

# Region-selective biosynthesis of artemisinic acid glycosides by crown galls of *Panax quinquefolium* and their *in vitro* antitumor activities

Jianhua Zhu<sup>1,2</sup>, Lijia Chen<sup>1</sup>, Xianjing Hu<sup>3</sup>, Liyan Song<sup>3</sup>, Mingxuan Wang<sup>1</sup>, Rongmin Yu<sup>1,2</sup>

<sup>1</sup>Biotechnological Institute of Chinese Materia Medica, Jinan University, <sup>2</sup>Departments of Natural Medicinal Chemistry and <sup>3</sup>Pharmacology, Jinan University, Guangzhou 510632, China

Submitted: 18-08-2014

Revised: 26-09-2014

Published: 10-07-2015

## ABSTRACT

**Background:** The biosynthesis of artemisinin derivatives is one of the interesting subjects. Artemisinic acid (AA) has been widely studied as a supposed intermediate in the biosynthetic pathway leading to artemisinin in *Artemisia annua*. **Objective:** To investigate the bioconversion of AA by transgenic crown galls of *Panax quinquefolium*. **Materials and Methods:** AA was administered into crown galls of *P. quinquefolium* and co-cultured for 2 days. The methanol extract was separated by column chromatography, and the structures of two biosynthesis products were elucidated by physicochemical and spectroscopic methods. Co-culture time curves on conversion were also established. In addition, the effects of AA on the growth and ginsenosides production of crown galls of *P. quinquefolium* were investigated. Furthermore, the *in vitro* antitumor activities of AA and two glycosides against HepG2 cell line were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. **Results:** Glycosylation of AA by crown galls of *P. quinquefolium* was observed, and two region-selectively glycosylated products were obtained (AA-1, AA-2), involving one new compound (AA-2). Their structures were elucidated to be AA  $\beta$ -D-glucopyranosyl ester (AA-1) and AA  $\beta$ -D-glucopyranosyl-(2  $\rightarrow$  1)- $\beta$ -D-glucopyranosyl ester (AA-2). The maximum yield of AA-1 was 19.3% on the 1<sup>st</sup> co-culture day while that of AA-2 was 59.1% on the 2<sup>nd</sup> day. MTT assay showed that the activity of monosaccharide glycoside (AA-1) was better than that of disaccharide glycoside (AA-2). **Conclusion:** Two AA glycosides involving one new compound with potential antitumor activity were obtained by region-selective biosynthesis with crown galls of *P. quinquefolium*.

**Key words:** Antitumor, artemisinic acid, Biosynthesis, crown galls of *Panax quinquefolium*, glycosylation

## INTRODUCTION

Artemisinin, a sesquiterpene lactone with a peroxide bridge extracted from *Artemisia annua*, has been studied extensively because of its significant biological activities, so have been its precursors in the biosynthetic pathway.<sup>[1-4]</sup> Artemisinic acid (AA), which has a similar chemical structure (cadinane-type sesquiterpene) to that of artemisinin, is widely investigated as a presumed intermediate in

the biosynthetic pathway of artemisinin in *A. annua*.<sup>[5-7]</sup> However, there is no other report on the biotransformation of AA by plant tissue cultures except our research group.<sup>[8]</sup> As we know, biotransformation is a useful tool to convert cheap and plentiful organic compounds into more useful ones.<sup>[9]</sup> Plant cultured cells have been studied as potential systems for biotransformation reactions especially for chiral alcohols as intermediates of pharmaceutical and other potential compounds to be used in industrial scale.<sup>[10]</sup> Contrary to calli or suspension culture cells (dedifferentiated cells), differentiated tissues of *Panax ginseng* cultures are very effective for secondary metabolites production. Additionally, tissue culture is found to be more stable in growth during long-term cultivation.<sup>[11]</sup> Therefore, biotransformation of AA by plant tissue cultures is of great interest.

