.86	$C_{26}H_{34}O_{11}$	522.210 1	545.200 6 (1.3)	521.201 7 (−1.2)	545.199 6 $[M+Na]^+$ (−0.6); 521.202 8 $[M-H]^-$ (1.0)	Isolarisiresinol-2*α*-*O*-*β*-*D*-glucoside or isolarisiresinol-3*α*-*O*-*β*-*D*-glucoside, isomer 2	
32	17.98	$C_{18}H_{26}O_9$	386.157 7	409.147 5 (0.0)	385.150 9 (2.6)	207.101 7 $[M+H-glc-H_2O]^+$ (−1.9)	Methylsyringin*	[21,22]
33	19.01	$C_{21}H_{20}O_{12}$	464.095 5	465.103 7 (0.9)	463.088 7 (2.2)	303.050 4 $[M+H-glc]^+$ [−0.3]; 301.034 2 $[M-H-glc]^-$ (2.0); 25 7.0450 $[M+H-glc-CH_2O_2]^+$ (0.0)	Quercetin-3-*O*-*β*-*D*-lucopyranoside	[23]
34	20.80	$C_{21}H_{20}O_{11}$	448.100 6	449.109 3 (2.0)	447.096 5 (1.8)	895.193 8 $[2M-H]^-$ (0.6); 303.051 2 $[M+H-rha]^+$ (2.3); 301.033 8 $[M-H-rha]^-$ (−3.3); 271.023 9 $[M-H-rha-H-CO]^-$ (−1.5); 255.029 1 $[M-H-rha-CO-OH]^-$ (−0.8); 229.052 7 $[C_{14}H_{13}O_3]^+$ (0.9); 151.002 2 $[M-H-rha-C_7HO_4]^-$ (−2.6); 109.029 4 $[C_6H_5O_2]^+$ (0.9)	Quercitrin*	[24]
35	21.04	$C_{20}H_{18}O_{11}$	434.084 9	435.092 8 (0.2)	433.077 6 (1.2)	867.160 9 $[2M-H]^-$ (1.0); 303.051 1 $[M+H-Xyl]^+$ (2.0); 301.034 1 $[M-H-Xyl]^-$ (−1.7); 135.044 3 $[C_8H_7O_2]^-$ (−2.2); 316.947 6 $[M-H-glc]^-$ (0.0); 261.133 8 $[M-H-glc-2CO]^-$ (0.0)	Hypolaetin-7-*O*-*β*-*D*-xylopyranoside*	[25]
36	22.77	$C_{20}H_{26}O_6$	362.172 9	385.162 7 (0.0)	361.163 3 (−5.0)	/	Unknown	
37	23.02	$C_{21}H_{20}O_{11}$	448.100 6	449.108 7 (0.7)	447.092 7 (0.0)	287.055 7 $[M+H-glc]^+$ (0.3); 285.039 9 $[M-H-glc]^-$ (0.0); 151.003 1 $[C_7H_3O_4]^-$ (0.0); 153.018 8 $[C_7H_5O_4]^+$ (0.0)	Cynaroside	[26]
38	24.26	$C_{15}H_{10}O_6$	286.047 7	287.056 0 (1.4)	285.039 7 (−0.7)	241.050 6 $[M-CO_2]^-$ (2.1); 153.018 8 $[M+H-C_8H_6O_2]^+$ (0.0); 151.039 0 $[M-H-C_8H_6O_2]^-$ (−3.3); 133.028 4 $[M-H-C_7H_4O_4]^-$ (−4.5)	Luteolin	[26]
39	24.73	$C_{33}H_{40}O_{19}$	740.216 4	/	739.205 0 (−4.9)	593.145 7 $[M-H-C_6H_{12}O_4]^-$ (1.5); 431.092 4 $[M-H-C_{12}H_{22}O_9]^-$ (1.2); 285.040 1 $[M-H-C_6H_{12}O_4-C_{12}H_{22}O_9]^-$ (0.7)	Robinin	[27]
40	26.64	$C_{36}H_{28}O_{15}$	700.142 8	701.150 9 (0.4)	699.135 2 (0.3)	539.098 2 [M+H−162 (hexose unit)]$^+$ (0.7); 537.083 0 [M−H−162 (hexose unit)]$^-$ (1.5)	Cupressuflavone 4'-*O*-*β*-*D*-glucopyranosides	[28]
41	29.01	$C_{15}H_{10}O_5$	270.052 8	271.060 5 (−0.4)	269.044 7 (−1.1)	225.056 0 $[M-H-CO_2]^-$ (3.6); 183.044 8 $[M-H-H_2O-C_3O_2]^-$ (1.1); 201.163 8 $[M-H-C_3O_2]^-$ (−2.5); 213.055 0 $[M-H-CO-CO]^-$ (−0.9); 241.048 9 $[M-H-CO]^-$ (−5.0)	Apigenin	[29]
42	29.37	$C_{36}H_{28}O_{15}$	700.142 8	701.150 6 (0.0)	699.133 4 (−2.3)	/	Unknown	
43	29.99	$C_{11}H_{10}O_3$	190.063 0	213.052 8 (0.0); 191.070 7 (−0.5)	/	213.052 8 $[M+Na]^+$ (0.0); 191.070 7 $[M+H]^+$ (−0.5)	Ekersenin*	[30]
44	31.36	$C_{30}H_{18}O_{10}$	538.090 0	539.097 4 (−0.7)	537.083 6 (2.6)	1 075.172 2 $[2M-H]^-$ (3.1); 377.066 3 $[M+H-C_9H_60_3]^+$ (0.5); 375.050 5 $[M-H-C_9H_6O_3]^-$ (0.0); 121.029 0 $[C_7H_5O_2]^+$ (0.0)	Cupressuflavone*	[31]

