Investigating the Catalytic Activity of Glycosyltransferase on Quercetin from *Tripterygium wilfordii*

Jie Gao, † Baowei Ma, † Yun Lu, †‡ Yifeng Zhang, †‡ Yuru Tong, †§ Siyuan Guo, † Wei Gao,*, †‡§ and Luqi Huang†∥

†School of Traditional Chinese Medicine, †School of Pharmaceutical Sciences, and §Advanced Innovation Center for Human Brain Protection, Capital Medical University, Beijing 100069, China
∥State Key Laboratory of Dao-di Herbs, National Resource Center for Chinese Materia Medica, Chinese Academy of Chinese Medical Sciences, Beijing 100700, China

ABSTRACT: Flavonoid glycosides have shown many pharmacological activities in clinical studies. However, the main way to obtain flavonoid glycosides is to extract and separate them from plants, which wastes both time and resources. Here, we identified the O-glycosyltransferase (UGTs) TwUGT3 from *Tripterygium wilfordii* and analyzed its bioinformatics. First, the enzyme was found to utilize phloretin and uridine diphosphate glucose (UDPG) as substrates to produce an acid-tolerant glucoside. Then, it also can use quercetin and UDPG as substrates to produce the corresponding O-glucoside. In addition, we further explored the substrate specificity of TwUGT3, which suggested that it also accepts luteolin, pinocembrin, and genistein to produce the corresponding O-glucosides. Subsequently, the optimum pH, reaction time, reaction temperature, and enzymatic kinetic parameters of TwUGT3 were determined.

INTRODUCTION

At the time of writing, more than 8000 flavonoids and their derivatives were known to be widely distributed in plants, many of which are associated with potential benefits for human health. They act as pigments or signal compounds in various parts of plant organs and have multiple functions. For example, they are involved in UV-B protection and plant—microbe interactions, while also providing antifungal and antibacterial activity. Furthermore, the antioxidant activity of flavonoids is closely related to human health, which has attracted widespread attention.

Glycosylation is one of the major modifications of phytochemicals. It plays vital roles in many physiological characteristics and functions of plants and is also a common modification of flavonoids. Glycosylases can change the solubility, stability, and other physical and physiological characteristics of their substrates and are consequently considered to play a role in the bioactivity of modified plant metabolites and the accumulation of flavonoids. Studies have indicated that there are two main types of glycosylation: O-glycosylation and C-glycosylation. At present, glycosylation is mainly achieved by glycosyltransferase catalysis, which has played a key role in the discovery and development of drugs. In addition, flavonoids have good biological activities after glycosylation, including antioxidant, antifungal, anti-inflammatory, and antihypertensive activities, and similar effects.

*Tripterygium wilfordii* Hook f. is a plant from the Euonymus family, Celastraceae, which had been used as a traditional medicinal plant for centuries in China. It is used to treat fever, chills, edema, and suppuration. Modern studies have shown that triptolide and its related substances are the main active constituents of *Tripterygium*. These substances showed broad-spectrum anti-inflammatory, immunosuppressive, anti-diuretic, and anticancer activities and were mainly used to treat autoimmune diseases, such as rheumatoid arthritis and systemic psoriasis.

In this study, we cloned the glycosyltransferase TwUGT3 from *T. wilfordii*, expressed it, and analyzed the substrate specificity of the recombinant enzyme.

RESULTS

Sequence and Molecular Phylogenetic Analysis. A full-length coding sequence for UDP-glycosyltransferase was identified by searching against the NCBI nonredundant protein sequence database, the NCBI nonredundant nucleotide sequence database, the Protein Family database, the
Through domain analysis, we obtained the conservative site molecular weight of 50.8 kDa and a theoretical pI of 5.41. TwUGT3 had an open reading frame (ORF) of 1380 bp, encoding a protein of 459 amino acids with a molecular weight of 50.8 kDa and a theoretical pI of 5.41. Subsequently, based on the full-length sequence, we designed primers and cloned the TwUGT3 glycosyltransferase gene. The sequence analysis indicated that TwUGT3 had an open reading frame (ORF) of 1380 bp, encoding a protein of 459 amino acids with a molecular weight of 50.8 kDa and a theoretical pI of 5.41. Through domain analysis, we obtained the conservative site (335−378) of the TwUGT3 gene. To analyze the specific triptophanolide glycosyltransferase, the PRABI-LG service was utilized to predict the secondary structure of TwUGT3. The result indicated that the protein had an alpha helix content of 43.57%, along with 15.47% extended strands and 40.96% random coils. Subsequently, a three-level model of TwUGT3 protein was constructed using SWISS-MODEL (Figure S1).