### Address for correspondence:

Dr. Rongmin Yu, Biotechnological Institute of Chinese Materia Medica, Jinan University, Guangzhou 510632, China.  
E-mail: tyrm@jnu.edu.cn  
Dr. Liyan Song, Department of Pharmacology, Jinan University, Guangzhou 510632, China.  
E-mail: tsly@jnu.edu.cn

### Access this article online

#### Website:

www.phcog.com

#### DOI:

10.4103/0973-1296.160460

#### Quick Response Code:



Glycosylation reaction is of special interest because it facilitates the conversion of water-insoluble compounds to those that are more water-soluble.<sup>[12]</sup> Plant tissue/cell cultures play an important role in this regard since it is difficult to perform this reaction by microorganisms or by chemical synthesis.<sup>[13]</sup> In recent years, increasing attention has been paid to glycosylation reactions in plant culture systems.

Crown galls of *Panax quinquefolium* were infected with *Agrobacterium tumefaciens* and the Ti plasmids were introduced into the *P. quinquefolium* cell nuclear genome.<sup>[14]</sup> Compared with callus and cell culture, the crown gall cultures grow faster, produce more active constituents, and are free of exogenous phytohormones.<sup>[11,15,16]</sup> The results of our previous studies demonstrated that the crown galls of *P. quinquefolium* had the capacities to catalyze glycosylation, hydroxylation and methylation reactions.<sup>[16,17]</sup> In order to explore new plant biotransformation system by feeding AA as precursor, and to find out novel artemisinin derivatives with good anti-malaria and/or antitumor activities, the biotransformation of AA by crown galls of *P. quinquefolium* was investigated in the present paper.

Our previously research indicated that AA may act as a kind of elicitor,<sup>[18]</sup> which could increase the production of alkaloids in culture cells of *Catharanthus roseus*. To find out whether AA affects the metabolism of crown galls of *P. quinquefolium*, the relationship between the growth and the production of total ginsenosides with the addition of AA was also investigated.

## MATERIALS AND METHODS

### General

<sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) and two-dimensional NMR spectra were recorded on a Bruker DRX-400 spectrometer, the chemical shifts ( $\delta$ ) were given in ppm relative to tetramethylsilane as an internal standard and coupling constants were given in Hz. Electrospray ionization/mass spectrometer (ESI-MS) data were obtained with a 4000 Q TRAP liquid chromatography/MS/MS (LC/MS/MS) system by direct inlet using MeOH as solvent. Time-of-flight mass spectrometry (HR-TOF-MS) were recorded on SYNAPT<sup>TM</sup> G2 HDMS, Waters, Manchester, U.K. Silica gel (100–200 mesh and 200–300 mesh) used for column chromatography (CC), and Silica GF<sub>254</sub> (10–40  $\mu$ ) for TLC were supplied by the Qingdao Marine Chemical Factory, China. ODS (YMC Co., LTD, Japan) and Sephadex LH-20 (Pharmacia Co.) were also used for the separation of biosynthesis products. High-performance liquid chromatography (HPLC) analysis was performed on a Agilent 1200 LC system (Palo Alto, CA, USA),

equipped with vacuum degasser, quaternary gradient pump, auto-sampler, and diode array detector (DAD), connected to a Agilent ChemStation software (Palo Alto, CA, USA). An Agilent Hypersil ODS column ( $\phi$ 4.6 mm  $\times$  250 mm, 5  $\mu$ m) and guard column (4.6 mm  $\times$  12.5 mm, 5  $\mu$ m) were used. A binary gradient elution system consisted of water (A) and methanol (B) and separation was achieved using the following gradient program: 0–5 min 40–50% B; 5–10 min 50–60% B; 10–15 min 60–70% B; 15–20 min 70–85% B; 20–25 min 100% B, and finally, reconditioning the column with 40% B isocratic for 2 min. The flow rate was 0.8 ml/min, and the system operated at 30°C. The detection wavelength was set at 230 nm.

### Chemical

AA was isolated from *A. annua* by our research group according to the reference.<sup>[19]</sup> The structure of AA was determined by MS and NMR. The purity was > 98% by HPLC analysis.

### Culture of crown galls

The crown galls of *P. quinquefolium* had been sub-cultured routinely every 3 weeks using MS medium. Prior to use for experiments, the cultured crown galls were transplanted to 500-ml Erlenmeyer flasks containing 200 ml of medium and cultured on a rotary shaker (110 rpm) for 14 days at 25°C.

