Supplementary Material

A Metabolomics Coupled with Chemometrics Strategy to Filter Combinatorial discriminatory Quality Markers of Crude and Salt-fired *Eucommiae Cortex*

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Table S1. The precision, repeatability, and stability of the qualitative method

| compounds | precision | | repeatability | | stability |
|-----------|-----------|-----------|--------------|-----------|-----------|
|           | Rt (%)    | m/z (%)   | Peak area (%)| Rt (%)    | m/z (%)   | Peak area (%)| Rt (%)    | m/z (%)   | Peak area (%)|
| M1        | 0.32      | 0.00      | 2.09         | 0.40      | 0.00      | 2.63         | 0.39      | 0.00      | 3.30         |
| M2        | 0.21      | 0.00      | 1.20         | 0.28      | 0.00      | 1.00         | 0.20      | 0.00      | 1.65         |
| M3        | 0.17      | 0.00      | 2.22         | 0.17      | 0.00      | 1.44         | 0.13      | 0.00      | 2.27         |
| M4        | 0.16      | 0.00      | 1.18         | 0.17      | 0.00      | 2.07         | 0.13      | 0.00      | 2.22         |
| M5        | 0.19      | 0.00      | 1.13         | 0.08      | 0.00      | 1.61         | 0.21      | 0.00      | 2.14         |
| M6        | 0.12      | 0.00      | 3.46         | 0.12      | 0.00      | 3.86         | 0.08      | 0.00      | 1.06         |
| M7        | 0.16      | 0.00      | 2.05         | 0.14      | 0.00      | 1.98         | 0.07      | 0.00      | 1.98         |
| M8        | 0.10      | 0.00      | 0.74         | 0.09      | 0.00      | 0.71         | 0.14      | 0.00      | 1.42         |
| M9        | 0.04      | 0.00      | 1.09         | 0.07      | 0.00      | 2.04         | 0.07      | 0.00      | 1.31         |
| M10       | 0.04      | 0.00      | 1.02         | 0.05      | 0.00      | 1.22         | 0.04      | 0.00      | 1.37         |
| M11       | 0.04      | 0.00      | 1.40         | 0.05      | 0.00      | 2.22         | 0.06      | 0.00      | 1.29         |

M1-11 represented geniposidic acid, neochlorogenic acid, chlorogenic acid, caffeic acid, geniposide, genipin, pinoresinol di-o-glucopyranoside, syringaresinol di-o-glucopyranoside, isochlorogenic acid A, pinoresinol o-glucopyranoside, and isochlorogenic acid C, respectively.
Table S2. The 72 candidate compounds found by formula

| Peak no. | Rt (min) | Formula | [M-H]$^-$ | [M+COOH]$^+$ | ∆ppm | Identification |
|----------|----------|---------|-----------|--------------|-------|----------------|
| 1        | 1.178    | C$_6$H$_8$O$_7$ | 191.0196  |              | 3.26  | isocitric acid |
| 2        | 1.45     | C$_{13}$H$_{22}$O$_9$ | 391.1231 |              | 4.29  | aucubin        |
| 3        | 1.534    | C$_{16}$H$_{22}$O$_{11}$ | 389.1090 |              | 0.47  | deacetylasperulosidic acid |
