**A comprehensive strategy integrating** **metabolomics** **with** **DNA barcoding for** **discovery of** **combinatorial** **discriminatory quality markers: A case of** ***Cimicifuga foetida* and *Cimicifuga dahurica***

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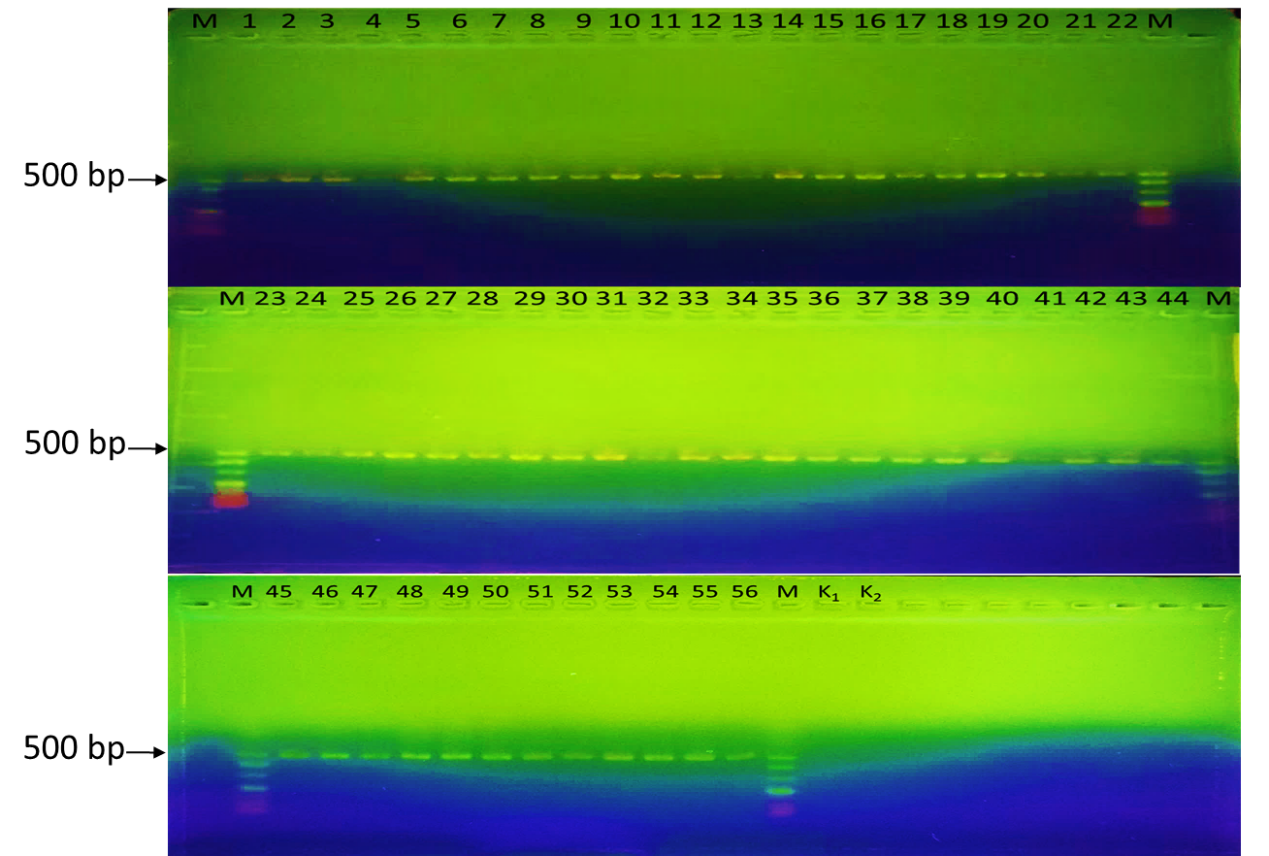
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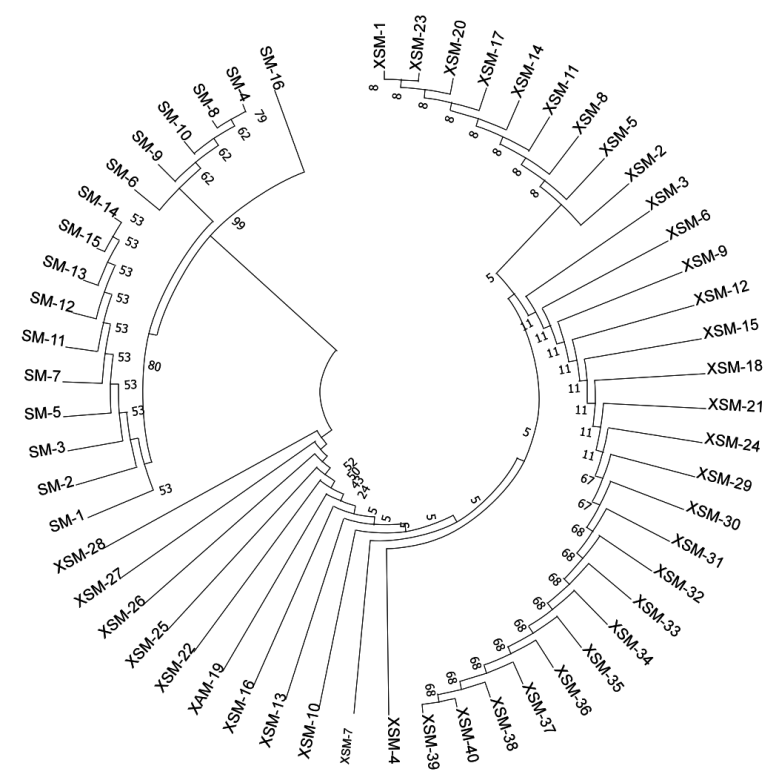
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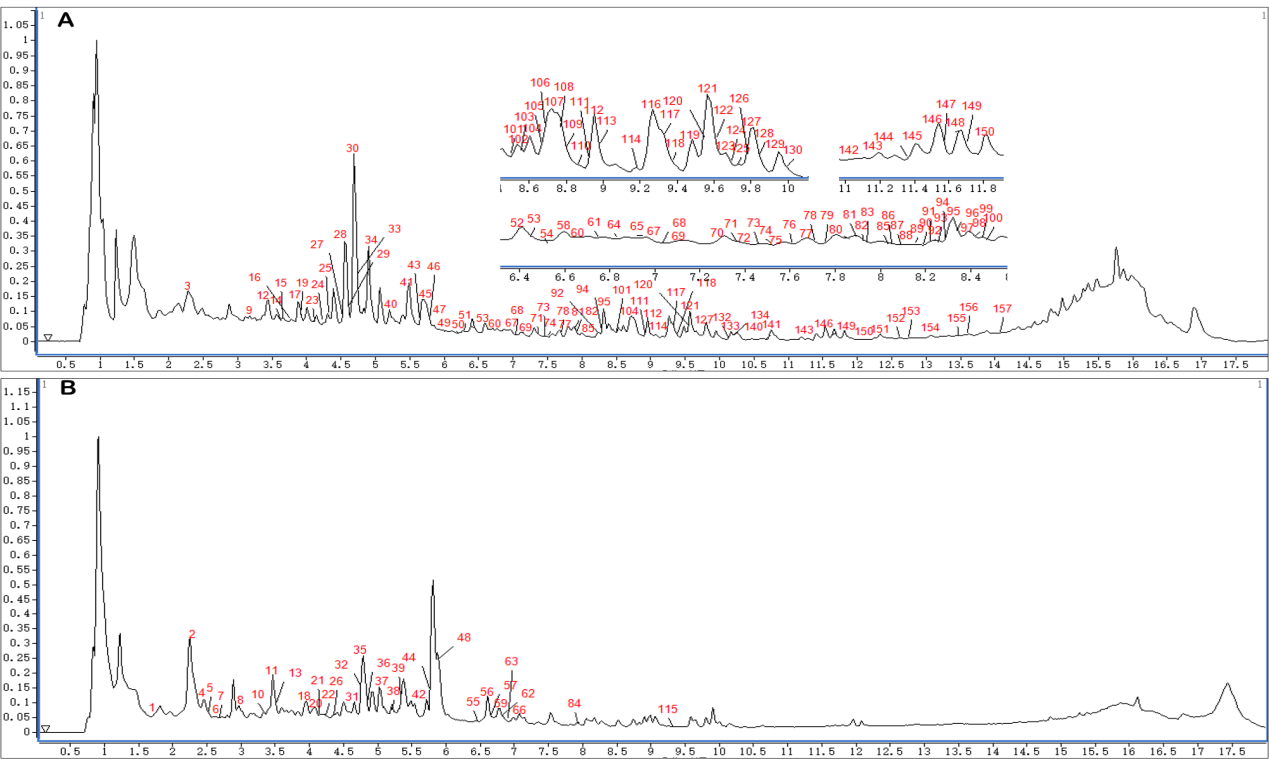
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**Fig S1** Specific PCR assay of 56 batches CR by primers ITS2F/ITS3R.