Continued

No.	t_R /min	Molecular formula	Theoretical mass (m/z)	Measured mass $[M+H]^+$/ $[M+Na]^+$ (ppm)	Measured mass $[M-H]^-$ (ppm)	MS/MS (m/z)	Compound	Ref.
45	32.83	$C_{30}H_{18}O_{10}$	538.090 0	539.098 3 (0.9)	537.082 9 (1.3)	1 075.176 5 $[2M-H]^-$ (0.8); 1 077.190 2 $[2M+H]^+$ (0.0); 521.087 7 $[M+H-H_2O]^+$ (0.8); 497.087 8 $[M+H-C_2H_2O]^+$ (1.0); 403.045 8 $[M+H-C_8H_6O-H_2O]^+$ (1.0); 379.045 8 $[M+H-C_8H_6O-C_2H_2O]^+$ (1.1); 377.066 5 $[M+H-C_9H_6O_3]^+$ (1.1); 311.055 9 $[M+H-C_8H_6O-C_3O_2]^+$ (1.0); 283.060 6 $[M+H-C_8H_6O-C_3O_2-CO]^+$ (0.0); 121.029 0 $[C_7H_5O_2]^+$ (0.0)	Amentoflavone*	[32]
46	34.33	$C_{30}H_{18}O_{10}$	538.090 0	539.097 6 (−0.4)	537.082 2 (0.0)	521.087 4 $[M+H-H_2O]^+$ (0.2); 413.068 8 $[M+H-C_6H_4O_3]^+$ (−1.9); 403.045 7 $[M+H-C_8H_6O-H_2O]^+$ (0.7); 387.087 0 $[M+H-C_7H_4O_4]^+$ (0.3); 375.050 2 $[M+H-C_8H_6O-H_2O-CO]^+$ (−0.8); 283.060 7 $[M+H-C_{14}H_9O_5]^+$ (0.4); 153.018 7 $[C_7H_5O_4]^+$ (−0.7); 121.028 9 $[C_7H_5O_2]^+$ (−0.8)	Robustaflavone	[32]
47	35.72	$C_{30}H_{20}O_{10}$	540.105 6	541.113 5 (0.0)	539.095 8 (2.8)	/	Unknown	
48	39.61	$C_{31}H_{20}O_{10}$	552.484 5	553.114 1 (1.1)	551.098 6 (1.5)	421.055 9 $[M+H-C_9H_8O]^+$ (−0.2); 403.045 7 $[M+H-C_9H_8O-H_2O]^+$ (0.7); 377.069 2 $[M+H-C_{10}H_8O_3]^+$ (−1.1); 375.054 0 $[M-H-C_{10}H_8O_3]^-$ (0.3); 135.044 1 $[C_8H_8O_2]^+$ (−3.7)	Podocarpusflavone A*	[32]
49	41.95	$C_{31}H_{20}O_{10}$	552.105 6	553.114 1 (1.1)	551.097 4 (−0.7)	1 105.222 2 $[2M+H]^+$ (2.8); 1 103.205 7 $[2M-H]^-$ (2.0); 403.046 0 $[M+H-C_8H_6O-CH_3OH]^+$ (1.5); 390.978 5 $[M+H-C_9H_6O_3]^+$ (2.6); 153.018 4 $[C_7H_5O_4]^+$ (−2.6)	4′-Methoxy robustaflavone	[31,32]
50	43.17	$C_{30}H_{18}O_{10}$	538.090 0	539.097 6 (−0.6)	537.081 8 (−0.7)	387.087 0 $[M+H-C_7H_4O_4]^+$ (0.3); 287.237 4 $[M+H-C_{15}H_9O_4]^+$ (−0.3); 285.221 3 $[M-H-C_{15}H_9O_4]^-$ (−1.8); 269.191 1 $[M+H-C_{15}H_9O_5]^+$ (2.2); 257.047 9 $[M+H-C_{15}H_9O_4-CO]^+$ (−2.3); 243.065 5 $[M+H-C_{15}H_9O_5-CO]^+$ (−0.8); 153.018 7 $[C_7H_5O_4]^+$ (−0.7); 121.028 9 $[C_7H_5O_2]^+$ (−0.8)	Hinokiflavone*	[32]
51	44.20	$C_{22}H_{34}O_5$	378.240 6	401.230 4(0.0)	377.231 7(−2.9)	/	Unknown	
52	46.67	$C_{35}H_{60}O_6$	576.439 0	577.446 8 (0.0)	/	577.446 8 $[M+H]^+$ (0.0)	β-Daucosterol	[33]
53	47.47	$C_{32}H_{22}O_{10}$	566.121 3	567.130 1 (1.8)	565.114 7 (2.1)	435.071 1 $[M+H-C_9H_8O]^+$ (−1.1); 417.061 2 $[M+H-C_9H_8O-H_2O]^+$ (0.5); 391.081 6 $[M+H-C_{10}H_8O_3]^+$ (−0.5); 389.066 1 $[M-H-C_{10}H_8O_3]^-$ (0.0); 167.128 6 $[C_8H_6O_4]^+$ (−1.8); 135.044 3 $[C_8H_8O_2]^+$ (−2.2)	Putraflavone B	[34]
54	52.99	$C_{20}H_{28}O_2$	300.208 9	301.218 0 (4.0)	299.200 3 (−3.0)	241.159 3 $[M+H-C_2H_6-C_2H_6]^+$ (0.4); 213.127 8 $[M+H-C_2H_6-C_2H_6-CO]^+$ (−0.5)	Sugiol	[35]
55	55.68	$C_{20}H_{26}O_4$	330.183 1	331.190 3 (−1.8)	329.172 9 (0.0)	331.190 3 $[M+H]^+$ (−1.8); 329.172 9 $[M-H]^-$ (0.0)	Carnosol	[36]
56	59.85	$C_{20}H_{30}O_2$	302.224 6	303.232 6 (0.7)	301.217 1 (1.0)	257.226 6 $[M-COOH]^+$ (1.2); 149.132 8 $[M-COOH-C_6H_6(CH_3)_2]^+$ (−1.3); 123.117 2 $[M+H-COOH-C_6H_6CH_2CH(CH_3)_2]^+$ (1.6)	Abietic acid	[37]

