

REVIEW ARTICLE

A Comprehensive Review on Botany, Phytochemistry, Traditional Uses, Pharmacology, Analytical Methods, Processing Methods, Pharmacokinetics and Toxicity of *Pulsatilla chinensis*

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ABSTRACT

Objective • To lay the foundation for further research and clinical application of *Pulsatilla chinensis* (Bge.) Regel (PR).

Method • The information presented in this paper is based on scientific databases (such as Baidu Academic, CNKI, Google Academic, PubMed, Science Direct, Web of Science, SciFinder Scholar), traditional Chinese medicine classics, Chinese Pharmacopoeia, doctoral and master's dissertations, etc. Various compounds were identified and isolated through HPLC analysis, including glycosides, polysaccharides, organic acids, phenylpropane, and flavonoids.

Results • These compounds have demonstrated a range of pharmacological effects such as anti-cancer, anti-inflammatory, insecticidal, immune-enhancing, antimutagenic, antioxidant, antiviral, antibacterial, hepatoprotective, and antidiarrheal properties. Its chemical composition and pharmacological effects are complex and diverse.

Conclusion • There is a need for further studies to investigate its mechanism, potential coordination, and antagonism. (*Altern Ther Health Med.* 2024;30(1):374-380).

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INTRODUCTION

Pulsatilla chinensis (PR) is the dry root of *Pulsatilla chinensis* of Ranunculaceae, also known as wild father, pink grass, old crown flower, and messenger of Hu Wang. It is born in Shan Ye, barren slopes and fields; it likes places with bright sunshine. There are more than 60 species of *Pulsatilla* in the world, and there are about 11 species in China, including one variety, mainly distributed in Hebei, Liaoning, Jiangsu, Anhui, Henan, and other places.

Pulsatilla chinensis has high medicinal value, and doctors have studied its medicinal value and historical origin in past dynasties. A comprehensive review of PAF is necessary to facilitate further study of the plant and its constituent compounds. Therefore, we reviewed and analyzed the available literature on aspects of botany, phytochemistry, traditional uses, pharmacological effects, analytical methods, pharmacokinetics, and toxicity of PR. This not only provides reference for the better development and utilization of PR, but also provides guidance for future PR research.

Botany

PR is a bewildering traditional Chinese medicine

because there will be a lot of adulteration in the market. Thus, PR can be distinguished from other artifacts by molecular identification. RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repetition), ISSR (Inter-SSR), AFLP (Amplified Fragment Length Polymorphism), and RFLP (Restrictive Fragment Length Polymorphism) are the early molecular marker identification methods that are widely used in species identification. Because these methods are time-consuming and expensive, they are unsuitable for rapid identification. DNA barcode is a rapid and standardized identification technique that can be used to identify PRS on the market. The pictures of PR is shown in Figure 1.

Traditional application

PR has been recorded in many ancient books. PR was first recorded in *Shen nong ben cao jing*, “*Pulsatilla chinensis*, with bitter and warm taste, is mainly used to warm malaria, and it is crazy, easy, cold and hot, with mass accumulation, gall, blood-expelling, pain-relieving, and cold sores.” *Ming yi bie lu*: “(Lord) epistaxis.” *Yao xing lun*: “It can stop abdominal pain and dysentery with red poison, treat toothache, and treat tumor furuncle under the main item.” “It hurts the joints.” *Ri hua zi ben cao*: “Cure all ethos, warm waist, and knees, improve eyesight, and eliminate redundancies.” *Zhou hou bei ji fang*: Treating “Pediatric Baldness.” The Dictionary of Traditional Chinese Medicine quotes *Ben cao bei yao*: “Treating alopecia, scrofula, hernia, bloody hemorrhoids, deviation, improving eyesight and eliminating warts.” *Tang ye ben cao*: “Cold qi, pungent and bitter taste.” *Lei gong pao*

Figure 1. The pictures of PR.