**Fig S2** Phylogenetic NJ tree of SM and XSM constructed with the ITS2 sequences

(1000 replicates).

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**Fig S3** The total ion chromatograms (TIC) of CR. (A) in positive ion mode and (B) in negative ion mode.

**Table S1** 56 batches sample information of CR collected from different origins.

|  |  |  |  |
| --- | --- | --- | --- |
| No. | Origin | No. | Origin |
| SM-1 | Hebei, China | XSM-13 | Inner Mongolia, China |
| SM-2 | Shanxi, China | XSM-14 | Inner Mongolia, China |
| SM-3 | Inner Mongolia, China | XSM-15 | Inner Mongolia, China |
| SM-4 | Jilin, China | XSM-16 | Heilongjiang, China |
| SM-5 | Jilin, China | XSM-17 | Heilongjiang, China |
| SM-6 | Jilin, China | XSM-18 | Heilongjiang, China |
| SM-7 | Liaoning, China | XSM-19 | Heilongjiang, China |
| SM-8 | Liaoning, China | XSM-20 | Heilongjiang, China |
| SM-9 | Liaoning, China | XSM-21 | Heilongjiang, China |
| SM-10 | Heilongjiang, China | XSM-22 | Heilongjiang, China |
| SM-11 | Heilongjiang, China | XSM-23 | Heilongjiang, China |
| SM-12 | Heilongjiang, China | XSM-24 | Heilongjiang, China |
| SM-13 | Heilongjiang, China | XSM-25 | Heilongjiang, China |
| SM-14 | Sichuan, China | XSM-26 | Heilongjiang, China |
| SM-15 | Sichuan, China | XSM-27 | Heilongjiang, China |
| SM-16 | Hubei, China | XSM-28 | Heilongjiang, China |
| XSM-1 | Anhui, China | XSM-29 | Heilongjiang, China |
| XSM-2 | Sichuan, China | XSM-30 | Heilongjiang, China |
| XSM-3 | Sichuan, China | XSM-31 | Heilongjiang, China |
| XSM-4 | Shannxi, China | XSM-32 | Heilongjiang, China |
| XSM-5 | Inner Mongolia, China | XSM-33 | Heilongjiang, China |
| XSM-6 | Inner Mongolia, China | XSM-34 | Heilongjiang, China |
| XSM-7 | Jilin, China | XSM-35 | Heilongjiang, China |
| XSM-8 | Liaoning, China | XSM-36 | Heilongjiang, China |
| XSM-9 | Liaoning, China | XSM-37 | Heilongjiang, China |
| XSM-10 | Liaoning, China | XSM-38 | Heilongjiang, China |
| XSM-11 | Inner Mongolia, China | XSM-39 | Heilongjiang, China |
| XSM-12 | Inner Mongolia, China | XSM-40 | Heilongjiang, China |

