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A new dammarane-type saponin from *Gynostemma pentaphyllum* induces apoptosis in A549 human lung carcinoma cells

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ABSTRACT

Gynostemma pentaphyllum has been widely used as a traditional herb for its antioxidant and immunostimulatory activities. We have previously reported several useful dammarane-type saponins with cytotoxicity against A549 human lung cancer cells from heat-processed *G. pentaphyllum*. In this study, a new dammarane-type saponin, 20(*S*)-2α,3β,12β-tetrahydroxydammar-3-*O*-β-D-glucopyranoside (namely gypenoside Jh1), was isolated from the ethanol extract of heat-processed *G. pentaphyllum* using column chromatography and semi-preparative HPLC. Gypenoside Jh1 exhibited strong cytotoxicity against A549 cells in a concentration-dependent manner, which was associated with apoptotic cell death characterized by morphological changes, Hoechst 33258 nuclear staining, Annexin V and propidium iodide binding and mitochondrial potentials assay. Quantitative analysis using flow cytometry also showed that the proportion of apoptotic cells was increased after gypenoside Jh1 treatment. These findings indicated that gypenoside Jh1 showed antiproliferative effects on A549 cells and mitochondrial-dependent pathway is involved in gypenoside Jh1-induced apoptosis.

Cancer has been a leading cause of global morbidity and mortality due to its rapid progression and poor prognosis.[1,2] For a long time, cancer chemoprevention and chemotherapy have been developed as a major field of scientific research. Although various chemical synthetic drugs have been put into use clinically, overall success in treating the disease is not satisfactory due to lots of serious side effects.[3] Thus, much attention has been focused on anticancer agents from natural resources to change the unsatisfactory situation.[4,5] Indeed, some natural products have been clinically used as anticancer drugs because of their high efficiency and low toxicity.[6,7]

The herb *Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae) has been used as popular folk medicine in Asia for centuries. It exhibits a variety of biological activities such as immune-stimulation,[8] regulation of liver functions,[9] inhibition of hyperlipoproteinemia,[10] and tumor suppression.[11] There are many dietary products containing *G. pentaphyllum* extract in China, such as Jiaogulan tea and Jiaogulan concentrated juice. *G. pentaphyllum* has been reported to contain saponins,[12] flavonoid,[13] polysaccharides,[14] vitamins and amino acids,[15] among which gypenosides, dammarane-type saponins, are the major active components.[16] Previous studies have shown pharmacological activities of gypenosides against human tongue cancer,[17] leukemia,[18] colon cancer,[19] and cervical cancer.[20] However, studies that examine anticancer effect of individual active components from *G. pentaphyllum* are sparse. In our previous study, we found that total gypenosides from ethanol extract of heat-processed *G. pentaphyllum* exhibited much stronger cytotoxic activity against human lung adenocarcinoma A549 cells than that of the raw plant.[21] Further, four dammarane-type saponins with greatly stronger anticancer activity than total gypenosides have been isolated from heat-processed *G. pentaphyllum*.[22] In the present study, a new compound, gypenoside Jh1 (**1**), was isolated from the ethanol extracts of heat-processed *G. pentaphyllum* (Fig. 1). Potential inhibitory activity of the isolated gypenoside on growth of human lung cancer cells was evaluated. In addition, basic mechanism underlying cytotoxicity of gypenoside Jh1 was also investigated with the aspect of inducing apoptosis.