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No.	t_R /min	Molecular formula	Theoretical mass (*m/z*)	Measured mass [M+H]$^+$/ [M+Na]$^+$ (ppm)	Measured mass [M−H]$^-$ (ppm)	MS/MS (*m/z*)	Compound	Ref.
57	62.38	$C_{29}H_{50}O$	414.386 2	415.394 0 (0.0)	/	397.383 4 [M−OH]$^+$ (3.5); 161.133 0 [M−OH−$C_{17}H_{33}$]$^+$ (2.1); 109.181 7 [M−OH−$C_{17}H_{33}$−C_4H_5]$^+$ (1.2); 81.070 4 [C_6H_9]$^+$ (1.5)	*β*-Sitosterol	[38]
58	63.19	$C_{20}H_{28}O_2$	300.208 9	323.198 6 (−0.3)	299.200 8 (−1.0)	/	Unknown	

碎片离子可推测黄酮类化合物及其同分异构体。以$C_{30}H_{18}O_{10}$同分异构体穗花衫双黄酮 (化合物**45**)、罗波斯塔双黄酮 (化合物**46**)、扁柏双黄酮 (化合物**50**) 为例, 其裂解途径如下。

化合物**45**, 其准分子离子峰为*m/z* 539.098 3 [M+H]$^+$, 537.082 9 [M−H]$^-$。MS^{2}中Ⅱ环中1/3 C环断裂生成特征碎片离子*m/z* 419.076 6 [M+H−C_8H_6O]$^+$、再失去H_2O生成特征碎片离子*m/z* 403.045 8 [M+H−C_8H_6O−H_2O]$^+$, 后失去CO, 生成特征碎片离子*m/z* 377.066 5 [M+H−$C_9H_6O_3$]$^+$; Ⅱ环中0/4 C环断裂生成特征碎片离子377.066 5 [M+H−$C_9H_6O_3$]$^+$, 结合文献[7,8,32,34]并通过MassLynx v 4.1 Elemental Composition拟合化学式为$C_{30}H_{18}O_{10}$, 经对照品比对鉴定该化合物为穗花杉双黄酮 (amentoflavone), 裂解途径如图2。

化合物**46**, 其准分子离子峰为*m/z* 539.097 6 [M+H]$^+$, 537.082 2 [M−H]$^-$。MS^{2}中Ⅰ环中1/3 C环断裂生成特征碎片离子*m/z* 387.087 2 [M+H−$C_7H_4O_4$]$^+$; Ⅰ环中1/4 C环断裂生成特征碎片离子413.068 8 [M+H−$C_6H_4O_3$]$^+$, 结合文献[7,8,32,34]并通过MassLynx v 4.1 Elemental Composition拟合化学式为$C_{30}H_{18}O_{10}$, 推测该化合物为罗波斯塔双黄酮 (robustaflavone)。