Abb: SM, *Cimicifuga foetida*; XSM, *Cimicifuga dahurica*

**Table S2** The precursor ion lists and static exclusion lists in different mass range.

|  |  |  |
| --- | --- | --- |
| Mass Range | Precursor ions lists | Static exclusion lists |
| (+)100-400 Da | 287.0555, 379.1002 | 121-122, 336-339, 288-288.5,316-316.5 |
| (+)400-600 Da | 487.3424, 509.3244, 525.3184, 527.3346, 529.3535 | 551-553, 526-526.5, 512.5-513, 465-465.5, 493-495 |
| (+)600-700 Da | 621.3989, 663.4103, 667.2476, 679.4058, 641.3664 | 628-628.5, 652-652.5, 673.5-674, 675.5-676, 610-610.5 |
| (+)700-800 Da | 701.3876, 703.4042,  787.4841 | 708.5-709, 776-776.5, 743-743.5 |
| (+)800-1200 Da | 803.4187, 805.4343, 865.4562 | **/** |
| (-)100-1500 Da | 461.1081, 609.1457,  635.3778 | 112-113, 116-117, 190-191, 307-308, 336.5-337, 416-416.5, 459-459.5, 601-602, 723.5-724, 965-966 |

**Table S3** The GC content, percent identify and genetic distance of ITS2 between *C. foetida* and *C. dahurica*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | Species name | GC (%) | Percent identify | | | Genetic distance |
| *C. foetida* | *C. dahurica* | *C. heracleifolia* |
| F1 | *C. foetida* | 52.5% | 100% | 95.43% | 96.35% | ­— |
| F2 | *C. foetida* | 52.1% | 99.54% | 95.43% | 95.89% | 0.0046 |
| F3 | *C. foetida* | 51.8% | 99.09% | 95.89% | 96.80% | 0.0046 |
| F4 | *C. foetida* | 53.0% | 99.54% | 94.98% | 95.89% | 0.0046 |
| D1 | *C. dahurica* | 50.5% | 94.98% | 99.54% | 97.72% | 0.0475 |
| D2 | *C. dahurica* | 50.2% | 94.98% | 99.54% | 98.60% | 0.0522 |
| D3 | *C. dahurica* | 50.7% | 95.43% | 100% | 98.17% | 0.0472 |

Note: \* it indicated the same base as the first row. Referring to *Molecular identification of DNA barcoding in traditional Chinese medicine*.