Subsequently, a molecular tree (Figure 1) was established based on the translated amino acid sequences of TwUGT3 and other reported genes screened from NCBI. The results of molecular phylogenetic analysis indicated that TwUGT3 belongs to the CGT (OG15) family. The enzymes from the OG15 family that convert 2′,4′,6′-trihydroxyacetophenone-like structures formed a clade. Subsequently, the PSPG-motif of TwUGT3 was found by multiple sequence alignment (Figure 1). Therefore, phloretin, which has a 2′,4′,6′-trihydroxyacetophenone-like structure, was selected as the initial substrate for the preliminary experiments. As illustrated in Figure 2a, there was a new product (peak 2) that was detected in the reaction using a crude enzyme (Figure 2a). Subsequently, the product was detected after incubation with hydrochloric acid for 3 h at 60 °C (Figure 2a). Therefore, it is speculated that the product is an acid-resistant hydrolyzed glycoside. The analysis results of UPLC/Q-TOF-MS (ultrahigh-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry) indicated that the molecular ion peak of the product may be 597.18, and the speculated product may be combined with two gluco-oses in phloretin. However, no products were detected in the reaction using a purified enzyme (Figure 2b). Related experiments such as enzymatic kinetics require pure enzymes to detect, so quercetin was chosen as a new substrate for subsequent experiments.

**Verification of Enzyme Activity.** To confirm the activity of the enzyme TwUGT3, we cloned the full-length cDNA into pMAL-c2x with a maltose-binding protein (MBP) tag and introduced it into Escherichia coli. By culturing the recombinant E. coli with the target gene, we obtained the crude enzyme for enzymatic reactions in vitro. The results (Figure 3a) indicated that the recombinant TwUGT3 had O-glycosylation activity.

We cultured strains of E. coli, respectively, harboring the recombinant plasmid and the blank vector and obtained corresponding protein extracts, which were then used to catalyze the glycosylation of quercetin in vitro, and the products were analyzed via UPLC. The chromatogram obtained using the recombinant strain (Figure 3a) contained a major peak with a retention time of 8.674 min (peak 2), which was similar to that of standard quercetin (8.658 min). Furthermore, the chromatogram contained a peak at 7.384 min which was similar to that of isoquercitrin, in addition to the major peak of quercetin (peak 2). Our results therefore revealed that the recombinant enzyme TwUGT3 displayed glycosyltransferase activity.

**Protein Purification and Characterization of the Catalytic Products of the Pure Enzyme.** After confirming the catalytic activity of the crude enzyme via UPLC analysis, we next attempted to purify the UGT enzyme using the solubility-promoting MBP tag and explored the catalytic properties of the recombinant TwUGT3. First, we obtained the pure enzyme, as confirmed by the results of sodium
dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis shown in Figure 3b.

As illustrated in Figure 3b, we analyzed the purified protein using markers with a molecular weight range from 35 to 180 kDa. The proteins of the blank vector strain were mainly concentrated in the parts with a molecular weight of less than 50 kDa. In contrast, the molecular weight of the recombinant protein was about 90 kDa. Analysis of SDS-PAGE showed that we obtained relatively pure recombinant protein and could further study the enzymatic reaction of the pure enzyme.

Subsequently, we carried out reactions using the pure enzyme in vitro with quercetin and UDPG as substrates and analyzed the structure of the product by UPLC/Q-TOF-MS. As expected, we discovered a peak that exhibited the $[M - H]^{-}$ at $m/z$ 463.0921, corresponding to a glucoside of quercetin (Figure 3c). A fragment ion of $[M - H - 162]^{-}$ at $m/z$ 301.0343 was also detected in the mass spectrum (Figure 3c), corresponding to the $[M - H]^{-}$ of quercetin. The retention time was similar to that of the standard compound, suggesting that the product is isoquercitrin.

**Enzyme Assays.** To confirm the $K_m$ value of TwUGT3, the factors that may affect enzyme activity were investigated, such as pH, temperature, reaction time, and so forth. The optimum temperature and pH of purified TwUGT3 was 35 °C and 7.0, respectively, under the conditions of glycosylation for 24 h (Figure 4). The kinetic parameters of recombinant TwUGT3 were measured by using a GraphPad Prism 7.00, and the $K_m$ and $V_{max}$ values were 64.1 $\mu$mol and $1.557 \times 10^{-2}$ $\mu$mol/min/mg, respectively.

