Phytochemistry, Pharmacology, Toxicology, and Structure-Cytotoxicity Relationship of *Paridis Rhizome* Saponin

MAN Shu-li[1, 2, 3], WANG Yan-li[4], LI Yuan-yuan[2, 3], GAO Wen-yuan[1, 2*], HUANG Xian-xiao[1], MA Chao-yi[1]

1. Tianjin Key Laboratory for Modern Drug Delivery and High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China
2. Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China
3. Tianjin Key Laboratory of Industry Microbiology, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China
4. Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China

Abstract: The rhizomes of *Paris polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* are used as traditional herbal medicines in many parts of China. The *Paridis Rhizome* saponin (PRS), as the active ingredient, has played an important role in hemostasis, antibacterial action, and inflammation counteraction, bearing some analogy to *Gongxuening* and *Yunnanbaiyao* in efficacy. Modern pharmacological experiments have proved that PRS possesses two main sapogenins: diosgenin and pennogenin, which could provide a lot of clinical treatment effects (anti-oxidation, anti-inflammation, anti-apoptosis, anti-metastasis, and immunostimulant, etc.). In the past, several main steroid saponins have been studied in a number of randomized controlled trials for their effects and mechanisms mainly on antitumor performance. The extensive results have demonstrated that PRS was an effective group of active components to antitumor clinical trials. In this article, we reviewed the reported phytochemical, pharmacological, and toxicological properties of PRS and compared the structure-cytotoxicity relationship of PRS in antitumor effects.

Key words: *Paridis Rhizoma* saponin; pharmacology; phytochemistry; structure-cytotoxicity relationship; toxicology
DOI: 10.7501/j.issn.1674-6384.2013.01.004

Introduction

Paris polyphylla Smith var. *yunnanensis* (Franch.) Hand.-Mazz. (PPY) and *Paris polyphylla* Smith var. *chinensis* (Franch.) Hara (PPC), commonly known as *Paridis Rhizoma* in China, grow primarily in the temperate zone and tropical regions of European and Asian continent, especially in Guangxi, Yunnan, and Guizhou provinces of China. The plants belong to *Paris* Linnaeus of Trilliaceae family. The *Paridis Rhizoma* was documented in the *Chinese Pharmacopoeia 1985* for the first time. It was first recorded in *Shennong Bencao Jing* named as *Zaoxiu*. It appeared with the same name in LI Shi-zhen's *Compendium of Materia*. In folk medicine, the dried *Paridis Rhizoma* (*Chonglou* in Chinese) has been used to treat fractures, parotitis, hemostasis, snake bite, and abscess for a long time. It also played an important role in the medicine development for antitumor, immunity adjustment, analgesia, and anti-inflammation (Yan *et al*, 2009). It is reported that steroidal saponins are the main and active components in *Paridis Rhizoma*.

After the ethanol extraction and macroporous resin purification, *Paridis Rhizoma* saponin (PRS) has been obtained. Due to wide and useful pharmacological effects of PRS, excessively excavation and use of this herb year by year have caused tremendous destruction, especially for its wild populations from different distribution places. In the present paper, recent advances

* Corresponding author: Gao WY Address: School of Pharmaceutical Science and Technology, Tianjin University, Weijin Road, Tianjin 300072, China E-mail: pharmgao@tju.edu.cn
Received: October 19, 2012; Revised: November 28, 2012; Accepted: December 16, 2012
Fund: Tianjin Municipal Science and Technology Commission (07JCZDJC05400); National Natural Science Foundation of China (30873378)

in its chemical constitute, pharmacological effects, and toxicological properties were summarized and some active constituents associated with this important plant were also discussed. The paper would introduce the main and active steroidal saponins isolated from this plant. Future prospects for the discovery of the precursor compounds and the research and development of the new anticancer drugs are also proposed in this paper.

Phytochemistry

There are many published reports on the constituents of different parts of *Paridis Rhizoma* (Deng *et al*, 2007; Kang *et al*, 2012). The constituents of *Paridis Rhizoma* is numerous and variable depending on place of the habitats and whether the rhizome is colloidal or powdery. The description of distinguishing pharmacognostic characteristics on nine species and varieties of *Paridis Rhizoma* have been published previously. According to the result of observation, the key to microscopical identification on 18 species and varieties was given by Wang *et al* (1990). This review has no intention to cover all the compounds reported in *Paridis Rhizoma*, but to summarize the major components that have been implicated in the pharmacological activities of the crude drug. Steroid saponins have been reported as major components of the activity ingredient in ethanol aqueous solution extracts from the rhizomes (Wu *et al*, 2004).

Saponins with diosgenin, pennogenin, or prosapogenin and their congeners as the aglycones constitute the most abundant types of steroid saponins in PRS. Then the structure diversity lies mainly in their glycoforms (Hostettmann and Marston, 1995). A common structural pattern of the glycoforms starts with a β-*D*-glucopyranosyl unit at the 3-OH of the steroid aglycones and extends mostly with α-*L*-rhamnopyranosyl residues. For example, diosgenin 3-*O*-β-*D*-glucopyranoside (trillin) is the simplest one in this family; Substitution of an α-*L*-rhamnopyranosyl residue at the 2-OH of the first glucose residue provides the disaccharide saponin ophiopogonin C'; And addition of an α-*L*-rhamnopyranosyl residue at 4-OH of the glucose residue produces the trisaccharide saponin dioscin (Hostettmann and Marston, 1995; Wang *et al*, 2007); And further addition of an α-*L*-rhamnopyranosyl residue at 4-OH of the rhamnose residue produces the tetrasaccharide saponin formosanin C.

There are about fifty kinds of compounds which have been characterized as diosgenyl, pennogenyl, and prototype saponins in Figs. 1—3 and Tables 1—3 (Man *et al*, 2009; 2010).

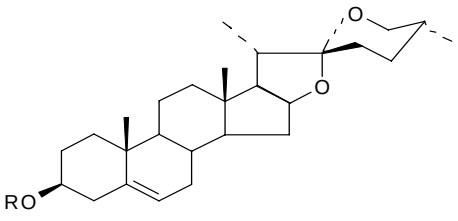

Fig. 1 Structure of diosgenin (R = H)

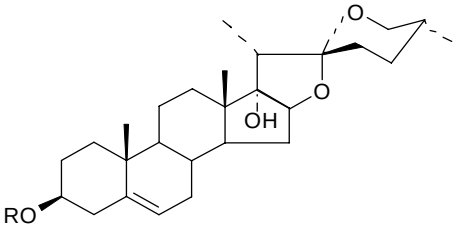

Fig. 2 Structure of pennogenin (R = H)

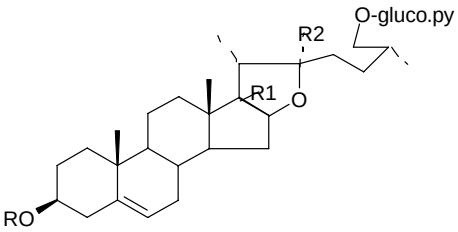

Fig. 3 Structure of prototype saponins

Pharmacological properties

PRS has attracted scientific attention because of their structural diversity and significant biological activities. In earlier studies, PRS exhibited depressant action on carotid pressure and inhibition on ethanol- or indomethacin-induced gastric mucosal lesions in rats (Matsuda *et al*, 2003). Its strong analgesic and sedative effects (Wang *et al*, 1990) and a moderate anti-mutagenic activity against picrolonic acid and benzo[α]pyrene-induced mutation (Lee and Lin, 1988) have also been reported. It also had some inhibitory effect on reverse transcriptase (Wang and Xu, 1987). In this paper, we discussed the immunity adjustment, hemostatic activity, and antitumor effects.