Table 1. Ancient Records of PR

Source	Record
<i>Shennong's Herbal Classic</i>	"A wild father-in-law, a messenger of King Hu."
<i>Wu pu ben cao</i>	"A wild father-in-law, a helpless grass." "Born in Songshan Valley, cure qi madness, cold and heat, and relieve pain."
<i>Ming yi bie lu</i>	"Poisonous, treating epistaxis. A helpless grass. Born in Songshan and the fields, picked in April."
<i>Ben cao jing ji zhu</i>	"A wild father-in-law, an angel of King Hu, and a helpless grass. High mountains, valleys and fields."
<i>Xin xiu ben cao</i>	"Its leaves are big like peony, and like wood pansy. Actually, there is white velvet, but pottery doesn't seem to know."
<i>Ben cao bei yao</i>	"There is a wind against the static. When there is no wind, it shakes, and there is white velvet near the root. Get good wine."
<i>Ben Cao Cheng Ya Ban Zhuo</i>	"A white-headed old man, a king's advocate, lives in the north, and this life is wuyue. Although you see it elsewhere, it's always less expensive than the two places, Luoyang, Henan, in Xin'an Mountain. Take this more, and the clouds make you live longer. Xiuji Good wine, flowers, seeds, stems, leaves, no difference in work."
<i>Ben cao meng quan</i>	"Valley fields are everywhere, Near the root, white hair is more than an inch, and he is an old man, hence his name. Harvest in autumn, dry in the sun, dry in the shade."
<i>Ben cao tu jing</i>	"Born in Songshan Valley, this is near Beijing County at the stem end of the leaf, there is white hair but not Hua Ze, and there is white velvet near the root, just like a white-headed old man, hence its name; The root is purple, as deep as a turnip."
<i>Ben cao xin bian</i>	"It is necessary to treat warm malaria, yang mania, cold and heat, and dysentery with red poison."
<i>Ben cao gang mu</i>	"Life in valleys and fields is everywhere. Spring-born seedlings, like Cynanchum paniculatum, are soft, slender and slightly longer, and the leaves have stem heads, such as apricot leaves with white hairs, and the roots are like cauliflower, with deep purple color. February is the core, and the color is yellow. Blossom purple, like hibiscus."

Table 2. Usage of PR in prescriptions in different periods

Period	Number of prescriptions/one	Number of Pulsatilla chinensis as monarch drug/piece	Proportion/%
Before and after Tang Dynasty	15	6	40.00
Song, Jin and Yuan Dynasties	48	12	25.00
Ming and Qing dynasties	21	9	42.86
modern times	14	4	28.57
total	98	31	31.63

zhi yao xing jie: "Entering the heart and kidney meridians." *Ben cao feng yuan*: "Bitter, slightly cold, non-toxic." *Chang sha yao jie*: "Enter the shaoyang gallbladder and Jueyin liver meridian." *Ben cao zheng yi*: "The taste is slightly bitter but light, the air is clear, and the quality is light."¹ The Ancient Records of PR are summarized in Table 1.

Among the searched prescriptions containing Pulsatilla chinensis, there are 31 prescriptions with Pulsatilla chinensis as the monarch drug. Before and after Tang Dynasty, a total of 15 prescriptions were retrieved, and the dosage of Pulsatilla was basically more than two. A total of 48 prescriptions were detected in Song, Jin and Yuan Dynasties, and the dosage was about one or two, among which the postpartum diseases of

Table 3. High-frequency drugs used in the prescriptions containing PR

No.	Medicines	Frequency of occurrence	Frequency/%
1	rhizome of Chinese goldthread	45	45.92
2	licorice	33	33.67
3	Chinese angelica	29	29.59
4	cork tree	24	24.49
5	rhizoma zingiberis	18	18.37
6	Qinpi	15	15.31
7	root of large-flowered skullcap	14	14.29
8	protect against the wind	14	14.29
9	pomegranate bark	14	14.29
10	rhinoceros horn	14	14.29
11	sternum/breastbone of a bird	12	12.24
12	rhizoma typhonii	12	12.24
13	Guixin	10	10.20
14	forsythia	10	10.20
15	E-gelatin	9	9.18
16	banksia rose	9	9.18
17	oyster	9	9.18
18	bark of magnolia	8	8.16
19	notopterygium	8	8.16
20	Chinese herbaceous peony	8	8.16
21	angelica	8	8.16
22	sanguisorba	8	8.16
23	Raw white peony root	8	8.16
24	fuling	7	7.14
25	folium artemisiae argyi	7	7.14
26	seaweed	7	7.14
27	large-leaved gentian	7	7.14

Table 4. Compatibility of PR

Combination	Application	Reference
Cortex Phellodendri	The Compatibility of the two has the effects of clearing away heat and dampness, cooling blood and detoxifying, and stopping dysentery.	(Chen, 2009b)
Sophora Flavescens	The Compatibility of the two can clear liver and cool blood, detoxify and relieve itching.	(Ha et al., 1997)
Cortex Fraxini	The two medicines are matched, one is to treat blood and the other is to treat qi, which complement each other, and have good effects of clearing heat and drying dampness, cooling blood and detoxifying, especially for dysentery.	(Chen, 2019)
Ejiao	The Compatibility of the two can clear away heat and toxic materials, nourish blood and nourish yin, and treat postpartum hypodynamia.	(Luo et al., 2020)
Scutellaria baicalensis Georgi and Lalang Grass Rhizome	two drugs are compatible to treat hemorrhoid bleeding.	(Yang, 2014)
Portulaca oleracea L.	The Compatibility of the two can clear damp-heat in large intestine, detoxify and stop dysentery, clear away heat and detoxify, and relieve stranguria and pain.	(Zeng, 2002)
dandelion	The Compatibility of the two can clear ying and detoxify, relieve swelling and itching, clear heat and cool blood to stop dysentery.	(Hu, 1985)
Smilax glabra	The Compatibility of the two can clear liver meridian damp-heat, kill insects, detoxify and relieve itching.	(Wang, 2001)
Sanguisorba officinalis	The Compatibility of the two can be used to treat various gynecological diseases of blood heat and blood stasis (such as menorrhagia, metrorrhagia, lochia after abortion, and recurrent menstruation).	(Xie et al., 2001)
Hedyotis diffusa	The Compatibility of the two can be used to treat acute pyelonephritis of damp-heat stagnation and downward flow of damp-heat.	(Liu et al., 2003)