化合物**50**, 其准分子离子峰为*m/z* 539.097 6 [M+H]$^+$, 537.081 8 [M−H]$^-$。MS^{2}中C-O键断裂生成特征碎片离子*m/z* 287.237 4 [M+H−$C_{15}H_9O_4$]$^+$, 269.191 1 [M+H−$C_{15}H_9O_5$]$^+$, 243.065 5 [M+H−$C_{15}H_9O_5$−CO]$^+$。结合文献[7,8,32,34]并通过、MassLynx v 4.1 Elemental Composition拟合化学式为$C_{30}H_{18}O_{10}$, 经对照品比对鉴定该化合物为扁柏双黄酮 (hinokiflavone)。

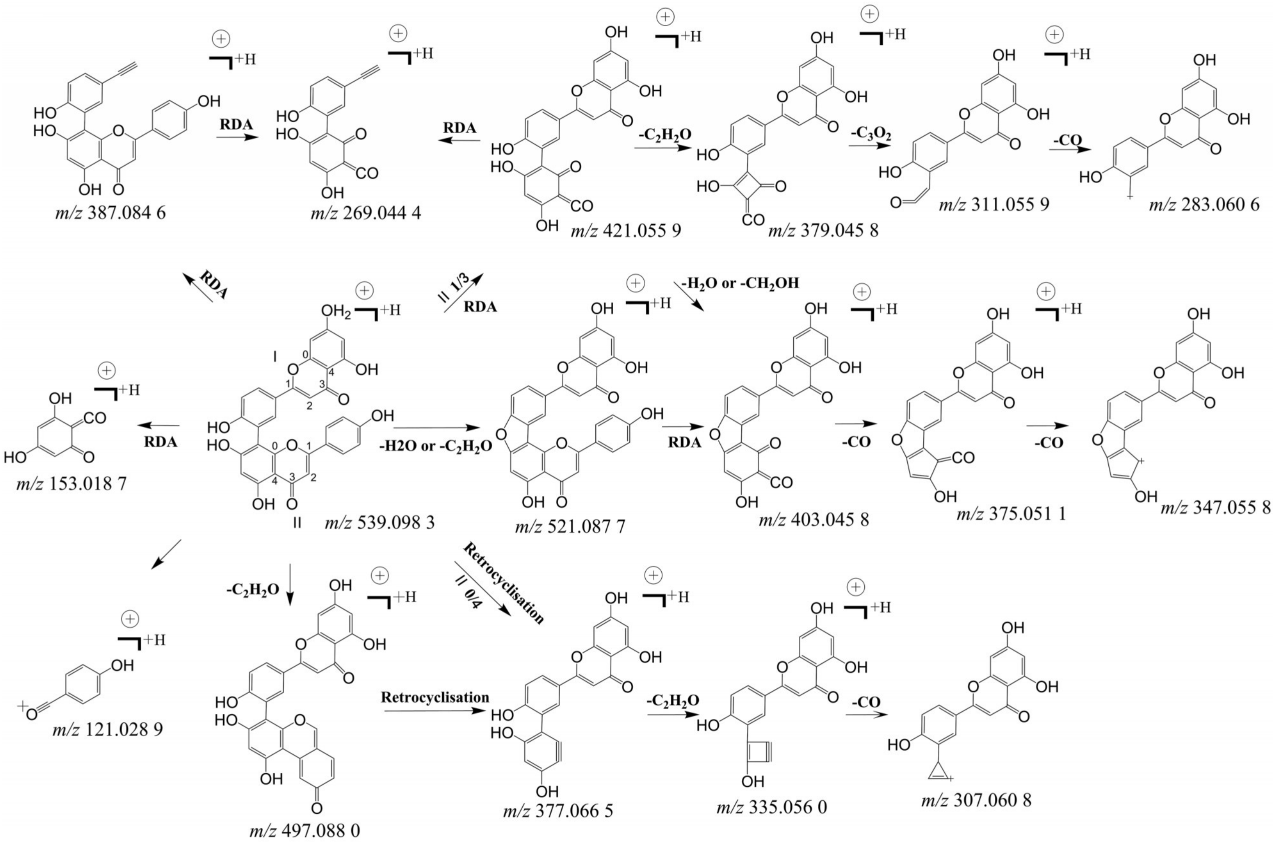

Figure 2 Fragmentation pathway of amentoflavone

1.2 有机酸类成分 有机酸类是分子结构中含有羧基 (−COOH) 的化合物, 其具有很强的抗氧化、抗菌消炎和调血脂等药理作用[12]。有机酸类化合物裂解规律一般是容易丢失CO、H_2O、C_2H_2等一系列中性基团, 从圆柏中初步推定8个有机酸类化合物, 以绿原酸 (化合物**11**) 为例, 其准分子离子峰为*m/z* 353.087 9 $[M-H]^-$, MS^2显示其组成部分咖啡酸*m/z* 161.023 2 $[M-H-C_7H_{12}O_6]^-$及奎宁酸*m/z* 191.055 8 $[M-H-C_9H_6O_3]^-$碎片离子峰, 后丢失CO、H_2O、C_2H_2等一系列中性基团生成特征碎片离子*m/z* 173.044 9 $[M-H-C_9H_6O_3-H_2O]^-$、135.044 4 $[M-H-C_7H_{10}O_6-CO]^-$、109.028 5 $[M-H-C_7H_{10}O_6-CO-C_2H_2]^-$等。结合文献[12,37]并通过UNIFI 1.8 (Waters, 美国) 数据库匹配以及MassLynx v 4.1 Elemental Composition拟合化学式为$C_{16}H_{18}O_9$, 推测该化合物为绿原酸, 裂解途径如图3。

1.3 苯丙素类成分 苯丙素是一类由C_6-C_3结构单元构成的一类化合物, 从圆柏中初步推定4个苯丙素类成分: 异落叶松脂素-3*α*-*O*-*β*-*D*-葡萄糖苷或其同分异构体 (化合物**30**、**31**) 以及2个首次鉴定在圆柏中鉴定出的苯丙素类成分methylsyringin (化合物**32**)、ekersenin (化合物**43**), 以methylsyringin (化合物**32**) 为例, 其准分子离子峰为*m/z* 385.150 9 $[M-H]^-$, MS^2中脱去葡萄糖及一分子的水生成特征碎片离子*m/z* 207.101 7 $[M+H-glc-H_2O]^+$, 结合文献[21,22]并通过MassLynx v 4.1 Elemental Composition拟合化学式为$C_{18}H_{26}O_9$, 经对照品比对鉴定该化合物为methylsyringin。