### Biotransformation of AA by crown galls of *Panax quinquefolium*

All experiments were performed in batch flask cultures in 500-ml Erlenmeyer flasks. The ethanol solution (0.1 ml) of AA was administered into a flask containing the suspended crown galls (pre-cultured for 14 days) of *P. quinquefolium* in the medium (200 ml). The cultures were incubated at 25°C on a rotary shaker (110 rpm) for another 2 days. The crown galls and media were separated by filtration with suction. Each filtered medium was extracted with EtOAc, and then extracted with n-BuOH. EtOAc and n-BuOH fractions were analyzed by TLC and HPLC. The crown galls were extracted with MeOH for 12 h and sonicated for 20 min. The MeOH fraction was concentrated and partitioned between H<sub>2</sub>O and EtOAc. EtOAc fractions were combined and analyzed by TLC and HPLC. Biosynthesis products (AA-1 and AA-2) were further purified on CC by silica gel, Sephadex LH-20, and ODS.

### Artemisinin acid $\beta$ -D-glucopyranosyl ester (AA-1)

Colorless needle crystal (MeOH). <sup>1</sup>H NMR (pyridine-d<sub>3</sub>, 400 MHz)  $\delta$  values: 0.78 (3H, d,  $J$  = 6 Hz, Me-14), 1.28 (1H, m, H-8 $\alpha$ ), 1.54 (3H, s, Me-15), 2.85 (1H, br s, H-6), 4.04–4.45 (5H, m, H-2', 3', 4', 5' and 6'), 5.12 (1H, s, H-5), 5.49 (1H, s, H-13 $\alpha$ ), 6.47 (1H, d,  $J$  = 7.6 Hz, H-1'), 6.58 (1H, s, H-13  $\beta$ ). <sup>13</sup>C-NMR (pyridine-d<sub>3</sub>, 100 MHz)

$\delta$  values: 41.3 (C-1), 25.4 (C-2), 26.2 (C-3), 134.4 (C-4), 120.6 (C-5), 37.9 (C-6), 42.5 (C-7), 25.9 (C-8), 35.2 (C-9), 27.4 (C-10), 143.3 (C-11), 168.0 (C-12), 125.3 (C-13), 19.6 (C-14), 23.5 (C-15), 96.2 (C-1'), 74.1 (C-2'), 78.4 (C-3'), 70.7 (C-4'), 79.3 (C-5'), 61.7 (C-6'). ESI-MS:  $M/z$  395 [M-H]<sup>-</sup>, 419 [M + Na]<sup>+</sup>. HR-TOF-MS:  $M/z$  397.3119 [M + H]<sup>+</sup>, calcd. for C<sub>21</sub>H<sub>33</sub>O<sub>7</sub>: 397.3107.

### Artemisinic acid $\beta$ -D-glucopyranosyl-(2 $\rightarrow$ 1)- $\beta$ -D-glucopyranosyl ester (AA-2)

Amorphous solid. <sup>1</sup>H NMR (pyridine-d<sub>5</sub>, 400 MHz)  $\delta$  values: 0.78 (3H, d,  $J$  = 6.4 Hz, Me-14), 1.31 (1H, m, H-8 $\alpha$ ), 1.55 (3H, s, Me-15), 2.71 (1H, br s, H-6), 3.9-4.49 (10H, m, H-2'~6', H-2''~6''), 5.16 (1H, s, H-5), 5.42 (1H, d,  $J$  = 8 Hz, H-1'), 5.73 (1H, s, H-13 $\alpha$ ), 6.38 (1H, d,  $J$  = 7.2 Hz, H-1'), 7.04 (1H, s, H-13  $\beta$ ). <sup>13</sup>C-NMR (pyridine-d<sub>5</sub>, 100 MHz)  $\delta$  values: 41.2 (C-1), 25.4 (C-2), 26.2 (C-3), 134.4 (C-4), 120.7 (C-5), 37.9 (C-6), 42.3 (C-7), 25.9 (C-8), 35.1 (C-9), 27.4 (C-10), 142.6 (C-11), 165.7 (C-12), 126.6 (C-13), 19.6 (C-14), 23.5 (C-15), 94.1 (C-1'), 82.4 (C-2'), 77.9 (C-3'), 70.1 (C-4'), 78.4 (C-5'), 61.4 (C-6'), 106.2 (C-1''), 76.1 (C-2''), 78.0 (C-3''), 71.1 (C-4''), 79.1 (C-5''), 62.3 (C-6''). ESI-MS:  $M/z$  557 [M-H]<sup>-</sup>, 581 [M + Na]<sup>+</sup>. HR-TOF-MS:  $M/z$  581.2579 [M + Na]<sup>+</sup>, calcd. for C<sub>27</sub>H<sub>42</sub>O<sub>12</sub>Na: 581.2574.

