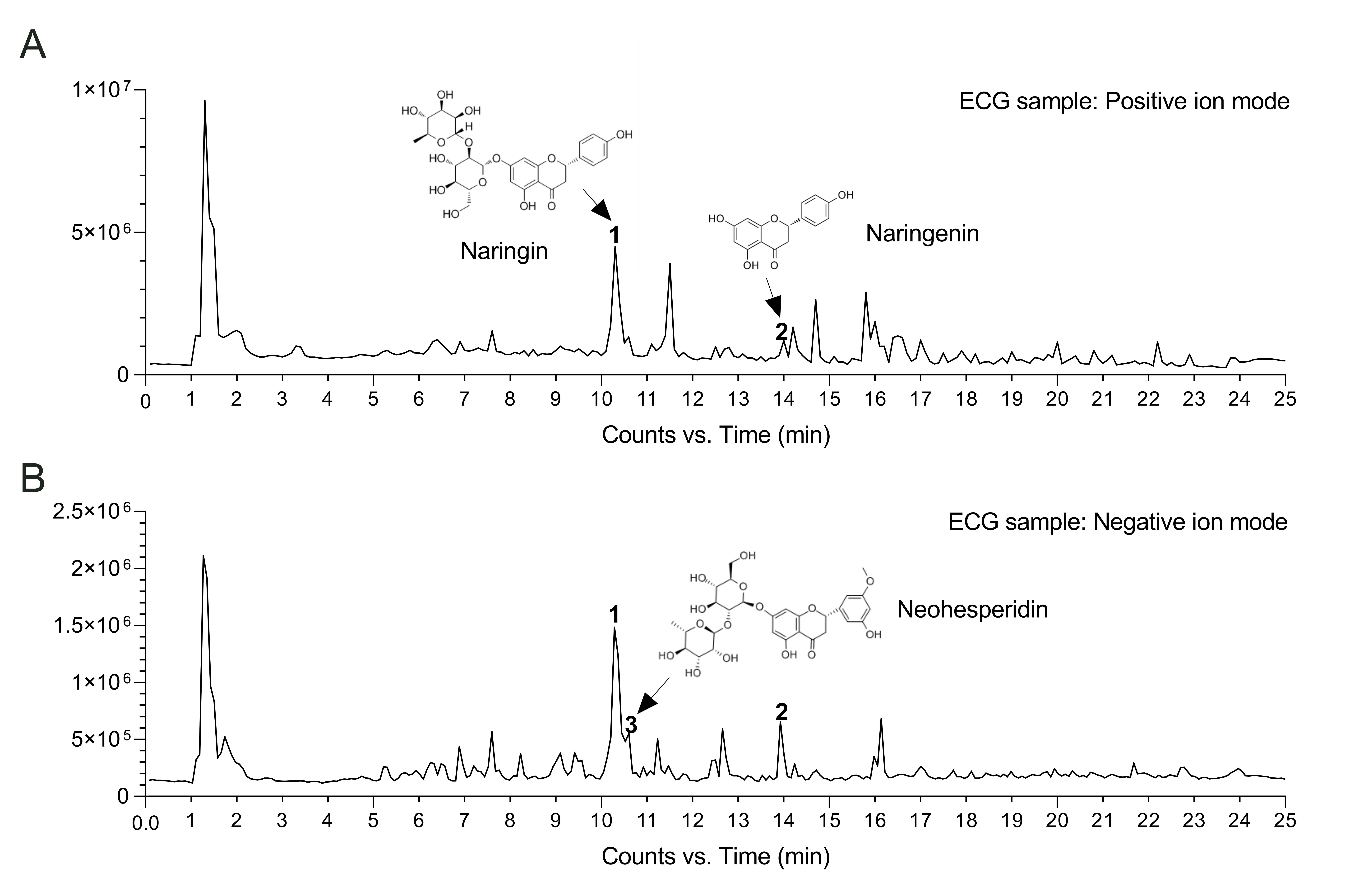
**Supplementary information**

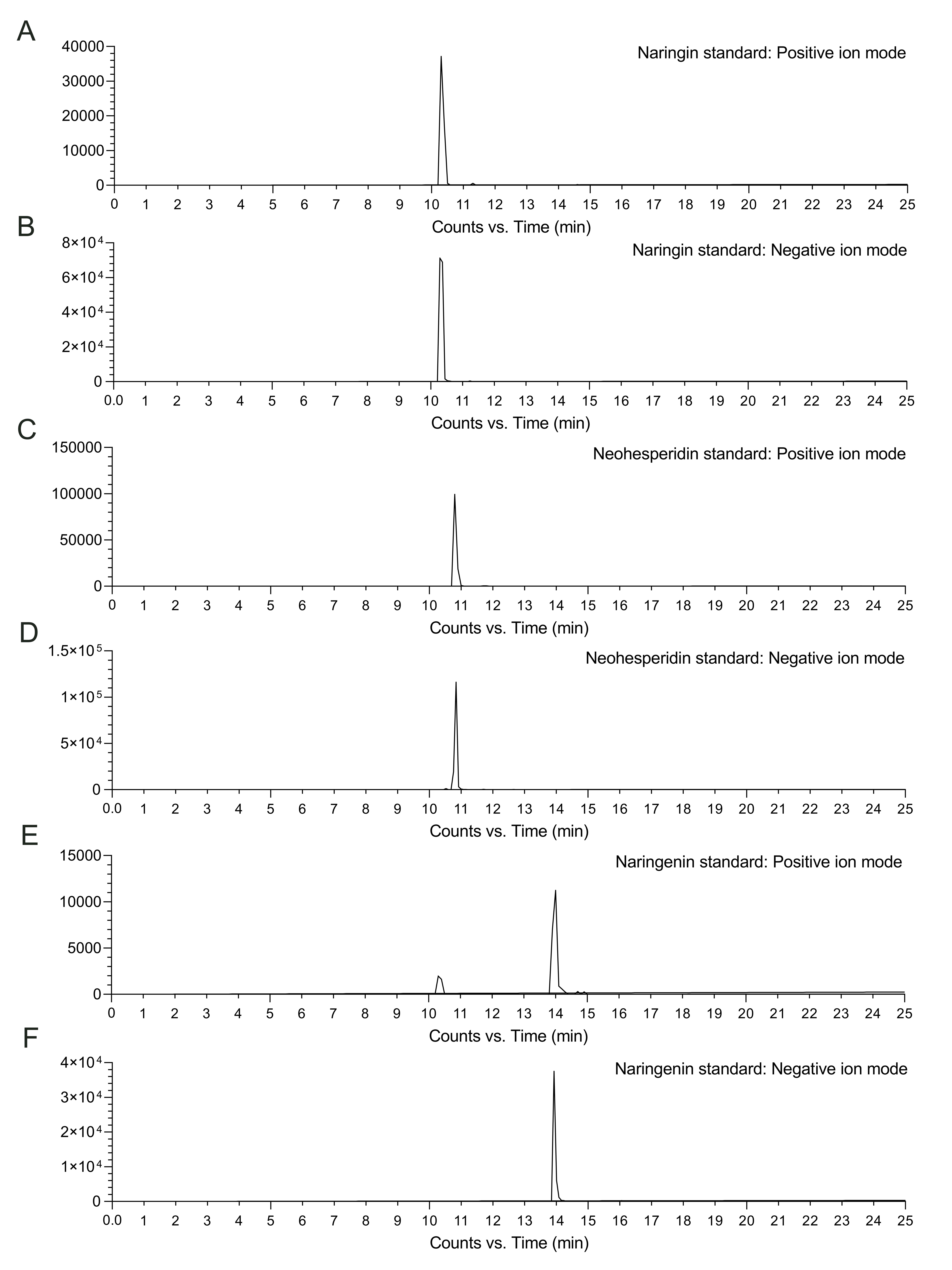
Supplementary methods

**UPLC-Q/TOF-MS/MS analysis**

The ECG sample (0.1001 g) was dissolved through sonication with 10 mL of 50% ethanol in water (v/v) and filtered using a 0.22 μm filter membrane. Chromatographic separation was performed using a Waters ACQUITY BEH C18 (2.1 × 100mm, 1.7μm) at 30 ℃ and a flow rate of 0.2 mL/min with 0.1% formic acid (A) and acetonitrile (B). The gradient elution was as follows: 0-2 min: 5% B; 2-10 min: 5-30% B; 10-15 min: 30-50% B; 15-20 min: 50-80% B; 20-22 min: 80% B; 22-25 min: 5% B. The MS analysis was carried out using a positive and negative ion mode equipped with an Agilent Dual AJS ESI source. The parameters of optimized MS were as follows: The drying gas (N2) temperature was 350 °C, the atomizing gas (N2) pressure was 35 psi, the dry gas (N2) flow rate was 8 L/min, the sheath gas temperature and flow rate were 350 °C and 11 L/min, respectively, the electrospray voltage was 3500 V, the capillary outlet voltage was 150 V, the cone hole voltage was 65 V, the octupole voltage was 750 V, the scanning range: *m/z* 100-1000, and the collision energy were 10, 20, 40 eV. In addition, naringin, neohesperidin, and naringenin (purity ≥ 98%) of reference standards were also detected.

