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Metabolic profiling of *Gynostemma pentaphyllum* extract in rat serum, urine and faeces after oral administration

Dao-Jin Chen [a], Hua-Gang Hu [a], Shao-Fang Xing [a], Ya-Jun Gao [b], Si-Fan Xu [a], Xiang-Lan Piao [a,*]

[a] *Institute of Chinese Minority Traditional Medicine, Minzu University of China, Beijing 100081, China*
[b] *Department of Breast Disease Prevention Center, Haidian Maternal and Child Health Hospital, Beijing 100080, China*

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ABSTRACT

Folk drug *Gynostemma pentaphyllum* (Thunb.) Makino contains many biologically active phytochemicals which have been demonstrated to be effective against chronic diseases. As in vivo anti-tumor experiments of *G. pentaphyllum* extract (GP) show much stronger antitumor activities than in vitro, it is important and necessary to understand the metabolic study of GP. A sensitive and specific U-HPLC–MS method was utilized for the first time to rapidly identify gypenosides and its possible metabolites in rat serum, urine, and faeces after oral administration. Solid phase extraction was utilized in the sample preparation. Negative Electrospray ionisation (ESI) mass spectrometry was used to discern gypenosides and its possible metabolites in rat samples. As a result, after oral administration, a total of seven metabolites of *G. pentaphyllum* extract were assigned, two from the rat serum and seven both from the rat urine and faeces. As metabolites of *G. pentaphyllum* extract, all of them have never been reported before.

1. Introduction

The use of ethnobotanical drugs as complementary medicine is provalent in Asia and is also gaining increasing popularity in the west. As one of the well-known traditional Chinese herbal medicines, *Gynostemma pentaphyllum* (Thunb.) Makino, a member of the Cucurbitaceae family, contains many biologically active phytochemicals [1]. Gypenosides, a group of dammarane-type triterpene saponins, are known to be the principal bioactive constituents of *G. pentaphyllum* [2]. As the gypenosides were reported structurally similar to ginsenosides from the expensive ginseng root, *G. pentaphyllum* has attracted much interest as a potential new plant drug. Pharmacological studies of gypenosides have shown a variety of interesting biological activities, such as anti-hyperlipidemic [3], hypoglycemic [4], anti-inflammatory [5], and anti-tumor activities [6,7]. But previous studies found that in vivo anti-tumor experiments of *G. pentaphyllum* extract (GP) showed much stronger antitumor activities than in vitro. These might suggest that in vivo metabolites of GP played a greater role in anti-tumor activity than GP [8–10]. Consequently, in order to understand the pharmacological effects of *G. pentaphyllum* and explore the real effective components in *G. pentaphyllum*, it is important and necessary to understand the metabolic study of GP.

The metabolism of ginseng and ginsenosides has been investigated extensively in recent years [11–13]. However, to the best of our knowledge, unlike ginsenosides, no data available described the metabolism and metabolites of gypenosides at present. Obviously, the study on metabolism of gypenosides will obtain valuable data and results to facilitate us to better understand the pharmacological or toxicological activities of gypenosides and play a crucial role in the development and clinical application of this potential folk drug.

According to previous metabolic studies on ginsenosides, the triterpene skeletons of both panaxadiol and panaxatriol were not changed at the process of metabolism [14–16]. Because of the similar structure with ginsenosides, the gypenosides may show the same process of metabolism. That is to say, neither parent compounds nor their possible metabolites should have any chromophore in chemical structures. As a result, gypenosides and their metabolites mainly show terminal absorptions in their UV spectra. Apparently UV detector is not really an optimal tool to detect gypenosides and their metabolites. Among the currently available analytical techniques, many analytical methods, like high performance liquid chromatography with ultraviolet detection (HPLC–UV), fluorescence detection (HPLC–FLD), evaporative light-scattering detection (HPLC–ELSD), have been reported for the

* Corresponding author. Tel.: +86 10 68939905; fax: +86 10 68939905.
E-mail address: xlpiao@163.com (X.-L. Piao).

