

## 基于UHPLC-QE-MS非靶向代谢组学的当归不同药用部位 差异代谢物分析

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**摘要:** 本研究利用UHPLC-QE-MS结合非靶向代谢组学技术鉴定当归不同药用部位(归头、归身、归尾)的差异性化合物,通过主成分分析和正交偏最小二乘-判别分析方法筛选差异代谢物,并分析相关代谢通路。结果在当归不同药用部位中共鉴定出18类1072个代谢物,主要为萜类、苯丙素类、脂质及其衍生物、生物碱、黄酮类、有机酸及其衍生物等,其中,归头对比归身映射530个差异代谢物、归头对比归尾映射565个差异代谢物、归身对比归尾映射474个差异代谢物,而山柰酚、绿原酸、阿魏酸、藜本内酯、甘草素、芦丁等成分主要富集于归尾,柚皮素、芹菜素等成分主要富集于归身,雌二醇、川芎嗪等成分主要富集于归头;差异代谢物主要富集在类黄酮、黄酮以及黄酮醇的生物合成、吲哚生物碱生物合成、色氨酸代谢、苯丙氨酸代谢等通路上。该研究通过非靶向代谢组学技术对当归不同药用部位的差异代谢物进行比较分析,为临床合理、精准应用当归不同药用部位提供科学支撑。

**关键词:** 当归; 不同药用部位; 代谢组学; 差异代谢物; UHPLC-QE-MS

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## Different medicinal parts of *Angelica sinensis* based on UHPLC-QE-MS non-targeted metabolomics differential metabolite analysis

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**Abstract:** In order to further distinguish the differences of compounds in different medicinal parts of *Angelica* (head, body and tail), our study used UHPLC-QE-MS combined with non-targeted metabolomics technology to identify the different compounds in different medicinal parts of *Angelica*. Principal component analysis (PCA) and OPLS-DA were used to screen the differential metabolites, and the related pathways were compared and analyzed. Eighteen types of 1072 metabolites were identified from different medicinal parts of *Angelica sinensis*, mainly terpenoids, phenylpropanes, lipids and their derivatives, alkaloids, flavonoids, organic acids and their derivatives, etc. The comparison between *Angelica* head and *Angelica* body, *Angelica* head and *Angelica* tail, *Angelica* body and

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*Angelica* tail showed 530, 565, 474 different metabolites, among which kaempferol, cinnamic acid, chlorogenic acid, ferulic acid, ligustilide, liquiritigenin, and rutin were mainly enriched in *Angelica* tail, naringin, apigenin were mainly enriched in *Angelica* body, estradiol, ligustrazine were mainly enriched in *Angelica* head; differential metabolites are concentrated in flavonoid biosynthesis, phenylalanine metabolism, indole alkaloid biosynthesis, tryptophan metabolism, flavonoid and flavonol biosynthesis and other pathways. In this study, UHPLC-QE-MS non-targeted metabolomics technology can effectively analyze the differential metabolites of different medicinal parts of *Angelica sinensis*, providing scientific support for clinical rational and accurate application of different medicinal parts of *Angelica sinensis*.

**Key words:** *Angelica sinensis*; different medicinal part; non-targeted metabolomics; differential metabolite; UHPLC-QE-MS

当归为伞形科植物当归 *Angelica sinensis* (Oliv.) Diels 的干燥根, 始载于《神农本草经》, 被列为中品<sup>[1]</sup>。性温味甘辛, 归心、脾、肝经, 能够补血活血、调经止痛、润肠通便, 临床可治疗血虚萎黄、月经不调、经闭痛经、肠燥便秘、虚寒腹痛、风湿痹痛等证<sup>[2]</sup>。当归因具有“气厚味薄, 可升可降”的特点, 在遣药组方中应用频率较高, 正所谓“十方九归”<sup>[3]</sup>。历代医家将当归按其药用部位分为当归头、当归身、当归尾。诸多本草文献记载当归用药部位不同, 则功、用侧重不同, “用者不分, 不如不使”<sup>[4]</sup>, 《脾胃论》曰: “当归头止血上行, 当归身补血中守, 当归尾破血下流, 全当归补血活血”<sup>[5]</sup>。《本草通玄》曰: “头止血而上行, 梢破血而下行, 身养血而守中, 全活血而不定”<sup>[6]</sup>。以上均提示医者: 全归长于和血(补血、活血), 归头长于止血, 归身长于养血, 归尾长于活血。

代谢组学是用于次生代谢物定量定性分析、基因功能分析等方面研究的新兴学科<sup>[7,8]</sup>, 具有覆盖范围广、灵敏度和准确性高等优点<sup>[9]</sup>。植物代谢组学是其分支之一, 可研究物种、基因或生态类型的植物在不同生长期所有代谢产物, 通过定性定量分析找出变化规律。目前, 已有研究者采用非靶代谢组学技术对草果<sup>[10]</sup>、苍耳<sup>[11]</sup>、天葵<sup>[12]</sup>等中药不同药用部位的差异代谢物进行了分析, 但对当归不同药用部位的差异代谢物的研究尚未有报道。

课题组前期已通过药效学<sup>[13,14]</sup>以及代谢组学<sup>[15]</sup>揭示了当归不同药用部位“身养血, 尾行血”的作用及机制, 发现归身可促进骨髓细胞DNA合成, 升高外周血象中红细胞(RBC)、红细胞压积(HCT)、血红蛋白浓度(HGB)的含量; 归尾能明显改善急性血瘀模型大鼠全血黏度(WBV)、血浆黏度(PV)、凝血酶原时间(PT)等血液流变学特征, 因而具有良好的活血作用。为进一步区分当归不同药用部位(归头、归身、归尾)的化合物种类和差异, 本研究采用UHPLC-QE-MS结合非靶

向代谢组学技术筛选当归不同药用部位的差异性化合物及代谢途径, 为临床合理、精准应用当归不同药用部位提供科学支撑。

## 材料与amp;方法

**仪器与试剂** Orbitrap Exploris 120 高分辨质谱仪和 Vanquish 超高效液相色谱系统(美国赛默飞公司); 天平(BSA124S-CW, 德国 Sartorius 公司); 研磨仪(JXFSTPRP-24, 上海净信科技有限公司); 超声仪(YM-080S, 深圳市方奥微电子有限公司); 色谱柱(ACQUITY UPLC BEH C18 1.7  $\mu\text{m}$ , 2.1 mm  $\times$  100 mm; 美国沃特世公司); 甲醇、乙腈(均为色谱纯)购自德国 CNW Technologies 公司; 甲酸(色谱纯)购自美国 Sigma 公司; L-2-氯苯丙氨酸(纯度  $\geq 98\%$ )来自上海恒柏生物科技有限公司。

