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REVIEW ARTICLE

Pulsatilla chinensis: A review of traditional uses, phytochemistry and pharmacology research progress

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KEYWORDS

Pulsatilla chinensis;
Triterpenoids;
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Review

Abstract This paper is intended to review advances in the botanical, traditional uses, phytochemical and pharmacological studies of the *Pulsatilla chinensis*. Up to date, 68 kinds of chemical constituents have been isolated and identified from *P. chinensis*. Among these compounds, triterpenoids, flavonoids, lignans and coumarins are the major constituents. Researches about the pharmacological properties of *P. chinensis* revealed that this plant exhibited therapeutic potential both *in vivo* and *in vitro*, including antitumor, anti-inflammatory, anti-microbial and antiviral activities. Further attention should be paid to gathering information about their toxicology data, quality-control measures, and the clinical value of the active compounds from *P. chinensis*.

Contents

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1. Introduction

Pulsatilla chinensis (Bunge) Regel, (a synonym of *Anemone chinensis* Bunge) belongs to the family of Ranunculaceae, a kind of perennial herbs with rhizomes, being used as a kind of medical herb for a long history. It is called as "Bai Tou Weng/白头翁" in China, はくとうおう (in Japanese), and 할미꽃 (in Korean), also be called as Milkweed, White-headed Grass, Old Aunt Grass. It is a kind of valuable plant with both medicinal potential and ornamental value, planted naturally in gardens to arrange flower beds, road sides, or interspersed in forest spaces (Fig. 1). In traditional Chinese medicine (TCM), the rhizome of *P. chinensis* is used to clear heat in body and detoxify, to cool blood and stop dysentery such as bacterial dysentery, amebic dysentery, especially good at clearing damp-heat of gastrointestinal tract and blood-heat toxin, in addition, it was reported to treat malaria and relieve spasm and pain as well. Modern pharmacological studies confirmed that the extracts or the active compounds isolated from *P. chinensis* played important roles in potencies of the *P. chinensis* including antitumor, anti-inflammatory, anti-microbial and antiviral (Cheng, et al., 2008, Li, et al., 2014, Sun, et al., 2010). Up to now, there is no comprehensive review on *P. chinensis*, herein, in this paper, we summarize the botany, traditional uses, phytochemistry, pharmacology, toxicity and chemical analysis of *P. chinensis* based on the research literature across the world over past decades. We expect this paper will make contributions to fill in the gaps in this field and develop potential clinical candidates from the constituents of *P. chinensis*.

2. Botany

To date, *P. chinensis* has been reported to distribute in East Asian region, especially in China. In China, *P. chinensis* is widely distributed in northeastern aera of China including Jilin, Liaoning, Hebei, Shandong, Henan, Shanxi, Shaanxi and Heilongjiang provinces (Fig. 1). The plant commonly prefers to grow on the low mountain slopes surrounded by grass and woodland or dry, stony slopes on plains. The height of *P. chinensis* is as high as 15–35 cm, with 0.8–1.5 cm rhizome underground in thickness. It grows 4–5 basal leaves with long petioles usually just emerging at flowering period; leaves are wide oval, 4.5–14 cm in length and 6.5–16 cm in width, trilobed, medium-deep lobes form as wedge-shaped inverted-ovate, with few narrow wedges or inverted trapezoids, toothed or not. The flowering stage ranges from March to May, and the fruit phase is typically from June to July.

3. Traditional uses

P. chinensis was first listed in the Chinese medical classic "*Shen Nong's herbal Classic*" (created by Shen Nong, 200 CE). It was described as cold and bitter in taste and attributive to the stomach, and large intestine meridians with potential in curing fever and physical trauma. According to "*Yao Xing Lun*" (created by Quan Zhen, around 700 CE), *P. chinensis* was listed as an herbal medicine with therapy effects on stomachache, dentalgia and joints pain. Based on "*Ri Hua Zi Ben Cao*" (created by Ri Hua Zi, around 700 CE), *P. chinensis* was described as a therapy for wind-dampnes and potential for weight loss and eyesight improvement. *P. chinensis* has been considered as an herbal medicine according to its wide spectrum of biological activities with a long history, especially its therapeutic effect on the gynopathy. Decocting with water or mashing for external application are the traditional possess methods of *P. chinensis*. As for the prescription of *P. chinensis*, the most commonly used formula was "*Bai Tou Weng Tang*" from "*Synopsis of the Golden Chamber*", which was composed of

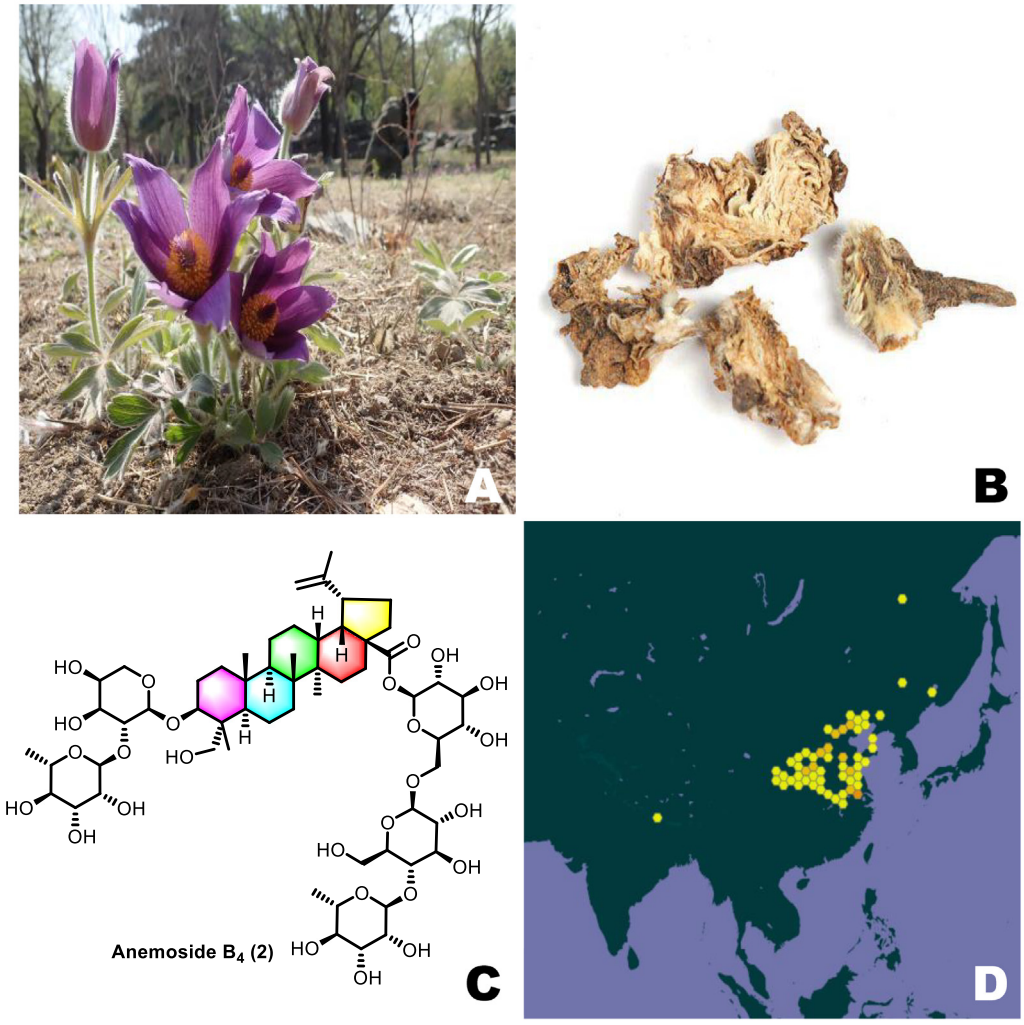

Fig. 1 *P. chinensis*: (**A**) *P. chinensis* bushes; (**B**) dry root of *P. chinensis*; (**C**) Chemcal structure of anemoside B_4; (D) distribution of *P. chinensis* (obtained from GBIF database: https://www.gbif.org).

P. chinensis, *Coptis chinensis*, *Phellodendron chinense* and *Fraxinus chinensis* with an equal proportion for the treatment of pyretic dysentery for more than one thousand years. The Chinese Pharmacopoeia recommends a dose of 9–15 g for *S. chinensis* (China Pharmacopoeia Commission, 2015).