Immunity adjustment

PRS could protect the rats subjected to multiple

Table 1 Diosgenyl saponins

No.	Name	Position of hydroxyl	R	References
1	trillin		3-*O*-β-*D*-Glu	Seshadri, Vydeeswaran, and Rao, 1972
2	prosapogenin A of dioscin or paris V or ophiopogonin C'		3-*O*-α-*L*-Rha (1→2)-β-*D*-Glu	Mimaki *et al*, 2000a
3	polyphyllin C		3-*O*-α-*L*-Rha (1→3)-β-*D*-Glu	Devkota *et al*, 2007; Singh, Thakur, and Schulten, 1980
4	prosapogenin B of dioscin		3-*O*-α-*L*-Rha (1→4)-β-*D*-Glu	Miyamura, Nakano, and Nohara, 1982; Seshadri, Vydeeswaran, and Rao, 1972
5	dioscin		3-*O*-α-*L*-Rha (1→4)[α-*L*-Rha (1→2)]-β-*D*-Glu	Chiang, Wang, and Wu, 1992
6	taccaoside		3-*O*-α-*L*-Rha (1→3)[α-*L*-Rha (1→2)]-β-*D*-Glu	
7	formosanin C or Pb		3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4) [α-*L*-Rha-(1→2)]-β-*D*-Glu	Kimiko, Kotaro, and Toshihiro, 1981; Mimaki *et al*, 2000a
8	polyphyllin E		3-*O*-α-*L*-Rha (1→2)-α-*L*-Rha (1→4)[α-*L*-Rha (1→3)]-β-*D*-Glu	Singh, Thakur, and Schulten, 1980
9			3-*O*-α-*L*-Ara(1→4)-β-*D*-Glu	Lin *et al*, 2007; Liu *et al*, 2006
10			3-*O*-α-*L*-Rha (1→2)[α-*L*-Ara (1→3)]-β-*D*-Glu	
11	polyphyllin D or Pa		3-*O*-α-*L*-Rha (1→2)[α-*L*-Ara (1→4)]-β-*D*-Glu	Devkota *et al*, 2007; Mimaki *et al*, 2000b
12			3-*O*-α-*L*-Rha (1→3)[α-*L*-Ara (1→4)]-β-*D*-Glu	Singh, Thakur, and Schulten, 1980
13			3-*O*-α-*L*-Rha (1→4)[α-*L*-Ara (1→3)]-β-*D*-Glu	Seshadri and Vydeeswaran, 1972
14	gracillin		3-*O*-α-*L*-Rha (1→2)[β-*D*-Glu (1→3)]-β-*D*-Glu	Chen and Zhou, 1984; Kang *et al*, 2005
15			3-*O*-β-*D*-Glu (1→3)-α-*L*-Rha (1→4)[α-*L*-Rha (1→3)]-β-*D*-Glu	Indresh, Seshadri, and Seshadri, 1975
16	reclinatoside		3-*O*-α-*L*-Rha (1→5)-α-*L*-Ara (1→4)[α-*L*-Rha (1→2)]-β-*D*-Glu	
17	loureiroside		3-*O*-β-*D*-Glu-(1→5)-α-*L*-Ara (1→4)[α-*L*-Rha (1→2)]-β-*D*-Glu	
18	polyphyllin F		3-*O*-α-*L*-Rha (1→4)[α-*L*-Rha (1→3)][β-*D*-Glu (1→2)]-α-*L*-Rha	Singh, Thakur, and Schulten, 1980
19		3β,7β-ol	3-*O*-α-*L*-Ara-(1→4)[α-*L*-Rha-(1→2)]-β-*D*-Glu	Zhao *et al*, 2007
20		3β,7α-ol	3-*O*-α-*L*-Ara-(1→4)[α-*L*-Rha-(1→2)]-β-*D*-Glu	Zhao *et al*, 2007
21		3β,23,27-diol	3-*O*-β-*D*-Glu-(1→6)-β-*D*-Glu	Liu *et al*, 2006

fractures, lipopolysaccharide, or heat-inactivated *Escherichia coli* strain by decreasing the levels of TNF-α, IL-6, and IL-1β in blood serum of rats and inhibit the acute lung injury (Zhou *et al*, 2008) (Table 4). PRS is used in traditional Chinese medicines for the treatment of chronic bronchitis, mastitis, and parotitis.

Hemostatic activity

On the basis of the excellent clinical results on 300 PRS-treated cases of uterine hemorrhage of various etiology (Tian *et al*, 1986), PRS has been developed into a drug for the treatment of abnormal uterine bleeding (Table 5). This drug is named as Gongxuening Capsule in Chinese market (Zhao and Shi, 2005). In recent years, many steroidal saponins have been isolated from the rhizomes of PPY and some were proposed to be responsible for its uterine contractile activity (Zhou, 1991). Their side effects are light and few (Fu *et al*, 2007; Yang, 2007).

Antitumor activity

P. polyphylla showed a predominant inhibitory effect on many kinds of cell lines with IC_{50} values ranging from 10 to 30 mg/mL (Sun *et al*, 2007). Patients with colon cancers were more sensitive to *Paridis Rhizoma* than patients with other cancers ($P < 0.05$). Eight cases of cancer cells resistant to *Paridis Rhizoma* had also increased the tolerance to docetaxel (Liu *et al*, 2008) (Table 6).

Table 2 Pennogenyl saponins

No.	Name	No. of hydroxyl	R	References
22			3-*O*-β-*D*-Glu	Mimaki *et al*, 2000a
23	paris-VI or Tb		3-*O*-α-*L*-Rha (1→2)-β-*D*-Glu	Chen, Zhang, and Zhou, 1983
24			3-*O*-α-*L*-Rha (1→4)-β-*D*-Glu	Mimaki *et al*, 2000a
25			3-*O*-α-*L*-Ara (1→4)-β-*D*-Glu	Lin *et al*, 2007; Miyamura, Nakano, and Nohara, 1982
26	chonglouoside H		3-*O*-α-*L*-Rha (1→2)[α-*L*-Ara (1→4)]-β-*D*-Glu	Guo *et al*, 2008; Mimaki *et al*, 2000a
27			3-*O*-α-*L*-Rha (1→2) [β-*D*-Glu (1→3)]-β-*D*-Glu	Mimaki *et al*, 2000a
28			3-*O*-α-*L*-Rha (1→2)[α-*L*-Rha (1→4)]-β-*D*-Glu	Chen and Zhou, 1990; Lin *et al*, 2007
29			3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4) -β-*D*-Glu	Mimaki *et al*, 2000a
30			3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→3)[α-*L*-Rha (1→2)]-β-*D*-Glu	Matsuda *et al*, 2003
31	paris-VII or Tg		3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4)[α-*L*-Rha (1→2)]-β-*D*-Glu	Chen *et al*, 1990; Matsuda *et al*, 2003
32	polyphylloside III	27-ol	3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4)[α-*L*-Rha (1→2)]-β-*D*-Glu	Chen *et al*, 1995
33	polyphylloside IV	23β,27-diol	3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4)[α-*L*-Rha (1→2)]-β-*D*-Glu	Chen *et al*, 1995

Table 3 Prototype saponins

No.	Name	R_1	R_2	R	References
34		OH	OCH_3	3-*O*-α-*L*-Rha (1→2) [α-*L*-Ara (1→4)]-β-*D*-Glu	Chen and Zhou, 1984
35		OH	OCH_3	3-*O*-α-*L*-Rha (1→2) [β-*D*-Glu(1→3)]-β-*D*-Glu	Chen and Zhou, 1987
36	methyl-Th	OH	OCH_3	3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4) [α-*L*-Rha (1→2)]-β-*D*-Glu	Chen and Zhou, 1984
37	parisyunanoside A	OH	OH	3-*O*-α-*L*-Rha (1→2) [α-*L*-Ara (1→4)]-β-*D*-Glu	Matsuda, *et al*, 2003; Singh and Thakur, 1982
38	Th	OH	OH	3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4) [α-*L*-Rha (1→2)]-β-*D*-Glu	Singh and Thakur, 1982
39		H	OCH_3	3-*O*-α-*L*-Rha (1→3) [α-*L*-Ara(1→4)]-β-*D*-Glu	Chen and Zhou, 1987
40	polyphyllin H	H	OCH_3	3-*O*-α-*L*-Rha (1→2) [α-*L*-Ara-(1→4)]-β-*D*-Glu	Chiang, Wang, and Wu, 1992; Singh and Thakur, 1982
41	methylprotogracillin	H	OCH_3	3-*O*-α-*L*-Rha (1→2) [β-*D*-Glu(1→3)]-β-*D*-Glu	Chen and Zhou, 1987
42	methyldichotomin	H	OCH_3	3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4) [α-*L*-Rha (1→2)]-β-*D*-Glu	Nambu *et al*, 1989
43	trigofoenoside A protobioside	H	OH	3-*O*-α-*L*-Rha (1→2)-β-*D*-Glu	Matsuda *et al*, 2003; Toshihiro, Yoshiko, and Haruko, 1982
44		H	OH	3-*O*-α-*L*-Rha (1→2) [α-*L*-Ara (1→3)]-β-*D*-Glu	Toshihiro, Yoshiko, and Haruko, 1982
45	parisaponin I	H	OH	3-*O*-α-*L*-Rha (1→2) [α-*L*-Ara (1→4)]-β-*D*-Glu	Matsuda *et al*, 2003
46		H	OH	3-*O*-α-*L*-Rha (1→3) [α-*L*-Ara (1→4)]-β-*D*-Glu	Wang, Xu, and Cheng, 1989
47	protogracillin	H	OH	3-*O*-α-*L*-Rha (1→2) [β-*D*-Glu (1→3)]-β-*D*-Glu	Matsuda *et al*, 2003; Toshihiro, Yoshiko, and Haruko, 1982
48	dichotomin	H	OH	3-*O*-α-*L*-Rha (1→4)-α-*L*-Rha (1→4) [α-*L*-Rha (1→2)]-β-*D*-Glu	Chiang, Wang, and Wu, 1992