**样品及其来源** 当归购于甘肃省定西市岷县中寨镇小寨村, 经甘肃中医药大学中药鉴定学教研室王明伟教授鉴定为伞形科植物 *Angelica sinensis* (Oliv) Diels 的根。取当年采挖当归, 洗净泥土后按照中国药典(2020版)当归项下规定, 将当归一次性分部位(归头、归身、归尾)切制, 自然阴干后备用, 如图1所示。

**供试品的制备** 取归头、归身、归尾各 100 mg (过三号筛), 分别加入 500  $\mu\text{L}$  提取液(甲醇:水 = 4:1, 内标浓度为 10  $\mu\text{g}\cdot\text{mL}^{-1}$ ), 涡旋 30 s, 匀浆 4 min (45 Hz), 超声 1 h (冰水浴), 静置 1 h ( $-4\text{ }^{\circ}\text{C}$ ), 12 000  $\text{r}\cdot\text{min}^{-1}$  离心 15 min ( $4\text{ }^{\circ}\text{C}$ ), 取上清液, 滤过(微孔滤膜), 上样分析(UPLC-MS/MS)。

**色谱条件** ACQUITY UPLC BEH C18 色谱柱(1.7  $\mu\text{m}$ , 2.1 mm  $\times$  100 mm); 流速: 400  $\mu\text{L}\cdot\text{min}^{-1}$ ; 进样体积: 5  $\mu\text{L}$ ; 流动相A为0.1%甲酸水溶液, 流动相B为0.1%甲酸-乙腈; 梯度洗脱条件: 0~3.5 min (5%~15% B), 3.5~6 min (15%~30% B), 6~6.5 min (保持 30% B), 6.5~12 min (30%~70% B), 12~2.5 min (保持 70% B), 12.5~18 min (70%~100% B); 18~25 min (保持 100% B); 25~26 min (100%~5% B); 26~30 min (保持 5% B)。



Figure 1 Decoction pieces of different medicinal parts of *Angelica sinensis*

**质谱条件** 对Orbitrap Exploris 120质谱进行一级、二级质谱数据采集。仪器详细参数如下:鞘气流速 (sheath gas flow rate), 30 Arb; 辅助气流速 (aux gas flow rate), 10 Arb; 离子转移管温度 (ion transfer tube temperature), 350 °C; 蒸发器温度 (vaporizer temperature), 350 °C; Full MS分辨率, 60 000; MS/MS分辨率, 15 000; 碰撞能量 (collision energy), 16/38/42 in NCE mode; 喷雾电压 (spray voltage), 5.5 kV (positive) 或 -4 kV (negative)。

#### 方法学考察

**精密度** 样品等量混合 (50  $\mu$ L) 用于制备QC样本, 对混样质控QC样品和QC样品中的内标L-2-氯苯丙氨酸进行检测, 连续进样, 所得离子流图中L-2-氯苯丙氨酸保留时间计算RSD值, 考察精密度。

**稳定性** 对QC样品在同日不同时间点进样, 对L-2-氯苯丙氨酸保留时间计算RSD值, 考察稳定性。

**重复性** 通过QC样本在一维和二维主成分分析 (principal component analysis, PCA) 图中的重叠度和偏差以及QC样本的相关性和QC中内标的RSD值来考察重复性。

**数据处理** 样品数据导入XCMS软件, 通过比对上海百趣公司自建二级质谱数据库进行峰鉴定。采用PCA和正交偏最小二乘法-判别分析 (orthogonal partial least squares discriminant analysis, OPLS-DA) 分析样品数据, 根据变量重要性投影 (variable importance projection, VIP) 大于1结合差异倍数 (fold change, FC)

值  $\geq 1.0$  或  $< 1.0$ , 且  $P$  值  $< 0.05$ , 筛选差异代谢物, 通过KEGG分析富集通路。

## 结果

### 1 方法学考察

**1.1 精密度** 对混合质控QC样品和QC样品中的内标L-2-氯苯丙氨酸进行检测, 质控样本TIC曲线重叠性高、质谱峰保留时间和强度一致, 且内标L-2-氯苯丙氨酸在样品中的保留时间RSD值均  $< 5\%$ , 说明仪器精密度良好, 结果见图2。

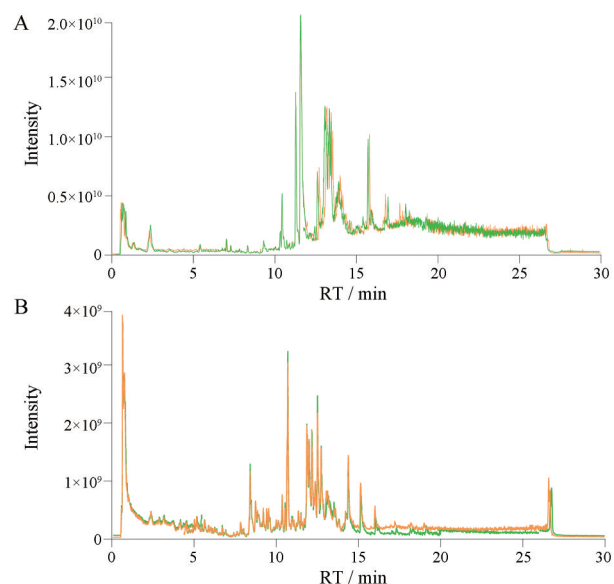


Figure 2 Total ion flow diagram of QC samples under different ion modes. A: Positive ion mode; B: Negative ion mode. RT: Retention time

**1.2 重复性和稳定性** 同一日连续进样4次所测内标的保留时间RSD值均小于5%, 表明样本稳定性良好。QC样本在二维PCA图中重叠良好且在一维图中在两倍的标准偏差范围内, 两个QC样本的相关性为0.954 (越接近1, 整个方法稳定性越好, 数据质量越高), 同时, 正、负离子模式下QC样本紧密聚集在一起, 重叠性高, 内标RSD值小于5%, 表明重复性良好。