**Table S4** Chemical components identified or tentatively characterized in SM and XSM.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Adduct | tR  (min) | Formula | Mass | m/z | Error  (ppm) | MS/MS Fragments | Identification | Source |
| 1 | [M-H]- | 7.71 | C11H12O8 | 272.0532 | 271.0459 | 0.89 | 271.0457,191.0346,179.0346,  135.0453 | Fukiic acid | SM, XSM |
| 2 | [M-H]- | 8.45 | C8H8O4 | 168.0423 | 167.035 | 1.68 | 167.0348,152.0110,108.0211 | 2-methoxy-5-hydroxybenzoic acid | SM, XSM |
| 3 | [M-H]- | 8.60 | C11H12O7 | 256.0583 | 255.051 | 0.49 | 255.0509,211.0609,193.0504,  179.0348,165.0555 | Piscidic acid | SM, XSM |
| 4 | [M+Na]+ | 8.63 | C14H20O8 | 316.1158 | 339.105 | 0.44 | 339.1049,177.0537,149.0247 | Cimidaurinine | SM, XSM |
| 5 | [M-H]- | 8.74 | C14H20O8 | 316.1158 | 315.1085 | 0.76 | 315.1083,153.0553,123.0449 | Cimidahurine | SM, XSM |
| 6 | [M-H]- | 9.09 | C15H18O9 | 342.0951 | 341.0878 | 3.82 | 341.0865,179.0354,135.0467 | Caffeic acid 4-*O*-*β*-D-glucopyranoside | SM, XSM |
| 7 | [M-H]- | 9.52 | C10H10O4 | 194.0579 | 193.0506 | -2.41 | 193.0511,178.0277,134.0377 | Methyl caffeate | SM, XSM |
| 8 | [M-H]- | 9.55 | C16H20O9 | 356.1107 | 355.1035 | -0.4 | 355.1036,193.0509,149.0599 | trans-isoferulic acid 3-*O*-*β*-D-allopyranoside | SM |
| 9 | [M+H]+ | 10.23 | C22H28O11 | 468.1632 | 469.1704 | 0.72 | 469.1701,397.0211,307.1163,  261.1129 | Cimifugin-4'-*O*-*β*-D- glucopyranoside | SM |
| 10 | [M-H]- | 10.54 | C27H30O16 | 610.1534 | 609.1461 | 0.67 | 609.1457,253.0349,193.0507,  163.0398 | Shomaside A | SM, XSM |
| 11 | [M-H]- | 10.76 | C16H20O9 | 356.1107 | 355.1035 | -0.69 | 355.1037,193.0498,149.0263 | *trans*-isoferulic acid 3-*O*-*β*-D-glucopyranosude | SM |
| 12 | [M-H]- | 10.90 | C7H6O3 | 138.0317 | 137.0244 | 1.58 | 137.0242,93.0353,65.0391 | 4-hydroxybenzoic acid | XSM |
| 13 | [M+H]+ | 10.90 | C22H28O11 | 468.1632 | 469.1704 | -0.99 | 469.1709,397.1129,307.1175,  289.1068,261.1121 | Cimicifugoside | SM |
| 14 | [M-H]- | 11.28 | C27H30O15 | 594.1585 | 593.1512 | 1.17 | 593.1505,237.0403,193.0502,  165.0555 | Shomaside G | SM,XSM |
| 15 | [M+H]+ | 11.29 | C25H31NO10 | 505.1948 | 506.2021 | 0.94 | 506.2016,344.1493,177.0541 | Isocimicifugamide | XSM |
| 16 | [M+H]+ | 11.41 | C24H29NO10 | 491.1791 | 492.1864 | -0.36 | 492.1866,330.1068,177.0542,  137.0607 | Cimicifugamide A | XSM |
| 17 | [M-H]- | 11.81 | C27H30O15 | 594.1585 | 593.1512 | 1.67 | 593.1502,355.1040,237.0413,  165.0553 | Shomaside B | SM, XSM |
| 18 | [M+H]+ | 12.24 | C24H29NO9 | 475.1842 | 476.1915 | 3.17 | 476.1900,314.1388,177.0548 | *trans*-Feruloyl tyramine-4-*O*-*β*-D-glucopyranoside | XSM |

continued

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Adduct | tR  (min) | Formula | Mass | m/z | Error  (ppm) | MS/MS Fragments | Identification | Source |
| 19 | [M-H]- | 12.28 | C9H8O4 | 180.0423 | 179.035 | 1.01 | 179.0348,135.0446 | Caffeic acid | SM, XSM |
| 20 | [M+H]+ | 12.35 | C21H26O11 | 454.1475 | 455.1548 | 0.63 | 455.1545,293.1018,275.0903 | Prim-*O*-glucosylangelicain | SM |
| 21 | [M+H]+ | 12.41 | C25H31NO10 | 505.1948 | 506.2021 | 0.74 | 506.2017,489.2515,344.1494,  177.0548,149.0611 | *trans*-Feruloyl-(3-*O*-methyl)-dopamine-4-*O*-*β*-D-allopyranoside | XSM |
| 22 | [M+H]+ | 12.67 | C16H18O6 | 306.1103 | 307.1176 | 0.05 | 307.1176,259.0598,235.0598,221.0441,177.0544 | Cimifugin | SM |
| 23 | [M-H]- | 12.91 | C20H18O10 | 418.09 | 417.0827 | 0.29 | 417.0826,237.0402,193.0505,  165.0555 | Cimicifugic acid C | SM, XSM |
| 24 | [M-H]- | 13.38 | C21H20O11 | 448.1006 | 447.0933 | -0.7 | 447.0936,253.0357,235.0251,  191.0352,165.056 | Cimicifugic acid A/B | SM, XSM |
| 25 | [M-H]- | 13.55 | C21H20O11 | 448.1006 | 447.0933 | 0.19 | 447.0932,253.0350,235.0244,  191.0346,181.0503 | Cimicifugic acid A/B | SM, XSM |
| 26 | [M-H]- | 14.26 | C22H22O11 | 462.1162 | 461.1089 | 1.59 | 461.1082,223.0607,193.0502,  165.0553 | 2-feruloyl fukinolic acid-1-metyl ester | XSM |
| 27 | [M-H]- | 14.45 | C21H20O10 | 432.1056 | 431.0984 | 1.78 | 431.0976,209.0451,193.0503,  178.0268,165.0554 | 2-feruloyl piscidic acid | SM, XSM |
| 28 | [M-H]- | 14.50 | C10H10O4 | 194.0579 | 193.0506 | 3.26 | 193.0500,178.0267,149.0604,  134.0370 | Ferulic acid | SM, XSM |
| 29 | [M-H]- | 14.61 | C21H20O10 | 432.1056 | 431.0984 | 1.32 | 431.0978,193.0503,165.0555,  149.0605 | Cimicifugic acid E/F | SM, XSM |
| 30 | [M-H]- | 14.71 | C10H10O4 | 194.0579 | 193.0506 | 2.74 | 193.0501,178.0272,149.0603,  134.0370 | Isoferulic acid | SM, XSM |
| 31 | [M+H]+ | 14.97 | C15H16O6 | 292.0947 | 293.102 | 1.25 | 293.1016,275.0912,233.0447,  221.0442 | Norcimifugin | SM |
| 32 | [M-H]- | 15.00 | C21H20O10 | 432.1056 | 431.0984 | 4.79 | 431.0963,209.0446,193.0503,  165.0552,149.0602 | Cimicifugic acid E/F | SM, XSM |
| 33 | [M-H]- | 15.32 | C18H16O7 | 344.0896 | 343.0823 | 1.24 | 343.0819,149.0603,134.0365 | 4'-methoxyl-3'-hydroxy-carboxybenzoyl isoferulic acid anhydride | SM, XSM |