Table 4 Effect on immunity adjustment

Object	Mechanism or Event	References
aarrow	↑cytokines	He *et al*, 2006
immunity of the mice	↑white blood cell and platelet	He *et al*, 2006
mouse lymphocytes to Con A	caused proliferative responses	Chiang, Wang, and Wu, 1992
mouse fibroblast cell L929 conditioned medium	↑mouse granulocyte/macrophage colony forming cells	Chiang, Wang, and Wu, 1992
mouse macrophage cells RAW 264.7	product phagocytosis; respiratory burst; nitric oxide	Zhan *et al*, 2007

↑ indicates increase, same as below

Table 5 Effect on hemostatic activity

Event	Object	Mechanism	References
platelet aggregation	↑platelet aggregation induced by ADP *ex vivo*	shape change; internal contractions; numerous pseudopodia	Fu *et al*, 2007
myometrial contractility	rat	↑ Ca^{2+}	Guo *et al*, 2008
		↑ PLA2 /AA signaling pathway	Bachran *et al*, 2008; Zhao *et al*, 2004
	endothelial cell	proliferation	Hu *et al*, 2008
		migration	Hu *et al*, 2008
		canaliculization	Hu *et al*, 2008
		↓ DNA synthesis	Hu *et al*, 2008

↓ indicates decrease, same as below

Table 6 Antitumor effect of PRS on some kinds of tumor

Species	Object	Mechanism or event	References
human digestive tumor cell lines	liver carcinoma cell lines (HepG-2 and SMMC-7721)	degeneration and necrosis of the tumor cells but not by inducing apoptosis; ↓ dUTPase, hnRNP K, GMPsynthase, etc	Huang *et al*, 2006; Jin *et al*, 2006; Sun *et al*, 2007
		↑ DNase gamma, Nucleoside diphosphate kinase A, Centrin-2, etc. (be associated with tumor initiation, promotion, and progression)	Cheng *et al*, 2008
	gastric cancer cell line (BGC-823)		Sun *et al*, 2007
	colon adenocarcinoma cell lines (LoVo and SW-116)		Liu *et al*, 2008; Sun *et al*, 2007
	esophagus adenocarcinoma cell line (CaEs-17)		Sun *et al*, 2007
ascites tumor	H_{22} tumor cells *in vivo*	biosynthesis of DNA, RNA and protein of tumor cells in mouse spleen tissues and tumors	Shi and Du, 1988
		incorporation of 3H-TdR, 3H-UR, 3H-Leucine into the tumor cells, especially 3H-TdR	Shi and Du, 1988
	S180, S37 tumor growth	↑ the life time	Su and Wei, 1983
leukemia	L759 cells	tumor inhibition rate was 61 to 65%	Su and Wei, 1983
uterine cervix cancer	Hela cells	↑ in-flow of extracellular Ca^{2+}	Gao *et al*, 2003
lung cancer	T739 mice	Induing apoptosis	Man *et al*, 2009
		↑ expression of TIMP-2	Man *et al*, 2011; Yan *et al*, 2009
		↓the level of MMP-2 and MMP-9	

The aqueous extracts of PRS, by gavage and ip injection could inhibit the growth of H_{22} tumor cells *in vivo* (Shi and Du, 1988) and S180 and S37 (Su and Wei, 1983) tumor growth. It prolonged the life span of H_{22} animal by 2 d with a four-day administration. PRS displayed a potent anticancer agent that elicited the programmed cell death and inhibited metastases in murine lung adenocarcinoma *in vivo* (Man *et al*, 2009), especially for its main diosgenin and pennogenin saponins (Man *et al*, 2011; Yan *et al*, 2009).

Structure-antitumor relationship

Previous research addressed that the main biological activity ascribed to saponin was their membrane permeabilizing property (Menin *et al*, 2001; Plock *et al*, 2001). The main actions were considered as changes in membrane permeability and pore formation (Melzig, 2001; Seeman *et al*, 1973). Meanwhile, the effect of saponins is independent on cell type (Bachran *et al*, 2006).

According to Table 7, the structure-cytotoxicity relationship has been discussed (Gonzalez *et al*, 2003; Song *et al*, 2004). No matter the aglycone is diosgenin or pennogenin, it exhibited some cytotoxicity effect (Gonzalez *et al*, 2003). With the same aglycone, the cytotoxicity depends on the type, length, linkage as well as the substitutes of the sugar (Wang *et al*, 2007; Yan *et al*, 2008). In general, diosgenyl saponin had more strongly cytotoxic activities than pennogenyl saponins. However, the hemostatic activity of pennogenin saponin with three glycones was stronger than that of diosgenyl saponin. Even at low concentration pennogenyl saponins played an intense role in hemostasis. Diosgenyl saponins with rhamnopyranoside exhibited stronger cytotoxicity than those with arabinofuranoside. This phenomenon was similar to pennogenyl saponins. It proved that the variety of glycosides in steroid saponins affected cytotoxicity. The structure of 3-*O*-aglycone chain may be the basis of the antitumor activity especially for tumor cell cycle (Trouillas *et al*, 2005).

For example, the antibacterial action of polyphyllin D was stronger than that of gracillin (Wang *et al*, 1989). And the cytotoxicity of gracillin was regarded to be lack of selectivity (Hu and Yao, 2003b). Hong *et al* (2005) confirmed that diosgenin, the sugar-free chain, had inhibitory effect on three kinds of tumor cells (U-2OS, SGC-7901, and ACCM) and two kinds of normal cells (HUCB and hRPE). It is shown that diosgenin has no selectivity on the tumor cells and the normal cells (Hong and Lin, 2005). This result proved that aglycone played an important role in pharmacological and biological activity, while the glycosyl chain influenced the cell recognition and regulation of biological activity.

Trillin and diosgenin showed no cytotoxic activity against HL-60 cells (Chiang *et al*, 1992) and the attachment of an α-*L*-rhamnosyl group at C-2 of the glucosyl moiety led to the appearance of considerable activity (IC_{50} 1.8 μg/mL). Further addition of an α-*L*-rhamnosyl, an α-*L*-arabinofuranosyl or a β-*D*-glucosyl to C-3 or C-4 of the inner glucosyl moiety either gave no influence on the activity or slightly increased the activity (IC_{50} 0.5—3.3 μg/mL). The attachment of others would lead to a decrease in the activity such as β-*D*-galactosyl (IC_{50} 9.2 μg/mL) (Gonzalez *et al*, 2003). Among α-*L*-rhamnosyl binding to different positions of the glucosyl moiety, only diosgenin-3-*O*-α-*L*-rhamnosyl-(1→2)-β-*D*-glucoside exhibited cytotoxic activity. Comparison of three molecular models of suggested that the three-dimensional structure of the diglycoside moiety contributed to the activity. In the cytotoxic ophiopogonin C, the diglycoside existed in a conformation having a vertical orientation against the steroid plane of aglycon, while others of diglycoside and steroid skeleton were on the same plane (Gonzalez *et al*, 2003). Dioscin and formosanin C (FC) also exerted the significant inhibitory effects on the growth of HL-60 human leukemia cells.