1.4 萜类成分 萜类化合物是指存在自然中, 分子式为异戊二烯倍数的化合物及其衍生物的总称[38], 在植物生命活动中, 萜类化合物具有重要的作用。目前, 从圆柏中初步推定3个萜类成分: *β*-谷甾醇 (化合物**52**)、柳杉酚 (化合物**54**)、鼠尾草酚 (化合物**55**)。以*β*-谷甾醇 (化合物**52**) 为例, 其准分子离子峰为*m/z* 415.394 0 $[M+H]^+$, MS^2中C_3-OH羟基易脱水生成*m/z* 397.383 4 $[M-H_2O+H]^+$的特征碎片离子, 进一步C环易发生裂解, 生成*m/z* 148.124 1、161.133 0等特征碎片离子。结合文献[9,38]并通过MassLynx v 4.1 Elemental Composition拟合化学式为$C_{29}H_{50}O$, 推测该化合物为*β*-谷甾醇。

1.5 其他成分 除了黄酮类、有机酸类及其衍生物、苯丙素类和萜类化合物, 本研究还鉴定出果糖 (化合物**2**)、蔗糖 (化合物**6**)、*L*-络氨酸 (化合物**3**)、腺苷 (化合物**4**) 和*β*-胡萝卜苷 (化合物**57**)。以腺苷 (化合物**4**) 为例, 推导其裂解过程, 其准分子离子峰为*m/z* 268.104 9 $[M+H]^+$, MS^2中N-糖苷键易裂解, 中性丢失1分子核糖 ($C_5H_{10}O_5$, *m/z* 132), 生成*m/z* 136.072 2 $[M+H-C_5H_8O_4]^+$的腺嘌呤碱基离子, 继续开环, 中性丢失NH_3、HCN等, 生成*m/z* 119.049 7、94.040 7的特征碎片离子。结合文献[9]并通过UNIFI 1.8 (Waters, 美国) 数据库匹配以及MassLynx v 4.1 Elemental Composition拟合化学式为$C_{10}H_{13}N_5O_4$, 推测该化合物为腺苷。

2 基于模式识别研究不同基原圆柏药材的化学成分差异

以预处理的所有化合物的峰面积 (正离子16 384个化合物、负离子16 842个化合物) 为指标, 利用SIMCA-P 14.1软件进行PCA和PLS-DA非靶向代谢组学分析。数据采用Par法进行预处理, 用PCA观察样品的自然聚集, 所有样品的PCA三维得分图如图4所示。在正离子 (ESI^+)、负离子 (ESI^-) 模式下, PCA模型的R^2X分别为0.859和0.842, Q^2分别为0.582和0.624, 表明该模型稳定性良好。PCA分析结果表明, 圆柏5个基原品种无法得到有效分离, 还需进一步采用有监督的识别模式分析。

为了减少组内差异的干扰, 更好地揭示圆柏不同品种之间的化学成分差异, 本研究进一步采用有监督的模式识别方法偏最小二乘判别分析 (PLS-DA) 对66批样品进行分析, 所有样品的PLS-DA三维得分图如图4所示。正、负离子数据的PLS-DA模型置换检验参数R^2X分别为0.836和0.801, 表明该模型稳定性良好。PLS-DA分析结果表明, 5个品种 (祁连圆柏、滇藏方枝柏、香柏、高山柏、大果圆柏) 在三维图谱中得到了较好的分离, 说明圆柏5个品种之间的化学成分存在一定的差异。