children and women would be reduced. In the Ming and Qing Dynasties, 21 prescriptions were detected, and the dosage was basically no more than half. There are 14 kinds of modern prescriptions, and the dosage of Pulsatilla chinensis is one or two or two. Combined with the historical standards of weights and measures, the dosage in Tang Dynasty was relatively large, and the dosage in other periods was basically the same.² The usage of PR in prescriptions in different periods are summarized in Table 2. The high-frequency drugs used in the prescriptions containing PR are summarized in Table 3.

Clinically, PR is often combined with other traditional Chinese medicines, mainly as in Table 4.

The commonly used clinical prescriptions include pulsating Decoction, gandihuang Decoction, modified pulsating Decoction, Lixue decoction, liquorice pulsating decoction, liquorice Ejiao decoction, pulsating Decoction and so on. Among them, pulsatile soup is the most commonly used one, which mainly treats dysentery diarrhea, blood abscesses, excessive redness, and reduced whiteness. The dosage forms involved include Decoction, pill, powder, wine, tea, tablet, capsule, etc. Traditional Chinese medicine formulations containing PR are summarized in Table 5. The prescription of pulsatile soups is summarized in Table 6.

Table 5. Analysis of the formulation of Chinese medicine containing PR

Form of a drug	Traditional Chinese medicine Prescription Containing <i>Pulsatilla chinensis</i>	Number/number of prescriptions
decoction of herbal medicine	Self-made Jianyin Decoction, Zhitong Siwu Decoction, Xijiao Decoction, Tongbian Pulsatilla Decoction, Tianshui Dichang Decoction, Qingre Daozhi Decoction, Qingmuxiang Decoction, Qinqi Decoction, Oyster Decoction, keel soup (2), Forsythia Decoction, Lixue Decoction, Jiawei Pulsatilla Decoction (3), Huoxiang Decoction, Huanglian Decoction (2), Huanglian Pulsatilla Decoction, Phellodendron Decoction, etc.	37
pill	Pulsatilla Pill (6), Forsythia Pill (4), Zhizhong Jieyang Pill, Zengkui Jianpi Pill, Xijiao Pill, Wujing Pill, Evodia rutaecarpa Pill, Aconitum Pill (2), Shegan Pill, Myrrha Pill, Dysentery Pill, Khumbu Pill, Jianpi Pill, Fangfeng Pill, Tiaohuali Pill, Dashenghuashe Niu Huang Pill and Aconitum chinense Pill.	29
powdered medicine	Huangsan, Wenbu Xiaoyao San, Soufeng San, Muli San, Longgu San, Huanglian San (3), Kochia scoparia San, Danggui San and Pulsatilla Powder (2)	12
vinum	Zuixianfang, Chuanqiong wine, Pulsatilla wine (3), and Decoction with wine.	6
Danji	Jiedu Dan, Huanglian Dan, orpiment Dan and Shenglingdan.	4
herbal mixture	Diarrhea tea	1
tablet	Compound Pulsatilla Tablet, Anti-osteomyelitis Tablet, Liyaning Tablet and Qinqao Zhili Tablet.	4
capsule	Lianzhili Capsule, Compound Pulsatilla Capsule	2
External preparation	Jade powder, Liuhui decoction and Pulsatilla root prescription.	3

Phytochemistry

To the best of our knowledge, multiple chemical components have been isolated from pr. Saponins are considered its main constituents, but other constituents are reported to be coumarins, lignans, polysaccharide, flavonoids, etc. Many parts of this plant have been studied phytochemically.

As the 2020 edition of Chinese Pharmacopoeia stipulates that the medicinal part of PR is the root, there have been some studies on the root components of PR, the main active components of which are saponins, but few studies on the aerial parts of PR. This also provides some new exploration directions for the study of PR, hoping to make new research progress from the aerial parts and make certain contributions to rational utilization of resources and more thorough study of PR. The Chemical constituents isolated from PR are summarized in Table 7. The structural formula of chemical constituents of PR are shown in Figure 2.