FC also had some effects on the immune responses. However, trillin and diosgenin obtained from the partial hydrolysis of FC had no effects on them. This demonstrated that the sugar moiety in the structure of FC displayed a very important pattern for the effect on the proliferative response of mouse lymphocytes to Con A (Chiang *et al*, 1992).

The existence of F ring and the three-dimensional configuration were the key factors in steroidal sapogenin. Spirostanol saponins with F ring exhibited stronger pharmacological activity than those without F ring.

Spirostanol saponin such as gracillin generally showed a stronger antitumor activity than methyl protoneogracillin belonging to furostanol saponin. In addition, methyl protoneogracillin (Fig. 4) is selective in

Table 7 Antitumor effects of PRS monomers on various kinds of cancer cells

Compounds	A431 [e]	A498 [r]	A2780 [o]	BEL7402 [l]	HepG2 [l]	Caco-2 [c]	HCT-15 [c]	Hela [u]
formosanin C	17.93 ± 3.45	10.47 ± 2.02		21.06 ± 4.31	30.39 ± 3.44	7.33 ± 0.57		3.19 ± 0.29
dioscin	>50	37.25 ± 3.49		45.74 ± 6.49	46.85 ± 4.54	17.91 ± 2.65		5.65 ± 1.01
ophiopogonin C’	9.33 ± 0.22		18.7 ± 0.16				5.86 ± 0.14	
prosapogenin B of dioscin								
polyphillin C								
trillin								
diosgenin				>50				
gracillin					>1000			>100
polyphyllin D	21.37 ± 2.56	17.56 ± 2.89		37.98 ± 4.74	34.61 ± 5.76	9.37 ± 0.93		4.03 ± 0.81
compound 9	>50	43.64 ± 1.58		>50	>50	22.37 ± 1.21		9.63 ± 0.80
compound 25	>1000	>1000		>1000		>1000		
paris H	>1000	>500		>1000	>1000	>400		>500
PPY-VII								
compound 28								
PPY-VI								

Compounds	HL-60 [a]	K562 [a]	KB [k]	NCI-H446 [n]	BF16	LA795 [n]	A549 [n]
formosanin C	11.73 ± 0.62		41.35 ± 3.11		6.79 ± 1.21	1.35 ± 0.19	1.16 ± 0.10
dioscin	2.0 ± 0.9		>50		6.90 ± 3.68	3.06 ± 0.33	4.76 ± 0.86
ophiopogonin C’	2.46	6.44 ± 0.10				9.92 ± 1.73	9.98 ± 0.38
prosapogenin B of dioscin		>27.7					
polyphillin C	>27.7						
trillin	>34.5					>50	
diosgenin	36.72 ± 0.19	>50		>50		>100	
gracillin	>100		>1000			39.00 ± 3.63	
polyphyllin D	12.93 ± 3.60		>50		6.05 ± 0.09	1.85 ± 0.11	2.17 ± 0.81
compound 9	37.06 ± 2.52		>150		4.08 ± 1.03	5.14 ± 0.29	7.03 ± 1.02
compound 25						>50	>500
paris H	>1000		>1000		9.16 ± 0.68	9.53 ± 2.77	>50
PPY-VII					5.46 ± 1.65	5.13 ± 1.85	
compound 28						2.26 ± 0.784	
PPY-VI						>50	

a: acute myeloid leukemia cell line: HL-60, K-562

c: colon cancer: Caco-2, HCT-15

e: epidermal cancer: A431

k: oral epithelial cell line: KB

l: liver cancer: BEL7402, HepG2

n: non small cancer lung cell (NSCLC): A549, LA795, NCI-H446

o: ovarian cancer: A2780

u: cervical cancer cells: Hela

r: renal adenocarcinoma cell line: A498

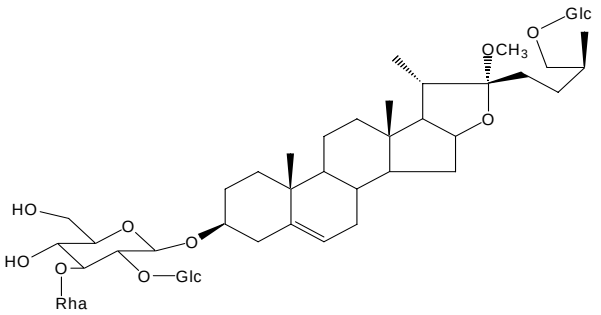

Fig. 4 Structure of methyl protoneogracillin

the antitumor activity (Hu and Yao, 2003b). Because of the different periods of incubation for cells and other reasons, different people showed different results. Wang *et al* (2001) reported that methyl protodioscin, which possesses a furostanol with 26-*O*-glycopyranoside, had no effect on HL-60 cells. In contrast to this result, researchers found that it could inhibit the growth of HL-60 cells (Gonzalez *et al*, 2003; Hu and Yao, 2003a). Protodioscin induced the cell death by apoptosis while there was no apoptosis effect on the gastric cancer cell line KATO, which demonstrated the cell line dependent results (Hibasami *et al*, 2003). The cytotoxicity observed on leukemia cell lines was 6- to 11-fold lower for methyl protogracillin compared with its stereoisomer (*R*/*S* configuration at C-25) methyl protoneogracillin, emphasizing distinct structural requirements for potent antitumorigenic activity (Hu and Yao, 2003b).

In addition, in our previous research, 17-α OH increased the sensitivity of diosgenyl saponins to the membrane-bound protease which could stimulate proMMP-2 activation, but it also decreased the anti-metastatic activity of diosgenyl saponin. Furthermore, their combination might provide a potential therapeutic modality for metastasis (Man *et al*, 2011).

As long as the chemical structure and the improved synthesis of polyphyllin D (PD, compound **11**) were ascertained, both *in vitro* and *in vivo* studies were performed (Table 8). Some researches indicated that PD was a potent anticancer agent that could overcome drug resistance in R-HepG2 cells and elicit programmed cell death via mitochondrial dysfunction (Cheung *et al*, 2005; Ong *et al*, 2008). Through the proteomic and transcriptomic analyses, it revealed that PD induced the cytotoxic effect through a mechanism initiated by endoplasmicreticulum (ER) stress followed by mitochondrial apoptotic pathway (Siu *et al*, 2008). *In vivo* study demonstrated that daily administration of PD (2.73 mg/kg) through iv injection for 10 d in nude mice bearing MCF-7 cells effectively reduced tumor growth for 50% in terms of tumor weight and size, and gave no significant toxicity in heart and liver to the host (Lee *et al*, 2005).

FC (compound **7**), a main constituent in PRS, has some effects on the immune responses (Table 9). Ip

Table 8 Antitumor effect of PD and its mechanisms

Object	Pathway	Mechanism or event
polyphyllin D	mitochondria dysfunction (Cheung *et al*, 2005; Ong *et al*, 2008; Siu *et al*, 2008)	↑ caspase-3 and -9
		↑ tumor suppressor p53
		DNA fragmentation
		phosphatidyl-serine (PS) externalization
		↑ H_2O_2
		↑ cytochrome c
		↑ apoptosis-inducing factor
		↑ depolarization of mitochondrial transmembrane potential (ΔΨm)
		↑ Bax
		↓ Bcl-2
	endoplasmic reticulum stress (Siu *et al*, 2008)	glucose-regulated protein 78 (BiP/GRP78)
		protein disulfide isomerase (PDI)
		↑ C/EBP homologous transcription factor (chop)
		↑ caspase-4 at early time point (8 h)

Table 9 Antitumor effect of FC and its mechanism

Object	Pathway	Mechanism or event
FC	mitochondria dysfunction (Lee *et al*, 2008)	↑ caspase-9, -3 and -2
		↑ cytochrome c
		cleavage of poly (ADP-ribose) polymerase (PARP)
		second mitochondria-derived activator of caspase/direct IAP binding protein with low pI (Smac/DIABLO)
		↑ Bax and Bak expressions
		↓ Bcl-X (L)
		↑ apoptosis-inducing factor
		change of mitochondrial membrane potential (ΔΨm)
		fragmentation of DNA
	cell cycle arrest	↑ cells at the sub-G1 phase
	nuclei changes	↑ endonuclease G expressions in nuclei
		change of nuclear morphology
	inhibition of expression of MMPs	↓expression of MMP-1, 2, 3, 9, 14
	immuno-modulate (Wu *et al*, 1990)	proliferative response of mouse lymphocytes to Con A
		blastogenic response of human peripheral blood cells to phytohemagglutinin (Chiang, Wang, and Wu, 1992)
		3H-thymidine incorporation of ConA-stimulated lymphocytes
		GM-CFC to mouse fibroblast cells L929 conditioned medium
		↑ natural killer cell activity
		↑ interferon

treatment with 1—2.5 mg/kg of FC would retard the growth of sc transplanted MH134 mouse hepatoma. The mechanism of the antitumor effect might be associated with the modification of the immune system (Wu *et al*, 1990). It could also enhance the antitumor effect of 5-fluorouracil. Activation of caspase-2 and the dysfunction of mitochondria might be also contributed to its antitumor effect in human colorectal cancer HT-29 cells (Lee *et al*, 2008). In our recent research, it showed a great antimetastatic effect on cancer cells through inhibiting MMP expression (Man *et al*, 2011).