3 筛选圆柏品种鉴别的化学标志物

为筛选可鉴别圆柏不同品种的化学标志物, 本研究VIP > 3、$P < 0.05$为阈值, 筛选差异代谢物 (表3)。本研究在ESI^+模式下筛选出12个潜在的种间差异成

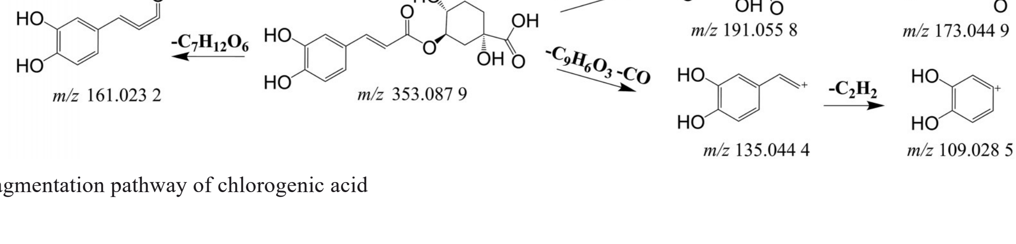

Figure 3 Fragmentation pathway of chlorogenic acid

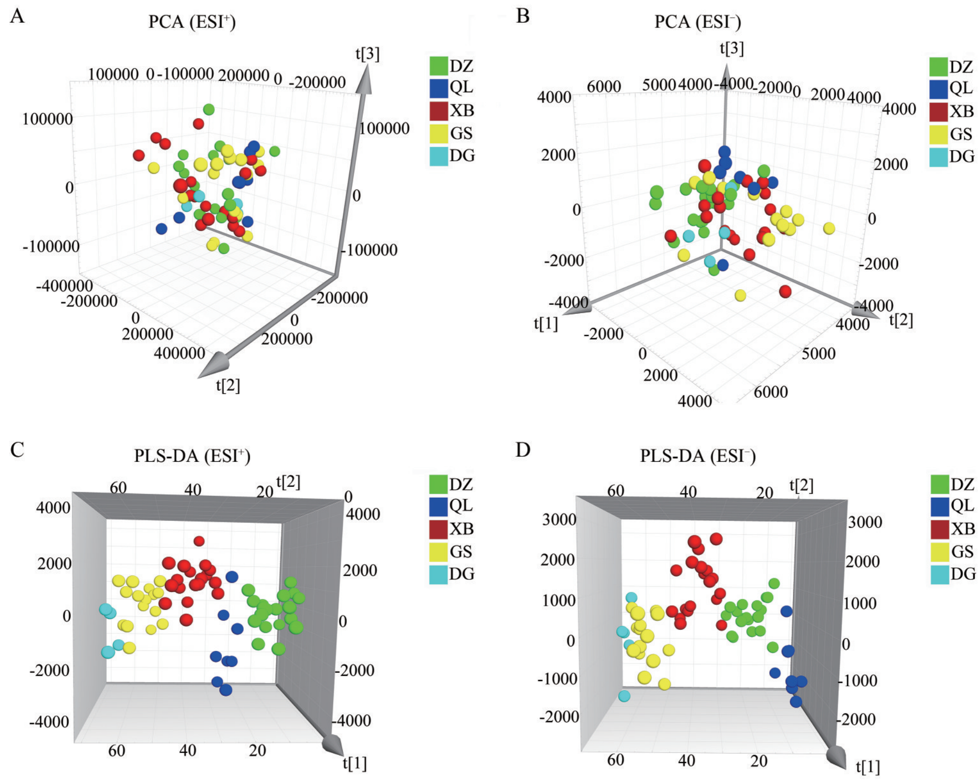

Figure 4　PCA and PLS-DA three-dimensional score plots of positive (A and C) and negative (B and D) ion modes of Juniperri Caulis et Folium. PCA: Principal components analysis; PLS-DA: Partial least squares discriminant analysis

分, 在 ESI^- 模式下筛选出 13 个潜在的种间差异成分。其中 4′-甲基-罗斯塔特双黄酮、穗花衫双黄酮、原花青素 B1、儿茶素 4 个化学成分为正负离子共有的潜在种间差异成分。

Table 3　Potential markers of positive (ESI^+) and negative (ESI^-) ion modes. VIP: Variable importance in the projection