G. pentaphyllum collected from Fujian Province in September 2011 were purchased from Tong Ren Tang (Beijing, China). Voucher specimen (No. GP2011-01) was deposited at the Isolation and Structure Identification Laboratory in Minzu University of China, China. The air dried whole *G. pentaphyllum* (10 kg) were

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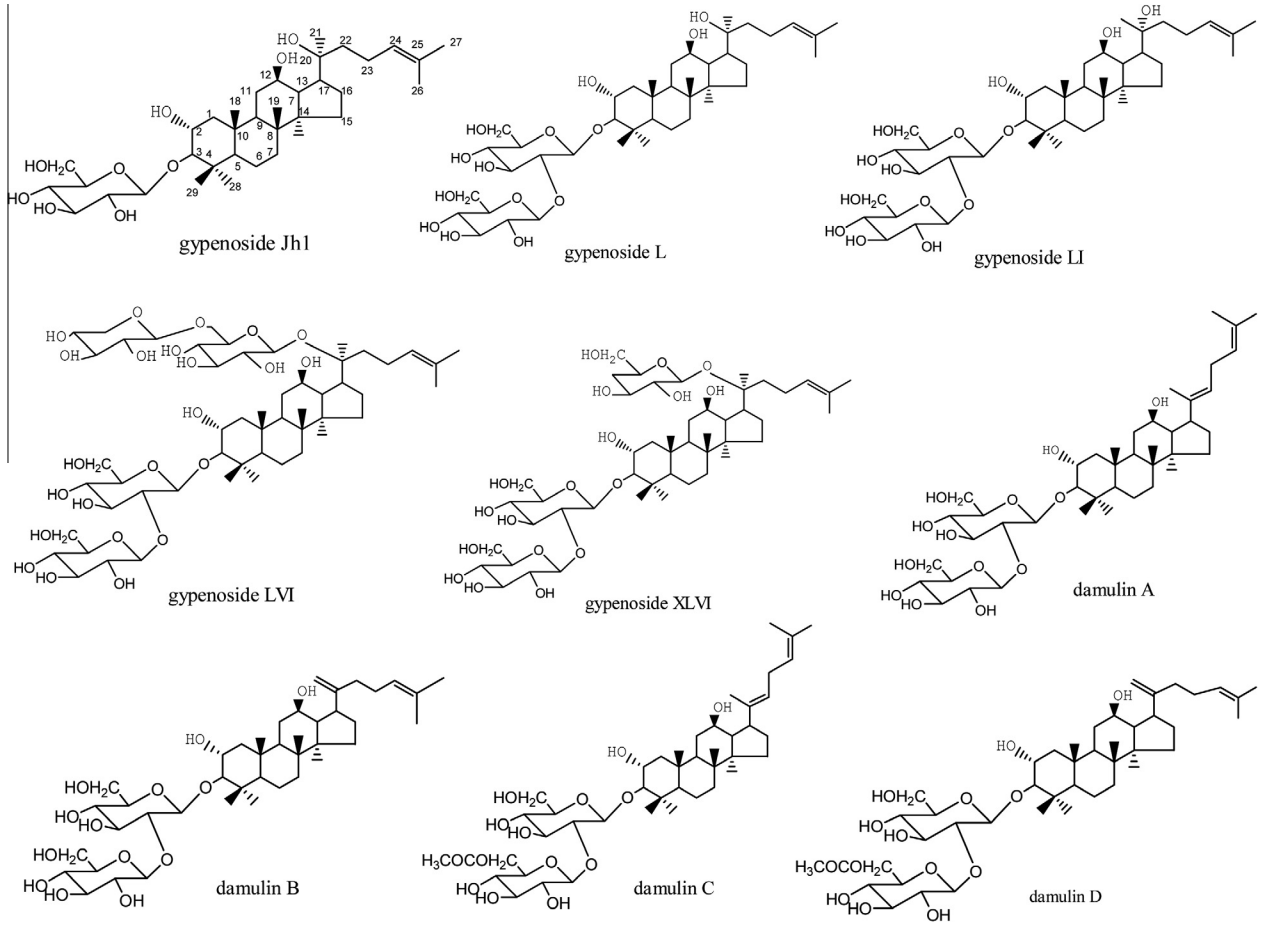

Figure 1. Chemical structures of dammarane-type saponins from raw and heat-processed *G. pentaphyllum*.