continued

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Adduct | tR  (min) | Formula | Mass | m/z | Error  (ppm) | MS/MS Fragments | Identification | Source |
| 34 | [M+H]+ | 15.53 | C18H19NO4 | 313.1314 | 314.1387 | 0.27 | 314.1386,177.0544,149.0597,  145.0281,117.0332 | Ferulyltyramine | SM, XSM |
| 35 | [M+H]+ | 16.13 | C32H48O9 | 576.3298 | 577.3371 | 1.06 | 577.3365,559.326,427.2839 | Cimicifugoside H-3 | SM |
| 36 | [M-H]- | 16.56 | C22H22O10 | 446.1213 | 445.114 | 2.96 | 445.1127,207.0657,193.0618,  165.0554,149.0604 | Cimicifugic acid L | XSM |
| 37 | [M-H]- | 16.56 | C37H58O11 | 678.3979 | 677.3906 | 0.2 | 677.3905,617.3685,485.3289,  59.0135 | 12*β*-acetylcimigenol-3-*O*-*β*-D-xylopyranoside | SM, XSM |
| 38 | [M+H]+ | 17.01 | C35H52O9 | 616.3611 | 617.3684 | 0.02 | 617.3684,467.3155,395.2592,  251.1801 | Cimicidanol-3-*O*-*α*-L-arabinoside | XSM |
| 39 | [M-H]- | 17.08 | C37H56O11 | 676.3823 | 675.375 | 1.01 | 675.3743,615.3565,579.3517,  541.2767,71.0133 | Cimiracemoside A | SM, XSM |
| 40 | [M-H]- | 17.09 | C37H58O11 | 678.3979 | 677.3906 | 1.23 | 677.3898,617.3681,599.3564,  559.3235,501.286 | 9,19-cyclocholest-7-en-16-one,23-(acetyloxy)-15,24,25-trihydroxy-4,4,14-trimethyl-3-(*β*-D-xylopyranoside) | XSM |
| 41 | [M-H]- | 17.44 | C35H54O10 | 634.3717 | 633.3644 | 0.35 | 633.3642,501.2848,369.2430 | Cimicifugoside H-2 | SM, XSM |
| 42 | [M-H]- | 17.65 | C19H18O7 | 358.1053 | 357.098 | 1.33 | 357.3975,193.0501 | Cimiracemate B | SM, XSM |
| 43 | [M-H]- | 17.65 | C19H18O7 | 358.1053 | 357.098 | 1.33 | 357.0975,193.0501,163.0397 | Cimiracemate A | SM, XSM |
| 44 | [M+H]+ | 17.72 | C37H54O10 | 658.3717 | 659.379 | 1.02 | 659.3783,599.3582,467.3177,  449.3052 | 26-dedoxycimifugoside | SM, XSM |
| 45 | [M-H]- | 17.90 | C37H56O10 | 660.3873 | 659.3801 | 1.77 | 659.3789,599.3592,581.3497,  559.3251，59.0135 | 23-*O*-aectyl-7,8-didehydroshengmanol 3-*O*-*α*-L-arabinopyranoside | XSM |
| 46 | [M+Na]+ | 17.90 | C30H48O5 | 488.3502 | 511.3394 | -4.72 | 511.3417,451.3205,433.3109,  379.2627 | Cimiacerin B | XSM |
| 47 | [M+H]+ | 18.18 | C30H44O5 | 484.3189 | 485.3262 | -0.31 | 485.3261,467.3156,449.3051,  431.2944,413.2691,395.2579 | Cimicidanol | SM, XSM |
| 48 | [M-H]- | 18.92 | C37H56O11 | 676.3823 | 675.375 | 1.01 | 675.3743,615.3538,543.2946,  131.0705,59.0136 | Actein | SM, XSM |