### 2 当归不同药用部位代谢组学分析

通过当归不同药用部位所获得的代谢物高分辨二级质谱数据与公共数据库比对, 共鉴定出1 072个化合物, 共有18类, 其中萜类165个、苯丙素类103个、脂质及其衍生物101个、生物碱94个、黄酮类89个、有机酸及其衍生物88个、有机杂环化合物52个、酚类48个、芳香族化合物32个、碳水化合物25个、醇类23个、氨基酸及其衍生物16个、酮类14个、酯类13个、醌类13个、醛类8个、核苷酸类4个, 其他类184个。结果表

明萜类、苯丙素类、脂质及其衍生物、生物碱、黄酮类、有机酸及其衍生物是主要的代谢物。

### 3 当归不同药用部位代谢组学差异分析

**3.1 主成分分析** 应用PCA对当归不同药用部位样品主成分进行分析,结果见图3A,主成分1的贡献率为55%,主成分2的贡献率为18.8%,虽有部分数据有所偏离,但总体各组样品有明显的分离趋势,不同部位样品分别聚为一类,结果表明当归不同药用部位组间存在显著差异。

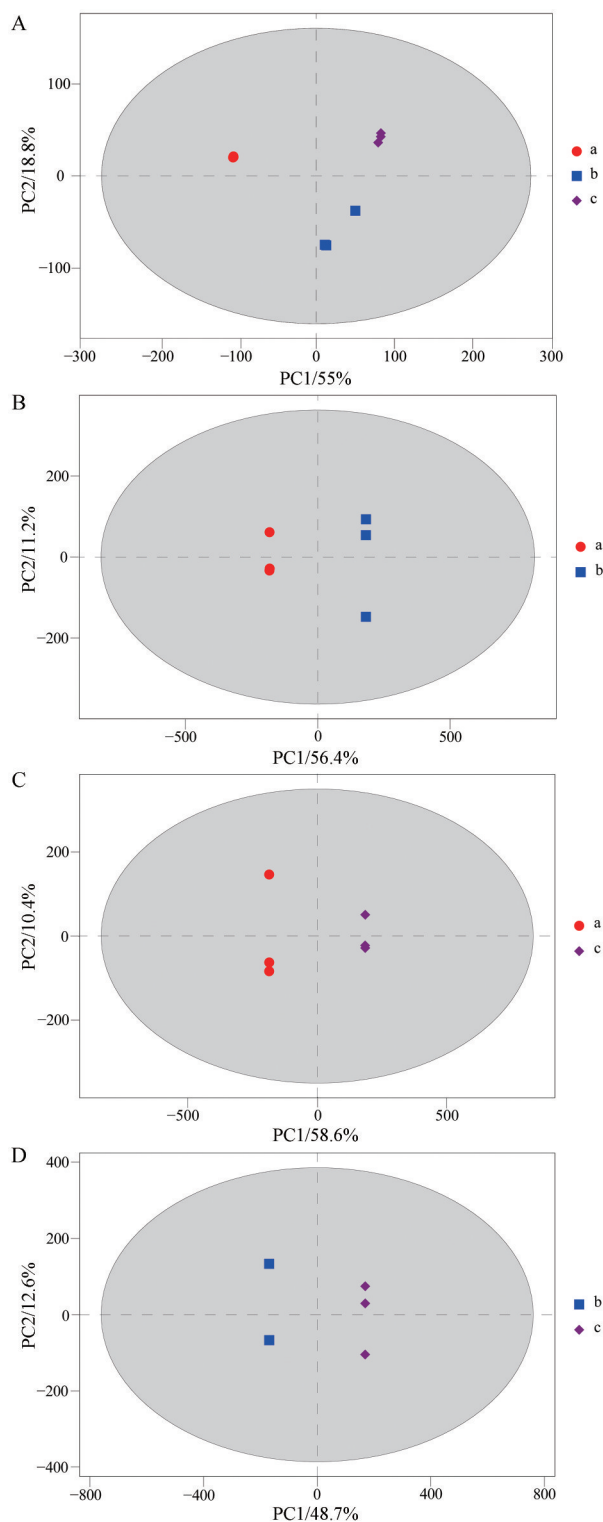
**3.2 正交偏最小二乘法-判别分析** 为进一步揭示当归不同部位代谢物的差异,采用OPLS-DA模型对当归不同药用部位代谢物进行差异分析。结果见图3B~D和表1所示,各组 $R^2X$ 均大于0.6、 $R^2Y$ 均等于1,  $Q^2$ 均大于0.9,说明所构建的模型有效,表明数学模型的区分度及预测效果较好,结果显示归头、归身、归尾完全分离,表明当归不同药用部位代谢物差异显著。OPLS-DA置换检验随机模型(次数 $n = 200$ )见表1,该模型 $Q^2$ 值均 < 原模型 $Q^2$ ,且随着置换保留度降低, $Q^2$ 下降,说明原模型不存在过拟合现象,表明OPLS-DA得分图结果准确可靠。

**Table 1** OPLS-DA model parameters and replacement tests

| Comparable group | OPLS-DA model parameter |            |           | OPLS-DA permutation test ( $n = 200$ ) |           |
|------------------|-------------------------|------------|-----------|--|-----------|
|                  | $R^2X/cum$              | $R^2Y/cum$ | $Q^2/cum$ | $R^2Y/cum$                             | $Q^2/cum$ |
| a vs b           | 0.676                   | 1          | 0.986     | 1                                      | -0.29     |
| a vs c           | 0.689                   | 1          | 0.991     | 1                                      | -0.29     |
| b vs c           | 0.612                   | 1          | 0.970     | 1                                      | -0.23     |

### 4 当归不同药用部位之间差异代谢分析

**4.1 当归不同药用部位差异代谢物鉴定与分析** 根据OPLS-DA结果,采用VIP值结合FC值进一步筛选当归不同药用部位间的差异代谢物,判定标准为 $VIP > 1$ 和 $FC \geq 1.0$ 或 $< 1.0$ ,且 $P < 0.05$ 。结果如图4所示,部分差异代谢物见表2。归头对比归身组共有530个差异代谢物,其中122个代谢物上调,如薯蓣皂苷、汉黄芩苷、精氨酸、白术内酯II等,408个代谢物下调,如异鼠李素、丁烯基苯酚、香草酚、苯甲酸等;归头对比归尾组共有565个差异代谢物,其中185个代谢物上调,如金丝桃苷、柚皮苷查尔酮、薯蓣皂苷、芦丁等,380个代谢物下调,如二氢槲皮素、洋蓟素、豆甾醇、雌二醇等;归身对比归尾组共有474个差异代谢物,其中291个代谢物上调,如藁本内酯、绿原酸、丁烯基苯酚、黄芩素等,183个代谢物下调,如龙胆苦苷、东莨菪碱、土大黄苷、银杏内酯B等。归头对比归身、归头对比归尾的差异代谢物以下调模式占主导,萜类、苯丙素类、黄酮类、生物碱等是主要的下调成分;归身对比归尾组