****

**Supplementary Fig. 1.** ECG sample was determined by UPLC-Q/TOF-MS/MS. (A) Positive ion mode. (B) Negative ion mode. (Identification No.: 1. Naringin; 2. Naringenin; 3. Neohesperidin).

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**Supplementary Fig. 2.** Naringin, neohesperidin, and naringenin of reference standards determined by UPLC-Q/TOF-MS/MS. (A-B) Positive and negative ion mode of naringin standard. (C-D) Positive and negative ion mode of neohesperidin standard. (E-F) Positive and negative ion mode of naringenin standard.

**Supplementary Table 1.** Primary antibodies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibodies** | **Host** | **Catalog No.** | **Dilution** | **Source** |
| TLR4 | Rabbit | 19811-1-AP | 1:500 | Proteintech |
| MyD88 | Rabbit | 4283 | 1:1000 | CST |
| NF-κB p65 | Rabbit | 8242 | 1:1000 | CST |
| NF-κB p-p65 (S536) | Rabbit | ab76302 | 1:1000 | Abcam |
| IκBα | Rabbit | ab32518 | 1:1000 | Abcam |
| p- IκBα (S36) | Rabbit | ab133462 | 1:1000 | Abcam |
| COX2 | Rabbit | 12282 | 1:1000 | CST |
| ICAM1 | Goat | AF796 | 1:1000 | R&D |
| iNOS | Rabbit | ab204017 | 1:1000 | Abcam |
| Bcl-2 | Rabbit | 2870 | 1:1000 | CST |
| Bax | Rabbit | 2772 | 1:1000 | CST |
| Caspase3 | Rabbit | 9662 | 1:1000 | CST |
| Caspase8 | Rabbit | WL03426 | 1:1000 | Wanleibio |
| Caspase9 | Mouse | 9508 | 1:1000 | CST |
| β-actin | Rabbit | YT0099 | 1:10000 | Immunoway |

**Supplementary Table 2.** Box sizes of Molecular docking

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **PDB\_ID** | **Protomol Grid** | **Atom number** |
| MyD88 | 4DOM | 21×23×22 | 220 |
| 4EO7 | 18×18×18 | 135 |
| 7BER | 19×18×24 | 204 |
| NF-κB p65 | 1NFI | 27×26×32 | 1185 |
| 5URN | 19×20×19 | 293 |
| 6QHM | 19×19×17 | 152 |
| Caspase9 | 1JXQ | 26×24×23 | 592 |
| 1NW9 | 22×19×21 | 199 |
| 4RHW | 33×31×30 | 2052 |
| Caspase3 | 1NME | 17×18×15 | 83 |
| 3KJF | 17×19×20 | 158 |
| 5IAE | 21×30×22 | 467 |

**Supplementary Table 3.** Active ingredients of ECG screened by the TCMSP.

|  |  |  |  |
| --- | --- | --- | --- |
| **MOL ID** | **Molecule Name** | **OB (%)** | **DL** |
| MOL013279 | 5,7,4'-Trimethylapigenin | 39.83 | 0.30 |
| MOL013277 | Isosinensetin | 51.15 | 0.44 |
| MOL013276 | Poncirin | 36.55 | 0.74 |
| MOL010267 | LYC | 32.57 | 0.51 |
| MOL005849 | Didymin | 38.55 | 0.24 |
| MOL005828 | Nobiletin | 61.67 | 0.52 |
| MOL004328 | Naringenin | 59.29 | 0.21 |
| MOL001803 | Sinensetin | 50.56 | 0.45 |
| MOL001798 | Neohesperidin\_qt | 71.17 | 0.27 |
| MOL000358 | beta-sitosterol | 36.91 | 0.75 |