**Substrate Specificity of TwUGT3.** To confirm the substrate specificity of the recombinant enzyme TwUGT3, we further screened different types of compounds as substrates. Several terpenoids from *T. wilfordii*, tetracyclic triterpenoids, coumarins, lignanoids, flavonoids, and stilbene (Table 1) were selected as substrates for in vitro enzymatic activity tests with the pure recombinant enzyme TwUGT3 and UDP-glucose. Seventeen compounds were selected as substrates for enzymatic reactions in vitro, but the UPLC/Q-TOF-MS analysis (Figure 5) indicated that the recombinant enzyme only catalyzed the production of luteolin 3-O-$\beta$-D-glucosidurano-
side, pinocembrin 7-O-β-D-glucoside, and sophoricoside from luteolin, pinocembrin, and genistein, respectively. The recombinant enzyme displayed signs of O-glycosylation activity toward some flavonoids, such as quercetin and luteolin. In contrast, it did not convert terpenoids, suggesting that flavone-like structures are necessary for substrates to be acceptable by TwUGT3. The catalytic activity of the enzyme TwUGT3 for luteolin, pinocembrin, and genistein was low. It could only be judged whether it has any catalytic activity by extracting the characteristic peak of the corresponding glycoside from the mass spectrum.

**MATERIALS AND METHODS**

**Plant Material.** *T. wilfordii* suspension cells were cultured in the Murashige and Skoog (MS) medium containing 0.5 mg·L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg·L⁻¹ indole-3-butyric acid, 0.1 mg·L⁻¹ kinetin, and 30 g·L⁻¹ sucrose at 25 °C in a rotary shaker at 120 rpm in the dark.

**Cloning and Sequence Analysis of TwUGT3.** Total RNA was extracted from *T. wilfordii* suspension cells utilizing the Eastep Super Total RNA Extraction Kit (Promega Biological Technology Co. Ltd., Beijing, China). RNAs were checked by electrophoresis on a 1.2% agarose gel and visualized using a Vilber Lourmat imaging system. The FastKing RT Kit (TIANGEN Biotech, Beijing, China) was used to synthesize cDNA according to manufacturer’s protocol. The primers TwUGT3R (CTAC-CAGTGTGTTCCACTACG) and TwUGT3F (ATGTCGGACTCCGGTGACTT) were designed using Primer Premier 5.0 software (Premier, BC, Canada) and synthesized by the Ruibo Biotech Company (Beijing, China). The glycosyltransferase gene was cloned from the cDNA library using the 2× KAPA HiFi HotStart ReadyMix. PCR amplification was performed with an initial 5 min step at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 58.5 °C, and 60 s at 72 °C, and a final 7 min step at 72 °C. The GeneJET Gel Extraction Kit (Thermo Fisher Scientific, USA) was used to purify the PCR products, which were ligated into the pEASY-Blunt Zero cloning vector (TransGen Biotech, Beijing, China). *E. coli* Trans1-T1 phase-resistant cells (TransGen

![Figure 3. Analysis of TwUGT3 recombinant protein reaction results of quercetin and purification of the enzyme. (a) UPLC analysis of the recombinant TwUGT3 reaction products of quercetin. Each panel shows a chromatogram from the following reaction conditions: with standard compounds; without enzyme; and with crude enzyme. The eluates were monitored at 350 nm using a diode array detector. (b) SDS-PAGE analysis of proteins from *T. wilfordii* purified O-glucosyltransferase. 1, protein marker; 2, purified recombinant enzyme TwUGT3. Arrow indicates the purified TwUGT3. (c) UPLC/Q-TOF-MS analysis of the recombinant TwUGT3 reaction products of quercetin. Each panel shows a chromatogram from the following reaction conditions: with standard compounds; without enzyme; and with pure enzyme. In vitro enzyme assay for determining the activity of the candidate TwUGT3 by UPLC/Q-TOF-MS using quercetin as a substrate, compared with retention time and mass spectrometry of authentic standards. 1, isoquercitrin and 2, quercetin.](image-url)
Biotech, Beijing, China) were transformed with the ligated vector and cultured in the Luria-Bertani (LB) medium with 100 mg/L ampicillin at 37 °C in the dark. Then, the Beijing Ruibo Biotech Company used an ABI 370XL automated sequencer (Applied Biosystems, USA) to sequence the positive individual bacterial clones, and the resulting sequence was aligned with the transcriptome sequence.

