



Qualitative and quantitative analysis of steroidal saponins in crude extracts from *Paris polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* by high performance liquid chromatography coupled with mass spectrometry

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ABSTRACT

High performance liquid chromatography coupled with electrospray ionization multi-stage tandem mass spectrometry (HPLC–ESI–MSⁿ) and triple quadrupole mass spectrometric detection (HPLC–ESI–MS/MS), respectively, had been employed for the simultaneous identification and quantification of steroidal saponins in the rhizomes of *Paris polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis*, which are the qualified plants of “Chonglou” in Chinese. The HPLC experiments were performed by means of a reversed-phase C-18 column and a binary mobile phase system consisting of 0.1% aqueous formic acid and acetonitrile under gradient elution conditions. The characteristic fragmentation patterns of diosgenin- and pennogenin-type steroidal saponins were investigated using ESI–MSⁿ in negative ion mode. The MSⁿ data of the [M–H][–] ions provided structural information on the sugar sequence of the oligosaccharide chains and the aglycones of steroidal saponins. As a result, ten and seven saponins were determined in *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis*, respectively, including four unknown compounds. One unknown compound was tentatively identified as diosgenin-3-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 4) [α -L-rhamnopyranosyl(1 $\rightarrow$ 2)]- β -D-glucopyranoside and the aglycones of the other three new compounds were reported from Chonglou for the first time. The developed HPLC–ESI–MS/MS method was validated and found to be satisfactorily linear, selective and robust. The limits of detection (LODs) and quantitation (LOQs) ranged, respectively, from 0.5 to 10 ng/mL and 2 to 34 ng/mL depending on six various compounds. The intra- and inter-day precisions of the method were evaluated and were less than 5.0%. Recoveries ranged from 92% to 104% for all compounds. The established quality evaluation method was successfully used for simultaneous quantification of six predominant steroidal saponins in the rhizomes of these two *Paris* species.

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1. Introduction

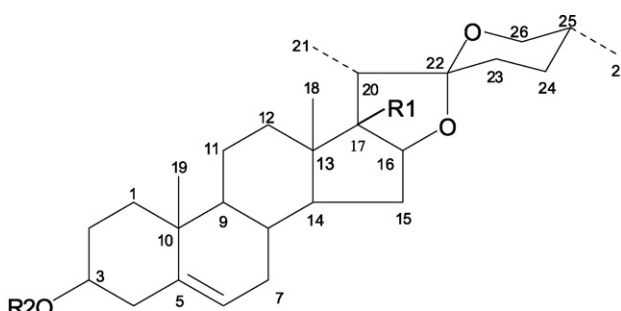
Rhizoma Paridis (“Chonglou” in Chinese), the dried rhizomes of both *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand. –Mazz. and *P. polyphylla* Smith var. *chinensis* (Franch.) Hara, is a famous and precious traditional Chinese medicine and well documented in the Chinese Pharmacopoeia [1]. It is one of the major components of many well-known prepared Chinese medicines like Yunnan Baiyao Powder, Gongxuening Capsules and Jidesheng Sheyao Tablet, and has frequently played an important role in clinics for immunity

adjustment and treating fractures, parotitis, tumours, analgesia and bleeding [2]. A lot of steroidal saponins have been isolated and identified from Chonglou and other species of the genus *Paris* [2], and they were considered as the major effective constituents for significant biological activities, such as antitumour [3,4], antifungal [5], inhibitory activities against abnormal uterine bleeding [6,7], chronotropic effects on spontaneous beating of myocardial cells [8], and protective effects on ethanol- or indomethacin-induced gastric mucosal lesions in rats [9]. The structures of these saponins were mainly classified into two types, pennogenin (PNA–PNE) and diosgenin (DSF–DSK), according to the differences of steroidal aglycones (Fig. 1).