**Supplementary Table 4.** Annotation of KEGG pathways.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Term ID** | **Description** | **Count** | **P value** | **Gene Name** |
| hsa04725 | Cholinergic synapse | 13 | 2.16E-13 | *CHRM2, CHRM3, ACHE, CHRM1, CHRM4, CHRNA7, PRKCA, PIK3CG, CREB1, BCL2, AKT1, MAPK1, MAPK3* |
| hsa05215 | Prostate cancer | 11 | 3.79E-11 | *AR, CREB1, HSP90AB1, GSTP1, BCL2, MAPK1, AKT1, TP53, MMP9, RELA, MAPK3* |
| hsa05200 | Pathways in cancer | 18 | 2.92E-10 | *TGFB1, HSP90AB1, NOS2, GSTP1, F2R, PRKCA, PTGS2, MMP9, RELA, AR, MAPK8, CASP3, BCL2, AKT1, MAPK1, PPARG, TP53, MAPK3* |
| hsa05161 | Hepatitis B | 12 | 3.40E-10 | *MAPK8, TGFB1, CREB1, CASP3, BCL2, MAPK1, AKT1, PRKCA, TP53, MMP9, RELA, MAPK3* |
| hsa05417 | Lipid and atherosclerosis | 13 | 4.57E-10 | *HSP90AB1, NOS3, PRKCA, MMP9, RELA, MAPK8, CASP3, BCL2, AKT1, MAPK1, PPARG, TP53, MAPK3* |
| hsa04926 | Relaxin signaling pathway | 11 | 6.67E-10 | *MAPK8, TGFB1, CREB1, NOS2, NOS3, MAPK1, AKT1, PRKCA, MMP9, RELA, MAPK3* |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 10 | 1.39E-09 | *MAPK8, TGFB1, NOS3, CASP3, BCL2, MAPK1, AKT1, PRKCA, RELA, MAPK3* |
| hsa05145 | Toxoplasmosis | 10 | 3.83E-09 | *MAPK8, TGFB1, NOS2, CASP3, BCL2, MAPK1, AKT1, RELA, PIK3CG, MAPK3* |
| hsa05207 | Chemical carcinogenesis - receptor activation | 12 | 5.96E-09 | *AR, CREB1, HSP90AB1, CHRNA7, BCL2, MAPK1, AKT1, PGR, PRKCA, ADRB2, RELA, MAPK3* |
| hsa04071 | Sphingolipid signaling pathway | 10 | 6.58E-09 | *ABCC1, MAPK8, NOS3, BCL2, MAPK1, AKT1, PRKCA, TP53, RELA, MAPK3* |
| hsa04151 | PI3K-Akt signaling pathway | 14 | 1.27E-08 | *CHRM2, CHRM1, HSP90AB1, NOS3, F2R, PRKCA, PIK3CG, RELA, CREB1, BCL2, AKT1, MAPK1, TP53, MAPK3* |
| hsa04915 | Estrogen signaling pathway | 10 | 2.43E-08 | *CREB1, HSP90AB1, NOS3, BCL2, MAPK1, AKT1, PGR, OPRM1, MMP9, MAPK3* |
| hsa04066 | HIF-1 signaling pathway | 9 | 6.36E-08 | *NOS2, NOS3, BCL2, MAPK1, AKT1, PRKCA, TIMP1, RELA, MAPK3* |
| hsa01524 | Platinum drug resistance | 8 | 7.21E-08 | *TOP2A, CASP3, GSTP1, BCL2, MAPK1, AKT1, TP53, MAPK3* |
| hsa04668 | TNF signaling pathway | 9 | 7.87E-08 | *MAPK8, CREB1, CASP3, MAPK1, AKT1, PTGS2, MMP9, RELA, MAPK3* |
| hsa05210 | Colorectal cancer | 8 | 2.26E-07 | *MAPK8, TGFB1, CASP3, BCL2, MAPK1, AKT1, TP53, MAPK3* |
| hsa05152 | Tuberculosis | 10 | 2.42E-07 | *MAPK8, TGFB1, CREB1, NOS2, CASP3, BCL2, MAPK1, AKT1, RELA, MAPK3* |
| hsa04657 | IL-17 signaling pathway | 8 | 4.19E-07 | *MAPK8, HSP90AB1, CASP3, MAPK1, PTGS2, MMP9, RELA, MAPK3* |
| hsa05418 | Fluid shear stress and atherosclerosis | 9 | 4.21E-07 | *MAPK8, HSP90AB1, NOS3, GSTP1, BCL2, AKT1, TP53, MMP9, RELA* |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection | 10 | 4.58E-07 | *MAPK8, CREB1, CASP3, MAPK1, AKT1, PTGS2, TP53, RELA, PIK3CG, MAPK3* |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **NO.** | **Time** | **Selected Ion** | **Elemental composition** | **Calculated mass** | **Mass error** | **(ESI+) MS/MS Fragmentation** | **(ESI-) MS/MS Fragmentation** | **Identification** |
| 1 | 10.26 | [M + H] + | C27H32O14 | 581.1865 | 0.7 | 527.1539, 435.1280, 419.1327, 315.0855, 273.0753, 129.0541 |  | Naringin |
| 2 | 10.59 | [M - H] - | C28H34O15 | 609.1825 | 1.3 |  | 301.0725 | Neohesperidin |
| 3 | 13.97 | [M + H] + | C15H12O5 | 273.0757 | 3.7 | 153.0180, 147.0439 |  | Naringenin |