Dioscin (Cai *et al*, 2002; Nguyen *et al*, 2008; Wang *et al*, 2007; Yun *et al*, 2007; Zhang *et al*, 2006) (compound **5**) is a preclinical drug with potent antiproliferative activities against most cell lines from leukemia and solid tumors (Table 10).

In cell culture experiments with Hela cervix carcinoma cells, it could dose- and time-dependently induce the apoptosis via the mitochondrial pathway. Proteomic analysis revealed that the expression of mitochondrial associated proteins was substantially altered in HL-60 cells corresponding to the dioscin

Table 10 Antitumor effect of dioscin and its mechanism

Object	Pathway	Mechanism or event
Dioscin	mitochondria dysfunction (Wang *et al*, 2006)	↑ caspase-3, -9
		↓ Bcl-2
		deregulation of oxidative stress
		alterations of phosphatases in cell signaling (Wang *et al*, 2006)
		↑ impairment in protein synthesis (Wang *et al*, 2006)
		enlargement of cell volume (Liu *et al*, 2004)
	cell cycle arrest (Liu *et al*, 2004)	↑ cells in the G_2/M phase
	mitotic arrest (Liu *et al*, 2004)	multinucleation
		phosphatidylserine externalization
		DNA hypodiploidy

treatment. Changes in proteome other than mitochondrial related proteins implicated that other mechanisms were also involved in dioscin-induced apoptosis in HL-60 cells (Wang *et al*, 2006).

Prosapogenin B of dioscin (compound **4**) also showed cytotoxicity on HCT-15 cells and it had stronger anticancer activity than that of the positive control cisplatin (Table 11). Compound **4** exerts its anticancer effect through inducing apoptosis on HCT-15 cells. Furthermore, it has been demonstrated that compound **4** triggered a mitochondria-controlled apoptotic pathway to induce apoptosis on HCT-15 cells (Wang *et al*, 2004).

Table 11 Antitumor effect and mechanism of prosapogenin B of dioscin

Object	Mechanism or event
prosapogenin B of dioscin (Wang *et al*, 2004)	↓ mitochondrial potential (ΔΨm) release of cytochrome C from mitochondria into the cytosol the ratio of Bcl-2/Bax expression level

Trillin (compound **1**) was one of the hydrolysates of dioscin. It could induce multinucleation in HL-60, K562, and human promyelocytic leukemia NB (4) cells (Liu *et al*, 2004), suggesting its extensive mitoticarresting effects. As the diosgenyl sapogenin, diosgenin was also shown to be able to induce multinucleation and apoptosis in K562 cells in a similar manner to dioscin. These findings suggested that diosgenyl saponins had the properties to induce mitotic arrest and apoptosis.

Diosgenin is the aglycone of diosgenyl saponins. It could be determined in the metabolites of the RS-treated rats. Diosgenin has been shown to suppress inflammation, inhibit proliferation, and induce apoptosis in a variety of tumor cells (Table 12).

Table 12 Antitumor effect and mechanism of diosgenin-treated cancer

Subject	Object	Mechanism or event
↓TNF-induced NF-κB activation (Shishodia and Aggarwal, 2006)	(NF-κB binding to DNA) NF-κB-regulated gene products; NF-κB-dependent reporter gene expression was also abrogated	cell proliferation (cyclin D1, COX-2, and c-myc) antiapoptosis (IAP1, Bcl-2, Bcl-X(L), Bfl-1/A1, TRAF1, and cFLIP) invasion (MMP-9)
↓ expression of survival factors (Leger *et al*, 2006)	NF-κB Bcl-Xl activation of caspase-3 PARP cleavage	
	↓ Akt activation	p65 phosphorylation and p65 nuclear translocation ↑IkappaBalpha kinase, IkappaBalpha phosphorylation, IkappaBalpha degradation
cell cycle (Leger *et al*, 2004)	G2/M arrest	
	↑ p21 in a p53-independent pathway	
Mitochondrial apoptosis (Raju and Bird, 2007)	↑ in Bax/Bcl-2 ratio PARP cleavage DNA fragmentation (Leger *et al*, 2004) provoked a collapse of mitochondrial membrane potential	
	↑ the intracellular calcium levels	major activators of cytosolic PLA2
	↑ p53 protein expression	
	nuclear localization of AIF; poly (ADP-ribose) polymerase cleavage (Corbiere *et al*, 2004)	
	cleavage of the 1.16×10^5 poly (ADP-ribose) polymerase protein to the 85kDa fragment (Raju and Bird, 2007)	
	induced cPLA2 activation through translocation to the cellular membrane	

(To be continued)

(Continued Table 12)

Subject	Object	Mechanism or event
Inhibit melanogenesis by PI3K pathway (Lee *et al*, 2007)	↓ the reduction of Akt and GSK 3beta phosphorylation	
	↑ MITF (microphthalmia-associated transcription factor) and tyrosinase	
megakaryocytic differentiation	↑ ERK (Leger *et al*, 2006)	
	↓ the p38 MAPK pathways (Leger *et al*, 2006)	
	↑ cyclooxygenase-2 and thromboxane synthase (Cailleteau *et al*, 2008)	
cholesterol homeostasis (Raju and Bird, 2007)	↓ HMG-CoA reductase at both mRNA and protein levels	
	↓ p21 ras and β-catenin lowed	

Diosgenin (10 mmol/L) induced megakaryocytic differentiation, while 40 mmol/L of it could induce apoptosis in HEL cells (Cailleteau *et al*, 2008). It played three kinds of roles on cells *in vitro*.

1. Megakaryocytic differentiation: The present report showed that diosgenin induced the megakaryocytic differentiation of HEL cells.

2. Apoptosis: Diosgenin had the antitumor effects on various cancer cells such as human osteosarcoma 1547, laryngocarcinoma Hep-2, and melanoma M4Beu cells. Moreover, arachidonic acid metabolism activation led to cyclooxygenase-2 (COX-2) which was associated with apoptosis induced by diosgenin (Leger *et al*, 2004).

3. Diosgenin could suppress proliferation, inhibit invasion, and suppress osteoclastogenesis through inhibiting the expression of NF-κB-regulated gene (Shishodia and Aggarwal, 2006).

Toxicological properties

The LD_{50} of *Paridis Rhizoma* to mice was 5.546 g/kg in the oral acute toxicity test and to domestic rabbit was over 2.2 g/kg in the skin acute toxicity test. It is indicated that PRS was a kind of slight toxic drug to untarget animal (Huang *et al*, 1996). But the continuous administration of PRS would cause some serious diarrhea. The LD_{50} to mice by ip injection was 111.3 mg/kg. Meanwhile, it possessed the sedative-hypnotic activity and gastric stimulus side effect in our recent research (Liu *et al*, 2012). The paridis polysaccharides had no cytotoxicity, 500 mg/kg with ip injection of L759, S37 or EAC showed no antitumor activity (Su and Wei, 1983).

Reference

Bachran C, Bachran S, Sutherland M, Bachran D, Fuchs H, 2008. Saponins in tumor therapy. *Mini Rev Med Chem* 8(6): 575-584.

Bachran C, Sutherland M, Heisler I, Hebestreit P, Melzig, MF, Fuchs H, 2006. The saponin-mediated enhanced uptake of targeted saporin-based drugs is strongly dependent on the saponin structure. *Exp Biol Med* 231(4): 412-420.