4. Phytochemistry

Till now, at least 68 kinds of constituents have been isolated and identified from the *P. chinensis,* which are mainly made up of triterpenoids, flavonoids, coumarins and lignans. All compounds are summarized and compiled in Table 1 with their details including name, CAS number and formula, and the chemical structure of listed compounds is shown in Fig. 2 and Fig. 3 (Bahramsoltani et al., 2019). The mostly contained in the plant were triterpenoids and their saponins, among them, anemoside B_4 (**2,** Fig. 1) has been comprehensively studied and used in China and regulated as the standard for quality control of *P. chinensis* as medicine by Chinese Pharmacopoeia, known as an active substance to treat cancer, nephrotoxicity, and it had other pharmacological effects including anti-inflammatory, antioxidant, antimicrobial, etc (He, et al., 2019, Xue, et al., 2019).

4.1. Triterpenoids

Triterpenoid saponins are the main and representative components of *P. chinensis*. At present, more than dozens of species have been isolated and identified, which belong to oleanolic and lupinane pentacyclic triterpenoids respectively. The main types of aglycones are oleanolic acid saponins (**29**–**40**, **48**, **51, 52**), lupane type saponins (**1**–**13**, **15**, **21**, **28**, **16**–**20, 49, 53**–**55**), ivy saponins (**22**–**27**, **41**–**45**) and 23-hydroxybutyric acid saponins (**14**, **50**). The positions of glycosides are mainly 3-hydroxy and 28-carboxyl groups of aglycones. The five types of glycosides are alpha-L-arabinose, alpha-L-rhamnose, beta-D-glucose, beta-D-galactose and beta-D-xylose.

4.2. Flavonoids

Up to date, only two kinds of flavonoid-derived compounds named tiliroside (**60**) and 5-hydroxy-2-(4-hydroxyphenyl)-7-[[

Table 1 Chemical constituents isolated from *P. chinensis*.

NO	Name	Type	Part/ Extract	CAS	Formula	Ref.
	Triterpenoids					
1	Anemoside A_3	A	Root/ EtOH	129724–84-1	$C_{41}H_{66}O_{12}$	(Chen, et al., 1990)
2	Anemoside B_4	A	Root/ EtOH	129741–57-7	$C_{59}H_{96}O_{26}$	(Chen, et al., 1990)
3	Pulchinenoside B	A	Root/ EtOH	135247–95-9	$C_{53}H_{86}O_{22}$	(Wu, et al., 1991)
4	Pulsatilloside A	A	Root/ MeOH	136684–41-8	$C_{35}H_{56}O_8$	(Wen et al., 1996)
5	Pulsatilloside B	A	Root/ MeOH	136684–40-7	$C_{30}H_{46}O_4$	(Wen et al., 1996)
6	Pulsatilloside C	A	Root/ MeOH	162341–28-8	$C_{48}H_{78}O_{18}$	(Ye, et al., 1998)
7	Pulsatilloside D	A	Root/ MeOH	439590–38-2	$C_{59}H_{96}O_{27}$	(Ye, et al., 2002)
8	Pulsatilloside E	A	Root/ MeOH	366814–43-9	$C_{65}H_{106}O_{31}$	(Ye, et al., 2002)
9	3-[(O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-O-[O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranosyl)oxy]-23–hydroxy-, O-6-deoxy-α-L-mannopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester	A	Root/ MeOH	366814–44-0	$C_{71}H_{116}O_{36}$	(Mimaki, et al., 2001)
10	(3β,4α)-3-[(O-6-Deoxy-α-L-mannopyranosyl-(1 → 2)-O-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranosyl)oxy]-23–hydroxylup-20(29)-en-28-oic acid	A	Root/ EtOH	848784–85-0	$C_{47}H_{76}O_{17}$	(Xu, 2013)
11	3-[(4-O-β-D-glucopyranosyl-α-L-arabinopyranosyl)oxy]-23–hydroxy-, 6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	A	Root/ EtOH	1476801–07-6	$C_{53}H_{86}O_{23}$	(Xu, 2013)
12	3-[(O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-O-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranosyl)oxy]-23–hydroxy-,6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	A	Root/ EtOH	1476801–08-7	$C_{59}H_{96}O_{27}$	(Xu, 2013)
13	(3β,4α)-23–(Acetyloxy)-3-[[3,4-di-O-acetyl-2-O-(2,3,4-tri-O-acetyl-6-deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy]lup-20(29)-en-28-oic acid	A	Root/ EtOH	133377–68-1	$C_{53}H_{78}O_{18}$	(Chen, et al., 1990)
14	Daucosterol	B	Root/ EtOH	474–58-8	$C_{35}H_{60}O_6$	(Wu, et al., 1991)
15	Betulinic acid	A	Root/ MeOH	85999–40-2	$C_{30}H_{48}O_4$	(Wen et al., 1996)
16	Cussosaponin C	A	Root/ MeOH	366814–42-8	$C_{59}H_{96}O_{25}$	(Mimaki, et al., 2001)
17	Betulinic acid 3β-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-α-L-arabinopyranoside	A	Root/ EtOH	848784–87-2	$C_{47}H_{76}O_{16}$	(Xu, 2013)
18	3-[[2-O-(6-deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy]-6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	A	Root/ EtOH	1257378–27-0	$C_{53}H_{86}O_{21}$	(Xu, 2013)
19	3-[(O-β-D-glucopyranosyl-(1 → 3)-O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl)oxy]-, 6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	A	Root/ EtOH	1476801–09-8	$C_{59}H_{96}O_{26}$	(Xu, 2013)
20	3-[(O-β-D-glucopyranosyl-(1 → 3)-O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl)oxy]-, O-6-deoxy-α-L-β-D-mannopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester	A	Root/ EtOH	1476801–10-1	$C_{65}H_{106}O_{30}$	(Xu, 2013)
21	Pulsatillic acid	A	Root/ EtOH	136684–40-7	$C_{30}H_{46}O_4$	(Xu, 2013)
22	Leontoside B	C	Root/ MeOH	17233–22-6	$C_{41}H_{66}O_{13}$	(Mimaki et al., 1999)
23	Pulsatilla saponin A	C	Root/ MeOH	27013–91-8	$C_{41}H_{66}O_{12}$	(Mimaki et al., 1999)
24	Pulsatilla saponin D	C	Root/ MeOH	68027–15-6	$C_{47}H_{76}O_{17}$	(Mimaki et al., 1999)
25	Kalopanaxsaponin H	C	Root/ MeOH	128730–82-5	$C_{47}H_{76}O_{17}$	(Mimaki et al., 1999)
26	3-[(O-β-D-glucopyranosyl-(1 → 3)-O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl)oxy]-23–hydroxy-, methyl ester	C	Root/ MeOH	244202–38-8	$C_{48}H_{78}O_{17}$	(Mimaki et al., 1999)
27	Pulsatilla saponin H	C	Root/ EtOH	68027–14-5	$C_{65}H_{106}O_{31}$	(Glebko et al., 2002)

Table 1 (*continued*)