**Figure 3** Principal component analysis (PCA) scores (A) and orthogonal partial least squares discriminant analysis (OPLS-DA) models (B-D) of different medicinal parts of *Angelica sinensis*. A: a vs b vs c; B: a vs b; C: a vs c; D: b vs c; a: *Angelica* head; b: *Angelica* body; c: *Angelica* tail

的差异代谢物以上调模式占主导,萜类、黄酮类、苯丙素类等是主要的上调组分。

**Table 2** Partial differential metabolites of different medicinal parts of *Angelica sinensis*

| Group  | Compound                                    | <i>m/z</i> | RT/min | VIP  | <i>P</i> -value | Up/Down | Type                              |
|--------|---|------------|--------|------|-----------------|---------|-----------------------------------|
| a vs b | Dioscin                                     | 869.495 4  | 14.30  | 1.32 | 0.007 65        | Up      | Terpene                           |
| a vs b | Wogonoside                                  | 461.107 8  | 8.10   | 1.32 | 0.011 92        | Up      | Flavonoid                         |
| a vs b | Arginine                                    | 175.118 6  | 1.64   | 1.32 | 0.000 08        | Up      | Amino acids and their derivatives |
| a vs b | Atractylenolide II                          | 233.153 0  | 12.08  | 1.27 | 0.002 03        | Up      | Terpene                           |
| a vs b | Verprosoid                                  | 499.141 9  | 4.19   | 1.33 | 0.000 01        | Up      | Other                             |
| a vs b | Apigenin                                    | 269.045 8  | 8.99   | 1.29 | 0.002 94        | Up      | Flavonoid                         |
| a vs b | Quercetin-3- <i>O</i> -beta-glucopyranoside | 465.101 9  | 5.56   | 1.31 | 0.000 82        | Up      | Flavonoid                         |
| a vs b | Lycodine                                    | 243.184 8  | 8.11   | 1.33 | 0.000 01        | Up      | Alkaloid                          |
| a vs b | Naringenin chalcone                         | 271.061 2  | 8.24   | 1.32 | 0.000 04        | Up      | Flavonoid                         |
| a vs b | Corydaline                                  | 370.199 8  | 4.07   | 1.32 | 0.000 01        | Up      | Alkaloid                          |
| a vs b | Estriol                                     | 271.168 6  | 16.07  | 1.30 | 0.001 60        | Up      | Alcohols                          |
| a vs b | Sweroside                                   | 381.115 4  | 3.33   | 1.25 | 0.013 64        | Up      | Terpene                           |
| a vs b | Atractylodin                                | 183.080 0  | 11.02  | 1.17 | 0.011 04        | Up      | Other                             |
| a vs b | Rutin                                       | 611.159 2  | 5.56   | 1.27 | 0.005 59        | Up      | Flavonoid                         |
| a vs b | Levistilide A                               | 381.204 9  | 14.70  | 1.30 | 0.001 01        | Up      | Terpene                           |
| a vs b | Phloretin                                   | 273.080 3  | 7.11   | 1.31 | 0.000 18        | Up      | Flavonoid                         |
| a vs b | Artemetin                                   | 389.122 6  | 5.64   | 1.32 | 0.000 19        | Up      | Flavonoid                         |
| a vs b | Wogonin                                     | 285.074 8  | 10.06  | 1.21 | 0.014 88        | Up      | Flavonoid                         |
| a vs b | Gentiopicroside                             | 401.109 4  | 2.80   | 1.33 | 0.000 04        | Up      | Terpene                           |
| a vs b | Tangeraxanthin                              | 485.340 6  | 12.71  | 1.31 | 0.000 43        | Up      | Lipids and their derivatives      |
| a vs b | Arctiin                                     | 573.172 0  | 10.17  | 1.05 | 0.048 11        | Up      | Phenylpropanoids                  |
| a vs b | Oripavine                                   | 298.140 6  | 9.80   | 1.20 | 0.014 47        | Up      | Alkaloid                          |
| a vs b | Palmitic acid                               | 255.232 9  | 18.78  | 1.11 | 0.040 08        | Up      | Lipids and their derivatives      |
| a vs b | Acetic anhydride                            | 103.038 7  | 3.39   | 1.20 | 0.013 13        | Down    | Other                             |
| a vs b | Licoricesaponin G2                          | 837.389 9  | 8.90   | 1.24 | 0.007 42        | Down    | Terpene                           |
| a vs b | Isorhamnetin                                | 315.051 1  | 8.64   | 1.24 | 0.006 01        | Down    | Flavonoid                         |
| a vs b | 3-Butylidenephthalide                       | 189.090 4  | 8.30   | 1.23 | 0.007 34        | Down    | Other                             |
| a vs b | Fragransol C                                | 357.168 2  | 10.35  | 1.13 | 0.032 15        | Down    | Phenylpropanoids                  |