**Supplementary Table 5.** Naringin, naringenin, and neohesperidin from ECG were identified by UPLC-Q/TOF-MS/MS analysis.

**Supplementary Table 6.** The total score of PDB\_ID of MyD88 binding to ECG active ingredients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | Total-score | | |
| 4DOM | 4EO7 | 7BER |
| Neohesperidin | MyD88 (Q99836) | 9.2265 | 7.9567 | 7.204 |
| Didymin | 7.1928 | 8.3729 | 7.6593 |
| Poncirin | 11.8925 | 8.2297 | 10.5308 |
| Isosinensetin | 7.8375 | 8.3152 | 8.4118 |
| Sinensetin | 7.3119 | 10.8304 | 6.0095 |
| Nobiletin | 8.1882 | 5.6779 | 7.0691 |
| 5,7,4'-Trimethylapigenin | 6.2752 | 8.7024 | 7.1334 |
| Naringenin | 5.3191 | 6.938 | 7.2349 |
| beta-sitosterol | 5.9223 | 5.9127 | 5.8175 |
| Naringin |  | 11.0242 | 8.3090 | 8.8077 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | PDB\_ID | Total-score | crash | polar | C-score |
| Neohesperidin | MyD88 (Q99836) | 4DOM | 9.2265 | -3.0512 | 2.9483 | 1 |
| Didymin | 4EO7 | 8.3729 | -1.6114 | 6.2194 | 3 |
| Poncirin | 4DOM | 11.8925 | -4.5916 | 5.027 | 4 |
| Isosinensetin | 7BER | 8.4118 | -1.6738 | 3.2961 | 2 |
| Sinensetin | 4EO7 | 10.8304 | -1.8573 | 3.9871 | 0 |
| Nobiletin | 4DOM | 8.1882 | -1.6714 | 1.077 | 0 |
| 5,7,4'-Trimethylapigenin | 4EO7 | 8.7024 | -1.7047 | 3.9026 | 0 |
| Naringenin | 7BER | 7.2349 | -0.9162 | 3.1372 | 0 |
| Naringin |  | 4DOM | 11.0242 | -4.0989 | 4.5135 | 3 |

**Supplementary Table 7.** The affinity of ECG active ingredients with MyD88.

**Supplementary Table 8.** The total score of PDB\_ID of NF-κB p65/RELA binding to ECG active ingredients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | Total-score | | |
| 1NFI | 5URN | 6QHM |
| Neohesperidin | NF-κB p65 (Q04206) | 10.4792 | 10.9476 | 9.0944 |
| Didymin | 12.1736 | 11.3985 | 8.1779 |
| Poncirin | 13.2215 | 10.6528 | 10.208 |
| Isosinensetin | 7.6416 | 7.2636 | 9.0543 |
| Sinensetin | 8.5939 | 7.7494 | 8.9273 |
| Nobiletin | 9.4246 | 7.6573 | 8.3364 |
| 5,7,4'-Trimethylapigenin | 5.8957 | 6.0821 | 7.9944 |
| Naringenin | 6.8948 | 8.0717 | 6.7958 |
| beta-sitosterol | 8.7535 | 8.2191 | 6.7255 |
| Naringin |  | 11.7323 | 13.5422 | 8.1704 |