Compound	Peak	VIP (ESI^+)	VIP (ESI^-)
Abietic acid	56	18.434 12	/
$C_{25}H_{34}O_{12}$	24	/	14.512 43
$C_{24}H_{32}O_{11}$	23	/	11.461 65
4'-Methoxy robustaflavone	49	10.240 51	10.806 80
$C_{22}H_{34}O_5$	51	/	10.211 32
Hinokiflavone	50	/	9.621 84
$C_{16}H_{26}O_8$	22	/	8.971 32
Hypolaetin-7-*O*-*β*-*D*-xylopyranoside	35	8.728 47	/
$C_{20}H_{28}O_2$	58	7.348 05	/
$C_{30}H_{20}O_{10}$	47	6.942 69	/
Podocarpusflavone A	48	/	6.908 60
$C_{36}H_{28}O_{15}$	42	6.447 10	/
Quercitrin	34	6.178 06	/
Amentoflavone	45	5.646 62	6.544 26
Proanthocyanidin B1	14	4.943 33	4.584 01
Sugiol	54	4.564 51	/
$C_{20}H_{26}O_6$	36	/	4.268 83
Catechin	16	3.996 22	3.509 34
$C_9H_6O_3$	21	3.627 38	/
Auinic acid	1	/	3.303 37
$C_{25}H_{32}O_{10}$	25	/	3.203 47

分别以正负离子模式下 21 个潜在标志物的峰面积为指标, 采用 SIMCA-P 14.1 软件对所有圆柏样品再进行 PCA 和 PLS-DA 分析。正离子和负离子 PCA 模型的 R^2X 分别为 0.999 和 0.999, Q^2 分别为 0.762 和 0.865。正离子和负离子 PLS-DA 模型的 R^2X 分别为 0.755 和 0.893, Q^2 分别为 0.772 和 0.875。结果圆柏 5 个品种 (祁连圆柏、滇藏方枝柏、香柏、高山柏、大果圆柏) 在 PCA 和 PLS-DA 中均得到了明显的分离 (图 5), 表明这 21 个差异化学成分可作为化学标志物, 用于圆柏药材的品种鉴别。

4　圆柏品种鉴别化学标志物的热图分析

为进一步观察化学标志物在不同品种中的分布情况, 采用 Metaboanalyst 5.0 对 66 批圆柏进行相关性分析, 以正离子 12 个和负离子 13 个标志物的峰面积为指标, 计算其 Pearson 相关系数, 并作聚类热图分析。因样本量较大, 以组间差异结果展示更为直观, 见图 6。图中深红棕色到深蓝色的颜色变化反映了代谢物在圆柏不同基原药材中的分布情况, 其中红棕色颜色越深代表其相对含量较高, 反之蓝色越深则代表其相对含量较低。从图 6 可以看出 4'-甲基-罗斯塔特双黄酮、穗花衫双黄酮在高山柏和香柏药材中含量较高, 儿茶素在高山柏药材中的含量明显低于其他 4 个品种, 原花

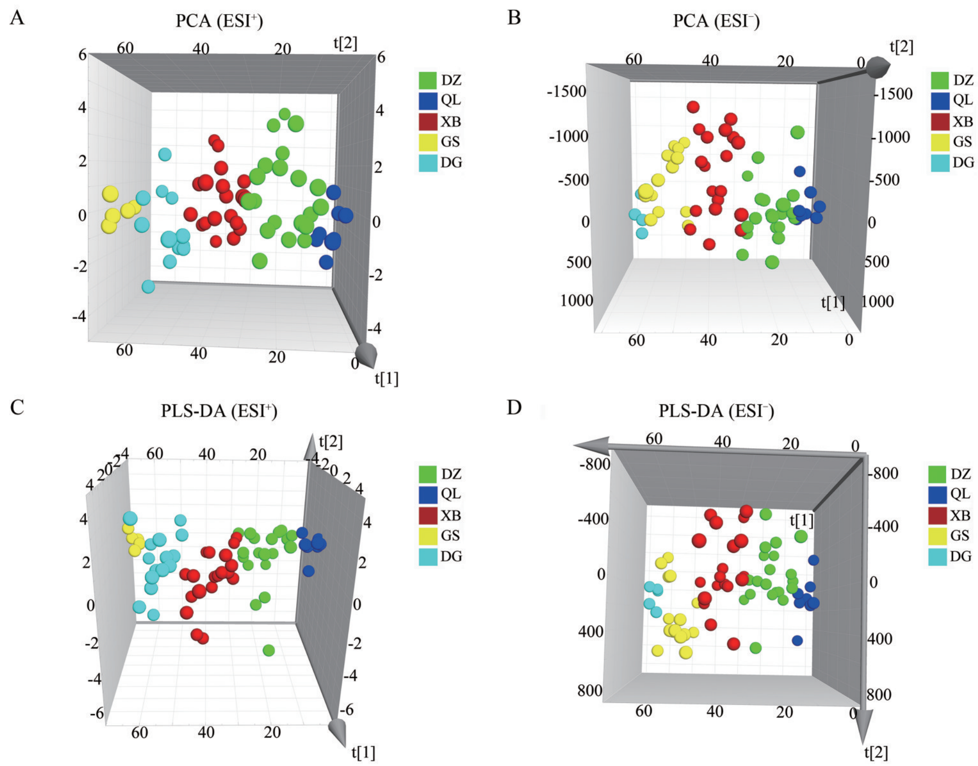

Figure 5 PCA and PLS-DA three-dimensional score plots of positive (A and C) and negative (B and D) ion modes of chemical markers