| a vs b | Benzoic acid                                | 123.043 8  | 3.65   | 1.26 | 0.006 17        | Down    | Phenols                           |
| a vs b | <i>L</i> -Valine                            | 118.086 1  | 27.86  | 1.20 | 0.017 37        | Down    | Alkaloid                          |
| a vs b | (-)-Scopolamine                             | 304.154 0  | 8.95   | 1.29 | 0.001 27        | Down    | Alkaloid                          |
| a vs b | Steviol                                     | 319.223 9  | 13.75  | 1.31 | 0.000 29        | Down    | Terpene                           |
| a vs b | Stigmasterol                                | 395.367 1  | 21.54  | 1.30 | 0.000 67        | Down    | Lipids and their derivatives      |
| a vs b | Ligustilide                                 | 191.106 0  | 10.33  | 1.28 | 0.003 35        | Down    | Other                             |
| a vs b | <i>L</i> -Cysteine                          | 120.012 4  | 6.62   | 1.28 | 0.000 94        | Down    | Lipids and their derivatives      |
| a vs b | 3- <i>n</i> -Butylphthalide                 | 191.106 0  | 10.86  | 1.32 | 0.000 43        | Down    | Aromatic compounds                |
| a vs b | Estradiol                                   | 273.183 8  | 12.81  | 1.32 | 0.000 06        | Down    | Terpene                           |
| a vs b | <i>L</i> -Asarinin                          | 355.117 1  | 9.56   | 1.32 | 0.000 03        | Down    | Phenylpropanoids                  |
| a vs b | Taxifolin                                   | 303.053 9  | 6.35   | 1.33 | 0.000 02        | Down    | Flavonoid                         |
| a vs b | Digitoxin                                   | 809.440 1  | 6.73   | 1.33 | 0.000 00        | Down    | Terpene                           |
| a vs b | Dioscorine                                  | 222.148 6  | 2.08   | 1.27 | 0.000 60        | Down    | Alkaloid                          |
| a vs b | Fraxinellone                                | 233.116 8  | 11.13  | 1.32 | 0.001 12        | Down    | Terpene                           |
| a vs b | Tetramethylpyrazine                         | 137.107 2  | 2.98   | 1.31 | 0.006 42        | Down    | Alkaloid                          |
| a vs c | Otobanone                                   | 339.122 2  | 8.40   | 1.31 | 0.000 69        | Up      | Phenylpropanoids                  |
| a vs c | Licorice saponin G2                         | 837.389 9  | 8.90   | 1.31 | 0.004 74        | Up      | Terpene                           |
| a vs c | Liquiritigenin                              | 257.080 7  | 5.66   | 1.29 | 0.008 11        | Up      | Flavonoid                         |
| a vs c | Atractylenolide II                          | 233.153 0  | 12.08  | 1.28 | 0.000 00        | Up      | Terpene                           |
| a vs c | Naringenin chalcone                         | 271.061 2  | 8.24   | 1.30 | 0.000 01        | Up      | Flavonoid                         |
| a vs c | Dioscin                                     | 869.495 4  | 14.30  | 1.29 | 0.020 23        | Up      | Terpene                           |
| a vs c | Rutin                                       | 611.159 2  | 5.56   | 1.31 | 0.000 00        | Up      | Flavonoid                         |
| a vs c | Narcissoside                                | 623.160 9  | 6.16   | 1.30 | 0.000 05        | Up      | Flavonoid                         |
| a vs c | Wogonoside                                  | 461.107 8  | 8.10   | 1.25 | 0.046 60        | Up      | Flavonoid                         |
| a vs c | Picraquassioside A                          | 399.128 0  | 7.53   | 1.30 | 0.000 01        | Up      | Carbohydrate                      |
| a vs c | Isorhamnetin                                | 315.051 1  | 8.64   | 1.31 | 0.000 00        | Up      | Flavonoid                         |
| a vs c | Tangeraxanthin                              | 485.340 6  | 12.71  | 1.30 | 0.000 04        | Up      | Lipids and their derivatives      |
| a vs c | Monocrotaline                               | 326.160 2  | 7.01   | 1.26 | 0.003 21        | Up      | Alkaloid                          |
| a vs c | Levistilide A                               | 381.204 9  | 14.70  | 1.27 | 0.001 85        | Up      | Terpene                           |
| a vs c | Oripavine                                   | 298.140 6  | 9.80   | 1.29 | 0.000 27        | Up      | Alkaloid                          |
| a vs c | Cinnamic acid                               | 149.059 3  | 10.29  | 1.25 | 0.003 55        | Up      | Phenylpropanoids                  |
| a vs c | Nardosinone                                 | 251.163 8  | 9.69   | 1.29 | 0.000 15        | Up      | Terpene                           |