**Supplementary Table 9.** The affinity of ECG active ingredients with NF-κB p65/RELA.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | PDB\_ID | Total-score | crash | polar | C-score |
| Neohesperidin | NF-κB p65 (Q04206) | 5URN | 10.9476 | -4.3416 | 5.8186 | 2 |
| Didymin | 1NFI | 12.1736 | -1.7904 | 9.5388 | 1 |
| Poncirin | 1NFI | 13.2215 | -2.3722 | 10.0005 | 3 |
| Isosinensetin | 6QHM | 9.0543 | -1.7031 | 3.0508 | 1 |
| Sinensetin | 6QHM | 8.9273 | -0.9834 | 4.1815 | 0 |
| Nobiletin | 1NFI | 9.4246 | -1.0811 | 2.1469 | 2 |
| 5,7,4'-Trimethylapigenin | 6QHM | 7.9944 | -1.3059 | 1.7809 | 0 |
| Naringenin | 5URN | 8.0717 | -1.2733 | 4.147 | 2 |
| beta-sitosterol | 1NFI | 8.7535 | -1.0456 | 1.4456 | 4 |
| Naringin |  | 5URN | 13.5422 | -2.0219 | 8.9128 | 3 |

**Supplementary Table 10.** The total score of PDB\_ID of Casepase9 binding to ECG active ingredients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | Total-score | | |
| 1NW9 | 4RHW | 1JXQ |
| Neohesperidin | Caspase9 (P55211) | 7.05 | 14.1072 | 15.4065 |
| Didymin | 9.2543 | 12.9809 | 16.4275 |
| Poncirin | 6.7177 | 14.0568 | 14.1671 |
| Isosinensetin | 6.6698 | 6.6295 | 7.8775 |
| Sinensetin | 5.0318 | 7.559 | 8.2425 |
| Nobiletin | 7.1891 | 8.5411 | 8.564 |
| 5,7,4'-Trimethylapigenin | 6.0238 | 6.77 | 8.5546 |
| Naringenin | 7.2965 | 8.905 | 7.3337 |
| beta-sitosterol | 4.0083 | 10.1386 | 9.308 |
| Naringin |  | 6.2394 | 16.3090 | 10.6933 |

**Supplementary Table 11.** The affinity of ECG active ingredients with Caspase9.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | PDB\_ID | Total-score | crash | polar | C-score |
| Neohesperidin | Caspase9 (P55211) | 1JXQ | 15.4065 | -2.745 | 10.9839 | 2 |
| Didymin | 1JXQ | 16.4275 | -2.8719 | 9.8484 | 5 |
| Poncirin | 1JXQ | 14.1671 | -3.4306 | 7.6517 | 2 |
| Isosinensetin | 1JXQ | 7.8775 | -1.2271 | 2.1406 | 1 |
| Sinensetin | 1JXQ | 8.2425 | -2.2573 | 1.0662 | 2 |
| Nobiletin | 1JXQ | 8.564 | -0.8674 | 2.0227 | 1 |
| 5,7,4'-Trimethylapigenin | 1JXQ | 8.5546 | -1.9672 | 1.9225 | 1 |
| Naringenin | 4RHW | 8.905 | -0.2909 | 6.091 | 3 |
| beta-sitosterol | 4RHW | 10.1386 | -1.7721 | 2.3762 | 4 |
| Naringin |  | 1JXQ | 16.3090 | -3.3116 | 13.6348 | 3 |

**Supplementary Table 12.** The total score of PDB\_ID of Casepase3 binding to ECG active ingredients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | Total-score | | |
| 3KJF | 1NME | 5IAE |
| Neohesperidin | Caspase3 (P42574) | 8.947 | 9.4456 | 8.4479 |
| Didymin | 9.0235 | 6.0213 | 11.5655 |
| Poncirin | 7.9688 | 7.5492 | 9.3318 |
| Isosinensetin | 8.6667 | 7.2326 | 6.018 |
| Sinensetin | 7.1348 | 6.0334 | 6.9857 |
| Nobiletin | 8.5085 | 8.418 | 6.0197 |
| 5,7,4'-Trimethylapigenin | 6.7836 | 6.3799 | 6.6918 |
| Naringenin | 7.6193 | 6.1121 | 7.9554 |
| beta-sitosterol | 5.6407 | 4.2199 | 10.2167 |
| Naringin |  | 8.6563 | 8.0762 | 10.6233 |