NO	Name	Type	Part/ Extract	CAS	Formula	Ref.
28	3-[[2-O-(6-deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy]-20,23–dihydroxy-, O-6-deoxy-α-L-mannopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester	A	Root/ MeOH	366814–41-7	$C_{59}H_{98}O_{27}$	(Mimaki, et al., 2001)
29	Tauroside C	D	Root/ MeOH	35790–95-5	$C_{41}H_{66}O_{11}$	(Mimaki et al., 1999)
30	Pulsatilla saponin I	D	Root/ MeOH	103956–33-8	$C_{47}H_{76}O_{16}$	(Mimaki et al., 1999)
31	Hederacolchiside A_1	D	Root/ MeOH	106577–39-3	$C_{47}H_{76}O_{16}$	(Mimaki et al., 1999)
32	(3β)-3-[(O-β-D-Glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 3)-O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid	D	Root/ MeOH	244202–36-6	$C_{53}H_{86}O_{21}$	(Mimaki et al., 1999)
33	(3β)-3-[(O-β-D-Glucopyranosyl-(1 → 4)-O-[O-β-D-glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 3)-6-deoxy-α-L-mannopyranosyl-(1 → 2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid	D	Root/ MeOH	244202–37-7	$C_{59}H_{96}O_{26}$	(Mimaki et al., 1999)
34	3-[(O-β-D-glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 3)-O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl)oxy]-methyl ester	D	Root/ MeOH	244202–39-9	$C_{54}H_{88}O_{21}$	(Mimaki et al., 1999)
35	Hederacolchiside E	D	Root/ EtOH	33783–82-3	$C_{65}H_{106}O_{30}$	(Xu et al., 2012)
36	Beesioside Q	D	Root/ EtOH	261767–91-3	$C_{65}H_{106}O_{30}$	(Xu et al., 2012)
37	(3β)-3-[(O-β-D-Glucopyranosyl-(1 → 4)-O-[O-β-D-glucopyranosyl-(1 → 3)-6-deoxy-α-L-mannopyranosyl]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid;	D	Root/ EtOH	106577–41-7	$C_{53}H_{86}O_{21}$	(Shu, et al., 2013)
38	Scabiosaponin D	D	Root/ EtOH	689257–60-1	$C_{59}H_{96}O_{26}$	(Shu, et al., 2013)
39	Lonimacranthoide II	D	Root/ EtOH	1201426–02-9	$C_{65}H_{106}O_{31}$	(Shu, et al., 2013)
40	(3β)-3-[(O-6-Deoxy-α-L-mannopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 3)-O-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid	D	Root/ EtOH	1415553–83-1	$C_{59}H_{96}O_{25}$	(Shu, et al., 2013)
41	Hederasaponin C	C	Root/ EtOH	14216–03-6	$C_{59}H_{96}O_{26}$	(Glebko et al., 2002)
42	Pulsatiloside C	C	Root/ EtOH	57539–70-5	$C_{48}H_{78}O_{18}$	(Glebko et al., 2002)
43	Hederacoside D	C	Root/ EtOH	760961–03-3	$C_{53}H_{86}O_{22}$	(Glebko et al., 2002)
44	2,3,23–trihydroxy-, O-6-deoxy-α-L-mannopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester	C	Root/ EtOH	1146973–76-3	$C_{48}H_{78}O_{19}$	(Glebko et al., 2002)
45	Leontoside D	C	Root/ EtOH	20830–84-6	$C_{59}H_{96}O_{27}$	(Xu et al., 2012)
46	Ursolic acid	E	Root/ EtOH	77–52-1	$C_{30}H_{48}O_3$	(Ding, et al., 2010)
47	β-Sitosterol	E	Root/ EtOH	83–46-5	$C_{29}H_{50}O$	(Ding, et al., 2010)
48	Hederagonic acid	D	Root/ EtOH	466–01-3	$C_{30}H_{46}O_4$	(Ding, et al., 2010)
49	23–Hydroxybetulinic acid	E	Root/ EtOH	472–15-1	$C_{30}H_{48}O_3$	(Ding, et al., 2010)
50	Daucosterin	B	Root/ EtOH	474–58-8	$C_{35}H_{60}O_6$	(Ding, et al., 2010)
51	Hederasaponin B	D	Root/ EtOH	36284–77-2	$C_{59}H_{96}O_{25}$	(Glebko et al., 2002)
52	Oleonolic acid	D	Root/ EtOH	508–02-1	$C_{30}H_{48}O_3$	(Ding, et al., 2010)
53	Betunolic acid	A	Root/ EtOH	4481–62-3	$C_{30}H_{46}O_3$	(Ding, et al., 2010)
54	Pulchinenoside E	A	Root/ EtOH	1310041–02-1	$C_{65}H_{106}O_{31}$	(Xu et al., 2012)
55	Pulsatilla triterpenic acid A	A	Root/ EtOH	1360878–33-6	$C_{35}H_{50}O_7$	(Shu, et al., 2011)

(*continued on next page*)

Table 1 (*continued*)

NO	Name	Type	Part/ Extract	CAS	Formula	Ref.
56	Pulsatilla triterpenic acid B	D	Root/ EtOH	1360878–34-7	$C_{36}H_{54}O_6$	(Shu, et al., 2011)
57	Pulsatilla triterpenic acid C	D	Root/ EtOH	1360878–35-8	$C_{36}H_{50}O_6$	(Shu, et al., 2011)
58	O-6-Deoxy-α-L-mannopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl(3β,4α)-3-hydroxy-23–(α-D-ribofuranosyloxy) ester	D	Root/ EtOH	342613–09-6	$C_{53}H_{86}O_{22}$	(Shu, et al., 2013)
59	(3β,4α)-3-(β-D-Glucopyranosyloxy)-23–(α-D-ribofuranosyloxy)olean-12-en-28-oic acid	D	Root/ EtOH	1500092–25-0	$C_{41}H_{66}O_{13}$	(Shu et al., 2013)
	Flavonoids					
60	Tiliroside		Root/ EtOH	20316–62-5	$C_{30}H_{26}O_{13}$	(Zhang, et al., 2008)
61	5-Hydroxy-2-(4-hydroxyphenyl)-7-[[3-O-[(2E)-3-(4-hydroxyphenyl)-1-oxo-2-propen-1-yl]-β-D-glucopyranosyl]oxy]-4H-1-benzopyran-4-one		Root/ EtOH	171367–93-4	$C_{30}H_{26}O_{12}$	(Zhang, et al., 2008)
	Coumarins					
62	Siderin		Root/ EtOH	53377–54-1	$C_{12}H_{12}O_4$	(Zhang, et al., 2008)
63	4,6,7-Trimethoxy-5-methylcoumarin		Root/ EtOH	62615–63-8	$C_{13}H_{14}O_5$	(Zhang, et al., 2008)
64	Xanthotoxine		Root/ MeOH	298–81-7	$C_{12}H_8O_4$	(Quan, et al., 2011)
	Lignans					
65	(+)-Pinoresinol		Root/ MeOH	487–36-5	$C_{20}H_{22}O_6$	(Mimaki et al., 1999)
66	beta-Podophyllin		Root/ MeOH	518–29-6	$C_{22}H_{22}O_8$	(Mimaki et al., 1999)
	Other compounds					
67	Anemonin		Root	508–44-1	$C_{10}H_8O_4$	(Duan, et al., 2006)
68	trans-Caffeoyltartaric acid		Root/ n-BuOH	70831–56-0	$C_{22}H_{18}O_{12}$	(Zhang, et al., 2008)

Note: type of triterpenoids: A: lupine; B: 23-hydroxybutyric acid; C: ivy; D: oleanolic acid; E: others.

3-O-[(2*E*)-3-(4-hydroxyphenyl)-1-oxo-2-propen-1-yl]-β-D-glucopyranosyl]oxy]-4H-1-benzopyran-4-one (**61**) have been characterized from *P. chinensis*, which are reported by (Zhang, et al., 2008), both the isolated flavonoids are kaempferol analogues, indicating potential research direction of this classes of constituents in *P. chinensis*.

4.3. Coumarins

Three kinds of benzofurans-type coumarins have been separated from *P. chinensis*. The structural characterization offer help to confirm their name as siderin (**62**), 4,6,7-trimethoxy-5-methylcoumarin (**63**) and xanthotoxine (**64**). The pharmacological properties of these monomers are still waiting for excavating.

4.4. Lignans

Investigations about lignans from *P. chinensis* are rare as well. Currently, only two kinds of lignans were separated from *P. chinensis*. They were (+)–pinoresinol (**65**) and beta-podophyllin (**66**). Among them, beta-podophyllin is the first derivative of podophyllotoxin isolated from Ranunculaceae plants.

4.5. Other compounds

Apart from the constituents mentioned above, anemonin (**67**) and *trans*-caffeoyltartaric acid (**68**) have also been isolated and identified from *P. chinensis*. The potential pharmacological potency of these compounds is a valuable task for researchers in the future.

5. Pharmacology

The traditional medicinal applications of *P. chinensis* have inspired various pharmacological investigations about it. The extracts and isolated compounds from *P. chinensis* displayed multiple bioactivities including antitumor, anti-inflammatory, anti-microbial and antiviral activities. The detailed pharmacological reviews were as follows.