**Phylogenetic and Bioinformatic Analysis.** The sequence of TwUGT3 was analyzed (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the ORFs were identified online (https://www.ncbi.nlm.nih.gov/orffinder/). The calculate molecular weight (Mw) and theoretical isoelectric point (pI) were analyzed using the Compute pI/Mw tool (http://Web.ExPASy.org/compute_pi/). PRABI-GERIAND (https://npsa-prabi.ibcp.fr/) was used to predict the secondary structure of the recombinant enzyme. SWISS-MODEL (https://swissmodel.expasy.org/) is used to construct the protein tertiary model. Amino acid sequences of similar enzymes were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). The multiple sequence alignment was carried out using DNAMAN, and the phylogenetic tree was established using the MEGA 6.0 with the neighbor-joining method, with 1000 replicates and the p-distance model.

**Heterologous Expression of TwUGT3.** The expression plasmid pMAL-c2x-TwUGT3 and a the pMAL-c2x control plasmid were used to transform E. coli BL21 (DE3) competent cells (TransGen Biotech, Beijing, China). Subsequently, 300 μL of an overnight culture of the cells were added into a 300 mL LB medium with 100 μg/L ampicillin. The bacterial cultures were shaken (250 rpm) at 37 °C until the OD600 reached 0.6 and 0.8. Then, 300 μL of 1 M isopropyl β-D-thiogalactoside (IPTG) was added to induce the E. coli, which was shaken (200 rpm) at 16 °C for 16 h. The cells were harvested by centrifugal separation (13 000 g, 2 min, 4 °C), the supernatant was discarded, the cells were shock-frozen in liquid nitrogen, and stored at −80 °C.

**Enzyme Purification.** The bacterial cells taken from the −80 °C freezer were thawed on ice and resuspended in 5 mL of resuspension buffer [50 mM Tris-HCl, 0.1 mM ethylene-diaminetetraacetic acid (EDTA), 150 mM NaCl, 1 mM diithiothreitol (DTT), 5% glycerol, and 1 mM phenylmethylsulfonyl fluoride, pH 7.5]. After resuspension, the cells were mixed with chicken egg white lysozyme (0.5 mg·mL⁻¹) and lysed on ice for 20 min. Triton X-100 (0.1%) and NaCl (0.5 mol·L⁻¹) were added to the mixture and sonicated in an ice bath for 10 s, with pausing for 10 s. Subsequently, the supernatant after centrifugation (12 000g, 4 °C) for 30 min was mixed with 1.0 mL of amylose resin (New England Biolabs, Beijing, China) and shaken for 2 h. Subsequently, the

---

**Figure 4.** Effects of various pH values (a), temperatures (b), and reaction time (c) on the optimum enzyme activity of TwUGT3, determination of kinetic parameters for TwUGT3 (d). UDPG was used as a sugar donor, and quercetin was used as a substrate. (a) I, II, III, and IV correspond to citric acid—sodium citrate buffer, Na2HPO4—NaH2PO4 buffer, Tris-HCl buffer and s Na2CO3—NaHCO3 buffer. X-axis shows that the highest activity of TwUGT3 is 100% at different temperature, reaction time, and pH value, and the other values are the relative activity of the enzyme.

| substrates               | enzyme activity |
|-------------------------|-----------------|
| terpenoids in *T. wilfordii* | ND⁴               |
| triptolide              | ND              |
| celastrol               | ND              |
| triptophenolide         | ND              |
| neotriptophenolide      | ND              |
| coumarin                | ND              |
| 4-methylumbelliferone   | ND              |
| marmesin                | ND              |
| lignanoid               | ND              |
| arctigenin              | ND              |
| stilbene               | ND              |
| raphontigenin           | ND              |
| tetracyclic triterpenoids | ND               |
| (20R)-protopanaxadiol   | ND              |
| (20S)-protopanaxadiol   | ND              |
| flavonoids              | ND              |
| daidzein                | ND              |
| kaempferol              | ND              |
| phloretin               | ND              |
| quercetin               | ND              |
| luteolin                | ND              |
| pinocembrin             | ND              |
| genistein               | ND              |