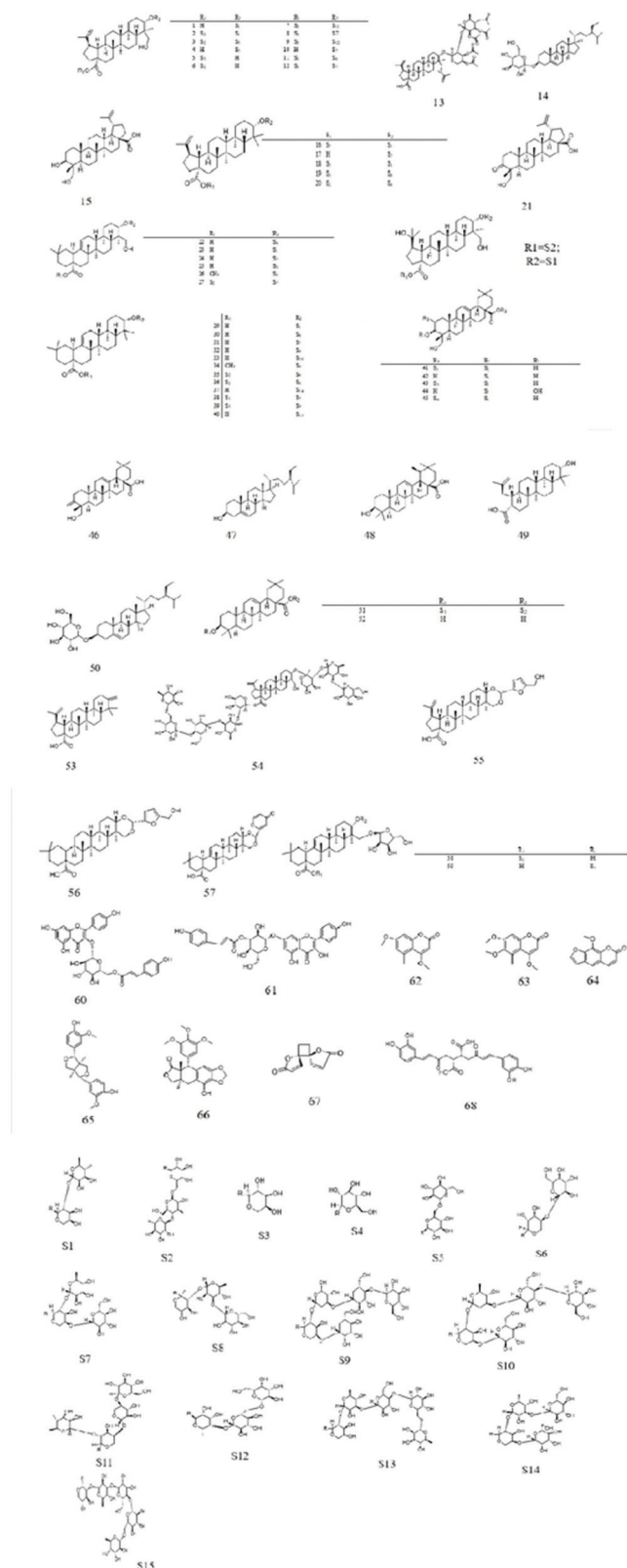
Pharmacological action

Modern pharmacological studies have shown that PR has the effects of antibacterial, antiviral, antitumor, insecticidal, immune enhancement, anti-inflammatory, antioxidant, antimutagenic, cell apoptosis, spermicidal, hepatoprotective, kidney-protective, diarrhea-resistant, and Blastocystis hominis inhibitory.³

PR also has the function of anti-biological enzyme activity. 3,4- hydroxycinnamic acid and 4-hydroxy -3-methoxy almond acid extracted from Pulsatilla koraiensis have strong anti-tyrosinase activity, and as a natural tyrosinase inhibitor, they may become new preparations for inhibiting the oxidation of L-dopa. In addition, Physalis alkekengi A