Cai J, Liu M, Wang Z, Ju Y, 2002. Apoptosis induced by dioscin in Hela cells. *Biol Pharm Bull* 25(2): 193-196.

Cailleteau C, Liagre B, Battu S, Jayat-Vignoles C, Beneytout JL, 2008. Increased cyclooxygenase-2 and thromboxane synthase expression is implicated in diosgenin-induced megakaryocytic differentiation in human erythroleukemia cells. *Anal Biochem* 380(1): 26-34.

Chen CX, Lian HB, Li YC, Zhou J, 1990. Steroidal saponins of the seed from *Paris polyphylla* var. *yunnanensise*. *Acta Bot Yunnan* 12(4): 452.

Chen CX, Zhang YT, Zhou J, 1983. Studies on the saponin components of plants in yunnan VI. Stesoid glycosides of *Paris polyphylla* Sm. var. *yunnanensis* (FR.) H-M. (2). *Acta Bot Yunnan* 5(1): 91-97.

Chen CX, Zhou J, 1984. Studies on the saponin components of plants in yunnan-X. Two new steroidal saponins of *Paris axialis* H, LI.(I). *Acta Bot Yunnan* 6(1): 111-117.

Chen CX, Zhou J, 1987. The steroidal saponins of *Paris axialis* (2). *Acta Bot Yunnan* 9(2): 239-245.

Chen CX, Zhou J, 1990. Steroid saponins of aerial parts of *Paris polyphylla* var. *yunnanensis*. *Acta Bot Yunnan* 12(3): 323-329.

Chen CX, Zhou J, Hiromichi N, Akinori S, 1995. Two minor steroidal saponins from the aerial parts of *Paris polyphylla* var. *yunnanensis*. *Acta Bot Yunnan* 17(2): 215-220.

Cheng ZX, Liu BR, QianXP, Ding YT, Hu WJ, Sun J, Yu LX, 2008. Proteomic analysis of anti-tumor effects by *Rhizoma Paridis* total saponin treatment in HepG2 cells. *J Ethnopharmacol* 120(2): 129-137.

Cheung JY, Ong RC, Suen YK, Ooi V, Wong HN, Mak TC, Fung KP, Yu B, Kong SK, 2005. Polyphyllin D is a potent apoptosis inducer in drug-resistant HepG2 cells. *Cancer Lett* 217(2): 203-211.

Chiang HC, Wang JJ, Wu RT, 1992a. Immunomodulating effects of the hydrolysis products of formosanin C and beta-ecdysone from *Paris formosana* Hayata. *Anticancer Res* 12(5): 1475-1478.

Chiang HC, Wang JJ, Wu RT, 1992b. Immunomodulators from *Paris formosana* Hayata. *Anticancer Res* 12(3): 949-957.

Corbiere C, Liagre B, Terro F, Beneytout JL, 2004. Induction of antiproliferative effect by diosgenin through activation of p53, release of apoptosis-inducing factor (AIF) and modulation of

caspase-3 activity in different human cancer cells. *Cell Res* 14(3): 188-196.

Deng ZC, Huang W, Zhang WS, Meng FY, Wang YY, 2007. Research progress on *Paris polyphylla* Smith var. *yunnanensis* (France.) Hand-Mazz. *Chin J Pharm Technol Economics Manag* 1(2): 57-63.

Devkota KP, Khan MT, Ranjit R, Lannang AM, Samreen, Choudhary MI, 2007. Tyrosinase inhibitory and antileishmanial constituents from the rhizomes of *Paris polyphylla*. *Nat Prod Res* 21(4): 321-327.

Fu YL, Zhao ZH, Shan YJ, Cong YW, 2007. Inducing effect of total steroid saponins from *Paris polyphylla* on platelet aggregation *in vitro* and its potential mechanism. *Bull Acad Mil Med Sci* 31(5): 416-419.

Gao D, Gao YL, Bai P, 2003. Influence of *Rhizoma Paridis* on calcium messenger system in Hela cells. *J Fujian Coll Tradit Chin Med* 13(4): 26-28.

Gonzalez AG, Hernandez JC, Leon F, Padron JI, Estevez F, Quintana J, Bermejo J, 2003. Steroidal saponins from the bark of *Dracaena draco* and their cytotoxic activities. *J Nat Prod* 66(6): 793-798.

Guo L, Su J, Deng BW,Yu ZY, Kang LP, Zhao ZH, Shan YJ, Chen JP, Ma BP, Cong YW, 2008. Active pharmaceutical ingredients and mechanisms underlying phasic myometrial contractions stimulated with the saponin extract from *Paris polyphylla* Sm. var. *yunnanensis* used for abnormal uterine bleeding. *Hum Reprod* 23(4): 964-971.

He X, Qiao A, Wang X, Liu B, Jiang M, Su L, Yao X, 2006. Structural identification of methyl protodioscin metabolites in rats' urine and their antiproliferative activities against human tumor cell lines. *Steroids* 71(9): 828-833.

Hibasami H, Moteki H, Ishikawa K, Katsuzaki H, Imai K, Yoshioka K, Ishii Y, Komiya T, 2003. Protodioscin isolated from fenugreek (*Trigonella foenumgraecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Int J Mol Med* 11(1): 23-26.

Hong ZQ, Lin JH, 2005. Antitumor activity of diosgenin *in vitro*. *J Fujian Coll Tradit Chin Med* 15(4): 35-37.

Hu J, Qian XP, Liu BR, Zhu LJ, Hu WJ, Sun J, Wang LF, Yu LX, 2008. Inhibitory effect of paridis on angiogenesis *in vitro*. *Modern Oncol* 16(8): 1273-1278.

Hu K, Yao X, 2003a. The cytotoxicity of methyl protodioscin against human cancer cell lines *in vitro*. *Cancer Invest* 21(3): 389-393.

Hu K, Yao X, 2003b. The cytotoxicity of methyl protoneogracillin (NSC-698793) and gracillin (NSC-698787), two steroidal saponins from the rhizomes of *Dioscorea collettii* var. *hypoglauca*, against human cancer cells *in vitro*. *Phytother Res* 17(6): 620-626.

Huang WT, Yang LJ, Tan PP, Hang S, 1996. Study on plant molluscicide of *Rhizoma Paridis* saponin. *Chin J Schistosomiasis Contral* 8(4): 216-218.

Huang Y, Cui LJ, Wang, Q, Ye WC, 2006. Separation and identification of active constituents of *Paris vietnamensis*. *Acta Pharm Sin* 41(4): 361-364.

Indresh K, Seshadri R, Seshadri TR, 1975. Constitution of pariphyllin-A and pariphyllin-B, the saponins isolated from the tubers of *Paris polyphylla*. *Indian J Chem* 13(8): 781-784.

Jin WD, Chen XP, Cai HJ, 2006. *In vitro* cytotoxicity of Paridis-extract on HepG2 cells. *Acta Med Univ Sci Technol Huazhong* 35(1): 103-106.

Kang LP, Ma BP, Zhang J, Xiong CQ, Tan DW, Cong YW, 2005. Isolation and identificiation of steroidal saponins from *Paris polyphylla* Smith. *Chin J Med Chem* 15(63): 25-31.

Kang LP, Yu K, Zhao Y, Liu YX, Yu HS, Pang X, Xiong CQ, Tan DW, Gao Y, Liu C, Ma BP, 2012. Characterization of steroidal glycosides from the extract of *Paris polyphylla* var. *yunnanensis* by UPLC/Q-TOF MSE. *J Pharm Biomed Anal* 62: 235-249.

Kimiko N, Kotaro M, Toshihiro N, 1981. The constituents of *Paris verticillata* M, V. Bieb. *Chem Pharm Bull* 29(5): 1445-1451.

Lee H, Lin JY, 1988. Antimutagenic activity of extracts from anticancer drugs in Chinese medicine. *Mutat Res* 204(2): 229-234.

Lee J, Jung K, Kim YS, Park D, 2007. Diosgenin inhibits melanogensis through the activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. *Life Sci* 81(3): 249-254.

Lee JC, Su CL, Chen LL, Won SJ, 2008. Formosanin C-induced apoptosis requires activation of caspase-2 and change of mitochondrial membrane potential. *Cancer Sci* 10(3): 503-513.