5.1. Antitumor activity

5.1.1. Saponin-enriched extracts of *P. chinensis* as antitumor agents

P. chinensis saponins, composed of 15 kinds of monomers, possessed anticancer effects with non-toxic side effects in human liver tumor 7402 cells *in vitro* (Xu, et al., 2012). On

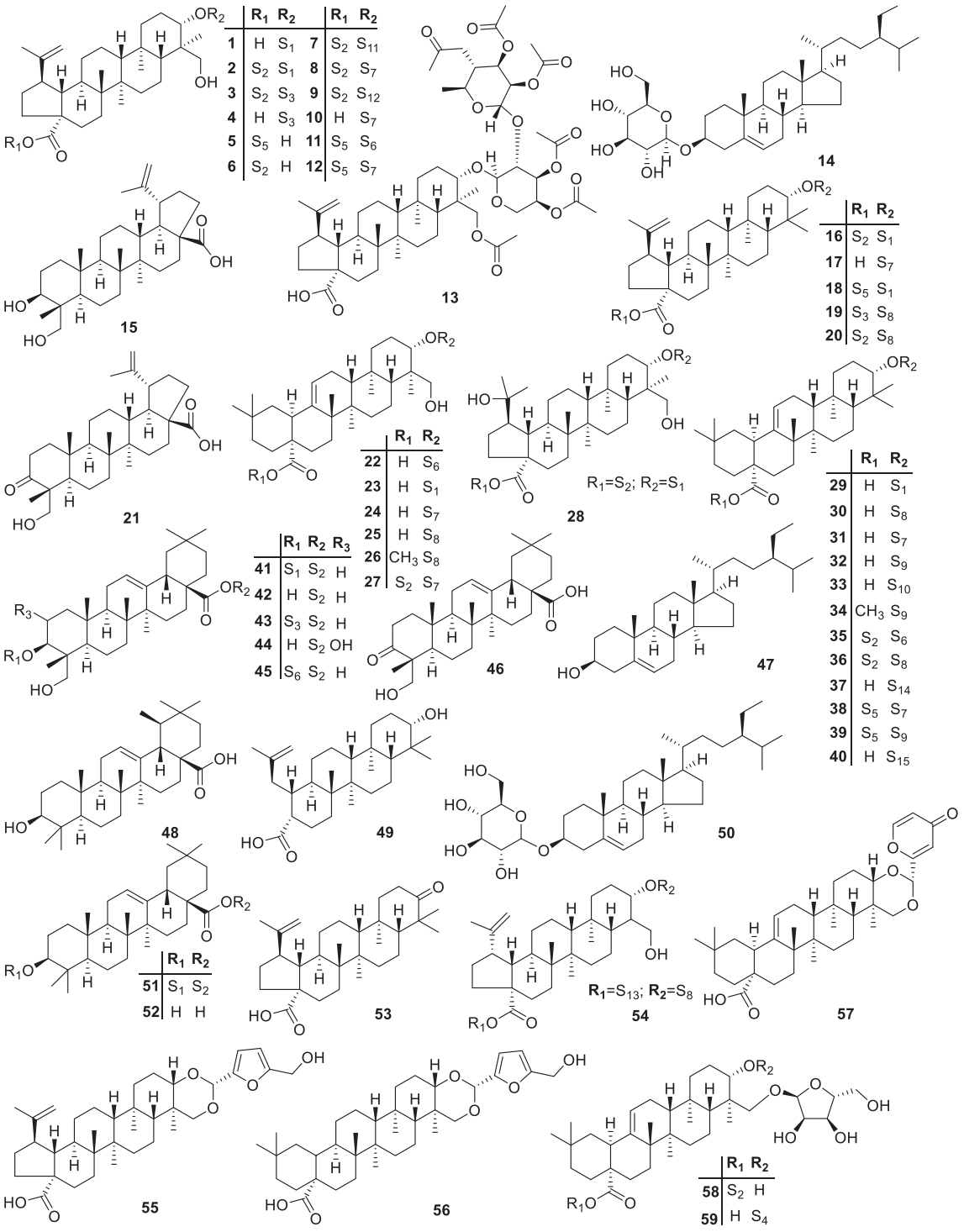

Fig. 2 Chemical structures of triterpenoids isolated from *P. chinensis*.

different concentrations (12.5 to 200 mg/mL), *P. chinensis* saponins inhibited the proliferation of human liver tumor 7402 cells *in vitro* by apoptosis. 19 days after administration of *P. chinensis* saponins (100, 200 mg/kg), the weight of tumor transplanted beneath the underarm skin of mice was markedly decreased in nude mice. The anti-tumor effect of *P. chinensis* saponins *in vivo* was associated with a significant increase in the tumor cell apoptosis rate. Although *P. chinensis* saponins inhibited the weight of mice, the extracts showed almost no effect on leukocyte number, liver and spleen weight index with no specific lesion in organ.

Total saponins from *P. chinensis* showed the potency to be effective therapy of human chronic myelogenous leukemia, and 23-hydroxybetulinic acid (23-HBA, **49**) was speculated to be the active substance (Liu, et al., 2015). As for the potential constituents, compared with other saponins with

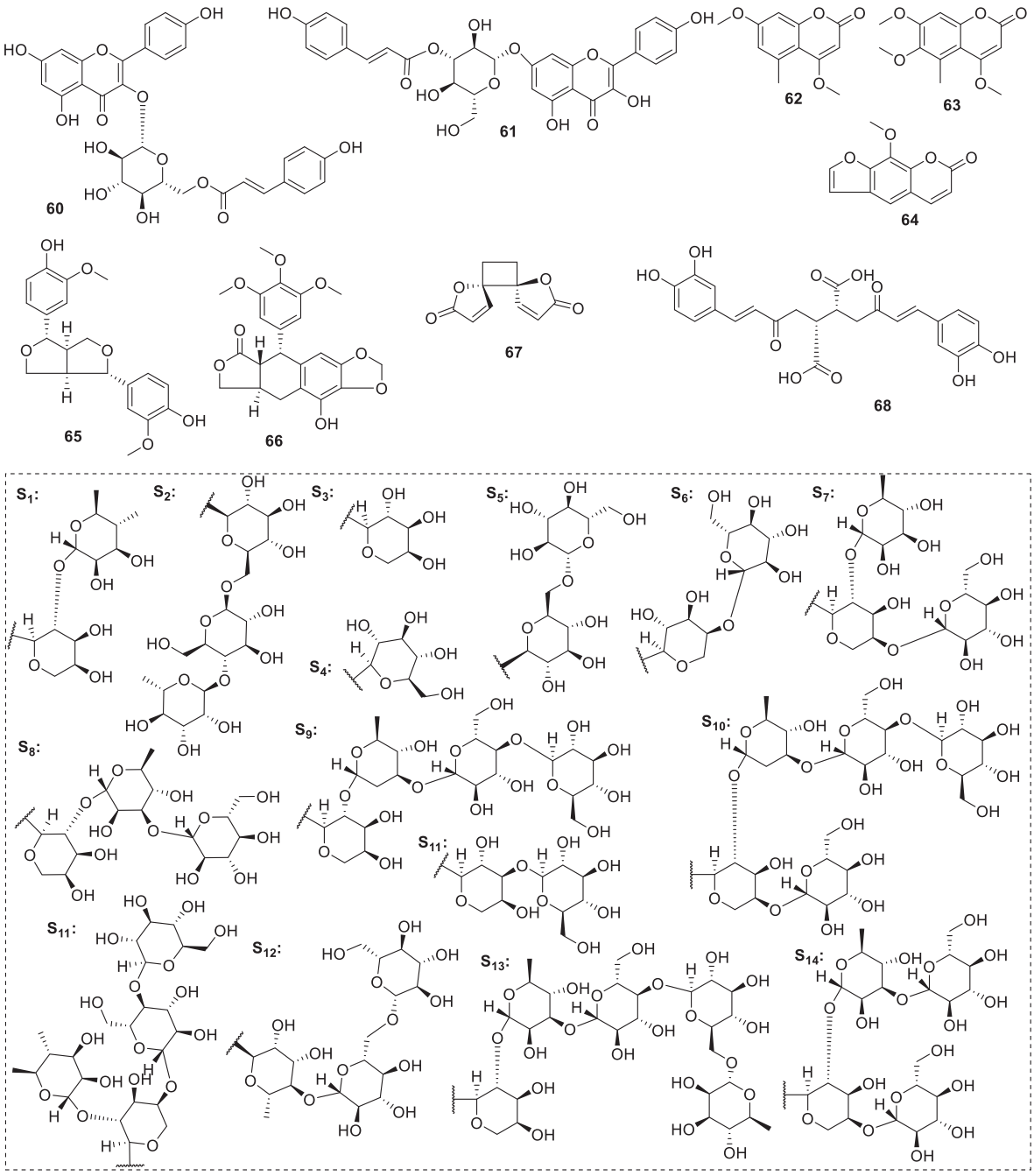

Fig. 3 Chemical structures of other constituents isolated from *P. chinensis*.

substituted glucosides on the C-3 or C-28 position, as an aglycone, compound **49** displayed better activities on multiple cancer cell lies including K562, B16, Hela and HUVEC with IC_{50} values for 18.7, 39.9, 78.5, 80 and 94.8 μM, respectively.