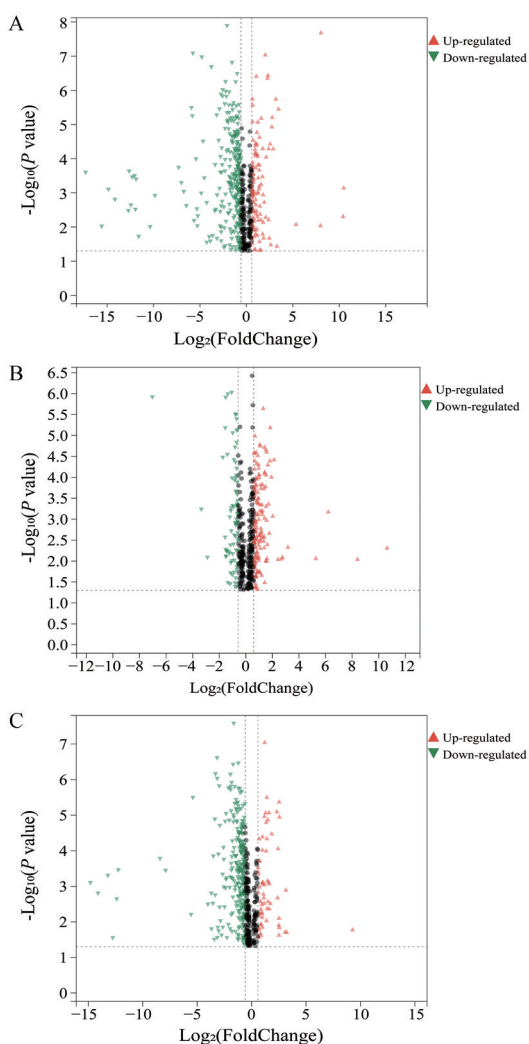
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| Group  | Compound                                    | <i>m/z</i> | RT/min | VIP  | <i>P</i> -value | Up/Down | Type                           |
|--------|---|------------|--------|------|-----------------|---------|--------------------------------|
| a vs c | Arctiin                                     | 573.172 0  | 10.17  | 1.20 | 0.004 98        | Up      | Phenylpropanoids               |
| a vs c | Panaxynol                                   | 245.189 5  | 12.48  | 1.31 | 0.000 00        | Up      | Lipids and their derivatives   |
| a vs c | Ginsenoside Ro                              | 955.487 0  | 10.48  | 1.29 | 0.000 21        | Up      | Terpene                        |
| a vs c | Curcumin II                                 | 367.153 4  | 10.59  | 1.09 | 0.029 54        | Up      | Phenols                        |
| a vs c | Camphor                                     | 153.127 0  | 5.90   | 1.28 | 0.000 63        | Up      | Terpene                        |
| a vs c | Gentiopicroside                             | 401.109 4  | 2.80   | 1.21 | 0.011 35        | Up      | Terpene                        |
| a vs c | Chlorogenic acid                            | 355.101 1  | 3.08   | 1.20 | 0.014 88        | Up      | Phenylpropanoids               |
| a vs c | Palmitic acid                               | 255.232 9  | 18.78  | 1.26 | 0.001 59        | Up      | Lipids and their derivatives   |
| a vs c | Ginkgolide A                                | 407.135 1  | 8.06   | 1.11 | 0.033 17        | Down    | Terpene                        |
| a vs c | Ginkgolide B                                | 425.141 5  | 4.07   | 1.23 | 0.006 43        | Down    | Terpene                        |
| a vs c | Taxifolin                                   | 303.053 9  | 6.35   | 1.10 | 0.034 90        | Down    | Flavonoid                      |
| a vs c | Dicaffeoyl quinic acid                      | 515.118 1  | 6.20   | 1.07 | 0.048 03        | Down    | Phenylpropanoids               |
| a vs c | Stigmasterol                                | 395.367 1  | 21.54  | 1.16 | 0.013 81        | Down    | Lipids and their derivatives   |
| a vs c | Estradiol                                   | 273.183 8  | 12.81  | 1.28 | 0.000 33        | Down    | Terpene                        |
| a vs c | 3- <i>n</i> -Butylphthalide                 | 191.106 0  | 10.86  | 1.29 | 0.000 54        | Down    | Aromatic compounds             |
| a vs c | Digitoxin                                   | 809.440 1  | 6.73   | 1.19 | 0.030 23        | Down    | Terpene                        |
| a vs c | Piperonaline                                | 364.186 5  | 4.31   | 1.30 | 0.000 02        | Down    | Organic heterocyclic compounds |
| a vs c | (-)-Scopolamine                             | 304.154 0  | 8.95   | 1.27 | 0.000 76        | Down    | Alkaloid                       |
| a vs c | Rhaponticin                                 | 421.145 8  | 6.57   | 1.30 | 0.000 05        | Down    | Phenols                        |
| a vs c | Tetramethylpyrazine                         | 137.107 2  | 2.98   | 1.31 | 0.006 72        | Down    | Alkaloid                       |
| a vs c | 17 $\alpha$ -Estradiol                      | 255.173 9  | 11.78  | 1.31 | 0.000 33        | Down    | Lipid derivatives              |
| a vs c | Ginsenoside Rh5                             | 653.453 2  | 11.59  | 1.31 | 0.009 83        | Down    | Lipids and their derivatives   |
| b vs c | Liquiritigenin                              | 257.080 7  | 5.66   | 1.43 | 0.008 38        | Up      | Flavonoid                      |
| b vs c | Tectochrysin                                | 269.080 6  | 9.32   | 1.03 | 0.004 53        | Up      | Flavonoid                      |
| b vs c | Formononetin                                | 267.066 2  | 9.31   | 1.11 | 0.007 91        | Up      | Flavonoid                      |
| b vs c | Quercetin-3- <i>O</i> -beta-glucopyranoside | 465.101 9  | 5.56   | 1.42 | 0.000 02        | Up      | Flavonoid                      |
| b vs c | Rutin                                       | 611.159 2  | 5.56   | 1.42 | 0.000 02        | Up      | Flavonoid                      |
| b vs c | Isorhamnetin                                | 315.051 1  | 8.64   | 1.43 | 0.000 00        | Up      | Flavonoid                      |
| b vs c | Monocrotaline                               | 326.160 2  | 7.01   | 1.39 | 0.002 49        | Up      | Alkaloid                       |
| b vs c | Taxifolin                                   | 303.053 9  | 6.35   | 1.42 | 0.000 14        | Up      | Flavonoid                      |
| b vs c | Digitoxin                                   | 809.440 1  | 6.73   | 1.27 | 0.038 47        | Up      | Terpene                        |
| b vs c | Tangeretin                                  | 373.128 0  | 9.08   | 1.24 | 0.023 02        | Up      | Flavonoid                      |
| b vs c | Narcissoside                                | 623.160 9  | 6.16   | 1.39 | 0.001 29        | Up      | Flavonoid                      |
| b vs c | Ligustilide                                 | 191.106 0  | 10.33  | 1.38 | 0.001 78        | Up      | Other                          |
| b vs c | Chlorogenic acid                            | 355.101 1  | 3.08   | 1.34 | 0.008 90        | Up      | Phenylpropanoids               |
| b vs c | 3-Butylidenephthalide                       | 189.090 4  | 8.30   | 1.31 | 0.011 20        | Up      | Other                          |
| b vs c | Tangeraxanthin                              | 485.340 6  | 12.71  | 1.38 | 0.002 03        | Up      | Lipids and their derivatives   |
| b vs c | Arctiin                                     | 573.172 0  | 10.17  | 1.38 | 0.003 02        | Up      | Phenylpropanoids               |
| b vs c | Gentiopicroside                             | 401.109 4  | 2.80   | 1.30 | 0.009 82        | Down    | Terpene                        |
| b vs c | (-)-Scopolamine                             | 304.154 0  | 8.95   | 1.21 | 0.029 85        | Down    | Alkaloid                       |
| b vs c | Rhaponticin                                 | 421.145 8  | 6.57   | 1.28 | 0.014 40        | Down    | Phenols                        |
| b vs c | Ginkgolide B                                | 425.141 5  | 4.07   | 1.43 | 0.000 04        | Down    | Terpene                        |
| b vs c | Artemetin                                   | 389.122 6  | 5.64   | 1.41 | 0.000 26        | Down    | Flavonoid                      |
| b vs c | Wogonoside                                  | 461.107 8  | 8.10   | 1.31 | 0.010 16        | Down    | Flavonoid                      |
| b vs c | Atractylodin                                | 183.080 0  | 11.02  | 1.39 | 0.000 64        | Down    | Other                          |
| b vs c | Dihydrosanguinarine                         | 334.107 9  | 7.59   | 1.40 | 0.019 75        | Down    | Alkaloid                       |
| b vs c | Corydaline                                  | 370.199 8  | 4.07   | 1.43 | 0.000 00        | Down    | Alkaloid                       |
| b vs c | Sweroside                                   | 381.115 4  | 3.33   | 1.38 | 0.005 82        | Down    | Terpene                        |