| 4        | 1.754    | C$_{13}$H$_{22}$O$_{10}$ | 361.1122 |              | 5.03  | catalpol       |
| 5        | 2.077    | C$_{13}$H$_{16}$O$_9$ | 315.0714  |              | 2.39  | protocatechuic acid-4-glucoside |
|          |          |         |           |              |       | 6-(4-formyl-2,6-dimethoxyphenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl methyl hydrogen sulfate |
| 6        | 2.194    | C$_{15}$H$_{20}$O$_{12}$S | 423.0598 |              | 1.11  | 2-glucopyranosyloxy-5-hydroxyphenyl acetic acid |
|          |          |         |           |              |       | methyl 3-(3,4-dihydroxyphenyl) propanoate |
| 7        | 2.212    | C$_{14}$H$_{18}$O$_9$ | 329.0873  |              | 1.53  | vanillic acid |
| 8        | 2.228    | C$_{10}$H$_{12}$O$_4$ | 241.0720  | -1.22        |       | geniposidic acid |
| 9        | 2.28     | C$_8$H$_6$O$_4$ | 167.0341  |              | 0.80  | 3,4-dihydroxy benzoic acid |
| 10       | 2.348    | C$_{16}$H$_{22}$O$_{10}$ | 373.1125 |              | 3.97  | 4-glucopyranosyloxy-3,5-dimethoxy benzoic acid |
| 11       | 2.534    | C$_7$H$_6$O$_4$ | 153.0186  |              | 4.78  | neochlorogenic acid |
| 12       | 2.753    | C$_{15}$H$_{20}$O$_{10}$ | 359.0981 |              | 0.75  | harpagide      |
| 13       | 2.874    | C$_{16}$H$_{18}$O$_9$ | 353.0883  | -1.40        |       | perioplobiose  |
| 14       | 3.008    | C$_{15}$H$_{24}$O$_{10}$ | 363.1281 |              | 4.31  | 3-hydroxybenzoic acid |
| 15       | 3.484    | C$_{13}$H$_{24}$O$_9$ | 323.1336  |              | 3.63  | 5-methoxy-guaiacylglycerol |
| 16       | 3.568    | C$_7$H$_6$O$_3$ | 137.0241  |              | 2.30  | 3-methoxybenzoic acid |
| 17       | 3.652    | C$_{11}$H$_{16}$O$_6$ | 243.0862  |              | 4.96  | catechol       |
| 18       | 3.652    | C$_6$H$_4$O$_2$ | 109.0292  |              | 2.75  | isochlorogenic acid A |
| 19       | 3.702    | C$_{22}$H$_{28}$O$_{14}$ | 515.1398 |              | 1.61  |                |
|    |     |       |           |          |                                              |
|----|-----|-------|-----------|---------|---------------------------------------------|
| 20 | 4.109 | C$_{32}$H$_{44}$O$_{17}$ | 745.2532 | 4.07    | olivil 4''-4''-di-o-glucopyranoside          |
| 21 | 4.16  | C$_{16}$H$_{18}$O$_{9}$ | 353.0883 | -1.4    | neochlorogenic acid                         |
| 22 | 4.492 | C$_{16}$H$_{18}$O$_{9}$ | 353.0876 | 0.58    | neochlorogenic acid                         |
| 23 | 4.636 | C$_{9}$H$_{8}$O$_{4}$  | 179.0355 | -2.88   | caffeic acid                                |
| 24 | 5.132 | C$_{17}$H$_{22}$O$_{10}$ | 385.1136 | 1.09    | 4-[3-glucopyranosyloxy-2-hydroxyphenyl]-3-methyl-4-oxobutanoic acid |
| 25 | 5.465 | C$_{18}$H$_{26}$O$_{10}$ | 447.1501 | 1.99    | 4-[2-(xylopyranosyloxy)ethyl] phenylxylopyranoside |