The extracts from *P. chinensis* were reported to inhibit the growth of hepatocellular carcinoma cells. Furthermore, to investigate the effective substance of *P. chinensis* extracts, the cytotoxic effect of anemoside B_4 (**2**) was evaluated with significantly inhibitory effects of the growth of SMMC7721 cell. Finally, a conclusion was draw that compound **2** induced apoptosis and autophagy of tumor cell lines through modulating the PI3K/Akt/mTOR signaling pathways Xue, et al. (2019).

5.1.2. Active monomers from P. chinensis as antitumor agents

Numerous previous studies provided evidences for therapeutic effects of active monomers isolated from *P. chinensis* against tumor cells. As a promising monomer, the privileged antitumor activity of compound **49** was proved again, it was demonstrated to activate cell cycle arrest at S phase to cause the apoptosis of tumor cells, with the selectively regulations of expression levels of significant proteins including Bcl-2, Bax, surviving, cytochrome *C* and caspase-9.

The 3-(4, 5-dimethylthiazol-2-yl)-2, 5diphenyltetrazolium-bromide (MTT) assay was implemented to test the antitumor potency of compounds isolated from *P. chinensis*, the results showed that multiple promising constituents including compound **27** (IC_{50}: 7.33 μM for A549, 7.13 μM for SGC-7901, 6.32 μM for HL-7702 cells), compound **37** (IC_{50}: 8.11 μM for A549, 5.41 μM for SGC-7901, 9.42 μM for HL-7702 cells), compound **40** (IC_{50}: 9.89 μM for A549, 7.15 μM for SGC-7901, 8.83 μM for HL-7702 cells) significantly inhibited the growth of cancer cells (Shu et al., 2013). Pulsatilla saponin D (**24**) was reported to show inhibitory effect on autophagic flux in some cancer cell lines to performed anticancer activities in diverse types of cancer.

Xu, et al. (2013) proposed that different types and structure of saponins induced disequilibrium effects towards the cancer cells including the human cancer cell lines (A549, SGC-7901) as well as the hepatic cell line (HL-7702). Multiple saponins from *P. chinensis* including compounds **8**–**11**, **23**, **29**–**33**, **38**, **39**, **42**, **43** were tested for the cytotoxicity by MTT assay. Among the test compounds, raddeanoside R_{13} (**31**) exhibited the strongest cytotoxic potency on multiple cell lines. Additionally, the results showed that a free carboxylic group on C-28 of saponins for example **31** (cytotoxic activity against A549, SGC-7901 cancer cell lines, and HL-7702 hepatic cell lines as IC_{50} values 5.81, 3.79 mM, and 8.23 mM, respectively) was indispensable for their cytotoxic activity, meanwhile, through the analysis of SAR demonstrated that the oleanane-type saponins had better cytotoxic effect than lupane-type saponins, also, the substituent of C-3 glycolic chain had an exact influence to the activity to kill the cancer cell.

Recently in 2020, pulchinenosides from *P. chinensis* such as compounds **23** and **24** were disclosed to stimulate P-glycoprotein functional activities and upregulate P-glycoprotein/ABCB1 (Liu et al., 2020). Moreover, both short-term and long-term dosing of pulchinenosides exerted the potential to reduce the oral bioavailability of P-glycoprotein substrates mRNA and protein expression, suggesting promising potential for *P. chinensis* using as antitumor agents.

Pulsatilla saponin A (**23**) exhibited expectable potential to develop as a novel differentiation inducer to treat the acute myeloid leukemia. Wang et al. (2016) picked U937 cells, K562 cells and HL-60 cells as well as the primary leukemia cells extracted from the AML patients to investigate the differentiation induced by compound **23**, the results illustrated compound **23** modified the differentiation effects of AML cells, possibly by modulating the MEK/ERK signaling pathway. It was reported that compound **23** also exhibited activity to against human colon cancer HT-29 cells, with a 9.5% ratio of apoptosis compared with 2% of control group. Additionally, compound **23** displayed synergy effects of exiting anticancer drug, it enhanced the activity of fluorouracil, a first line chemotherapy drug for colon cancer (Yang, et al., 2017).

5.1.3. Structural optimization of anti-tumor P. chinensis compounds with their SAR

Nowadays, structural optimization of precursors excavating from natural products has become an important task for the development of natural compound chemists, which matters the potential yields for researchers and convenience for patients. Rational design of the synthesis of lead compounds can reduce the cost during the extraction and isolation, and the SAR analysis can provide perspective to both chemists and biologists. To date, the structural optimization investigations were mainly possessed on betulinic acid (**15**), pulsatilla saponin A (**23**), pulsatilla saponin D (**24**), hederacolchiside A_1 (**31**), herein, we introduce the research progress in this field according to this sequence in order to give more evidence to develop clinical candidates from constituents in *P. chinensis*.

In 2009, Gauthier et al. (Gauthier, et al., 2009) synthesized a sequence of betulinic acid (**15**) bidesmosidic derivatives as antitumor agents. Among the synthesized derivatives, compound **S1** (synthetic derivative 1, Fig. 4.) showed the most significant antitumor potency on cancer cell lines including A549, DLD-1, MCF7, PC-3 and WS1 with IC_{50} values for 1.9, 1.9, 1.7, 1.8 and 1.3 μM, respectively. The SAR analysis was speculated as that the relative cytotoxicity of bidesmosidic betulin and betulinic acid saponins were strongly influenced by the nature of both the aglycone and the ranosyl moieties at both C-3 and C-28 positions were highly cytotoxic sugar moieties.

In 2015, Shilin Yang task group carried out the structural optimization jobs on pulsatilla saponin A and D and evaluated their antitumor potency (Chen, et al., 2015), among the synthetized derivatives, compound **S2** (Fig. 5) exhibited the most significant antitumor activities *in vitro* against cancer cell lines A-549, Bel-7402 and SMMC-7721 with IC_{50} values for 1.0, 0.9 and 3.2 μM and good selectivity with the cancer cell lines to normal cell line with IC_{50} value for more than 100 μM on normal cell lines HL-7702. The concentration inducing fifty percent of erythrocytes haemolysis for **S2** (Fig. 5.) was more than 500 μM, indicating a favorable safety with low-risk of haemolysis problem. The antitumor effects on mentioned cell lines were better than both pulsatilla saponin A and D, moreover, *in vivo* in the acute toxicity test, the administration of **S2** was detected to obtain the tolerance dose for more than 160 mg/g in mice, which was 16-folds to the value of pulsatilla saponin A and D.

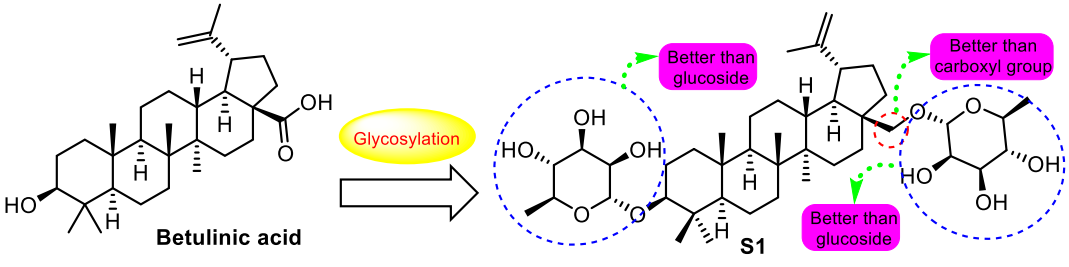

Fig. 4 SAR study of betulinic acid analogue **S1** as antitumor agent.