Table 6. Prescriptions of Pulsatilla Decoction

Composition and proportion of prescription	Source	Function indication	Dosage
Pulsatilla chinensis, yellow tiller, yellow bark and cortex fraxini are in twos and threes.	Treatise on Febrile Diseases and Miscellaneous Diseases	"Clearing away heat and toxic materials, cooling blood to stop dysentery, and toxic heat to dysentery. Abdominal pain, severe pain in the stomach, burning anus, diarrhea, purulent blood, redness and whiteness, thirst for water, red tongue and yellow coating, and pulse string."	"Take four flavors of medicine, seven liters of water, two liters of boiled water, go to me, take one liter warm, and take another liter if you don't heal. If there is exterior pathogenic factors, aversion to cold and fever, kudzu root, forsythia suspensa and honeysuckle are added to relieve fever through exterior surface; After being urgent, it is very heavy, adding aucklandia root, betel nut and bitter orange to regulate qi; For those with excessive pus, add Radix Paeoniae Rubra, Cortex Moutan and Radix Sanguisorbae to cool blood and blood; There is food stagnation, adding burnt hawthorn, and fructus aurantii immaturus to eliminate food stagnation."
Pulsatilla chinensis, thick cypress, donkey-hide gelatin, coptis chinensis, Cortex Fraxini, Radix Aconiti Lateralis Preparata, Cortex Phellodendri, Poria, Radix Paeoniae (pairwise each), Zingiberis Rhizoma, Radix Angelicae Sinensis, Halloysitum rubrum, Glycyrrhizae Radix, Os Draconis (pairwise each), Jujube (30 pieces), and japonica rice (one liter)	Be prepared for a thousand dollars.	Clearing heat and stopping dysentery. When treating dysentery with red blood, it is urgent and then heavy, and it won't heal for months.	Take 1.2 liters of water, boil rice until cooked, take out Mina medicine, boil 300 ml, and take it three times.
Pulsatilla chinensis 30g, Coptidis Rhizoma 9g, Cortex Phellodendri 9g, Cortex Fraxini 9g.	Fang Li Wenliang	Clearing away heat and dampness, detoxifying and stopping dysentery. Damp-heat in the stomach and intestines is dominated, and blood is divided into heat toxin, and damp-heat toxin and evil are aggregated.	Decoct with water, one dose per day, twice a day.
2 Liang of peony, 3 Liang of dried ginger, 3 Liang of halloysite, 3 Liang of licorice, 3 Liang of keel, 30 jujubes, 1 liter of Pulsatilla japonica, 2 Liang of dried ginger, 1 Liang of licorice (roasted), 1 Liang of angelica, 1 Liang of coptis chinensis, 1 Liang of pomegranate peel (2 Liang of the living)	"Waitai" Volume 25 cited "Ancient and Modern Records and Tests"	Acute and stagnation of cold dysentery	Take 8 liters of water, boil 3 liters, and divide into 4 servings.
Pulsatilla chinensis (Herba Artemisiae Anomalaе can also be used) 2 Liang, Glycyrrhiza uralensis Fisch. 2 Liang, Ejiao 2 Liang, Coptidis Rhizoma 3 Liang, Chinese cypress bark 3 Liang and dried tangerine peel 3 Liang.	Puji Fang Volume 355	The breeze calms the liver. Deficiency of main postpartum diarrhea	On the mouth (father's mouth) mouth, each take four yuan, water bowl and a half, fry until 7 points, remove the dregs, take empty clothes three times a day.
Pulsatilla chinensis 2 Liang, Coptis chinensis 3 Liang, Cupressus chinensis 3 Liang, Toona sinensis 3 Liang.	Puji Fang, Volume 212	Diarrhea due to stagnation of heat, even months of bleeding will not heal.	For every 4 bucks, take 1 cup of water, fry for 7 cents, and take it away.
Pulsatilla chinensis, Cortex Fraxini and Rhizoma Coptidis are equally divided.	Ming medicine at the palm of your hand	Self-interest due to diarrhea, red and astringent urination, and severe diarrhea due to heat.	
Pulsatilla chinensis 2 Liang, Coptidis Rhizoma 3 Liang, Cortex Phellodendri 2 Liang, Pericarpium Citri Tangerinae 2 Liang	Xingyuan	Damp-heat dysentery	Go to the mouth. 1 bucket of water, boil 5 liters, remove me, and take 1 liter each time.

Figure2. The structural formula of chemical constituents of PR.**Table 7.** Chemical constituents isolated from PR.