“ND: no detected. †: catalytic activity.”
resin was washed six times with 15 mL of washing buffer (50 mM Tris-HCl, 0.1 mM EDTA, 500 mM NaCl, 1 mM DTT, and 5% glycerol, pH 7.5) and 15 mL of resuspension buffer (50 mM Tris-HCl, 0.1 mM EDTA, 150 mM NaCl, 1 mM DTT, and 5% glycerol, pH 7.5). In the next step, 2 mL of elution buffer A (50 mM Tris-HCl, 0.1 mM EDTA, 150 mM NaCl, 1 mM DTT, 2 mM maltose, and 5% glycerol, pH 7.5) and 5 mL of elution buffer B (50 mM Tris-HCl, 0.1 mM EDTA, 150 mM NaCl, 1 mM DTT, 10 mM maltose, and 5% glycerol, pH 7.5) were used to wash the resin. Finally, the elution was concentrated to less than 500 μL using Amicon Ultra-30K filters (Millipore, Merck KGaA, USA) and stored at −80 °C. The enzyme purity was determined by SDS-PAGE, and the concentration was measured using a modified Bradford Protein Assay Kit (Sangon Biotech, Shanghai, China).

**In Vitro Enzyme Assays of TwUGT3.** The in vitro enzymatic reaction system was composed of a 100 μL reaction solution comprising 100 mM Tris-HCl buffer (pH 7.5), 1 mM sugar donor (uridine diphosphate glucose, UDPG), 100 μM substrate, and 50 μg of purified recombinant TwUGT3 protein. The reaction system was incubated at 35 °C for 24 h, and the reaction was stopped by adding 200 μL of HPLC-grade methanol. Finally, the products were detected by UPLC-molecular weight determination/electrospray ionization-mass spectrometry (UPLC-MWD/ESI-MS/MS).

To determine the optimal pH, we routinely conducted three parallel assays. The reactions mixtures containing 50 μg of purified recombinant TwUGT3 protein, 1 mM UDPG, and 100 μM quercetin were prepared in buffers with various pH values (4.0–6.0: 100 mM citric acid–sodium citrate buffer; 6.0–8.0: 100 mM Na2HPO4–NaH2PO4 buffer; 7.0–9.0: 100 mM Tris-HCl buffer; and 9.0–11.0: 100 mM Na2CO3–NaHCO3 buffer) and incubated at 35 °C for 24 h.

To confirm the optimal temperature, we routinely conducted three parallel assays. The enzymatic reactions were performed at different temperatures (20, 25, 30, 35, 40, 45, 50, 55, 60, and 70 °C) for 24 h; the reaction mixtures consisted of 100 mM Tris-HCl buffer (pH 7.0), 1 mM UDPG, and 100 μM quercetin.

To indicate the optimal reaction times, we routinely conducted three parallel assays. The reaction times were set at 1, 2, 3, 4, 5, 8, 10, 24, and 48 h; the mixture contained 100 mM Tris-HCl buffer (pH 7.0), 1 mM UDPG, and 100 μM quercetin.

To analyze the kinetic parameters, hyperbolic Michaelis–Menten saturation curves for the substrates were analyzed using the GraphPad Prism 7.0 with the peak areas of the compounds. The reaction was conducted in Tris-HCl buffer (pH 7.0) with 1 mM UDPG and quercetin (40–400 μM) at 35 °C for 13 h. The reactions were stopped with 200 μL of
MeOH, and the reaction products of TwUGT3 catalysis were quantified by UPLC-diode array detector (UPLC-DAD).

**DISCUSSION**

Isoquercitrin, a flavonoid derived from the medicinal plant *Apocynum venetum* L., is widely distributed in plants, fruits, and flowers.38,39 Isoquercitrin possesses many biological activities, such as antioxidant activities, free radical scavenging activities, neuroprotective effects, anti-inflammatory effects, antihypertensive effects, antitumor effects, diuretic effects, and so on.36–38 At present, isoquercitrin mainly removes rhamnose from rutin by hydrolysis of Venus kinase. However, many byproducts and other small metabolites will be produced in this way, which will make the subsequent separation and purification process more difficult.36–38 Therefore, the gene obtained in this experiment can be used to construct the corresponding biosynthetic pathway for isoquercitrin.