### Time-course of biotransformation

Cultured crown galls of *P. quinquefolium* (10 g) were transferred to a 500-ml Erlenmeyer flask containing 200 ml of medium and cultured by continuous shaking for 14 days at 25°C. AA (5 mg/flask) was added to the suspension cultures and incubated at 25°C in a rotary shaker (110 rpm). At 1-day interval, three of the flasks were taken out from the rotary shaker, and the crown galls and media were separated by filtration. The extraction and analysis procedures were the same as those described above. The yields of the products were calculated on the basis of the peak area from HPLC using calibration curves prepared by HPLC-DAD and were expressed as relative percentages to the total amount of whole biosynthesis products.

### Effect of AA on the growth and the production of total ginsenosides of the crown galls

Cultured crown galls of *P. quinquefolium* (10 g) were transferred to a 500-ml Erlenmeyer flask containing 200 ml of medium and cultured by continuous shaking for 14 days at 25°C. AA with different concentrations (10, 20, 30, 40 mg/l) was added to the suspension cultures and incubated at 25°C in a rotary shaker (110 rpm) for 2 days. After 2 days of co-culture, the crown galls and media were separated by filtration. Crown galls were dried under 55°C in the oven. After total elimination of water was achieved, the waterless crown galls were weighed. For the analysis of total ginsenosides, 1 g of crown galls of each group was

taken out and extracted with MeOH for 12 h and sonicated for 20 min. The MeOH fraction was concentrated and defatted by n-hexane and then partitioned between H<sub>2</sub>O and water-saturated n-BuOH. n-BuOH fractions were combined. After evaporation under vacuum, the residue was dissolved in MeOH (10 ml). Total ginsenosides were determined by spectrophotometry using ginsenoside Rb<sub>1</sub> as standard.

### 3 -(4,5 -dimethylthiazol -2 -yl) -2,5 -diphenyl -tetrazolium bromide cell proliferation assay

The inhibitory effects of compounds (AA, AA -1, AA -2) on the growth of HepG2 cells were evaluated *in vitro* by 3 -(4,5 -dimethylthiazol -2 -yl) -2,5 -diphenyl -tetrazolium bromide (MTT) assay, which was performed as described in the literature.<sup>[20]</sup> The concentrations of compounds on the selected cell line were in the range of 0.0185–0.5000  $\mu$ mol/ml.

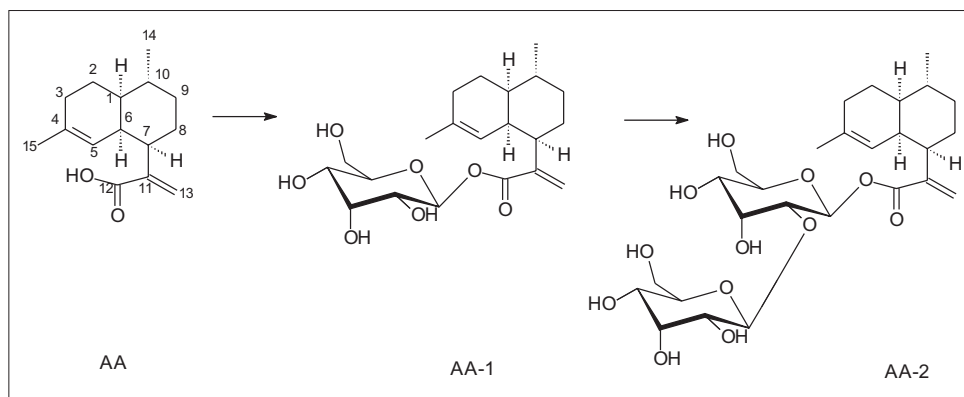
## RESULTS

### Biotransformation of AA by crown galls of *Panax quinquefolium*

Two glycosylated products were obtained (AA-1, AA-2) after AA was administrated into the culture system of crown galls of *P. quinquefolium*.