**4.2 当归不同药用部位差异代谢物通路分析** 为进一步探究当归不同药用部位代谢途径中差异代谢物的变化,通过KEGG对差异代谢物进行关键代谢通路富集。其中归头对比归身组共有68种差异代谢物富集在37条通路中,归头对比归尾组共有77种差异代谢物富集在37条通路中,归身对比归尾组共有62种差异代谢物富集在32条通路中,主要通路如图5所示。

由图可知,苯丙氨酸代谢、类黄酮生物合成是归头

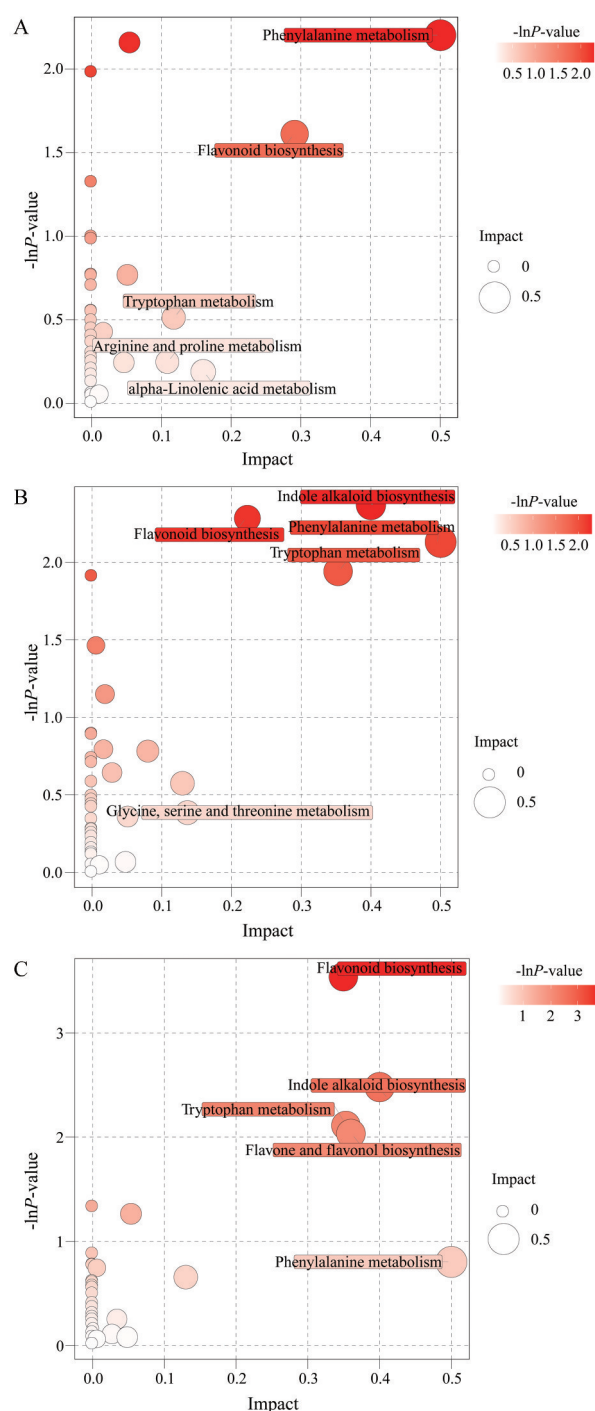
对比归身的重要代谢通路,其中*L*-苯丙氨酸、4-羟基肉桂酸等化合物被注释到苯丙氨酸代谢通路上,花翠素、柚皮素、柚皮素查尔酮、芹菜素、二氢槲皮素等化合物被注释到类黄酮生物合成通路上;吲哚生物碱、类黄酮生物合成和苯丙氨酸代谢、色氨酸代谢等通路对归头对比归尾组的差异影响最大,其中左旋色氨酸、色胺被注释到吲哚生物碱生物合成通路上,花翠素、甘草素、柚皮素查尔酮、芹菜素、二氢槲皮素、绿原酸等被注释



**Figure 4** Metabolic volcano maps of different medicinal parts of *Angelica sinensis*. A: a vs b; B: a vs c; C: b vs c

到类黄酮生物合成通路上, 左旋色氨酸、3-羟基苯甲酸、色胺、吲哚乙酸被注释到色氨酸代谢途径上; 类黄酮生物合成、吲哚生物碱生物合成、色氨酸代谢、黄酮和黄酮醇的生物合成等通路对归身对比归尾组的差异影响最大, 其中山柰酚、花翠素、柚皮素、甘草素、柚皮素查尔酮、二氢槲皮素、绿原酸等化合物被注释到类黄酮生物合成途径上, 山柰酚、芦丁被注释到黄酮和黄酮醇的生物合成途径上。

类黄酮生物合成通路是归头对比归尾、归头对比归尾和归身对比归尾共有的通路, 花翠素、芹菜素、山柰酚、柚皮素、甘草素、柚皮素查尔酮、二氢槲皮素、绿原酸 8 个差异代谢物被注释到该通路上。与归头相比, 花翠素、芹菜素、柚皮查尔酮在归身和归尾中含量呈上升趋势, 二氢槲皮素呈下降趋势, 柚皮素在归身中呈上升趋势, 甘草素、绿原酸在归尾中呈上升趋势; 与归身相比, 归尾中山柰酚、花翠素、甘草素、柚皮查尔



**Figure 5** Bubble map of metabolic pathways in different parts of *Angelica sinensis*. A: a vs b; B: a vs c; C: b vs c

耳酮、二氢槲皮素、绿原酸含量呈上升趋势, 柚皮素含量呈下降趋势。苯丙氨酸代谢是归头对比归尾、归头对比归尾共有的通路, 吲哚生物碱生物合成、色氨酸代谢是归头对比归尾和归身对比归尾共有的通路。其中, 苯丙氨酸代谢途径共有 *L*-苯丙氨酸、4-羟基肉桂酸 2 个代谢物被注释, 与归头相比, *L*-苯丙氨酸和 4-羟基肉桂酸在归身、归尾中均呈下降趋势; 吲哚生物碱生物

合成途径共有左旋色氨酸、色胺 2 个代谢物被注释该通路上,与归头、归身相比,这两种化合物在归尾中呈下降趋势;色氨酸代谢途径共有左旋色氨酸、3-羟基苯甲酸、色胺、吲哚乙酸 4 个代谢物被注释到该通路上,与归头相比,左旋色氨酸、3-羟基苯甲酸、色胺、吲哚乙酸在归尾中均呈下降趋势,与归身相比,左旋色氨酸、色胺在归尾中呈下降趋势,3-羟基苯甲酸、吲哚乙酸呈上升趋势。黄酮和黄酮醇的生物合成通路为归身对比归尾组具有显著差异的代谢通路,该通路上共有山柰酚、芦丁 2 个代谢差异物被注释,与归身相比,山柰酚、芦丁在归尾中均呈上升趋势。