NO.	Name	CAS	Formula	Type	Part/Extract
1	Anemoside A3	129724- 84-1	C ₂₆ H ₄₀ O ₁₃	A	Root/ EtOH
2	Anemoside B4	129741- 57-7	C ₂₆ H ₄₀ O ₁₃	A	Root/ EtOH
3	Pulchinoside B	135247- 95-9	C ₂₆ H ₄₀ O ₁₃	A	Root/ EtOH
4	Pulsatilloside A	136684- 41-8	C ₂₆ H ₄₀ O ₁₃	A	Root/ MeOH
5	Pulsatilloside B	136684- 40-7	C ₂₆ H ₄₀ O ₁₃	A	Root/ MeOH
6	Pulsatilloside C	162341- 28-8	C ₂₆ H ₄₀ O ₁₃	A	Root/ MeOH
7	Pulsatilloside D	439590- 38-2	C ₂₆ H ₄₀ O ₁₃	A	Root/ MeOH
8	Pulsatilloside E	366814- 43-9	C ₂₆ H ₄₀ O ₁₃	A	Root/ MeOH
9	3-[(O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[O-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	366814- 44-0	C ₅₁ H ₁₁₆ O ₃₆	A	Root/ MeOH
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	848784- 85-0	C ₄₇ H ₇₆ O ₁₇	A	Root/ EtOH
11	3-[(4-O--D-glucopyranosyl-α-L-arabinopyranosyl)oxy]-23-hydroxy-, 6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	1476801- 07-6	C ₅₃ H ₈₆ O ₂₃	A	Root/ EtOH
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	1476801- 08-7	C ₅₃ H ₈₆ O ₂₇	A	Root/ EtOH
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	133377- 68-1	C ₅₃ H ₇₈ O ₁₈	A	Root/ EtOH
14	Daucosterol	474-58-8	C ₂₈ H ₄₆ O ₆	B	Root/ EtOH
15	Betulinic acid	85999- 40-2	C ₂₆ H ₄₀ O ₁₃	A	Root/ MeOH
16	Cusosaponin C	366814- 42-8	C ₅₃ H ₈₆ O ₂₃	A	Root/ MeOH
17	Betulinic acid 3β-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside	848784- 87-2	C ₄₇ H ₇₆ O ₁₆	A	Root/ EtOH
18	3-[[2-O-(6-deoxy-α-L-mannopyranosyl)-α-L-arabinopyranosyl]oxy]-6-O-β-D-glucopyranosyl-β-D-glucopyranosyl ester	1257378- 27-0	C ₅₃ H ₈₆ O ₂₁	A	Root/ EtOH
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	1476801- 09-8	C ₅₉ H ₉₆ O ₂₆	A	Root/ EtOH
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	1476801- 10-1	C ₆₅ H ₁₀₈ O ₃₀	A	Root/ EtOH
21	Pulsatillac acid	136684- 40-7	C ₂₆ H ₄₀ O ₁₃	A	Root/ EtOH
22	Leontoside B	17233- 22-6	C ₄₁ H ₆₆ O ₁₃	C	Root/ MeOH
23	Pulsatilla saponin A	27013- 91-8	C ₄₁ H ₆₆ O ₁₃	C	Root/ MeOH
24	Pulsatilla saponin D	68027- 15-6	C ₄₁ H ₆₆ O ₁₃	C	Root/ MeOH
25	Kalopanaxsaponin H	128730- 82-5	C ₄₈ H ₇₈ O ₁₇	C	Root/ MeOH
26	3-[(O-β-D-glucopyranosyl-(1→3)-O-6-deoxy-α-L-mannopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-23-hydroxy-, methyl ester	244202- 38-8	C ₄₈ H ₇₈ O ₁₇	C	Root/ MeOH
27	Pulsatilla saponin H	68027- 14-5	C ₄₁ H ₆₆ O ₁₃	C	Root/ EtOH
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	366814- 41-7	C ₅₉ H ₉₈ O ₂₇	A	Root/ MeOH
29	Tauroside C	35790- 95-5	C ₄₁ H ₆₆ O ₁₄	D	Root/ MeOH
30	Pulsatilla saponin I	103956- 33-8	C ₄₁ H ₆₆ O ₁₆	D	Root/ MeOH
31	Hederacolchiside A1	106577- 39-3	C ₄₁ H ₆₆ O ₁₆	D	Root/ MeOH
32	(3β)-3-[(O-β-D-Glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)-O-6-deoxy-α-L-mannopyranosyl-(12)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid	244202- 36-6	C ₅₃ H ₈₆ O ₂₁	D	Root/ MeOH
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	244202- 37-7	C ₅₉ H ₉₆ O ₂₆	D	Root/ MeOH
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	244202- 39-9	C ₅₄ H ₈₈ O ₂₁	D	Root/ MeOH
35	Hederacolchiside E	33783- 82-3	C ₄₁ H ₆₆ O ₁₆	D	Root/ EtOH
36	Beesioside Q	261767- 91-3	C ₆₆ H ₁₀₆ O ₃₀	D	Root/ EtOH

Table 7. (continued)