According to the result of the crude enzyme reaction, TwUGT3 has a strong activity on phloretin, and after terminating the reaction, phloretin can be completely converted into a product. The product did not change after terminating the reaction, and TwUGT3 can efficiently catalyze phlorizin to produce acid-tolerant glucosides. This is the first discovery of a glycosyltransferase from *T. wilfordii*, which can catalyze the production of acid-resistant glucoside from flavonoids. His-tag and MBP tag are two commonly used in the prokaryotic expression system of protein. pET-32a (+) with His-tag was used to express TwUGT3 recombinant protein. However, because of the continuous formation of protein inclusion body, it was decided to use pMAL-c2x with MBP-tag to express TwUGT3. MBP-tag is a soluble protein label, which can increase the solubility of fusion protein. However, because of its high molecular weight, about 40 kDa, it has a certain impact on the structure of protein. TwUGT3 was found to bind to MBP tag to form fusion protein, which may affect its activity to potential flavonoids. In addition, when exploring the optimum reaction conditions of TwUGT3, we found that TwUGT3 was too sensitive to the change of the pH value, possibly because MBP tags affected the structure of fusion protein, so the pH value had a great influence on protein activity. Comparing the enzymatic reaction results of pure enzyme and crude enzyme, it is speculated that it may be due to the MBP-tag, which affects the structure of TwUGT3 protein resulting in that the product cannot be detected. So far, the glycosyltransferase in *T. wilfordii* is rarely reported. Therefore, the extraction of glucosides in *T. wilfordii* is still in the exploration stage. At present, the main glucosides that can be formed in *T. wilfordii* are terpenoids, such as triptolide.49 The C-glycosides in *T. wilfordii* have not been found. Therefore, although flavonoids are rarely found in *T. wilfordii*, TwUGT3 obtained from *T. wilfordii* has a strong specificity for flavonoids and is of great significance.49 Therefore, the construction of a compound biosynthetic chassis cell for industrial production should not be limited to the species in which the compound is located. This knowledge can expand the research horizon to identify the right genes.

**ASSOCIATED CONTENT**

# Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02919.

Abbreviations and genbank accession numbers of the gene used in bioinformatics analysis, PCR primers used in this study, UPLC conditions of detecting sugar acceptors and corresponding glycosylated products in this study, 3D structure prediction of TwUGT3 using SWISS-MODEL, UPLC/Q-TOF-MS analysis of the recombinant TwUGT3 reaction products of luteolin, UPLC/Q-TOF-MS analysis of the recombinant TwUGT3 reaction products of pinocembrin, and UPLC/Q-TOF-MS analysis of the recombinant TwUGT3 reaction products of genistein (PDF)

**Accession Codes**

Sequence data from this article can be found in the GenBank data libraries with the accession number: TwUGT3 (MN165635).

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: weigao@ccmu.edu.cn (W.G.).
*E-mail: huangluqi01@126.com (L.H.).

**ORCID**

Jie Gao: 0000-0002-8434-1069
Wei Gao: 0000-0003-9819-9642

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

Author W.G. received funding from the High-level Teachers in Beijing Municipal Universities in the Period of 13th Five-year Plan (CIT&TCD201707324) and the National Program for Special Support of Eminent Professionals. Author L.H. received funding from the Key Project at a central government level: The ability establishment of sustainable use for valuable Chinese medicine resources (2060302).

**REFERENCES**

(1) Pietta, P.-G. Flavonoids as antioxidants. *J. Nat. Prod.* 2000, 63, 1035–1042.
(2) Jiang, N.; Joseff, A.; Grote, A.; Groten, E. Flavones: From Biosynthesis to Health Benefits. *Plants* 2016, 5, 27.
(3) Orhan, D. D.; Özel, B.; Orgen, S.; Ergun, F. Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiol. Res.* 2010, 165, 496–504.
(4) Pollastri, S.; Tattini, M. Flavonols: old compounds for old roles. *Ann. Bot.* 2011, 108, 1225–1233.
(5) Hassan, S.; Mathesius, U. The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant-microbe interactions. *J. Exp. Bot.* 2012, 63, 3429–3444.
(6) Bowles, D.; Isayenkova, J.; Lim, E.-K.; Poppenberger, B. Glycosyltransferases: managers of small molecules. *Curr. Opin. Plant Biol.* 2005, 8, 254–263.
(7) Gachon, C. M. M.; Langlois-Meurinne, M.; Saindrenan, P. Plant secondary metabolism glycosylation transferases: the emerging functional analysis. *Trends Plant Sci.* 2005, 10, 542–549.
(8) Xiao, J.; Muzashvili, T. S.; Georgiev, M. I. Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnol. Adv.* 2014, 32, 1145–1156.
(9) Koirala, N.; Pandey, R. P.; Parajuli, P.; Jung, H. H.; Sohn, J. K. Methylation and subsequent glycosylation of 7,8-dihydroxyflavone. *J. Biotechnol.* 2014, 184, 128–137.
(10) Lee, H. S.; Thorson, J. S. Development of a universal glycosyltransferase assay amenable to high-throughput formats. *Anal. Biochem.* 2011, 418, 85–88.
(11) Williams, G. J.; Zhang, C.; Thorson, J. S. Expanding the promiscuity of a natural-product glycosyltransferase by directed evolution. Nat. Chem. Biol. 2007, 3, 657–662.