## 讨论

本研究利用 UHPLC-QE-MS 非靶向代谢组学对当归不同药用部位代谢物进行分析,共鉴定出 18 类 1 072 个化合物,主要为萜类 (165 个)、苯丙素类 (103 个)、脂质及其衍生物 (101 个)、生物碱 (94 个)、黄酮类 (89 个)、有机酸及其衍生物 (88 个) 等成分。通过主成分分析发现,当归不同药用部位各组样品有明显的分离趋势,不同部位样品分别聚为一类,结果表明当归不同药用部位组间组分存在显著差异。为进一步揭示当归不同部位代谢物的差异,采用 OPLS-DA 模型对当归不同药用部位代谢物进行分析,根据 VIP 值结合 FC 值筛选当归不同药用部位间的差异代谢物,结果显示归头对比归身、归头对比归尾和归身对比归尾之间的差异代谢物分别为 530、565、474 个。生物体内的复杂代谢反应通常不是一个基因或蛋白单独调控,而是不同基因和蛋白质形成复杂的通路,它们的相互影响和相互调控从而影响着代谢组发生系统性的变化。将当归不同药用部位筛选出的差异代谢物通过 KEGG 数据库进行富集,并对代谢通路进行分析,寻找与代谢物差异相关性最高的关键代谢通路。本研究显示与差异代谢物相关性最高的关键通路有类黄酮生物合成、苯丙氨酸代谢、吲哚生物碱生物合成、色氨酸代谢、黄酮和黄酮醇的生物合成等。

人体的生长发育和生理机能的维持均需要通过苯丙氨酸和色氨酸代谢来完成。本研究发现 *L*-苯丙氨酸、4-羟基肉桂酸、左旋色氨酸、3-羟基苯甲酸、色胺、吲哚乙酸等代谢物主要富集在这两条通路上,且主要富集于归头。5-羟色胺 (5-hydroxytryptamine, 5-HT) 是神经调节物质和血小板凝血因子,主要负责收缩血管<sup>[16]</sup>,色氨酸作为合成 5-HT 的前体化合物,具有调节血管功能的作用。此外,雌二醇和川芎嗪也在归头中上调积累,研究表明雌二醇可以提高人体的凝血功能,有利于止血<sup>[17]</sup>,升高大鼠体内雌二醇质量浓度,缩短凝

血酶原时间和凝血酶时间,可促进子宫复旧及子宫内膜修复<sup>[18]</sup>。在动物实验中,常用雌二醇联合其他药物建立寒凝血瘀痛经大鼠模型<sup>[19,20]</sup>。川芎嗪有扩张血管、增加血流、诱导血小板聚集和抗纤维素蛋白形成的作用,正常状态下人体血液中凝血系统纤溶系统相拮抗保持动态平衡,促凝血因素川芎嗪成分的加入可激活凝血系统,解聚伤口血小板<sup>[21]</sup>。这可能与“头止血”的作用机制有关。

此外,本研究发现归身中的部分化合物显著上调积累,如柚皮素、芹菜素等。柚皮素具有明显的抗凝血作用,随着柚皮素浓度的增大,抑制凝血酶诱导的血小板聚集能力增强<sup>[22]</sup>。柚皮素可提升脑微血管内皮细胞的迁移,加快脑微血管新生<sup>[23]</sup>,亦促进造血细胞分化和增殖,保护肝损伤,还提高心脏抗缺氧能力,保护心脑血管<sup>[24]</sup>。芹菜素可通过上调血管内皮生长因子 (vascular endothelial growth factor, VEGF) 表达改善血流动力学、血液流变学<sup>[25]</sup>,亦通过调节血脂来改善局部微循环来促进血管新生<sup>[26]</sup>。以上两种化合物均被富集到类黄酮生物合成通路上,表明“身养血”的作用机制可能与此有关。

类黄酮生物合成通路是三个组共同调节的代谢通路,有花翠素、芹菜素、山柰酚、柚皮素、甘草素、柚皮素查尔酮、二氢槲皮素、绿原酸 8 个差异代谢物被注释到该通路上,其中山柰酚可通过干扰血小板中的 MAPK 和 AKT 信号通路,有效抑制凝血酶和活化的凝血因子 X (factor Xa, Fxa) 的活性,实现抗血小板凝集,从而改善血流动力学,促进血液循环<sup>[27]</sup>,还可通过降低白细胞介素 6 (interleukin, IL-6)、肿瘤坏死因子- $\alpha$  (tumor necrosis factor, TNF- $\alpha$ ) 的水平减轻心肌炎症损伤,增加血管数量,改善血流动力学指标<sup>[28]</sup>,抑制血栓的形成及血小板的活化<sup>[29,30]</sup>。绿原酸、阿魏酸均能通过抑制血小板活化<sup>[31]</sup>,达到抗血小板聚集的目的<sup>[32]</sup>,进而抑制血栓的形成<sup>[33]</sup>。藜本内酯通过改善血液流态和红细胞聚集状态,加快血流流速,从而达到舒张血管作用<sup>[34]</sup>。甘草素可以显著改善血液动力学参数<sup>[35]</sup>,能够靶向凝血酶影响血栓形成的动态过程<sup>[36]</sup>,发挥抗血小板聚集作用<sup>[37]</sup>。芦丁能阻断血小板膜糖蛋白 GPIIb/IIIa 受体<sup>[38]</sup>,抑制血小板活化<sup>[39]</sup>和聚集<sup>[40]</sup>,缩短凝血时间<sup>[41]</sup>,防止血栓的产生<sup>[42]</sup>。同时,山柰酚和芦丁还被注释到了黄酮和黄酮醇的生物合成通路上。这些化合物在归尾中均上调积累,这表明“尾行血”的机制可能与其所含的山柰酚、绿原酸、阿魏酸、藜本内酯、甘草素、芦丁等调节类黄酮生物合成以及黄酮和黄酮醇的生物合成等通路,从而发挥抗血小板聚集、改善血液动力学、促进血液循环等有关。

综上,本研究基于UHPLC-QE-MS结合非靶向代谢组学技术,通过PCA、OPLS-DA对差异代谢物的表达及分类、代谢通路富集等进行系统分析,探讨当归不同药用部位代谢物的差异,为当归临床合理、精准用药提供参考。

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