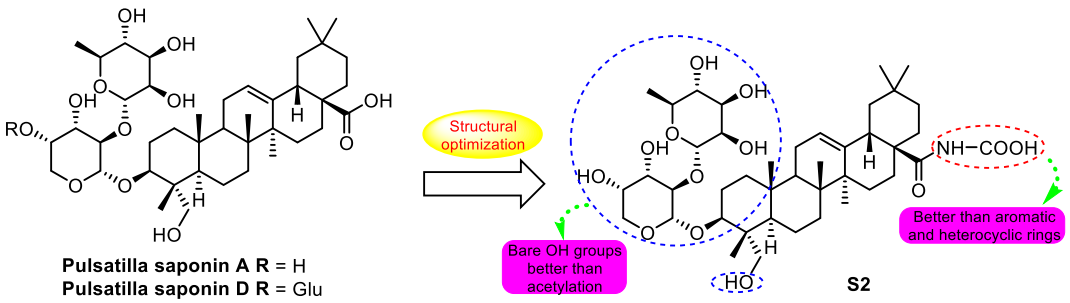

Fig. 5 SAR study of pulsatilla saponin A and D analogue **S2** as antitumor agent.

In 2016, the same task group published their further research. Based on the structure of hederacolchiside A1 (**31**), a series of analogues were synthesized as antitumor agents (Fang, et al., 2016), among which most promising compound **S3** (Fig. 6) displayed the most remarkable antitumor activity. Compound **S3** showed cytotoxicity against six human cancer cell lines including SMMC-7721, NCI-H460, U251, SK-OV-3, HCT-116 and SGC-790 with IC_{50} values ranging from 1.1 to 4.6 μM after 48 h incubation. In addition, in mice acute toxicity test, **S3** promoted the tolerance dose and reduced the death number of test mice compared with the precursor hederacolchiside A1.

Subsequently, more pulsatilla saponin D-based derivatives were synthesized and determined the antitumor activity (Fang, et al., 2019). Compound **S4** (Fig. 7) showed strongest antitumor activity among the productions against cancer cell lines such as SMMC-772, MCF-7, NCI-H460, A549 and HCT-116 with IC_{50} values for 2.4, 4.5, 3.2, 3.7 and 1.7 μM, respectively. Same as the results of **S3** in mice acute toxicity test, **S4** promoted the tolerance dose and reduced the death number of test mice compared with the lead compound.

In 2017, Zhong Chen and co-workers (Tong, et al., 2017) were deeply involved with the structural modification of pulsatilla saponin A. Compound **S5** (Fig. 8) was considered to be the most potent antitumor derivative on cancer cell lines including A549, MDA-MB-231, KB, KB-VIN and MCF-7 with IC_{50} values for 4.685, 5.540, 5.075, 5.248 and 10.737 μM. Additionally, compound **S5** showed a better balance between haemolytic toxicity (HD_{50} > 500 μM) and cytotoxicity toward lung cancer cells A549. Molecular studies indicated that **S5** was liked to lead to G1 cell cycle arrest.

In 2018, they reported new progress in this area. Directing against the strong hemolytic toxicity of pulsatilla saponin D (HD_{50}: 6.3 μM), a cluster of derivatives were designed and prepared (Chen, et al., 2018). Compound **S6** (Fig. 9) was selected for its significant antitumor activity (IC_{50} values for A549, MDA-MB-231, KB, KB-VIN and MCF-7 cells: 2.8–8.6 μM) and weak hemolytic toxicity (HD_{50} > 500 μM). Molecular studies indicated that **S6** induced typical G1 cell cycle arrest and apoptosis in A549 cells, and Western blot assays suggested that both intrinsic and extrinsic apoptosis pathways were activated by **S6**.

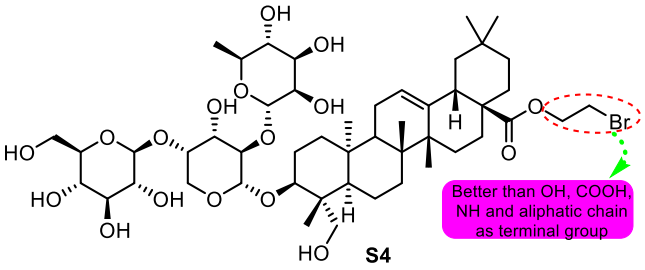

Fig. 7 SAR study of pulsatilla saponin D analogue **S4** as antitumor agent.

The task group also possessed the structural optimization from the aspect of carbohydrate chemistry. Based on the structure of compound **31** and β-hederin, multiple substituted saponins were synthesized (Wang, et al., 2017), among which compound **30** (Fig. 10) exhibited strongest antitumor potential than other derivatives against cancer cell lines including SMMC-7721, Bel-7402 and A-549 with IC_{50} values for 3.07, 4.57 and 2.43 μM, respectively. The results showed that type of terminal monosaccharides and linkage position had apparent effects on cytotoxicity and selectivity of the synthesized against cancer cell lines tested.

5.1.4. Effective polysaccharides isolated from P. chinensis as antitumor agents

Polysaccharides from *P. chinensis* were disclosed to be promising antitumor agents as well. *P. chinensis* polysaccharides (PCPs) showed significant anti-proliferative effect on C6 glioma *in vitro* (Zhou, et al., 2012), meanwhile *P. chinensis*

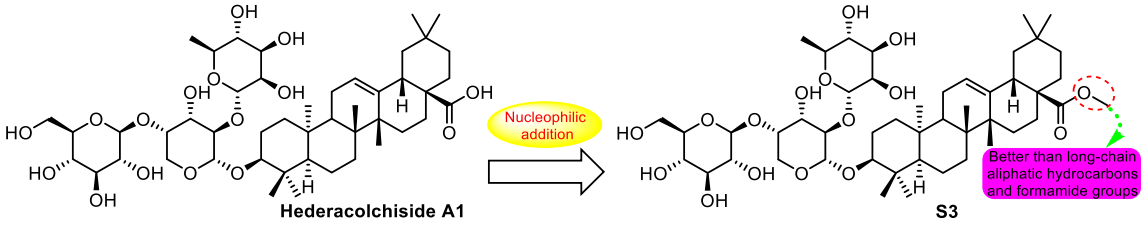

Fig. 6 SAR study of hederacolchiside A1 analogue **S3** as antitumor agent.

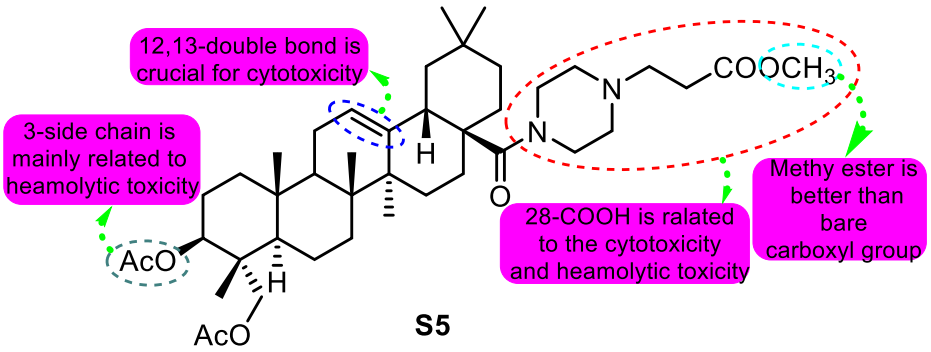

Fig. 8 SAR study of pulsatilla saponin A analogue **S5** as antitumor agent.

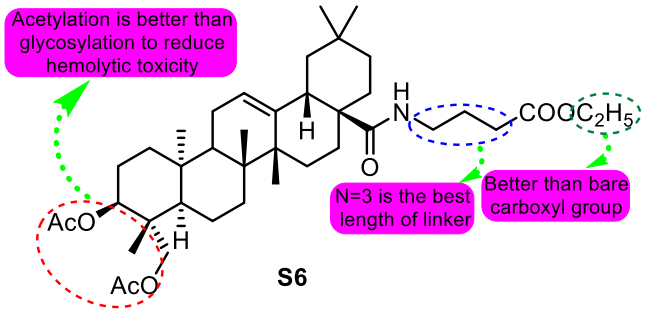

Fig. 9 SAR study of pulsatilla saponin D analogue **S6** as antitumor agent.