AA-2 was isolated as a white amorphous solid. The  $m/z$  values at 557.2 [M-H]<sup>-</sup> and 581 [M + Na]<sup>+</sup>, detected by ESI-MS, were used to propose the molecular weight as 558. Its molecular formula was determined as C<sub>27</sub>H<sub>42</sub>O<sub>12</sub> on the basis of HR-TOF-MS ( $m/z$  = 581.2579 [M + Na]<sup>+</sup>, calcd. for C<sub>27</sub>H<sub>42</sub>O<sub>12</sub>Na, 581.2574). The <sup>1</sup>H NMR spectrum exhibited two set of  $\beta$ -glucopyranosyl signals with the resonance for the anomeric protons at  $\delta$  6.38 (1H, d,  $J$  = 7.2 Hz) and  $\delta$  5.42 (1H, d,  $J$  = 8.0 Hz). A comparison of <sup>13</sup>C NMR of AA-2 with that of AA showed that carboxyl carbon signal was shifted by 6.9 ppm (172.6172t. 7), suggesting that AA-2 was an ester. The HMBC spectrum implied the sugar moiety was linked to the carbonyl carbon [ $\delta$  165.7 (C<sub>12</sub>) was correlated with  $\delta_{\text{H}}$  7.04 (1H, s, H-13  $\beta$ ), 6.38 (1H, d,  $J$  = 7.2 Hz) and  $\delta$  5.73 (1H, s, H-13 $\alpha$ )], and the linkage of glucoses was 2 $\rightarrow$ 1 [ $\delta_{\text{C}}$  82.4 (Glc C-2') was correlated with  $\delta_{\text{H}}$  5.42 (1H, d,  $J$  = 8.0 Hz)]. Therefore, the structure of AA-2 was established as AA  $\beta$ -D-glucopyranosyl-(2  $\rightarrow$  1)- $\beta$ -D-glucopyranosyl ester [Figure 1]. AA-2 is a new compound.

Based on the similar structural elucidation procedure, AA-1 was determined to be AA  $\beta$ -D-glucopyranosyl ester [Figure 1] according to the spectra of ESI-MS, HR-TOF-MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR and by comparing the data with those from the literature.<sup>[8]</sup>



**Figure 1:** Biotransformation of artemisinin acid by crown galls of *Panax quinquefolium*

### Time-course of biotransformation

To provide direct evidences for the biotransformation pathway of AA in crown galls of *P. quinquefolium*, the time-courses were established. As indicated in Figure 2, AA disappeared after 2 days of co-culture, meaning that AA was completely transformed. As shown in Figure 2, the yield of AA-1 reached its maximum biotransformation rate (19.3%) on the 1<sup>st</sup> day, and then decreased gradually, indicating that AA-1 might act as an intermediate. For AA-2, the maximum yield (59.1%) appeared on the 2<sup>nd</sup> day, and then reduced in the following 2 days and maintained the balance since the 4<sup>th</sup> day. A possible biosynthetic pathway in the cultures is proposed in Figure 1. As shown in Figure 1, one molecule of glucose was firstly attached to the carboxyl carbon of AA to generate AA-1. Then, another molecule of glucose attached to the C-2' of AA-1 to form AA-2. AA glycosides could be more easily obtained if the reactions stopped at a specific time. The optimal co-culture time for AA-1 and AA-2 was the 1<sup>st</sup> day and the 2<sup>nd</sup> day, respectively after AA was administrated.

### Effect of AA on the growth and the production of total ginsenosides of the crown galls

As shown in Figure 3, AA could accelerate the growth of crown galls of *P. quinquefolium* when the concentration of AA was under 30 mg/l. But AA in higher concentration seemed to have a negative effect on the growth of crown galls.

As shown in Figure 4, the significant effect of AA was not observed in the production of total ginsenosides. Though slight increase in the yield of total ginsenosides was observed after the addition of AA, there were no significant differences between the treated and control groups.