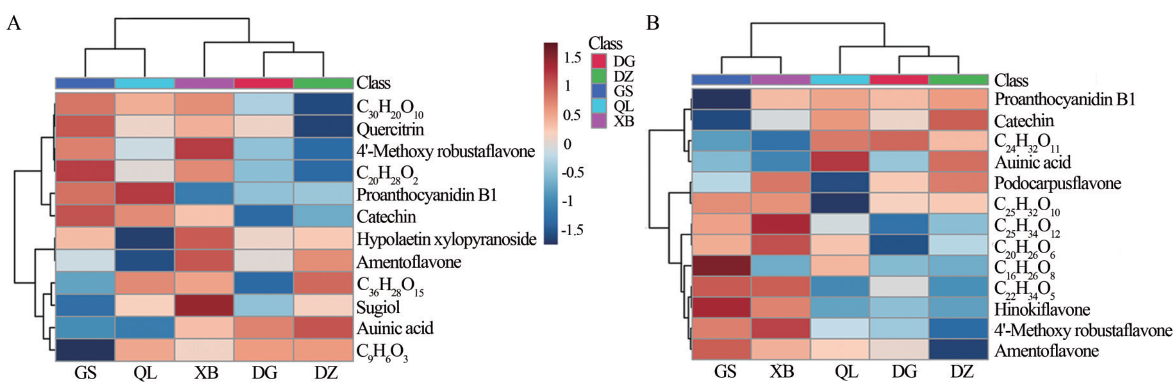

Figure 6 Heatmap of chemical markers in positive (A) and negative (B) ion modes

青素B1在滇藏方枝柏药材中含量较高。热图分析结果进一步说明了这21个化学标志物在圆柏5个品种中具有明显的差异。

讨论

本实验采用UPLC-Q-TOF-MS/MS分析技术检测出圆柏58个化学成分，鉴定了其中46个化学成分。结果表明，圆柏药材主要含有黄酮类、有机酸类、苯丙素类等化学成分。其中，9个化学成分经对照品比对确定，包括海波拉亭-7-*O*-*β*-*D*-吡喃葡萄糖苷 (化合物**27**)、methylsyringin (化合物**32**)、槲皮苷 (化合物**34**)、海波拉亭-7-*O*-*β*-*D*-吡喃木糖苷 (化合物35)、ekersenin (化合物**43**)、柏木双黄酮 (化合物**44**)、穗花杉双黄酮 (化合物**46**)、罗汉松双黄酮A (化合物**48**)、扁柏双黄酮 (化合物**50**)。此外，methylsyringin和ekersenin为首次在圆柏中鉴定的成分。结果表明，UPLC-Q-TOF-MS/MS技术可以快速、准确地对圆柏中的化学成分进行定性表征。

采用PCA、PLS-DA等模式识别方法，发现高山柏、滇藏方枝柏、香柏、祁连圆柏和大果圆柏之间的化学成分存在差异，结合VIP > 3和$P < 0.05$筛选出21个可用于圆柏品种鉴别的化学标志物，这21个标志物多为黄酮类成分，如穗花杉双黄酮、槲皮苷、扁柏双黄酮、原花青素B1等。现代研究表明，黄酮类化合物具有较好的

抗炎, 抗肿瘤等生物活性[39], 如穗花杉双黄酮具有抗炎[34]、抗肿瘤[40]、抗心肌缺血[41]等作用; 槲皮苷具有抗炎[42]、抗氧化[43]、抗肿瘤[44]、抗病毒[45]等药理作用, 这与圆柏药材的传统功效有较好的相关性。因此, 本研究结果也可为今后圆柏不同品种的质量评价提供参考。

尽管 UPLC-Q-TOF-MS/MS 技术具有分析速度快、分辨率高, 可以精确检测相对分子质量且二级质谱能提示特征离子碎片信息等[46]优点, 但目前未知化学成分的鉴定仍具有挑战。目前, 藏药还存在基础研究薄弱, 大多化学成分不明确等问题, 且药材中存在许多同分异构体, 其二级碎片离子和裂解规律极为相近, 增加了化学成分精准定性的困难, 因此今后还需结合二维核磁共振、植物化学分离等技术和方法不断完善圆柏的化学成分数据库, 促进其质量控制和药效物质基础研究。

作者贡献: 赵紫薇、彭芳负责实验研究、数据分析及文稿撰写, 张琨、罗玉婷负责实验操作, 曾宇骄、武鑫玥负责圆柏样本采集, 通讯作者唐策、范刚负责项目总体设计、规划、指导写作以及稿件修改。

利益冲突: 所有作者均不存在任何利益冲突。

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