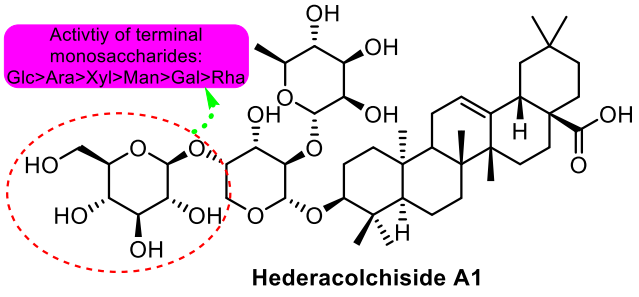

Fig. 10 SAR study of hederacolchiside A_1 (**30**) as antitumor agent.

polysaccharides inhibited the growth of C6 glioma and prolonged the life survival *in vivo*, comparable to carmustine administration. In addition, PCPs treatment to tumor bearing mice could not only reduce the body weight loss, but also elevate the thymus and spleen indices. In addition, PCPs administration to tumor bearing mice could relieve the liver and kidney damage with decreased levels of aspartate aminotransferase, alanine aminotransferase and urea, and promote superoxide dismutase (SOD) and catalase enzyme activities.

A water-soluble *P. chinensis* polysaccharide, which consisted of five different monosaccharides, including rhamnose, arabinose, xylose, galactose, mannose with the molar ratio of 1.00: 7.85: 0.37: 0.65: 3.01 (molecular weight: 7.8×10^5 Da), inhibited the growth of transplantable tumor on bearing mice *in vivo* Liu, et al. (2013). Moreover, the polysaccharide promoted the concanavalin A, lipopolysaccharide (LPS)-stimulated splenocytes proliferation, the serum lysozyme level and 2,4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity reactions at the dosage of 100 mg/kg, meanwhile, significant improvements in peripheral blood abnormality and anemia were observed in *P. chinensis* polysaccharide treatment, indicating both cellular and humoral immune response were improved.

5.2. Anti-inflammatory activity

The anti-inflammation activity of 16 compounds isolated from *Pulsatilla koreana* was investigated Yang, et al. (2010), almost all the compounds exhibted the activity of anti-inflammation. Thereinto, pulsatilloside E (**8**) also isolated from *P. chinensis*, and the results showed that compound **8** exerted strong inhibitory activity against NO production in LPS-stimulated RAW 264.7 cells, the inhibitions were 67.7%, 49.2%, 5.3% respectively at concentrations of 1, 10 and 100 μM. In the isolated compounds, the in monosaccharide or trisaccharide substituted triterpenoids were better than disaccharide substituted triterpenoids in general.

Anemonin (**67**), isolated from *P. chinensis*, at the dosage of 10 μg/mL, prevented intestinal microvascular dysfunction and exerted beneficial therapeutic action in intestinal inflammation (Duan, et al., 2006). Compound **67** inhibited LPS-induced rat intestinal microvascular endothelial cells (RIMECs) damage *in vitro*, the expression of cell adhesion molecules and secretory products in RIMECs were also regulated by compound **67.** Compound **67** may also have applications for various other diseases, including cardiovascular diseases and arthritis where ET-1 and NO activation has been shown to mediate pathogenesis.

Anemoside B_4 (**2**), a representative constituent from *P. chinensis*, was reported to exhibit anti-inflammatory and immunemodulatory activities *in vivo* in mice models (Kang, et al., 2019). At the dosages 12.5–50 mg/kg, compound **2** suppressed xylene-induced mice ear edema remarkedly. Furthermore, it ameliorated LPS-induced kidney and lung inflammation damage, which inhibited pro-inflammatory response by NF-κB pathway in mice. In addition, compound **2** reduced $CD4^+/CD8^+$ ratio, inhibited splenic lymphocyte proliferation and decreased DNFB-induced changes of ear thickness, exerting potential to be a novel natural anti-inflammatory drug candidate for treating inflammatory disorder. Lately in 2020, compound **2** was reported to prevent acute ulcerative colitis

through inhibiting of TLR4/NF-κB/MAPK signaling pathway (Ma, et al., 2020). Compound **2** was injected in the C57BL/6 mice model of ulcerative colitis and the DSS-induced colitis mice from losing weight, shortening colon length and improving pathological changes of colon tissues was prevented by compound **2**. Moreover, compound **2** significantly reduced levels of inflammatory cytokines interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) in colon tissues. *In vitro*, compound **2** was almost nontoxic to RAW264.7 cells and could protect cells against LPS. Furthermore, compound **2** significantly inhibited the activation of the TLR4 signaling pathway induced by DSS and down-regulated the expression of key proteins in the TLR4/NF-κB/MAPK signaling pathway in RAW264.7 cells induced by LPS.

5.3. Anti-microbial activity

Infectious diseases induced by microbial trouble both human and animals both in health and economics (Bao, et al., 2017, Dao Ngoc Hien, et al., 2018). The exploration and development of *P. chinensis* as anti-microbial agent gives inspiration in the development of natural products as health productions. *P. chinensis* has been recorded to be used in the veterinary medicine for the treatment of enteritis induced by parasite according to the Chinese Veterinary Pharmacopoeia. The dosages of *P. chinensis* are ranging from 5 g to 100 g based on the body type of animals. The common formula so called Baitouweng oral liquid is composed of *P. chinensis*, *C. chinensis*, *P. chinense* and *F. rhynchophylla*, which is similar to the composition of the prescription Bai Tou Weng Tang.

P. chinensis extracts were reported to inhibit the growth of *Giardia intestinalis* Li, et al. (2012). The ethyl acetate fraction was the most effective part on parasite growth, cell viability, adherence and morphology among the sub-fractionsn according to the pharmacological screening results. In addition, the growth of *G. intestinalis* could also be moderately inhibited by the ethyl acetate fraction (IC_{50}: 257.081 μg/mL). Changes in *G. intestinalis* morphology induced by *P. chinensis* were observed to some degree, providing evidence that *P. chinensis* can be develop potential anti-giardiasis agent.

Schistosomiasis, a parasitic disease mainly caused by *Schistosoma japonicum*, *S. mansoni* and *S. haematobium*, affects public health potentially (Kang, et al., 2018). Pulsatilla saponin A (**23**) extracted from the *P. chinensis* was proved to be a potential candidate to schistosomiasis infected by the *S. japonicum* and *S. mansoni*. After injecting compound **23** to *S. japonicum*-infected mice, the female and total worm were decreased by 97.2% and 89.5% *in vivo*, respectively, as well as in the mice infected by *S. mansoni* with the reductions of the female and total worms were 88.6% to 80.7%, respectively. *In vitro*, at the concentration of 8.93 μM, compound **23** killed *S. japonicum* worms with a ratio of 100% on newly transformed schistosomula, which was equivalent to the currently used drugs praziquantel and artesunate at the doses of 96.03 and 78.04 μM, respectively.

5.4. Antiviral activity

P. chinensis was used as an herbal substitutive therapy for hepatitis B patient, with a great clearance of hepatitis B virus (HBV) Yao, et al. (2009). Betulinic acid (**15**) extracted from *P. chinensis* exhibited hopeful inhibitory potency on hepatitis B virus (HBV). Compound **15** downregulated the manganese SOD expression to inhibit the HBV, additionally, compound **15** suppressed the HBV X protein and translocated into the mitochondria followed by cytochrome *C* release. Both compound **15** and the *P. chinensis* crude extracts increased the clearance of HBV. Herein, compound **15** was proved to be a potential candidate for the development of anti-HBV drug. It was worth mentioning that as a potential monomer, compound **15** had been reported for antiviral activities not limited in HBV, refractory diseases including human immunodeficiency virus (HIV) and herpes viruses HSV-1 were also introduced into the treatment (Aiken and Chen, 2005, Bildziukevich, et al., 2019, Rios and Manez, 2018), suggesting the feasibility for it to develop as antiviral agent. The antiviral potency of *P. chinensis* was disclosed to preserve plant against plant virus. After screening for 126 kinds of plants grown in the Qinling region of China for the antiviral effects against infection by *Tobacco mosaic virus*, the extracts of *P. chinensis* stand out through the half-leaf method Jing, et al. (2012), the results showed a good activity to against *T. mosaic* virus with the inhibition rate of 61.25% at the concentration of 20 μg/mL.