(12) Watanabe, M. An anthocyanin compound in buckwheat sprouts and its contribution to antioxidant capacity. BioSci, Biotechnol., Biochem. 2007, 71, 579–582.

(13) McNally, D. J.; Wurm, V. Y.; Labbé, C.; Quideau, S.; Bélanger, R. B. Complex C-glycosyl flavonoid phytoalexins from Cucumis sativus. J. Nat. Prod. 2003, 66, 1280–1283.

(14) Zhang, Y.; Bao, B.; Lu, B.; Ren, Y.; Tie, X.; Zhang, Y. Determination of flavone C-glucosides in antioxidant of bamboo leaves (AOB) fortified foods by reversed-phase high-performance liquid chromatography with ultraviolet diode array detection. J. Chromatogr. A 2005, 1070, 177–185.

(15) Prabhakar, M.; Bano, H.; Kumar, I.; Shamsi, M.; Khan, S. Pharmacological Investigations on Vxinix. Planta Med. 2007, 43, 396–403.

(16) Tu, S. H. [Difficulties and countermeasures in treatment of rheumatoid arthritis with Tripterygium]. Zhongguo Zhongxiyi Jiehe Zazhi 2009, 29, 104–105.

(17) Helmstädt, A. Tripterygium wilfordii Hook. f. - how a traditional Taiwanese medicinal plant found its way to the West. Pharmazie 2013, 68, 643–646.

(18) Tao, X.; Lipsky, P. E. The Chinese anti-inflammatory and immunosuppressive remedy Tripterygium wilfordii Hook F. Rheum. Dis. Clin. North Am. 2000, 26, 29–50.

(19) Wu, C.; Jin, H.-Z.; Shu, D.; Li, F.; He, C.-X.; Qiao, J.; Yu, X.-L.; Zhang, Y.; He, Y.-B.; Liu, T.-J. Efficacy and Safety of Tripterygium wilfordii Hook F Versus Acitretin in Moderate Severe Psoriasis Vulgaris: A Randomized Clinical Trial. Chin. Med. J. 2015, 128, 443–449.

(20) Liu, Y.; Tu, S.; Gao, W.; Wang, Y.; Liu, P.; Hu, Y.; Dong, H. Extracts of Tripterygium wilfordii Hook F in the Treatment of Rheumatoid Arthritis: A Systemic Review and Meta-Analysis of Randomised Controlled Trials. Evidence-Based Complementary Altern. Med. 2013, 2013, 410793.

(21) Manzo, S. G.; Zhou, Z.-L.; Wang, Y.-Q.; Marinello, J.; He, J.-X.; Li, Y.-C.; Ding, J.; Caprancio, G.; Miao, Z.-H. Natural product triptolide mediates cancer cell death by triggering CKD7-dependent degradation of RNA polymerase II. Cancer Res. 2012, 72, 5363–5373.

(22) Chugh, R.; Sangwan, V.; Patil, S. P.; Dudge, V.; Dawra, R. K.; Bhat, S.; Schmacher, R. J.; Blazar, B. R.; Georg, G. L.; Vickers, S. M.; Saluja, A. K. A preclinical evaluation of Minneldle as a therapeutic agent against pancreatic cancer. Sci. Transl. Med. 2012, 4, 156ra139.

(23) Chen, Y.-W.; Lin, G.; Jia, W.; Lin, C.-K.; Chen, D.; Sytwu, H.-K. Triptolide exerts anti-tumor effect on oral cancer and its contribution to antioxidant capacity. 2007 Biochem. Sobreiro, M. A.; Conrado, M. A.; Priolli, D. G.; Frankland Sawaya, A. C. H.; Ruiz, A. L. T. G.; de Carvalho, J. E.; de Oliveira Carvalho, P. Enzymatic de-glycosylation of rutin improves its antioxidant and antiproliferative effects. Food Chem. 2013, 141, 266–273.