To sum up from Figures 3 and 4, AA at high concentration (40 mg/l) stimulated the production of ginsenosides but

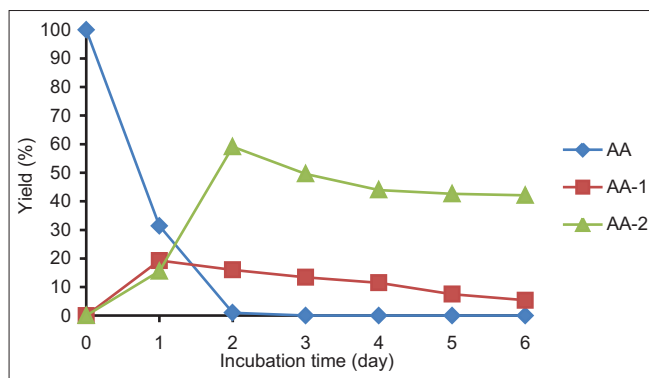
caused less biomass. To achieve the maximum production of AA glycosides without harming the growth of crown galls of *P. quinquefolium*, 30 mg/l of AA might be the optimal concentration.

### 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide cell proliferation assay

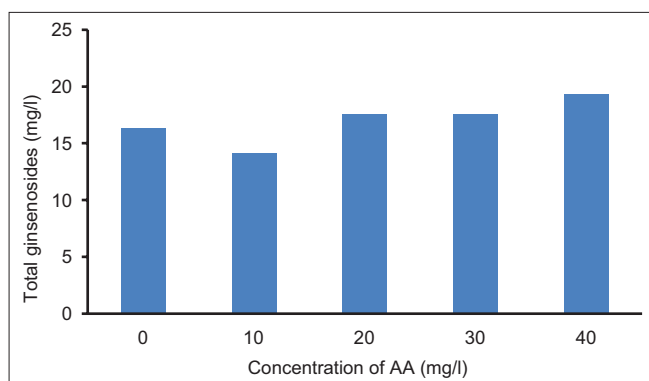
Antitumor activities of AA and its glycosides were investigated against HepG2 cell line [Figure 5]. The IC<sub>50</sub> values of AA and AA-1 were 0.27 and 0.13  $\mu\text{mol/ml}$ , respectively. And the inhibitory rate of AA-2 was 32.2% at the concentration of 0.5  $\mu\text{mol/ml}$ . The results indicated that AA and its glycosides possessed certain ability of antitumor. In addition, mono-glycoside of AA (AA-1) exhibited the strongest antitumor activity among the compounds tested.

## DISCUSSION

In our research, only glycosylation products were obtained, which might be due to the presence of high concentration glycosyltransferase in crown galls of *P. quinquefolium*. According to the previous report, the most abundant chemical constituents in crown galls of *P. quinquefolium* were ginsenosides.<sup>[15]</sup> The way of sugar linkage in the obtained glycosides was similar to that of ginsenosides, which demonstrated that the transformed manner would be affected by the secondary metabolic pathway. And this was consistent with those reported.<sup>[21]</sup> The sugar residues of glycosylation products obtained in this paper were all linked to the carboxyl group, indicating that the glycosyltransferase of crown galls of *P. quinquefolium* functioned as a high region-selective enzyme. More importantly, results showed that crown galls of *P. quinquefolium* could tolerate the high concentration of exogenous compound. This proposed that crown galls of *P. quinquefolium* possessed the potential to be developed as excellent biocatalysts that could be used in industrial scale.