| 26 | 5.742 | C$_{16}$H$_{18}$O$_{8}$ | 337.0933 | -1.21   | 3-p-coumaroylquinic acid                    |
| 27 | 5.929 | C$_{17}$H$_{24}$O$_{10}$ | 433.1341 | -2.70   | geniposide                                  |
| 28 | 6.07  | C$_{26}$H$_{34}$O$_{12}$ | 537.1956 | 3.99    | olivil 4'-o-glucopyranoside                 |
| 29 | 6.552 | C$_{22}$H$_{42}$O$_{17}$ | 697.2336 | 1.90    | l-hydroxypinoresinol di-o-glucopyranoside    |
| 30 | 6.62  | C$_{33}$H$_{44}$O$_{19}$ | 743.2380 | 3.23    | naringin DHC 4-α-β-d-glucopyranoside        |
| 31 | 7.62  | C$_{11}$H$_{14}$O$_{5}$ | 225.0767 | 0.65    | genipin                                     |
| 32 | 7.907 | C$_{26}$H$_{34}$O$_{12}$ | 537.1956 | 3.99    | olivil 4'-o-glucopyranoside                 |
| 33 | 8.162 | C$_{32}$H$_{42}$O$_{16}$ | 681.2373 | 3.97    | pinoresinol di-o-glucopyranoside            |
| 34 | 8.450 | C$_{10}$H$_{10}$O$_{4}$ | 193.0497 | 4.80    | methyl 3-phenylacrylate                     |
| 35 | 8.705 | C$_{32}$H$_{42}$O$_{16}$ | 681.2376 | 3.97    | pinoresinol di-o-glucopyranoside            |
| 36 | 8.974 | C$_{17}$H$_{20}$O$_{9}$ | 367.1028 | 1.78    | 5-o-feruloylquinic acid                     |
| 37 | 9.112 | C$_{23}$H$_{26}$O$_{13}$ | 509.1295 | 1.11    | 4,8,9,10-tetrahydroxy-3,6,7-trimethoxy-2-anthryl-glucopyranoside |
| 38 | 9.196 | C$_{33}$H$_{46}$O$_{18}$ | 729.2584 | 3.75    | 3-[4-(2-[4-glucopyranosyloxy-3-methoxyphenyl]-2-hydroxy-1-(hydroxymethyl)ethoxy]- |
|   | Molecular Formula | Molecular Weight | Retention Time | Compound Name |
|---|------------------|------------------|---------------|---------------|
| 39 | C_{33}H_{44}O_{17} | 711.2487         | 9.315         | 3,5-dimethoxyphenyl-2-propen-1-ylglucopyranoside |
| 40 | C_{26}H_{32}O_{12} | 535.1800         | 9.738         | medioresinol di-o-glucopyranoside |
| 41 | C_{26}H_{32}O_{12} | 535.1800         | 10.196        | l-hydroxypinoresinol 4’-o-glucopyranoside |
| 42 | C_{34}H_{46}O_{18} | 741.2593         | 10.281        | syringaresinol di-o-glucopyranoside |
| 43 | C_{10}H_{18}O_{5}  | 217.1078         | 10.671        | l-hydroxypinoresinol 4’-o-glucopyranoside |
| 44 | C_{23}H_{26}O_{13} | 509.1292         | 11.838        | 4,8,9,10-tetrahydroxy-3,6,7-trimethoxy-2-anthryl-glucopyranoside |
| 45 | C_{25}H_{31}NO_{11}| 520.1811         | 12.094        | eucomoside B |
| 46 | C_{25}H_{24}O_{12} | 515.1189         | 12.705        | isochlorogenic acid A |
| 47 | C_{20}H_{22}O_{7}  | 373.1291         | 12.774        | erythro-guaiacylglycerol-β-conifery aldehyde ether |
| 48 | C_{43}H_{56}O_{21} | 907.3220         | 12.79         | hedyotol C di-o-glucopyranoside |
| 49 | C_{43}H_{54}O_{22} | 921.3000         | 12.858        | unknown |
| 50 | C_{20}H_{22}O_{6}  | 357.1342         | 12.991        | pinoresinol |
| 51 | C_{26}H_{32}O_{11} | 519.1862         | 12.994        | pinoresinol-o-glucopyranoside |