Lee MS, Yuet-Wa JC, Kong SK, Yu B, Eng-Choon VO, Nai-Ching HW, Chung-Wai TM, Fung KP, 2005. Effects of polyphyllin D, a steroidal saponin in *Paris polyphylla*, in growth inhibition of human breast cancer cells and in xenograft. *Cancer Biol Ther* 4(11): 1248-1254.

Leger DY, Liagre B, Beneytout JL, 2006. Role of MAPKs and NF-kappaB in diosgenin-induced megakaryocytic differentiation and subsequent apoptosis in HEL cells. *Int J Oncol* 28(1): 201-207.

Leger DY, Liagre B, Corbiere C, Cook-Moreau J, Beneytout JL, 2004. Diosgenin induces cell cycle arrest and apoptosis in HEL cells with increase in intracellular calcium level, activation of cPLA2 and COX-2 overexpression. *Int J Oncol* 25(3): 555-562.

Lin S, Wang D, Yang D, Yao J, Tong, Y, Chen J, 2007. Characterization of steroidal saponins in crude extract from *Dioscorea nipponica* Makino by liquid chromatography tandem multi-stage mass spectrometry. *Anal Chim Acta* 599(1): 98-106.

Liu GX, Wang TT, Hu WJ, Qian XP, Yu LX, Liu BR, 2008. Anticancer effect of *Rhizoma Paridis* on primary cancer cells isolated from malignant pleural effusion and ascites. *Pract Geriatr* 22(2): 101-104.

Liu H, Zhang T, Chen XQ, Huang Y, Wang Q, 2006. Steroidal saponins of *Paris polyphylla* Smith var. *yunnanensis*. *Chin J Nat Med* 4(4): 264-267.

Liu MJ, Wang Z, Ju Y, Zhou JB, Wang Y, Wong RN, 2004. The mitotic-arresting and apoptosis-inducing effects of diosgenyl saponins on human leukemia cell lines. *Biol Pharm Bull* 27(7): 1059-1065.

Liu Z , Gao WY, Man SL, Wang JY, Li N, Yin SS, Wu SS, Liu CX, 2012. Pharmacological evaluation of sedative-hypnotic activity and gastro-intestinal toxicity of *Rhizoma Paridis* saponins. *J Ethnopharmacol* 144: 67-72.

Man SL, Gao WY, Yan YJ, Liu Z, Liu CX, 2011. Inhibition of matrix metalloproteinases related to metastasis by diosgenyl and pennogenyl saponins. *J Ethnopharmacol* 137(3): 1221-1227.

Man SL, Gao WY, Zhang YJ, Jin XH, Ma CY, Huang XX, Li QY, 2009. Characterization of steroidal saponins in saponin extract

from *Paris polyphylla* by liquid chromatography tandem multi-stage mass spectrometry. *Anal Bioanal Chem* 395(2): 495-505.

Man SL, Gao WY, Zhang YJ, Liu Z ,Yan L, Huang LQ, Liu CX, 2011. Formosanin C-inhibited pulmonary metastasis through repression of matrix metalloproteinases on mouse lung adenocarcinoma. *Cancer Biol Ther* 11(6): 592-598.

Man SL, Gao WY, Zhang YJ, Ma CY, Yang L, Li YW, 2011. Paridis saponins inhibiting carcinoma growth and metastasis *in vitro* and *in vivo*. *Arch Pharm Res* 34(1): 43-50.

Man SL, Gao WY, Zhang YJ, Wang JY, Zhao WS, Huang LJ, Liu CX, 2010. Qualitative and quantitative determination of major saponins in Paris and Trillium by HPLC-ELSD and HPLC-MS/MS. *J Chromatogr B* 878(29): 2943-2948.

Man SL, Gao WY, Zhang YJ, Yan LL, Ma CY, Liu CX, Huang LQ, 2009. Antitumor and antimetastatic activities of *Rhizoma Paridis* saponins. *Steroids* 74(13/14): 1051-1056.

Matsuda H, Pongpiriyadacha Y, Morikawa T, Kishi A, Kataoka, S, Yoshikawa M, 2003. Protective effects of steroid saponins from *Paris polyphylla* var. *yunnanensis* on ethanol- or indomethacin-induced gastric mucosal lesions in rats: Structural requirement for activity and mode of action. *Bioorg Med Chem Lett* 13(6): 1101-1106.

Melzig MF, Bader G., Loose R, 2001. Investigations of the mechanism of membrane activity of selected triterpenoid saponins. *Planta Med* 67(1): 43-48.

Menin L, Panchichkina M, Keriel C, Olivares J, Braun U, Seppet EK, Saks VA, 2001. Macrocompartmentation of total creatine in cardiomyocytes revisited. *Mol Cell Biochem* 220(1/2): 149-159.

Mimaki Y, Kuroda M, Obata Y, Sashida Y, Kitahara M, Yasuda A, Naoi N, Xu ZW, Li MR, Lao AN, 2000a. Steroidal saponins from the rhizomes of *Paris polyphylla* var. *chinensis* and cytotoxic activity on HL-60 cells. *Nat Prod Lett* 14(5): 357-364.

Mimaki Y, Kuroda M, Obata Y, Sashida Y, Kitahara M, Yasuda A, Naoi N, Xu ZW, Li MR, Lao AN, 2000b. Steroidal saponins from the rhizomes of *Paris polyphylla* var. *chinensis* and their cytotoxic activity on HL-60 cells. *Nat Prod Lett* 14(5): 357-364.

Miyamura M, Nakano K, Nohara T, 1982. Steroid saponins from *Paris polyphylla* sm. supplement. *Chem Pharm Bull* 30(2): 712-718.

Nambu T, Huang X, Shu Y, Huang S, Hattori M, Kakiuchi N, Wang Q, Xu GJ, 1989. Chronotropic effect of the methanolic extracts of the plants of the *Paris* species and steroidal glycosides isolated from *P. vietnamensis* on spontaneous beating of myocardial cells. *Planta Med* 55(6): 501-505.

Nguyen VT, Darbour N, Bayet C, Doreau A, Raad I, Phung BH, Dumontet C, Di Pietro A, Dijoux-Franca MG, Guilet D, 2009. Selective modulation of P-glycoprotein activity by steroidal saponines from *Paris polyphylla*. *Fitoterapia* 80(1): 39-42.

Ong RC, Lei J, Lee RK, Cheung JY, Fung KP, Lin C, Ho HP, Yu B, Li M, Kong SK, 2008. Polyphyllin D induces mitochondrial fragmentation and acts directly on the mitochondria to induce apoptosis in drug-resistant HepG2 cells. *Cancer Lett* 261(2): 158-164.

Plock A, Sokolowska-Kohler W, Presber W, 2001. Application of flow cytometry and microscopical methods to characterize the effect of herbal drugs on *Leishmania* Spp. *Exp Parasitol* 97(3): 141-153.

Raju J, Bird RP, 2007. Diosgenin, a naturally occurring steroid [corrected] saponin suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells. *Cancer Lett* 255(2): 194-204.

Seeman P, Cheng D, Iles GH, 1973. Structure of membrane holes in osmotic and saponin hemolysis. *J Cell Biol* 56(2): 519-527.

Seshadri TR, Vydeeswaran S, 1972. Constitution of pariphyllin, a saponin isolated from the tubers of *Paris polyphylla*. *Indian J Chem* 10(6): 589-591.

Seshadri TR, Vydeeswaran S, Rao PR, 1972. Saponins from *Paris polyphylla*. *Indian J Chem* 10(4): 377-378.

Shi XF, Du DJ, 1988. The effect of paris saponin on synthesis of nuclear acid and protein of tumour cells in mice. *Pharmacol Clin Chin Mater Med* 4(4): 30-31.

Shishodia S, Aggarwal BB, 2006. Diosgenin inhibits osteoclastogenesis, invasion and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. *Oncogene* 25(10): 1463-1473.

Singh SB, Thakur RS, 1982. Furostanol saponins from *Paris polyphylla*: Structures of polyphyllin G and H. *Phytochemistry* 21(8): 2079-2082.

Singh SB, Thakur RS, Schulten HR, 1980. Spirostanol saponins from *Paris polyphylla*, structures of polyphyllin C, D, E and F. *Phytochemistry* 21(12): 2925-2929.