The isolation and identification of these steroidal saponins by conventional procedures are tedious and time-consuming for their unfavorable inherent features, such as high polarities, poor volatilities and low contents in plant materials [10]. Therefore, there is a

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Standards	Aglycone	Type	R1	R2	Molecular mass
PNA	Pennogenin	-OH	Rha (1→4)-	Rha (1→4) [Rha (1→2)] -Glc-	1030
PNB	Pennogenin	-OH		Rha (1→2)- [Glc (1→3)] -Glc-	900
PNC	Pennogenin	-OH		Rha (1→2)- [Ara (1→4)] -Glc-	870
PND	Pennogenin	-OH		Rha (1→2)-Glc-	738
PNE	Pennogenin	-OH		Ara(1→4)-Glc-	724
DSF	Diosgenin	-H	Rha (1→4)-	Rha (1→4) [Rha (1→2)] -Glc-	1014
DSG	Diosgenin	-H		Rha (1→2)- [Glc (1→3)] -Glc-	884
DSH	Diosgenin	-H		Rha (1→2)- [Ara (1→4)] -Glc-	854
DSI	Diosgenin	-H		Rha (1→2)-Glc-	722
DSJ	Diosgenin	-H		Ara(1→4)-Glc-	708
DSK	Diosgenin	-H		Glc-	576

Abbreviations: Glc: β-D-glucopyranosyl; Rha: α-L-rhamnopyranosyl; Ara: α-L-arabinofuranosyl

Fig. 1. Structures of eleven steroidal saponins used in this study.

great need for a sensitive, selective and practical method to structurally analyze steroidal saponins in complex mixture. With its superior performances in sensitivity and selectivity, the coupling of mass spectrometry with high performance liquid chromatography (HPLC–MS) has opened up the door to chemical research and quality control of traditional Chinese medicine. Especially, electrospray ionization mass spectrometry (ESI–MS) is a soft ionization technique that forms mainly molecular ion peaks, and the data from multi-stage tandem mass spectrometry (MSⁿ) with collision induced dissociation (CID) reactions on the molecular ions can provide more rich structural information [11–13]. The combinatorial HPLC–ESI–MSⁿ technique, which complements the excellent separating ability of liquid chromatography with the power of ESI–MSⁿ, is widely employed in characterization and quantification of saponins [14,15], differentiation of some specific saponin isomers [16,17], and investigations on the fragmentation behavior of saponins, such as saponin molecular species, aglycones, the saccharide sequence [18,19]. Therefore, it has been applied not only for separation and elucidation of the known steroidal saponins [20–22], but also for identification of unknown compounds in many botanical extracts [23,24]. Moreover, it is worthwhile to mention that the introduction of triple quadrupole mass spectrometric detection (MS/MS) has also led to a significant improvement in detectability and selectivity by employing multiple reactions monitoring (MRM) data acquisition [25,26].

In our previous study, the steroidal saponins in extracts from the rhizomes of *Paris* species and prepared Chinese medicines had been detected by HPLC–ESI–MS [27], whereas there were no methods available for elucidation of the structural characteristics by HPLC–ESI–MSⁿ and quantitative analysis of the steroidal saponins in Chonglou by LC–MS/MS. In this study, the fragmentation behavior, corresponding mechanisms and quantitative analysis of the steroidal saponins in methanol extracts, prepared from the dried rhizomes of *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis*, had been investigated by HPLC–ESI–MSⁿ and HPLC–ESI–MS/MS, respectively. The interpretation of their fragmentation pathways and major diagnostic fragment ions had been accessed to the differentiation of the two types of steroidal saponins, i.e. diosgenin- and pennogenin-type. As a result, six saponins were unambiguously determined in both *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* by comparison with reference compounds while the structure of one compound was elucidated and the aglycones of other three new constituents were firstly reported from *P. polyphylla* var. *yunnanensis*. In addition, a simple and effective analytical method using HPLC–ESI–MS/MS had been established for the simultaneous quantification of six predominant steroidal saponins in the rhizomes of these two *Paris* species. The presented strategy herein allows a rapid, selective and sensitive analysis of saponin distribution and composition in crude extracts of Chonglou.

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