**Figure 2:** Time-course of biotransformation of AA by crown galls of *P. quinquefolium*

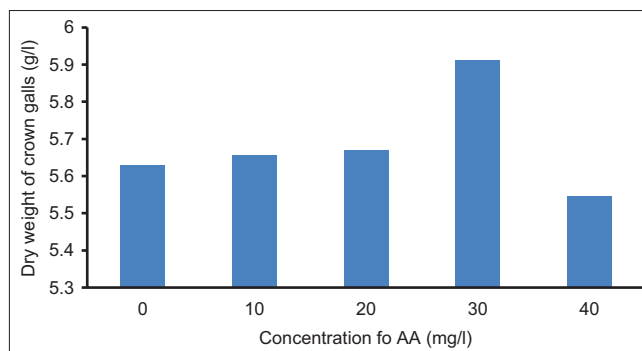


**Figure 4:** Effect of AA on the production of total ginsenosides in crown galls of *P. quinquefolium*

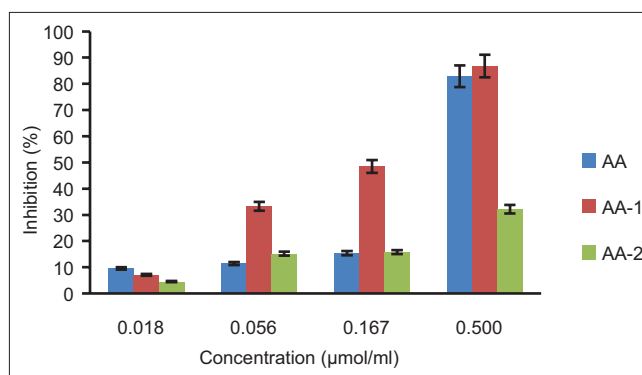
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide cell proliferation assay showed that the inhibition rate of AA-1 was much higher than that of AA against HepG2 cell line, indicating that one unit of glucose linked to the structure of AA was beneficial to the enhancement of antitumor activity. This may be due to the improvement of water solubility by the sugar residue. However, the antitumor activity of disaccharide glycoside of AA (AA-2), was lower than that of AA obviously. This might be due to the enlargement of molecular weight, like the relationship between ginsenosides Rg<sub>3</sub> and Rb<sub>1</sub>, in which Rg<sub>3</sub> shows better pharmacological activities than Rb<sub>1</sub> because of smaller molecular weight.<sup>[22]</sup>

## CONCLUSIONS

Two AA glycosides were biosynthesized by crown galls of *P. quinquefolium*, including one new compound (AA-2). The glycosyltransferase in crown galls of *P. quinquefolium* functioned as a highly region-selective enzyme that only catalyzed glycosylation on the carboxyl carbon of AA. In addition, crown galls of *P. quinquefolium* could tolerate the high concentration of AA. As a new biocatalyst system, crown galls of *P. quinquefolium* have the advantages of fast



**Figure 3:** Effect of AA on the growth of crown galls of *P. quinquefolium*



**Figure 5:** Inhibitory effect of AA and the glycosylated products on the growth of HepG2 cells. Values are given as means ± standard deviation of three separate experiments

growth, hormone free, selective reactions and tolerance to high concentration exogenous compound. Therefore, crown galls of *P. quinquefolium* are a potential agent for glycosylation reactions in industrial use.

## ACKNOWLEDGMENTS

This research work was financially supported by National Natural Sciences Foundation of China (No. 81073004, 81102771 and 81274045), Pearl River Scientific and Technological New Star Program of Guangzhou (No. 2014J2200004), the Natural Science Foundation of Guangdong Province (No. 2014A030313385) and Science and Technology Specific Project of Guangzhou (No.201300000138).

## REFERENCES

- Baldi A, Dixit VK. Yield enhancement strategies for artemisinin production by suspension cultures of *Artemisia annua*. *Bioresour Technol* 2008;99:4609-14.
- Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 2006;440:940-3.
- Zhang Y, Nowak G, Reed DW, Covello PS. The production of artemisinin precursors in tobacco. *Plant Biotechnol J* 2011;9:445-54.