continued

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Adduct | tR  (min) | Formula | Mass | m/z | Error  (ppm) | MS/MS Fragments | Identification | Source |
| 49 | [M+H]+ | 19.07 | C37H54O9 | 642.3768 | 643.3841 | 0.09 | 643.3840,583.363,451.3209,  73.0284 | Asiaticoside B | XSM |
| 50 | [M-H]- | 20.45 | C35H56O10 | 636.3873 | 635.3801 | 0.58 | 635.3797,577.3375,445.295 | 7*β*-hydroxycimigenol-3-*O*-*β*-D-xylopyranoside | SM, XSM |
| 51 | [M+H]+ | 20.45 | C35H52O8 | 600.3662 | 601.3735 | -0.34 | 601.3737,529.3167,451.3207,  397.2736,379.2631 | CimiracemosideⅠ | SM, XSM |
| 52 | [M-H]- | 20.71 | C35H56O10 | 636.3873 | 635.3801 | 0.43 | 635.3798,577.3386,445.2976 | 12*β*-hydroxycimigenol-3-*O*-*β*-D-xylopyranoside | SM, XSM |
| 53 | [M-H]- | 21.34 | C38H60O12 | 708.4085 | 707.4012 | -0.28 | 707.4014,647.4202,603.3539,  59.0139 | 24-*epi*-24-*O*-acetyl-7,8-didehydroshengmanol-3-*O*-*β*-D-galactopyranoside | SM |
| 54 | [M-H]- | 22.09 | C35H54O9 | 618.3768 | 617.3695 | 0.5 | 617.3692,599.3578,559.3270,  541.3163,467.2797 | 7,8-didehydrocimigenol 3-*O*-*β*-D-xyloside | SM, XSM |
| 55 | [M-H]- | 22.14 | C35H56O10 | 636.3873 | 635.3801 | 0.58 | 635.3797,577.3381,445.2970 | 7*β*-hydroxycimigenol-3-*O*-*α*-L-arabinopyranoside | XSM |
| 56 | [M-H]- | 22.32 | C35H56O10 | 636.3873 | 635.3801 | 0.58 | 635.3797,577.3379,445.2958 | (22R)-22*β*-hydroxycimigenol 3-*O*-*β*-D-xylopyranoside | SM, XSM |
| 57 | [M+H]+ | 22.40 | C37H54O10 | 658.3717 | 659.379 | 2.54 | 659.3773,641.3666,599.3580,  581.3475,467.3155,449.3048 | 26-deoxycimicifugoside | SM, XSM |
| 58 | [M-H]- | 22.72 | C35H56O10 | 636.3873 | 635.3801 | 1.84 | 635.3789,577.3377,445.2972 | 12*β*-hydroxycimigenol-3-*O*-*α*-L-arabinopyranoside | SM, XSM |
| 59 | [M-H]- | 22.78 | C35H54O9 | 618.3768 | 617.3695 | 0.33 | 617.3694,599.3474,559.3274,  541.3163 | 7,8-didehydroshengmanol-3-*O*-*β*-D-xylranoside | SM, XSM |
| 60 | [M+H]+ | 23.20 | C37H56O10 | 660.3873 | 661.3946 | -0.42 | 661.3949,529.3162,469.3303,  451.3209,397.2741,379.2641 | Cimiricaside A | XSM |
| 61 | [M-H]- | 23.35 | C37H60O11 | 680.4136 | 679.4063 | 2.63 | 679.4045,619.3850,601.3803,  487.3438 | 24-*epi*-*O*-acetylhydroshengmanol-3-*O*-*α*-L-arabinopyranoside | SM, XSM |

continued

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Adduct | tR  (min) | Formula | Mass | m/z | Error  (ppm) | MS/MS Fragments | Identification | Source |
| 62 | [M+H]+ | 23.35 | C13H13NO | 199.0997 | 200.107 | -1.55 | 200.1073,158.0603,130.0654 | (*E*)/(*Z*)-3-(3'-methyl-2'-butenylidene)-2-indolinone | XSM |
| 63 | [M+H]+ | 23.67 | C35H54O8 | 602.3819 | 603.3891 | -0.42 | 603.3894,585.3791,471.3482,  453.3368,435.3268,73.0282 | 25-anhydrocimigenol 3-*O*-*α*-L-arabinopyranoside | XSM |
| 64 | [M-H]- | 23.99 | C37H56O10 | 660.3873 | 659.3801 | -0.19 | 659.3802,641.3697,509.2882,  59.0136 | 27-deoxyactein | SM, XSM |
| 65 | [M+Na]+ | 24.41 | C30H48O5 | 488.3502 | 511.3394 | -5.54 | 511.3421,451.3212,433.3103,  379.2632 | Cimigenol | XSM |
| 66 | [M-H]- | 25.47 | C37H58O10 | 662.403 | 661.3957 | 0.79 | 661.3952,601.3279,469.2872,  59.0135 | 25-*O*-acetylcimigenol-3-*O*-*α*-L-arabinoside | SM, XSM |
| 67 | [M+Na]+ | 25.72 | C35H56O9 | 620.3924 | 643.3817 | -2.81 | 643.3834,583.3633,451.3197,  379.265,361.2516 | Cimigenol-3-*O*-*α*-L-arabinoside | SM, XSM |
| 68 | [M+H]+ | 26.29 | C35H52O8 | 600.3662 | 601.3735 | -1.17 | 601.3742,583.3636,511.3073,  469.332,451.3209 | 7,8-didehydro-25-anhydrocimigenol-3-*O*-*β*-D-xyloside | SM, XSM |
| 69 | [M-H]- | 26.46 | C39H58O11 | 702.3979 | 701.3906 | -0.23 | 701.3908,641.3695,623.3593,  601.3384,583.3278 | Cimiricaside C | XSM |
| 70 | [M-H]- | 26.64 | C37H58O11 | 678.3979 | 677.3906 | -0.09 | 677.3907,617.3695,599.358,  485.255,71.0138 | 24-*O*-acetyl-7,8-didehydroshengmanol-3-*O*-*β*-D-xylopyranoside | SM, XSM |
| 71 | [M+Na]+ | 26.64 | C35H56O9 | 620.3924 | 643.3817 | -4.91 | 643.3847,583.3637,511.3423,  451.3211,433.3107 | Cimigenol-3-*O*-*β*-D-xylopyranoside | SM, XSM |
| 72 | [M+H]+ | 26.68 | C37H56O10 | 660.3873 | 661.3946 | -0.87 | 661.3952,643.3847,583.3636,  529.3163,469.3317,451.3210,  397.2741 | 25-*O*-aectyl-7,8-didehydroshengmanol 3-*O*-*α*-L-arabinopyranoside | SM, XSM |
| 73 | [M+Na]+ | 26.71 | C33H52O7 | 560.3713 | 583.3605 | -5.13 | 583.3634,565.3527,451.3216,  423.3106,415.2999 | 24-*O*-acetyl-25-*O*-methyl-7，8-didehydrohydro-shengmanol | SM, XSM |
| 74 | [M+H]+ | 26.85 | C37H56O10 | 660.3873 | 661.3946 | -1.48 | 661.3956,643.3849,583.3637,  529.3174,451.3209,397.2743 | 25-*O*-aectyl-7,8-didehydroshengmanol 3-*O*-*β*-D-xylranoside | SM, XSM |

