**Supporting information**

Table S1. PCR primers used in this study

|  |  |
| --- | --- |
| Primers  | Sequences (5′ to 3′)  |
| TwUGT2-F | ATGACTCTGTTAGGCACGGGAACC |
| TwUGT2-R | TCATTCCAAAGCAGCCAAAAGATTT |

Table S2. Other plant UGTs included in phylogenetic tree.

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Species | Type of Synthase | Genebank accession number |
| Am4CGT | *Antirrhinum majus* | chalcone 4'-O-glucosyltransferase | BAE48239 |
| Lv4CGT | *Linaria vulgaris* | chalcone 4'-glucosyltransferase | BAE48240 |
| PlUGT1 | *Pueraria montana var. lobata* | isoflavone 7-O-glucosyltransferase | AGZ84545 |
| PlUGT2 | *Pueraria montana var. lobata* | isoflavone 4' 7-O-glucosyltransferase | AMQ26112 |
| GT04F14 | *Pueraria montana var. lobata* | isoflavone 7-O-glucosyltransferase | ADV71364 |
| UBGT | *Scutellaria baicalensis* | flavonoid 7-O-glucosyltransferase | BAA83484 |
| FaGT7 | *Fragaria x ananassa* | flavonol 3-O-glucosyltransferase | Q2V6J9 |
| PpUFGT | *Prunus persica* | flavonoid 3-O-glucosyltransferase | ARW73634 |
| pNgt2 | *Ipomoea nil* | coumarin glucosyltransferase | BAM63146 |
| CsUGT2 | *Citrus sinensis* | terpenoid glycosyltransferase | ACS87991 |
| EPGT1 | *Eucalyptus perriniana* | monoterpene glucosyltransferase | BAD90934 |
| EPGT2 | *Eucalyptus perriniana* | monoterpene glucosyltransferase | BAD90935 |
| UGT74M1 | *Gypsophila vaccaria* | triterpene carboxylic acid glucosyltransferase | ABK76266 |
| UGT74T1 | *Linum usitatissimum* | lignan glucosyltransferase | AGD95008 |
| UGT74S1 | *Linum usitatissimum* | lignan glucosyltransferase | AGD95005 |
| AtSGT | *Arabidopsis thaliana* | sterol glucosyltransferase | CAB06082 |
| SGTL1 | *Withania somnifera* | sterol glucosyltransferase | ABC96116 |
| UGT52 | *Dictyostelium discoideum* | sterol glucosyltransferase | AAD28546 |
| UGT51C1 | *Candida albicans* | sterol glucosyltransferase | AAD29571 |
| UGT51B1 | *Komagataella pastoris* | sterol glucosyltransferase | AAD29570 |

Table S3. UPLC conditions of detecting sugar acceptors and corresponding glycosylated products in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| Chemical Component | Solvent A | Solvent B | Gradient |
| Quercetin and Isoquercitrin | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Pinocembrin and Pinocembrin 7-O-beta-D-glucoside | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Genistein | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Liquiritigenin | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Kaempferol | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Phloretin | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Daidzein | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Luteolin | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| 4-Methylumbelliferone | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-90%(A)4 - 8 min: 90%(A)-70%(A)8 -12 min: 70%(A)-30%(A) |
|  |  |  |  |
| Nodakenitin | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-80%(A)4 -10 min:80%(A)-40%(A)10-15 min:40%(A)-20%(A) |
|  |  |  |  |
| Arctigenin  | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Rhapontigenin | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-75%(A)4 - 8 min: 75%(A)-45%(A)8 -12 min: 45%(A)-40%(A) |
|  |  |  |  |
| Triptolide | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-90%(A)4 - 7 min: 90%(A)-70%(A)7 -12 min: 70%(A)-40%(A)12 -13 min: 40%(A)-0%(A)13 -18 min: 0%(A)-0%(A) |
|  |  |  |  |
| Celastrol | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-90%(A)4 - 7 min: 90%(A)-30%(A)7 - 9 min: 30%(A) - 0%(A)9 -15 min: 0%(A)-0%(A) |
|  |  |  |  |
| Triptophenolide | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-70%(A)4 - 7 min: 70%(A)-65%(A)7 - 9 min: 65%(A) -25%(A)9 -10 min: 25%(A)-0%(A)10 -15 min: 0%(A)-0%(A) |
|  |  |  |  |
| Neotriptophenolide | 0.1% trifluoroacetic acid-water | Acetonitrile | 0-3.5 min: 95%(A)-95%(A)3.5-4 min: 95%(A)-70%(A)4 - 7 min: 70%(A)-65%(A)7 – 9 min: 65%(A) -25%(A)9 -10 min: 25%(A)-0%(A)10 -15 min: 0%(A)-0%(A) |



Fig. S1. The 3D structure prediction of TwUGT2 using homology-based modelling. The protein 5nlm.1. A (indoxyl UDP-glucosyltransferase) was used as a template for the 3D modelling of TwUGT2.



Fig. S2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electropherogram of recombinant protein expressed in *E. coli* BL21(DE3). M: Protein MW standard; TwUGT2: purified recombinant protein.



Fig. S3. MS and MS2 of the authentic chemical standards: A (isoquercitrin) and B (pinocembrin 7-O-beta-D-glucoside). (a) MS of the authentic chemical standard; (b) MS2 of the authentic chemical standard.