(25) Leuenroth, S. J.; Bencivenga, N.; Chahbourn, H.; Hyde, F.; Crews, C. M. Triptolide reduces cyst formation in a neonatal to adult transition Pkd1 model of ADPKD. Nephrol., Dial., Transplant. 2010, 25, 2187–2194.

(26) Titov, D. V.; Gilman, B.; He, Q.-L.; Bhat, S.; Low, W.-K.; Dang, Y.; Smeaton, M.; Demain, A. L.; Miller, P. S.; Kugel, J. F.; Goodrich, J. A.; Liu, J. O. XPB, a subunit of TFIIH, is a target of the natural product triptolide. Nat. Chem. Biol. 2011, 7, 182–188.

(27) Reuten, R.; Nikodemus, D.; Oliveira, M. B.; Patel, T. R.; Brachvogel, B.; Breloy, I.; Stetefeld, J.; Koch, M. Maltoose-Binding Protein (MBP), a Secretion-Enhancing Tag for Mammalian Protein Expression Systems. PLoS One 2016, 11, No. e0152386.

(28) Valentičová, K.; Vrbá, J.; Bencivová, M.; Ultrichová, J.; Kien, V. Isoquercitrin: Pharmacology, toxicology, and metabolism. Food Chem. Toxicol. 2014, 68, 267–282.

(29) Xie, W.; Zhang, X.; Wang, T.; Hu, J. Botany, traditional uses, phytochemistry and pharmacology of Apocynum venustum L. (Luobuma): A review. J. Ethnopharmacol. 2012, 141, 1–8.

(30) Hassan, W.; Rongyin, G.; Daoud, A.; Ding, L.; Wang, L.; Liu, J.; Shang, J. Reduced oxidative stress contributes to the lipid lowering effects of isoquercitrin in free fatty acids induced hepatocytes. Oxid. Med. Cell. Longevity 2015, 2015, 313602.

(31) Junior, A. G.; Gasparotto, F. M.; Lourenço, E. L. B.; Crestani, S.; Stefanello, M. E. A.; Salvador, M. J.; da Silva-Santos, J. E.; Marques, M. C. A.; Kugel, J. F.; Goodrich, J. A. Reduced oxidative stress contributes to the lipid lowering effects of isoquercitrin and extracts from Tropaeolum majus L: Evidence for the inhibition of angiogenin converting enzyme. J. Ethnopharmacol. 2011, 134, 363–372.

(32) Junior, A. G.; Prando, T. B. L.; Leme, T. D. S. V.; Gasparotto, F. M.; Lourenço, E. L. B.; Rattmann, Y. D.; Da Silva-Santos, J. E.; Kassuya, C. A. L.; Marques, M. C. A. Mechanisms underlying the diuretic effects of Tropaeolum majus L. extracts and its main component isoquercitrin. J. Ethnopharmacol. 2012, 141, 501–509.

(33) Magalingam, K.; Rajakrishnan, A.; Haleagrana, N. Protective effects of flavonol isoquercitrin, against 6-hydroxy dopamine (6-OHDA) - induced toxicity in PC12 cells. BMC Res. Notes 2014, 7, 49.

(34) Chen, Q.; Li, P.; Li, P.; Yu, X.; Li, Y.; Tang, B. Isoquercitrin inhibits the progression of pancreatic cancer in vivo and in vitro by regulating opioid receptors and the mitogen-activated protein kinase signalling pathway. Oncol. Rep. 2015, 33, 840–848.

(35) Rogerto, A. P.; Kanashiro, A.; Fontanari, C.; da Silva, E. V. G.; Lucisano-Valim, Y. M.; Soares, E. G.; Faccioli, L. H. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflammation Res. 2007, 56, 402–408.

(36) de Araújo, M. E. M. B.; Moreira Franco, Y. E.; Alves, O. T. G.; Sobreiro, M. A.; Conrado, M. A.; Priolli, D. G.; Frankland Sawaya, A. C. H.; Ruiz, A. L. T. G.; de Carvalho, J. E.; de Oliveira Carvalho, P. Enzymatic de-glycosylation of rutin improves its antioxidant and antiproliferative effects. Food Chem. 2013, 141, 266–273.