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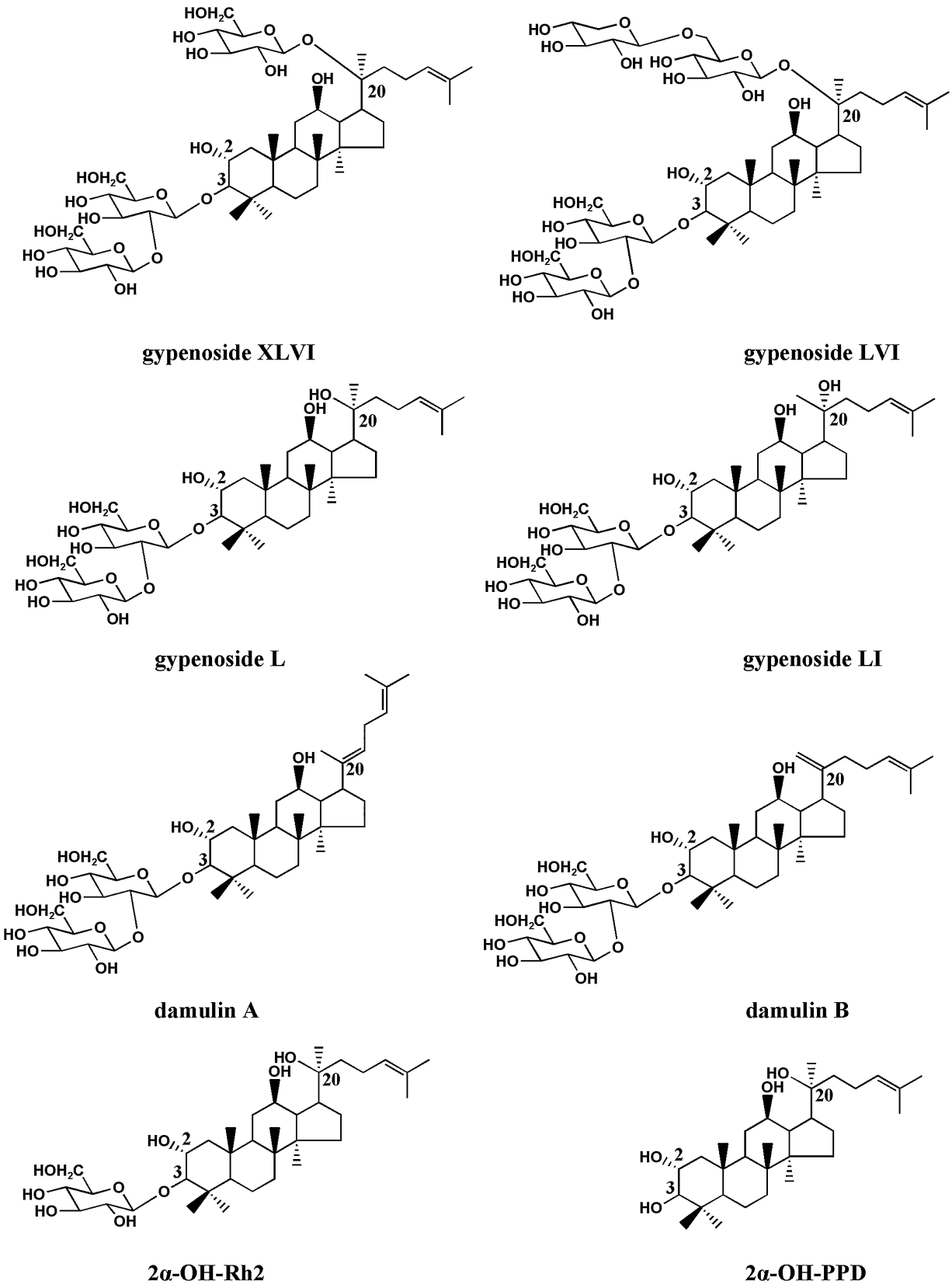

Fig. 1. Structures of possible metabolites of *G. pentaphyllum* extract.

detection of saponins [17–19]. However, most of these methods have such disadvantages as long run time, low sensitivity, low resolution and high detection limit, failing to meet the requirement of speedy, accurate and high throughput analysis of samples in laboratories. Most of all, they all can hardly obtain necessary information about chemical structures of analytes, which causes difficulty in identifying possible metabolites of parent compound. Ultra performance liquid chromatography coupled with tandem