NO.	Name	CAS	Formula	Type	Part/Extract
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	106577- 41-7	C ₅₃ H ₈₆ O ₂₁	D	Root/ EtOH
38	Scabiosaponin D	689257- 60-1	C ₅₉ H ₉₆ O ₂₆	D	Root/ EtOH
39	Lonimacranthoide II	1201426- 02-9	C ₆₅ H ₁₀₆ O ₃₁	D	Root/ EtOH
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	1415553- 83-1	C ₅₉ H ₉₆ O ₂₅	D	Root/ EtOH
41	Hederasaponin C	14216- 03-6	C ₅₉ H ₉₆ O ₂₆	C	Root/ EtOH
42	Pulsatilloside C	57539- 70-5	C ₄₈ H ₇₈ O ₁₈	C	Root/ EtOH
43	Hederacoside D	760961- 03-3	C ₄₈ H ₇₈ O ₁₉	C	Root/ EtOH
44	2,3,23-trihydroxy-, O-6-deoxy-α-L-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester	1146973- 76-3	C ₄₈ H ₇₈ O ₁₉	C	Root/ EtOH
45	Leontoside D	20830-84-6	C ₄₈ H ₇₈ O ₁₉	C	Root/ EtOH
46	Ursolic acid	77-52-1	C ₃₀ H ₄₈ O ₅	E	Root/ EtOH
47	b-Sitostero	83-46-5	C ₂₇ H ₄₈ O	E	Root/ EtOH
48	Hederagonic acid	466-01-3	C ₃₀ H ₄₈ O ₅	D	Root/ EtOH
49	23-Hydroxybetulinic acid	472-15-1	C ₃₀ H ₄₈ O ₅	E	Root/ EtOH
50	Daucosterin	474-58-8	C ₂₈ H ₄₆ O ₄	B	Root/ EtOH
51	Hederasaponin B	36284-77-2	C ₅₉ H ₉₆ O ₂₅	D	Root/ EtOH
52	Oleonic acid	508-02-1	C ₃₀ H ₄₈ O ₃	D	Root/ EtOH
53	Betulinic acid	4481-62-3	C ₃₀ H ₄₈ O ₅	A	Root/ EtOH
54	Pulchinoside E	1310041- 02-1	C ₅₉ H ₉₆ O ₃₁	A	Root/ EtOH
55	Pulsatilla triterpenic acid A	1360878- 33-6	C ₄₈ H ₇₈ O ₁₈	A	Root/ EtOH
56	Pulsatilla triterpenic acid B	1360878- 34-7	C ₄₈ H ₇₈ O ₁₈	D	Root/ EtOH
57	Pulsatilla triterpenic acid C	1360878- 35-8	C ₄₈ H ₇₈ O ₁₈	D	Root/ EtOH
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	342613- 09-6	C ₅₃ H ₈₆ O ₂₂	D	Root/ EtOH
59	(3β,4α)-3-(β-D-Glucopyranosyloxy)-23-(α-D-ribofuranosyloxy)olean-12-en-28-oic acid	1500092- 25-0	C ₄₁ H ₆₆ O ₁₃	D	Root/ EtOH
60	Tiliroside	20316- 62-5	C ₃₀ H ₄₈ O ₁₃		Root/ EtOH
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	171367- 93-4	C ₃₀ H ₂₆ O ₁₂		Root/ EtOH
62	Siderin	53377- 54-1	C ₁₅ H ₁₂ O ₁		Root/ EtOH
63	4,6,7-Trimethoxy-5-methylcoumarin	62615- 63-8	C ₁₅ H ₁₂ O ₅		Root/ EtOH
64	Xanthotoxine	298-81-7	C ₁₅ H ₁₀ O ₄		Root/ MeOH
65	(+)-Pinoresinol	487-36-5	C ₂₀ H ₂₆ O ₄		Root/ MeOH
66	beta-Podophyllin	518-29-6	C ₂₀ H ₂₆ O ₄		Root/ MeOH
67	Anemonin	508-44-1	C ₁₀ H ₆ O ₄		Root
68	trans-Caffeoyltartaric acid	70831- 56-0	C ₂₀ H ₂₆ O ₁₂		Root/ nBuOH

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

and *Physalis alkekengi* B extracted from *Pulsatilla koreana* have anti-β-fructofuranosidase activity.^{4,5}

It was found that PRS has a strong spermicidal effect, which is stronger than terpene phenoxypolyoxyethylene ethanol (TS-88) but slightly weaker than nonyl phenoxypolyoxyethylene ethanol (NP-10).⁶

PR can protect the liver. Currently, isoniazid and rifampicin are still irreplaceable first-line anti-tuberculosis drugs, which often need to be combined. However, the hepatotoxicity of the combined use of them is obviously increased. It was found that PR can resist the increase of serum glutamic-pyruvic transaminase (SGPT) caused by isoniazid and rifampicin, and resist the hepatocyte death caused by hepatotoxicity caused by isoniazid and rifampicin, and has a protective effect on hepatocytes. The mechanism of action may be that PR contains free radical scavenging components such as flavonoids, which can scavenge free radicals, causing damage to dry cell membranes.^{7,8}

Besides the above effects, PR also has an anti-diarrhea effect,⁹ which can protect the kidney from nephrotoxicity caused by cisplatin,¹⁰ reduce neointimal hyperplasia after arterial injury, and inhibit proliferation, migration and

dedifferentiation of VSMC induced by growth factors. The pharmacology functions of PR are shown in table 8.

Processing method

The processing methods of PR are stir-fry PR, and *Tai ping Sheng hui Recipe*: “File and stir-fry slightly.” *Pu ji fang*: “File, fry, steamed Pulsatilla”, *Tai ping Sheng hui fang*: “Go to the reed head, steam it five times, and bake it.” *Tai ping Sheng hui fang*: “Put Pulsatilla slices in a pot, stir-fry them with strong fire until the outside is black and the inside is black and brown, spray a proper amount of cold water, kill all the sparks, take them out, and let them dry.” Now, the processing procedure of PR is removing impurities-washing-moistening-slicing-drying. Characteristics of Pulsatilla decoction pieces: round pieces, yellow-brown or tan skin, irregular longitudinal wrinkles or grooves. Pieces of decoction near the root have white fluff. The cut surface is yellowish white or yellowish brown, and the wood is yellowish. Slight gas a slightly bitter taste.