Siu FM, Ma DL, Cheung YW, Lok CN, Yan K, Yang Z, Yang M, Xu S, Ko BC, He QY, Che CM, 2008. Proteomic and transcriptomic study on the action of a cytotoxic saponin (polyphyllin D): induction of endoplasmic reticulum stress and mitochondria-mediated apoptotic pathways. *Proteomics* 8(15): 3105-3117.

Song Y, Liang CQ, He ZM, Zhang ZG, Li JH, Liu DP, Peng C, 2004. The study of anti-tumor effect of diosgenin *in vitro*. *Bull Chin Cancer* 13(10): 651-653.

Su SY, Wei SX, 1983. The antitumor effects of paris saponin and polysaccharide research. *J Dali Med Coll* 5: 1-4.

Sun J, Liu BR, Hu WJ, Yu LX, Qian XP, 2007. *In vitro* anticancer activity of aqueous extracts and ethanol extracts of fifteen traditional Chinese medicines on human digestive tumor cell lines. *Phytother Res* 21(11): 1102-1104.

Tian Y, Zheng LH, Xu ZY, Sun LQ, Gao CK, Zheng QZ, Zhang ZH, Shu Y, 1986. Clinical and pharmacological study of the hemostatic action of *Rhizoma Paridis* by contraction of uterus. *J Tradit Chin Med* 6(3): 178-182.

Toshihiro N, Yoshiko I, Haruko S, 1982. Study on the constituents of *Paris quadriforia* L. *Chem Pharm Bull* 30(5): 1851-1856.

Trouillas P, Corbiere C, Liagre B, Duroux JL, Beneytout JL, 2005. Structure-function relationship for saponin effects on cell cycle arrest and apoptosis in the human 1547 osteosarcoma cells: A molecular modelling approach of natural molecules structurally close to diosgenin. *Bioorg Med Chem* 13(4): 1141-1149.

Wang Q, Xu G, Cheng YB, 1989. Study on antibacterial and hemostatic activities of *Rhizoma Paridis*. *J China Pharm Univ* 20(4): 251-253.

Wang Q, Xu GJ, 1987. Inhibitory effect of the Chinese drug qiyeyizhihua (*Rhizoma Paridis*) on reverse transcriptase. *J China Pharm Univ* 18(3): 195-198.

Wang Q, Xu GJ, Jiang Y, 1990. Analgesic and sedative effects of Chinese drug *Rhizoma Paridis*. *China J Chin Mater Med* 15(2):

45-47.

Wang Q, Xu GJ, Li H, Zheng XZ, 1990. Microscopic identification on the tissues of Chinese drug Chonglou (*Rhizoma Paridis*) II. *J China Pharm Univ* 21(5): 298-301.

Wang SL, Cai B, Cui CB, Liu HW, Wu CF, Yao XS, 2004. Diosgenin-3-*O*-alpha-*L*-rhamnopyranosyl-(1-->4)-beta-*D*-glucopyranoside obtained as a new anticancer agent from *Dioscorea futschauensis* induces apoptosis on human colon carcinoma HCT-15 cells via mitochondria-controlled apoptotic pathway. *J Asian Nat Prod Res* 6(2): 115-125.

Wang T, Liu Z, Li J, Zhong M, Li J, Chen X, Bi K, 2007. Determination of protodioscin in rat plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B* 848(2): 363-368.

Wang Y, Cheung YH, Yang Z, Chiu JF, Che CM, He QY, 2006. Proteomic approach to study the cytotoxicity of dioscin (saponin). *Proteomics* 6(8): 2422-2432.

Wang Y, Zhang Y, Zhu Z, Zhu S, Li Y, Li M, Yu B, 2007. Exploration of the correlation between the structure, hemolytic activity and cytotoxicity of steroid saponins. *Bioorg Med Chem* 15(7): 2528-2532.

Wang Z, Zhou J, Ju Y, Zhang H, Liu M, Li X, 2001. Effects of two saponins extracted from the *Polygonatum zanlanscianense* pamp on the human leukemia (HL-60) cells. *Biol Pharm Bull* 24(2): 159-162.

Wu RT, Chiang HC, Fu WC, Chien KY, Chung YM, Horng LY, 1990. Formosanin-C, an immunomodulator with antitumor activity. *Int J Immunopharmacol* 12(7): 777-786.

Wu SS, Gao WY, Duan, HQ, Jia W, 2004. Advances in studies on chemical constituents and pharmacological activities of *Rhizoma Paridis*. *Chin Tradit Herb Drugs* 35(3): 344-347.

Yan LL, Zhang YJ, Gao WY, Man SL, 2008. The relationship between cytotoxicity and structure-activity of Yunnanensis paris saponins on 10 kinds of tumor cell lines. *China J Chin Mater Med* 33(16): 2057-2060.

Yan LL, Zhang YJ, Gao WY, Man SL, 2009. Antitumor activity of steroid saponins extracted from *Paris polyphylla* var. *yunnanensis* against lung adenocarcinoma cells *in vitro* and *in vivo*. *Chin Tradit Herb Drugs* 40: 424-428.

Yan LL, Zhang YJ, Gao WY, Man SL, Wang Y, 2009. *In vitro* and *in vivo* anticancer activity of steroid saponins of *Paris polyphylla* var. *yunnanensis*. *Exp Oncol* 31(1): 27-32.

Yang Y, 2007. Clinical study of Gongxuening Capsule for treatment of uterine bleeding caused by interuterine device. *China Pharm* 16(23): 57-58.

Yun H, Li JC, Wen HZ, Yu HD, Yong LW, Qiang W, Ding Z, 2007. Separation and identification of steroidal compounds with cytotoxic activity against human gastric cancer cell lines in vitro from the rhizomes of *Paris polyphylla* var. *chinensis*. *Chem Nat Compd* 43(6): 672-677.

Zhang XF, Cui Y, Huang JJ, Zhang YZ, Nie Z, Wang LF, Yan BZ, Tang YL, Liu Y, 2007. Immuno-stimulating properties of diosgenyl saponins isolated from *Paris polyphylla*. *Bioorg Med Chem Lett* 17(9): 2408-2413.

Zhang Y, Li HZ, Zhang YJ, Jacob MR, Khan SI, Li XC, Yang CR, 2006. Atropurosides A — G, new steroidal saponins from *Smilacina atropurpurea*. *Steroids* 71(8): 712-719.

Zhao L, Shi Q, 2005. Analysis on the therapeutic effect on colporrhagia due to drug abortion (240 cases) treated by Gongxuening. *J Pract Tradit Chin Med* 21: 455-456.

Zhao Y, Kang LP, Liu YX, Zhao Y, Xiong CQ, Ma BP, Dong FT, 2007. Three new steroidal saponins from the rhizome of *Paris polyphylla*. *Magn Reson Chem* 45(9): 739-744.

Zhao ZH, Li JY, Shan YJ, Yuan XL, Tang SM, Cong YW, 2004. Effects of Gongxuening on phasic contractions in isolated rat uterus. *Pharm J Chin People's Liberat Army* 20(2): 93-95.

Zhou J, 1991. Bioactive glycosides from Chinese medicines. *Mem Inst Oswaldo Cruz* 86(Suppl 2): 231-234.

Zhou MH, Du WS, Long SS, Han JH, Shen Y, Li TJ, 2008. Effect of *Rhizoma Paridis* total saponins on TNF-α and IL-1β secretion in rat peritoneal macrophages induced by lipopolysaccharide. *J Sichuan Tradit Chin Med* 26(3): 14-16.

Zhou MH, He HJ, Pan Y, Shan LJ, Ma Y, Liu Y, 2008a. Effects of *Rhizoma Paridis* total saponins on levels of cytokines in blood serum of two-hit rat model induced by multiple fractures and lipopolysaccharide. *China J Orthop Traumatol* 21(9): 662-663.

Zhou MH, He JH, Pan Y, Shan LQ, Ma Y, Liu Y, 2008b. Effects of *Rhizoma Paridis* total saponins on levels of cytokines in blood serum of two-hit rat model induced by multiple fractures and lipopolysaccharide. *China J Orthop Traumatol* 21(9): 662-664.

Zhou MH, Wang S, Ma XL, Li TJ, Liu TY, Liu AP, 2008c. Protective effects of *Rhizoma Paridis* total saponins on septic rats. *Chin Crit Care Med* 20(9): 568-570.