cut into pieces and steamed at 125 °C and 0.24 MPa for 3 h. The steamed *G. pentaphyllum* were then refluxed with 80% ethanol three times (2 h, 2 h and 1 h, respectively). The organic solvent was collected and then removed under a vacuum to give ethanol extract (1.35 kg). Using various chromatographic and spectroscopic techniques, compound **1** was isolated and identified from the extract.[23]

Compound **1** was obtained as white powder. In the negative mode ESI-MS of **1**, a quasimolecular ion peak at *m*/*z* 637.4 $[M-H]^-$ was appeared. The HRESIMS analysis indicated a deprotonated ion peak at *m*/*z* 637.4316 (Error: +2.29 ppm) which corresponds to the molecular formula $C_{36}H_{61}O_9$. Under the optimized collision conditions, the $[M-H-162]^-$ ion at *m*/*z* 475.5 clearly resulted from the loss of glucosyl moiety of C3 position. The IR spectrum revealed the presence of hydroxyl functional groups at 3435 cm^-, olefinic C=C double bond at 1631 cm^{-1}, and C—O stretching vibrations at 1082 cm^{-1}. The ^1H NMR (CD_3OD, 600 MHz) spectrum of compound **1** indicated the presence of 8 methyl signals at δ 0.92 (3H, s, H-29), 0.95 (3H, s, H-30), 1.00 (3H, s, H-19), 1.04 (3H, s, H-18), 1.14 (3H, s, H-28), 1.16 (3H, s, H-21), 1.62 (3H, s, H-27) and 1.68 (3H, s, H-26), and an anomeric proton signal at δ 4.34 (1H, d, J = 7.8 Hz, H-1′). According to the anomeric proton coupling constant value of 7.8 Hz, the configuration of the sugar could be identified as β-type (Table 1). The ^{13}C NMR (CD_3OD, 125 MHz), DEPT and HMQC spectra showed 36 carbon signals, among which 30 carbons were assigned to aglycon. The ^1H NMR data and the chemical shift of two olefinic carbon signals at δ 124.8 (C-24) and 130.6 (C-25) supported the fact that compound **1** has a dammarane skeleton. The ^1H and ^{13}C NMR spectra of compound **1** also showed signals for an anomeric proton at δ 4.34 (H-1′) and an anomeric carbon at δ 105.0 (C-1′). These results indicated the presence of one monosaccaride moiety.[24] Combined use of COSY, HSQC, and HMBC experiments allowed the sequential assignments of all resonances for the monosaccharide, starting from the anomeric proton (Table 1). The sequence and linkages between the sugar moiety and the aglycon were revealed by HMBC spectrum. The H-3 of the aglycon (δ 3.02) showed a correlation with the anomeric carbon of glucose (C-1′, δ 105.0). On the basis of the obtained data, the structure of compound **1** was assigned as 20(*S*)-2α,3β,12β-tetrahydroxydammar-3-*O*-β-D-glucopyranoside, namely gypenoside Jh1 (Fig. 1).

In our previous study, we found that gypenoside LVI and gypenoside XLVI (Fig. 1) were main saponins of raw *G. pentaphyllum* and their contents were decreased in the extract from heat-processed *G. pentaphyllum*.[25] A newly isolated gypenoside Jh1 was not detected in raw *G. pentaphyllum* and it can be inferred that gypenoside Jh1 was produced from gypenoside LVI and gypenoside XLVI by the loss of glucose and xylose moiety during heat treatment.

The isolated compound was evaluated for its ability to inhibit growth of A549 cells to search for anticancer agent against lung cancer. To investigate cytotoxicity of gypenoside Jh1 in A549 cells, we employed CCK-8 assay method.[26] In previous study, gypenoside L and gypenoside LI, which are structurally similar to gypenoside Jh1, have been reported to exert cytotoxic effect on A549 cells,[21] thus they were also tested to compare the cytotoxicity. As shown in Figure 2, all tested gypenosides induced a decrease in the viable A549 cells in a concentration-dependent manner. Gypenoside Jh1 showed a significantly stronger inhibitory effect against A549 cells growth than gypenoside L and gypenoside LI over the concentra-