5.5. Other biological activities

Apart from the broad bioactivities mentioned above, cardiovascular system activity was also related to the pharmacological potential of *P. chinensis*. 23-Hydroxybetulinic acid (**49**) isolated from *P. chinensis* was reported to prevent heart from cardiotoxicity induced by the doxorubicin with a correlation on the inhibition carbonyl reductase mediated metabolism (Zhou, et al., 2015). Compound **49** alleviated the doxorubicin-induced cardiotoxicity in mice, and this was accompanied by inhibition of the metabolism of doxorubicin and reduced accumulation of doxorubicinol selectively in hearts. In H9c2 cells, the protective effect of compound **49** was shown to be closely associated with a decreased rate and extent of accumulation of doxorubicinol in mitochondria and nuclei. siRNA and docking analysis (Fig. 11) demonstrated that carbonyl reductase 1 has a crucial role in doxorubicin-mediated cardiotoxicity and compound **49** inhibiting this metabolic pathway.

With the development of molecular biology, more and more investigations have been carried out about the biocative constituents isolated from TCM with their potential molecular mechanism, providing evidence for elucidating the effective substances. Anemoside B_4 (**2**), a representative and major component with a content of up to 10% in root of *P. chinensis*, was reported to show protecting effects against cisplatin-induced nephrotoxicity through NF-κB and MAPK mediated apoptosis signaling pathways in mice recently (Wang, et al., 2020). Compound **2** mainly act on NF-κB signaling pathway to reduce the levels of TNF-α, IL-1β, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) in the kidney after CP exposure. Furthermore, compound **2** regulated MAPK signaling pathway and its downstream apoptotic factors to inhibit the occurrence of apoptosis including Bax, Bcl-2, caspase-3 and caspase-9. Notably, the activations of caspase-3 induced by cisplatin were strikingly reduced in compound **2**-treated mice.

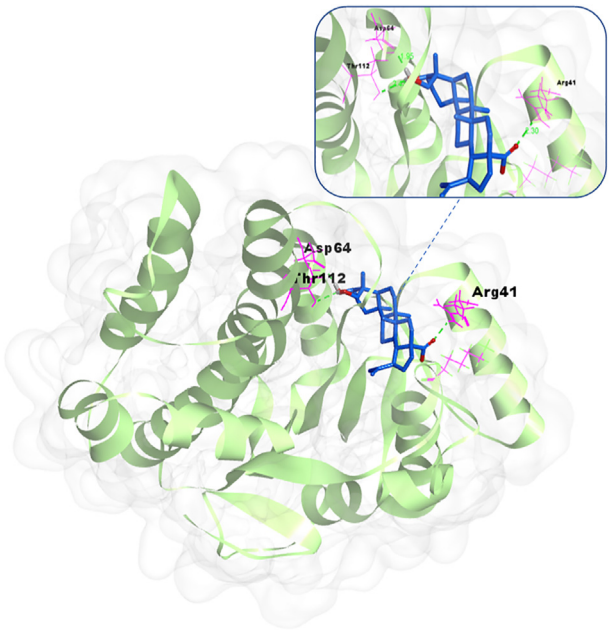

Fig. 11 Molecular docking between the potential target carbonyl reductase 1 and active compound 23-hydroxybetulinic acid (**48**) from *P. chinensis*.

6. Toxicity

Toxicity, especially hemolytic toxicity and liver injury, induced by the constituents from *P. chinensis* should be vigilant. Chronic liver injury, caused by crude *P. chinensis* saponin for a long time taking was disclosed along with the elevation of biochemical indexes including alkaline phosphatase (from 30 to 65 U/L), cereal third transaminase (from 90 to 105 U/L) and alkaline phosphatase (from 100 to 115 U/L) in patients, indicating a potential chronic liver toxicity of *P. chinensis* (Song, et al., 2019). As for the molecular biology level, recently in 2020, (Su, et al., 2020) reported the liver injury induced by *P. chinensis* saponins through interfering ceramide/sphingomyelin balance that promoted lipid metabolism dysregulation *in vivo* and apoptosis *in vitro*. Further investigations revealed that *P. chinensis* saponins caused lipid metabolism dysregulation and apoptosis through bile acid-mediated sphingolipid pathway which ultimately induced liver injury. Extrinsic and intrinsic apoptosis were observed on hepatocytes.

7. Quality control and metabolism analysis

Quality control of TCM is essential to ensure their efficiency and safety. Advanced analytical techniques provide reliable and efficient methods for the quality control of *P. chinensis* (Zhao, et al., 2019). According to the 2015 edition of Chinese Pharmacopoeia, the content of anemoside B_4 (**2**) in *P. chinensis* must be no less than 4.6 % based on HPLC calibration Standard Operating Procedure. The chromatographic separation should be performed on a C18 silica gel column, with methanol and water (65:35, V/V) as the mobile phase, and the wavelength for detection is at 201 nm.

The HPLC-MS fingerprint method has also been prevalently used in the quality control of compounds isolated from *P. chinensis*. Multiple analysis methods have been developed, such like liquid chromatography-mass spectrometry (LC-MS). Some main compounds including pulsatilla saponin A (**23**), anemoside B_4 (**2**), anemoside A_3 (**1**) and 23-hydroxybetulinic acid (**49**) have been quantifed as internal standard substances. Comprehensive methods for the quality control of *P. chinensis* are being set up. Pulsatilla saponin A (**23**) were separated by gradient elution with the mobile phase of methanol and water (25–15%) through the HPLC, moreover, the oral bioavailability of the compound **23** was obtained as 1.16 through the calculation between each major pharmacokinetic parameters (Liu, et al., 2013).

As for the metabolism analysis of the effective substances from *P. chinensis*, several studies have been published, giving inspirations for the further investigations for *P. chinensis*. Ouyang and co-workers totally identified 18 kinds of metabolites including prototype in plasm, urine and feces of rat after pulsatilla saponin D (**24**) orally administered to the rat through liquid chromatography-mass spectrometry (Ouyang, et al., 2014). The results of this study indicated that compound **24** was mainly metabolized through deglycosylation, dehydrogenation, hydroxylation and sulfation in rat.

In 2018, an research was published to investigate the dynamic condition of Gouteng-Baitouweng (GB), a pair of traditional Chinese medicine (Tian, et al., 2018). After orally administrated GB with the dosage of 25 g/kg to rats, a sensitive, selective and accurate LC-MS/MS analysis method result revealed that three contents in *P. chinensis*, anemoside B_4 (**2**), anemoside A_3(**1**) and 23-hydroxybetulinic acid (**49**) were main effective substances through the brain according to the organ distribution test results, in which compound **49** had the highest values for AUC and C_{max} at 3301.8 ng/g * h and 187.2 ng/g, respectively, thus the three saponins should be paid more attention in Parkinson treatments.

8. Conclusion

Numerous plants are traditionally and ethnopharmacologically used to treat multiple disorders, and have been proven effective (Liu et al., 2020, Zhao, et al., 2018, Zhao, et al., 2017, Zhao, et al., 2020). Therefore, researches in the area of natural medicines have improved opportunities for finding newer and safer alternatives to fight diseases. *P. chinensis* is excellent medicinal plant rich in bioactive constituents, which have been extensively studied in recent years. The traditional pharmacological activities of *P. chinensis* focused on the treatment of cool blood and stop dysentery. Modern investigation proved that triterpenoids with the prominent antitumor and anti-inflammatory activities were the main contributors to the traditional pharmacological activities. Constituents from *P. chinensis* are more promising for the treatment of inflammatory-immune related diseases in the future. While the toxicity on of the plant liver and kindey is notable as well, according to the Chinese pharmacopoeia, the suggested dosage of *P. chinensis* ranges from 9 to 15 g, thus it is vigilant when the dosage is beyond the safe range. However, there are still unclear issues about this plant. Firstly, the pharmacological activities of only a few potential compounds including typical compounds betulinic acid (**15**), pulsatilla saponin A (**23**), pulsatilla saponin D (**24**), hederacolchiside A_1 (**31**), have been investigated, further stud-

ies should pay attention to more constituents in order to discover promising precursors for the clinical drug development. Moreover, the information on randomized clinical trials (RCTs) of *P. chinensis* is limited, although *P. chinensis* is frequently used in TCM. Therefore, further studies on the effective substances, toxicity, and clinical investigations of *P. chinensis* are indispensable to meet the requirements of evidence-based medicine. It is also expected that new skeletons and new active molecules will be found from *P. chinensis*. This review brings together the most recent studies in the field of the *P. chinensis* research; therefore, it will help to promote further investigation of *P. chinensis* for the development of new herbal medicine and health products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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