| 52 | C_{27}H_{34}O_{12} | 549.1978         | 13.059        | eucommin A |
| 53 | C_{9}H_{16}O_{4}   | 187.0972         | 13.113        | eucommiol |
| 54 | C_{44}H_{58}O_{22} | 937.3316         | 13.333        | glycerol-syringaresinol ether di-glucopyranoside |
| 55 | C_{28}H_{36}O_{13} | 579.2027         | 13.516        | syringaresinol 4’-o-glucopyranoside |
| 56 | C_{27}H_{34}O_{12} | 549.1978         | 13.604        | eucommin A |
| 57 | C_{20}H_{22}O_{7}  | 373.1278         | 13.859        | erythro-guaiacylglycerol-β-conifery aldehyde ether |
| No. | M & ESI | CAS | MW | Name |
|-----|---------|-----|-----|------|
| 58  | 13.875  | C₂₅H₂₄O₁₂ | 515.1189 | isochlorogenic acid A |
| 59  | 14.063  | C₄₂H₅₂O₂₁ | 891.2918 | syringaresinol vanillic acid ether diglucopyranoside |
| 60  | 14.195  | C₄₀H₄₈O₁₉ | 831.2689 | pinoresinol vanillic acid ether diglucopyranoside |
| 61  | 14.264  | C₁₃H₂₆O₇  | 317.1601 | 2-(5-hydroxy-2,3-dimethyl-2-cyclopenten-1-yl)ethylglucopyranoside |
| 62  | 14.265  | C₄₁H₅₀O₂₀ | 861.2794 | medioresinol vanillic acid ether diglucopyranoside |
| 63  | 14.334  | C₂₀H₂₂O₇  | 373.1295 | erythro-guaiacylglycerol-β-conifery aldehyde ether |
| 64  | 14.398  | C₂₁H₂₄O₇  | 433.1499 | medioresinol |
| 65  | 14.401  | C₄₀H₄₈O₁₉ | 831.2686 | pinoresinol vanillic acid ether diglucopyranoside |
| 66  | 14.604  | C₃₇H₄₆O₁₆ | 745.2690 | glycerol-medioresinol ether 4”-glucopyranoside |
| 67  | 15.094  | C₃₇H₄₆O₁₆ | 745.2688 | glycerol-medioresinol ether 4”-glucopyranoside |
| 68  | 16.3    | C₃₆H₄₂O₁₆ | 729.23937 | syringaresinol vanillic acid ether glucopyranoside |
| 69  | 16.383  | C₃₅H₄₀O₁₅ | 699.2292 | medioresinol vanillic acid ether glucopyranoside |
| 70  | 16.586  | C₃₄H₃₈O₁₄ | 669.2182 | pinoresinol vanillic acid ether glucopyranoside |
| 71  | 16.638  | C₉H₁₆O₃   | 171.1023 | 1-deoxyeucommiol |
| 72  | 17.873  | C₁₂H₂₀O₄  | 227.1281 | 5,6,7,8-tetrahydro-7-hydroxy-3,3-dimethyl-1Hcyclopenta[1,3]dioxepin-6-ethanol |
**Table S3.** The regressive equations, linear ranges, LODs, LOQs, repeatability, and recoveries of 11 compounds

| analytes | regressive equation | r²  | linear range (g/mL) | LOQ (μg/mL) | LOD (μg/mL) | recovery average (%) | RSD (%) | repeatability RSD (%) |
|----------|---------------------|-----|---------------------|------------|-------------|----------------------|---------|----------------------|
| M1       | y=14242.94x+1974.73 | 0.9991 | 1-250 | 0.4 | 0.1 | 95.9±3.6 | 3.19 | 1.69 |
| M2       | y=25774.93x+58.91   | 0.9994 | 0.04-10 | 0.04 | 0.01 | 104±4 | 3.46 | 1.87 |
| M3       | y=26005.17x-2756.75 | 0.9995 | 0.4-100 | 0.08 | 0.025 | 97.4±4.8 | 4.88 | 2.00 |
| M4       | y=60808.33x-438.04  | 0.9996 | 0.04-10 | 0.03 | 0.01 | 96.2±4.1 | 4.09 | 3.52 |
| M5       | y=18072.88x+855.45  | 0.9999 | 0.4-100 | 0.3 | 0.01 | 95.9±3.9 | 3.92 | 1.07 |