**Supplementary Table 13.** The affinity of ECG active ingredients with Caspase3.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Molecule Name | Protein (Uniprot\_ID) | PDB\_ID | Total-score | crash | polar | C-score |
| Neohesperidin | Caspase3 (P42574) | 5IAE | 8.4479 | -1.3979 | 3.6832 | 4 |
| Didymin | 5IAE | 11.5655 | -2.508 | 6.9908 | 0 |
| Poncirin | 5IAE | 9.3318 | -1.9024 | 2.7801 | 5 |
| Isosinensetin | 3KJF | 8.6667 | -2.8045 | 1.8825 | 1 |
| Sinensetin | 3KJF | 7.1348 | -2.3315 | 1.7317 | 3 |
| Nobiletin | 3KJF | 8.5085 | -2.161 | 2.0806 | 2 |
| 5,7,4’-Trimethylapigenin | 3KJF | 6.7836 | -0.9624 | 1.8234 | 1 |
| Naringenin | 5IAE | 7.9554 | -1.4026 | 4.638 | 2 |
| beta-sitosterol | 5IAE | 10.2167 | -1.1761 | 0 | 0 |
| Naringin |  | 5IAE | 10.6233 | -3.0502 | 3.9337 | 0 |

**Supplementary Table 14.** The energy contribution of the amino acid residues in neohesperidin-NF-κB p65 complex (kcal/mol).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Residue | MM Energy | Polar Energy | APolar Energy | Total Binding energy |
| ILE 8 | -5.1652 | 1.901 | -0.1986 | -3.4713 |
| GLU 10 | -36.4242 | 63.4202 | -1.1452 | 25.7635 |
| LYS 11 | -20.4165 | 25.9011 | -0.9186 | 4.5747 |
| MET 58 | -0.8228 | 0.9628 | -0.0629 | 0.0713 |
| ARG 86 | -3.4601 | 5.5367 | -0.0392 | 2.0351 |
| HIS 87 | 0.085 | -0.555 | -0.0057 | -0.4768 |
| MET 88 | -10.9183 | 8.6636 | -1.165 | -3.427 |
| SER 535 | -13.1808 | 7.7945 | -0.8958 | -6.2828 |
| ALA 538 | -5.5889 | -0.1005 | -0.4884 | -6.1824 |

**Supplementary Table 15.** The energy contribution of the amino acid residues in the naringenin-NF-κB p65 complex (kcal/mol).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Residue | MM Energy | Polar Energy | APolar Energy | Total Binding energy |
| GLU10 | -6.5918 | 7.1605 | -0.6695 | -0.1422 |
| MET88 | -13.2582 | 6.1838 | -1.1914 | -8.258 |
| ASP531 | -1.0888 | 0.2481 | 0 | -0.8299 |

**Supplementary Table 16.** The energy contribution of the amino acid residues in neohesperidin-Caspase3 complex (kcal/mol).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Residue | MM Energy | Polar Energy | APolar Energy | Total Binding energy |
| GLU 124 | -7.8276 | 16.8005 | -0.8736 | 8.1054 |
| 1.736 | 0.8107 | -0.4942 | 2.0309 |
| GLY 125 | -1.8699 | 0.4082 | -0.2831 | -1.7426 |
| -1.8888 | 0.8688 | -0.2268 | -1.2437 |
| LYS 137 | -0.8766 | -0.0434 | -0.4287 | -1.367 |
| GLU 167 | -0.5384 | 0.852 | -0.0336 | 0.2912 |
| TYR 195 | -11.0548 | 4.2523 | -0.6707 | -7.4419 |
| TYR 197 | -3.3409 | 4.7668 | -0.3271 | 1.1066 |
| PRO 201 | -14.15 | 7.5177 | -1.2704 | -7.932 |
| -5.2973 | 1.3014 | -0.5665 | -4.5642 |

**Supplementary Table 17.** The energy contribution of the amino acid residues in the naringenin-Caspase3 complex (kcal/mol).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Residue | MM Energy | Polar Energy | APolar Energy | Total Binding energy |
| GLU 124 | -16.5042 | 27.1338 | -0.5084 | 10.1101 |
| GLY 125 | -2.5541 | 1.3247 | -0.2976 | -1.5287 |
| LEU 136 | -8.3707 | 1.7725 | -0.3337 | -6.9455 |
| LYS 137 | -15.5161 | 34.0438 | -1.1915 | 17.3003 |
| GLU 190 | -3.212 | 12.0639 | -0.1558 | 8.7066 |
| TYR 195 | -7.3428 | 3.3882 | -0.4201 | -4.3589 |
| PRO 201 | -12.6917 | 7.3716 | -0.7853 | -6.1003 |