continued

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Adduct | tR  (min) | Formula | Mass | m/z | Error  (ppm) | MS/MS Fragments | Identification | Source |
| 75 | [M-H]- | 27.77 | C37H56O10 | 660.3873 | 659.3801 | -0.04 | 659.3801,599.3584,59.0136 | 23-*O*-aectyl-7,8-didehydroshengmanol 3-*O*-*β*-D-xylranoside | SM, XSM |
| 76 | [M+H]+ | 27.80 | C30H46O5 | 486.3345 | 487.3418 | -0.41 | 487.3420,451.3210,433.3096,  397.2738 | Cimigenol-3-one | XSM |
| 77 | [M+H]+ | 28.33 | C40H58O13 | 746.3877 | 747.395 | -0.51 | 747.3954,729.3852,669.3639,  651.3532,451.3208,397.2740 | 23-*O*-acetyl-7,8-didehydroshengmanol-3-*O*-(2'-*O*-malonyl)-xylopyranoside | XSM |
| 78 | [M+Na]+ | 28.55 | C30H48O5 | 488.3502 | 511.3394 | -5.95 | 511.3423,451.3213,433.3103,  379.2622 | 24-*epi*-cimigenol | XSM |
| 79 | [M-H]- | 30.36 | C39H58O11 | 702.3979 | 701.3906 | 0.05 | 701.3906,641.3696,623.3593,  583.3278,59.0137 | 15,23-*O*-diacetyl-7(8)-ene-shengmanol-3-*O*-*α*-L-arabinopyranoside | SM, XSM |
| 80 | [M-H]- | 31.21 | C30H48O6 | 504.3451 | 503.3378 | 0.42 | 503.3376,445.2960 | 11*β*-hydroxy-24-*epi*-cimigenol | SM, XSM |
| 81 | [M+H]+ | 31.72 | C32H48O6 | 528.3451 | 529.3524 | 3.72 | 529.3504,511.3407,469.3308,  451.3202 | 24-*O*-acetylshengmanol-7(8)-en-isodahurinol | SM |
| 82 | [M+H]+ | 32.27 | C32H48O6 | 528.3451 | 529.3524 | 3.72 | 529.3504,511.3416,493.3317,  469.3308,451.3203,397.2728 | 27-deoxyacetylacteol | SM |
| 83 | [M+H]+ | 32.66 | C32H48O6 | 528.3451 | 529.3524 | 5.8 | 529.3493,511.3393,451.3198,  379.2602 | 25-*O*-acetyl-7,8-didehydrocimigenol | SM |
| 84 | [M+Na]+ | 33.61 | C30H46O6 | 502.3294 | 525.3187 | 0.32 | 525.3185,469.3328,451.3201 | 25-*O*-methylisodahurinol | XSM |
| 85 | [M+Na]+ | 36.02 | C30H46O5 | 486.3345 | 509.3237 | -0.52 | 509.3240,451.3195 | 7,8-didehydrocimigenol | SM, XSM |
| 86 | [M+H]+ | 37.15 | C30H46O4 | 470.3396 | 471.3469 | 0.61 | 471.3466,453.3362,435.3251,  363.2676 | 25-dehydrocimigenol | SM |
| 87 | [M-H]- | 37.53 | C32H50O7 | 546.3557 | 545.3484 | -0.59 | 545.3487,485.3265,59.0136 | 12*β*-acetoxycimigenol | SM, XSM |
| 88 | [M+H]+ | 39.48 | C30H42O5 | 482.3032 | 483.3105 | -1.24 | 483.3111,467.3098,449.3051,  411.2536,395.2562 | (20R,24R)-24,25-epoxy-11*β*-hydroxy-7-en-9,19-cyclolanost-3,16,23-trione | XSM |

**Table S5** Regression formula, correlation coefficient, range of concentration, LOQ and recovery rate of the four markers.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compounds | Regression formula | Coefficient | Range/(μg/mL) | LOQ  (μg/mL) | Recovery | RSD(%) |
| Caffeic acid | y=0.6451x+1.1813 | 0.9998 | 0.08-500 | 0.08 | 97.7% | 4.57% |
| Cimifugin | y=0.2493x+0.5499 | 0.9998 | 0.08-500 | 0.08 | 103% | 5.44% |
| Ferulic acid | y=0.6307x+0.6669 | 0.9998 | 0.048-300 | 0.048 | 103% | 3.70% |
| Isoferulic acid | y=0.6616x+2.4382 | 0.9998 | 0.16-1000 | 0.16 | 96.1% | 3.30% |