| M6       | y=24973.82x+2116.83 | 0.9999 | 0.4-100 | 0.35 | 0.15 | 97.0±4.3 | 4.38 | 0.86 |
| M7       | y=15837.93x-2843.27 | 0.9992 | 1-200 | 1 | 0.3 | 100±3 | 3.23 | 2.00 |
| M8       | y=15163.16x-629.57  | 0.9999 | 0.4-100 | 1 | 0.3 | 103±3 | 3.21 | 2.76 |
| M9       | y=23407.61x+2085.57 | 0.9991 | 0.1-25 | 0.04 | 0.01 | 100±3 | 3.17 | 3.35 |
| M10      | y=30106.82x-17.81   | 0.9998 | 0.4-100 | 0.4 | 0.16 | 94.3±4.1 | 4.32 | 1.49 |
| M11      | y=25787.85x-199.09  | 0.9997 | 0.04-10 | 0.04 | 0.16 | 98.1±4.3 | 4.56 | 4.23 |

M1-11 represented geniposidic acid, neochlorogenic acid, chlorogenic acid, caffeic acid, geniposide, genipin, pinoresinol di-o-glucopyranoside, syringaresinol di-o-glucopyranoside, isochlorogenic acid A, pinoresinol o-glucopyranoside, and isochlorogenic acid C, respectively.
Table S4. The Intra-day and Inter-day accuracy and precision, and stability of 11 markers (n = 6).

| Analytes | Concentration (μg/mL) | Inter-day | Intra-day | Stability |
|----------|-----------------------|-----------|-----------|-----------|
|          |                       | Accuracy (%) | RSD (%) | Accuracy (%) | RSD (%) | Accuracy (%) | RSD (%) |
| 2        | 93.3±2.4              | 2.30       | 97.8±2.6  | 2.60       | 96.4±2.2  | 2.16       |
| M1       | 104±0                 | 0.10       | 104±1     | 0.72       | 104±0     | 0.49       |
|          | 103±0                 | 0.39       | 103±1     | 1.54       | 99.7±1.5  | 1.52       |
|          | 92.9±4.1              | 3.70       | 96.4±0.8  | 0.79       | 97.1±3.0  | 2.98       |
| M2       | 97.2±1.2              | 1.17       | 99.6±2.1  | 2.06       | 98.5±1.0  | 0.97       |
|          | 80                   | 90.8±1.9   | 1.70       | 95.1±3.3   | 3.07       | 99.1±1.3   | 1.30       |
|          | 0.8                  | 93.1±4.4   | 3.88       | 96.9±2.3   | 2.21       | 99.8±0.9   | 0.86       |
| M3       | 91.9±0.8              | 0.76       | 94.2±2.2  | 2.09       | 91.3±0.6  | 0.58       |
|          | 80                   | 92.1±0.7   | 0.63       | 96.6±3.3   | 3.07       | 97.9±1.3   | 1.32       |
|          | 0.08                 | 104±1      | 1.13       | 102±1      | 1.27       | 101±3      | 3.32       |
| M4       | 96.4±0.6              | 0.56       | 97.5±1.1  | 1.04       | 96.8±0.7  | 0.71       |
|          | 80                   | 98.7±0.4   | 0.41       | 101±1      | 1.35       | 97.6±3.0   | 2.83       |
|          | 0.8                  | 94.8±3.7   | 3.54       | 98.0±1.4   | 1.30       | 99.1±2.6   | 2.56       |
| M5       | 96.3±0.4              | 0.42       | 99.6±2.5  | 2.43       | 100±0     | 0.48       |
|          | 80                   | 94.6±0.3   | 0.27       | 96.1±1.5   | 1.48       | 97.4±2.8   | 2.60       |
|          | 0.8                  | 94.8±0.0   | 3.90       | 95.0±0.8   | 0.79       | 95.5±3.8   | 3.55       |