4. Aftab T, Khan MM, Naeem M, Idrees M, Siddiqi TO, Moinuddin, *et al.* Effect of irradiated sodium alginate and phosphorus on biomass and artemisinin production in *Artemisia annua*. *Carbohydr Polym* 2014;110:396-404.
5. Zhang Y, Teoh KH, Reed DW, Maes L, Goossens A, Olson DJ, *et al.* The molecular cloning of artemisinic aldehyde Delta11 (13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in *Artemisia annua*. *J Biol Chem* 2008;283:21501-8.
6. Sangwan RS, Agarwal K, Luthra R, Thakur RS, Singh-Sangwan N. Biotransformation of artemisinic acid into arteannuin-B and artemisinin in *Artemisia annua*. *Phytochemistry* 1993;34:1301-2.
7. Brown GD, Sy LK. *In vivo* transformations of artemisinic acid in *Artemisia annua* plants. *Tetrahedron* 2007;63:9548-66.
8. Kawamoto H, Asada Y, Sekine H, Furuya T. Biotransformation of artemisinic acid by cultured cells of *Artemisia annua*. *Phytochemistry* 1998;48:1329-33.
9. Utsukihara T, Sato M, Kawamoto M, Itoh K, Sakamaki H, Kuniyoshi M, *et al.* Biotransformation of aromatic heterocyclic compounds by *Caragana chamlagu* and *Wasabia japonica*. *J Mol Catal B Enzym* 2007;48:59-63.
10. Machado LL, Monte FJ, de Oliveira MF, de Mattos MC, Lemos TL, Gotor-Fernández V, *et al.* Bioreduction of aromatic aldehydes and ketones by fruits' barks of *Passiflora edulis*. *J Mol Catal B Enzym* 2008;54:130-3.
11. Langhansova L, Marsik P, Vanek T. Regulation of tissue differentiation by plant growth regulators on tTCLs of *Panax ginseng* adventitious roots. *Ind Crops Prod* 2012; 35:154-9.
12. Chen X, Zhang J, Liu JH, Yu BY. Biotransformation of p-, m-, and o-hydroxybenzoic acids by *Panax ginseng* hairy root cultures. *J Mol Catal B Enzym* 2008;54:72-5.
13. Giri A, Dhingra V, Giri CC, Singh A, Ward OP, Narasu ML. Biotransformations using plant cells, organ cultures and enzyme systems: Current trends and future prospects. *Biotechnol Adv* 2001;19:175-99.
14. Yu RM, Song YB, Zhang H, Ye WC, Zhang YL, Yao XS. Study on the culture of crown gall from *Panax quinquefolium* and the prouction of its secondary metabolites – Ginsenosides Re and Rg1. *Sheng Wu Gong Cheng Xue Bao* 2003;19:372-5.
15. Zhu JH, Yu RM, Yan CY, Song LY, Zhao Y. Study on isolation and structural elucidation of ginsenosides from transgenic crown gall of ginsenosides from transgenic crown gall of *Panax quinquefolium*. *Chin Pharm J* 2008;43:412-5.
16. Ma WL, Yan CY, Zhu JH, Duan GY, Yu RM. Biotransformation of paeonol and emodin by transgenic crown galls of *Panax quinquefolium*. *Appl Biochem Biotechnol* 2010;160:1301-8.
17. Zhu JH, Yu RM, Yang L, Hu YS, Song LY, Huang YJ, *et al.* Novel biotransformation processes of dihydroartemisinic acid and artemisinic acid to their hydroxylated derivatives by two plant cell culture systems. *Process Biochem* 2010;45:1652-6.
18. Wen W, Zhu JH, Liu JW, Yu YM. Impact of artemisinic acid on the growth and catharanthine production in *Catharanthus roseus* cultured cells. *J Med Plant Res* 2012;6:2019-28.
19. Vonwiller SC, Haynes RK, King G, Wang HJ. An improved method for the isolation of qinghao (Artemisinic) Acid from *Artemisia annua*. *Planta Med* 1993;59:562-3.
20. Ye H, Wang K, Zhou C, Liu J, Zeng X. Purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassum pallidum*. *Food Chem* 2008;111:428-32.
21. Kawaguchi K, Watanabe T, Hirotani M, Furuya T. Biotransformation of digitoxigenin by cultured ginseng cells. *Phytochemistry* 1996;42:667-9.
22. Cheng LQ, Na JR, Bang MH, Kim MK, Yang DC. Conversion of major ginsenoside Rb1 to 20(S)-ginsenoside Rg3 by *Microbacterium* sp. GS514. *Phytochemistry* 2008;69:218-24.

**Cite this article as:** Zhu J, Chen L, Hu X, Song L, Wang M, Yu R. Region-selective biosynthesis of artemisinic acid glycosides by crown galls of *Panax quinquefolium* and their *in vitro* antitumor activities. *Phcog Mag* 2015;11:518-23.

**Source of Support:** Nil, **Conflict of Interest:** None declared.