**Table S****6** The contents of four markers in all batches of samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Caffeic acid  (mg/g) | Cimifugin  (mg/g) | Ferulic acid  (mg/g) | Isoferulic acid  (mg/g) |
| SM-1 | 0.298 | 1.607 | 0.311 | 0.557 |
| SM-2 | 0.381 | 2.206 | 0.197 | 0.050 |
| SM-3 | 0.176 | 1.611 | 0.231 | 0.287 |
| SM-4 | 0.134 | 3.648 | 0.157 | 0.519 |
| SM-5 | 0.173 | 3.611 | 0.169 | 0.582 |
| SM-6 | 0.291 | 2.811 | 0.276 | 0.294 |
| SM-7 | 0.182 | 4.227 | 0.178 | 0.656 |
| SM-8 | 0.160 | 3.375 | 0.173 | 0.625 |
| SM-9 | 0.209 | 4.234 | 0.196 | 0.695 |
| SM-10 | 0.227 | 2.003 | 0.257 | 0.046 |
| SM-11 | 0.281 | 2.590 | 0.275 | 5.105 |
| SM-12 | 0.405 | 2.225 | 0.377 | 4.569 |
| SM-13 | 0.648 | 2.521 | 0.296 | 4.011 |
| SM-14 | 0.311 | 3.000 | 0.689 | 1.910 |
| SM-15 | 0.197 | 0.007 | 0.296 | 1.397 |
| SM-16 | 0.153 | 0.065 | 0.111 | 1.252 |
| XSM-1 | 0.093 | 0.101 | 0.317 | 0.794 |
| XSM-2 | 0.058 | 0.257 | 0.242 | 1.223 |
| XSM-3 | 0.172 | 0.092 | 0.354 | 1.146 |
| XSM-4 | 0.023 | 0.012 | 0.158 | 1.122 |
| XSM-5 | 0.436 | 0.379 | 0.845 | 4.487 |
| XSM-6 | 0.086 | 0.046 | 0.195 | 0.992 |
| XSM-7 | 0.170 | 0.336 | 0.251 | 1.076 |
| XSM-8 | 0.090 | 0.090 | 0.257 | 1.401 |
| XSM-9 | 0.069 | 0.119 | 0.230 | 0.738 |
| XSM-10 | 0.035 | 0.038 | 0.291 | 1.571 |
| XSM-11 | 0.043 | 0.037 | 0.229 | 0.723 |
| XSM-12 | 0.038 | 0.046 | 0.235 | 0.616 |
| XSM-13 | 0.050 | 0.021 | 0.248 | 0.738 |
| XSM-14 | 0.062 | 0.042 | 0.152 | 0.537 |
| XSM-15 | 0.049 | 0.033 | 0.223 | 0.729 |
| XSM-16 | 0.007 | 0.001 | 0.157 | 0.648 |
| XSM-17 | 0.048 | 0.024 | 0.301 | 0.763 |
| XSM-18 | 0.034 | 0.004 | 0.251 | 0.895 |
| XSM-19 | 0.049 | 0.014 | 0.259 | 0.738 |
| XSM-20 | 0.048 | 0.015 | 0.225 | 0.746 |
| XSM-21 | 0.083 | 0.030 | 0.259 | 0.750 |
| XSM-22 | 0.086 | 0.045 | 0.359 | 1.035 |
| XSM-23 | 0.049 | 0.004 | 0.265 | 0.874 |
| XSM-24 | 0.038 | 0.059 | 0.279 | 0.914 |
| XSM-25 | 0.034 | 0.085 | 0.195 | 0.570 |
| XSM-26 | 0.119 | 0.480 | 0.224 | 1.155 |
| XSM-27 | 0.014 | 0.221 | 0.222 | 1.384 |
| XSM-28 | 0.039 | 0.180 | 0.323 | 1.053 |
| XSM-29 | 0.056 | 0.096 | 0.019 | 0.074 |
| XSM-30 | 0.065 | 0.195 | 0.154 | 0.696 |
| XSM-31 | 0.129 | 0.282 | 0.051 | 0.163 |
| XSM-32 | 0.085 | 0.277 | 0.108 | 0.228 |
| XSM-33 | 0.039 | 0.230 | 0.082 | 0.537 |
| XSM-34 | 0.060 | 0.209 | 0.091 | 0.185 |
| XSM-35 | 0.107 | 0.334 | 0.041 | 0.093 |
| XSM-36 | 0.093 | 0.244 | 0.020 | 0.085 |

continued

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Caffeic acid  (mg/g) | Cimifugin  (mg/g) | Ferulic acid  (mg/g) | Isoferulic acid  (mg/g) |
| XSM-37 | 0.108 | 0.407 | 0.044 | 0.087 |
| XSM-38 | 0.126 | 0.472 | 0.073 | 0.211 |
| XSM-39 | 0.082 | 0.282 | 0.097 | 0.364 |
| XSM-40 | 0.075 | 0.307 | 0.101 | 0.492 |