| M6       | 94.3±1.9              | 1.76       | 96.3±1.1  | 1.07       | 93.6±1.2  | 1.08       |
|          | 80                   | 92.4±0.2   | 0.20       | 94.4±2.1   | 1.48       | 92.3±3.9   | 3.47       |
|          | 1.6                  | 105±4      | 4.21       | 101±1      | 1.35       | 107±6      | 4.18       |
| M7       | 97.1±1.1              | 1.31       | 100±2     | 2.30       | 103±1     | 1.01       |
|          | 160                  | 97.5±0.1   | 0.11       | 98.8±1.6   | 1.58       | 101±3      | 2.66       |
|          | 0.8                  | 90.1±2.7   | 2.44       | 95.3±2.5   | 2.43       | 94.2±2.0   | 2.02       |
| M8 | 8   | 96.8±0.6 | 0.59 | 99.9±2.7 | 2.05 | 99.5±0.8 | 0.82 |
|----|-----|----------|------|----------|------|----------|------|
|    | 80  | 97.5±0.2 | 0.19 | 100±2   | 1.79 | 96.9±2.7 | 2.57 |
|    | 0.2 | 88.2±3.5 | 3.14 | 92.8±3.2 | 2.97 | 88.3±3.0 | 2.74 |
| M9 | 2   | 94.6±1.0 | 0.95 | 96.7±1.3 | 1.22 | 97.1±1.1 | 1.07 |
|    | 20  | 105±0    | 0.40 | 101±3   | 2.86 | 103±1    | 1.29 |
|    | 0.8 | 88.6±4.3 | 3.93 | 92.7±2.4 | 2.24 | 88.9±3.2 | 2.90 |
| M10| 8   | 95.1±1.0 | 0.97 | 97.3±1.4 | 1.38 | 97.0±1.5 | 1.43 |
|    | 80  | 96.6±0.2 | 0.23 | 98.2±1.8 | 1.77 | 96.4±2.8 | 2.68 |
|    | 0.08| 97.8±4.9 | 4.69 | 97.1±2.5 | 2.40 | 97.0±2.3 | 2.19 |
| M11| 0.8 | 94.1±2.0 | 1.85 | 95.8±1.6 | 1.48 | 96.7±0.6 | 0.62 |
|    | 8   | 93.5±1.4 | 1.28 | 97.7±3.1 | 2.91 | 96.3±2.8 | 2.68 |

M1-11 represented geniposidic acid, neochlorogenic acid, chlorogenic acid, caffeic acid, geniposide, genipin, pinoresinol di-α-glucopyranoside, syringaresinol di-α-glucopyranoside, isochlorogenic acid A, pinoresinol α-glucopyranoside, and isochlorogenic acid C, respectively.
Table S5. The variable importance parameter (VIP) of 11 CdQMs between two types of *Eucommiae Cortex* from same and different origin places by OPLS-DA analysis.

| compounds                        | VIP         | same origin places | different origin places |
|----------------------------------|-------------|--------------------|-------------------------|
| genipin                          | 5.41589     | 6.75122            |
| pinoresinol di-<i>o</i>-glucopyranoside | 5.05593     | 6.27523            |
| geniposide                       | 5.57087     | 5.94117            |
| chlorogenic acid                 | 4.81987     | 4.97134            |
| caffeic acid                     | 4.06297     | 2.84501            |
| pinoresinol <i>o</i>-glucopyranoside | 3.48873     | 3.26732            |
| isochlorogenic acid C            | 2.75739     | 2.55568            |
| geniposidic acid                | 2.02841     | 5.41920            |
| neochlorogenic acid              | 1.87003     | 1.84273            |
| syringaresinol di-<i>o</i>-glucopyranoside | 1.77705     | 2.18564            |
| isochlorogenic acid A            | 1.60547     | 1.48496            |