Processing effect. Pulsatilla tastes bitter and cold. Stomach and large intestine meridians entered. It has the functions of clearing away heat and toxic materials, cooling blood, stopping dysentery, eliminating dampness, and killing insects. It can be used for treating bloody dysentery due to toxic heat, vaginal itching, vaginal discharge, epistaxis, metrorrhagia, bloody hemorrhoids, carbuncle, sore eyes and eyes, etc. PR used cooling blood to stop bleeding. Such as Pulsatilla Decoction (Treatise on Febrile Diseases) for treating damp-heat and toxic dysentery; Pulsatilla plus Glycyrrhiza Ejiao Decoction for treating postpartum diarrhea due to blood deficiency (Synopsis of the Golden Chamber); For scrofula, it is combined with Angelica sinensis, Cortex Moutan and Prunellae Spica (Materia Medica Huiyan). Stir-fried charcoal enhances the hemostatic effect, such as treating metrorrhagia and metrorrhagia, and it is combined with lotus charcoal and palm charcoal (Materia Medica Huiyan). The processing methods of PR are shown in table 9.

Analysis methods and Quality control

There are more than 60 species of Pulsatilla in the world, and there are about 11 species in China. There will inevitably be some Pulsatilla-like plants for sale in the market, which requires relevant analysis methods for researchers to analyze. However, most methods have some disadvantages, such as complicated sample pretreatment, high-end instruments and equipment, high prices, toxic organic solvents, environmental soil pollution, and insufficient environmental protection. However, we believe that with the development of science and technology, more and more low-cost and high-efficiency instruments and green reagents will be developed.

Toxicity

As a natural medicine, PR has complex active ingredients. Because the ingredients of PR are complex, it is difficult to thoroughly study the therapeutic effects and side effects of PR, but on the whole, the side effects of PR will be much smaller than most Western medicines.

Table 8. Pharmacology of PR

Pharmacological action	Tested substance	Model	Tested living system/Organ/cell	Dose range	Result	Reference
resist neoplasia	Pulsatilla saponin d	H22 hepatocellular carcinoma cell tumor	ICRmice	3.125-25.50µg/ml	Pulsatilla saponin D inhibited the proliferation of SMMC-7721 cells in different degrees at 24 h, 48 h and 72 h after administration. Pulsatilla saponin D has obvious inhibitory effect on transplanted hepatoma H22 in tumor-bearing mice.	(Xu et al., 2014)
resist inflammation	anemonin	Rat intestinal microvascular endothelial cells	Rat	1.5-10g/ml	Pulsatillin may play some beneficial therapeutic roles in intestinal inflammation, at least in part by inhibiting the production of NO, ET-1 and ICAM-1 in RMECs, thus preventing intestinal microvascular dysfunction.	(Duan et al., 2006)
Antivirus and antibacterial	anemoside b4	Mouse enterovirus 71	Mice	12.5-200µM	B can enhance the antiviral effect of IFN-β by coordinating Hippo and RLRs pathways.	(Kang et al., 2022)
Anti-schistosomiasis	Pulsatilla saponin	Schistosoma japonicum cercariae	hauschka	0-8µg/ml	After the larvae were incubated with PRS of 1, 2, 3, 4, 5, 6, 7 and 8 µg/ml for 30 min, the mortality rates of the larvae were 13.47, 26.05, 60.99, 90.84, 100, 100, 100 and 100% respectively. After cercariae were incubated with PRS of 1, 2, 3, 4, 5, 6, 7 and 8 µg/ml for 30 min, the mortality of cercariae was 5.32, 18.81, 44.7, 76.87, 98.28, 100, 100 and 100% respectively. PRS has the effect of resisting Schistosoma japonicum eggs, cercariae and cercariae in vitro.	(Chen et al., 2013)
Enhance immunity	Pulsatilla saponin A3	multiple sclerosis	EAE mice		AA3 inhibited the response of T cells to encephalitis epitope caused by myelin oligodendrocyte glycoprotein (MOG). Aa significantly down-regulated the expression of some Th1 and Th17 cytokines in activated T cells stimulated by MOG. In addition, AA3 inhibits the activation of STAT4 and STAT3, which are the key factors to activate Th1 and Th17 lineage differentiation in T cells, respectively.	(F et al., 2017)
Anti-mutation and anti-oxidation	Water extract of Pulsatilla chinensis	Keywords bone marrow tuberculosis rate, serum superoxide dismutase activity, total antioxidant capacity	Mice		PWE has antimutagenic and antioxidative effects, and its mechanism may be as follows: inhibiting mutagenic effect of mutagens, reducing genetic damage of DNA by mutagens, antioxidation, eliminating free radicals, interrupting or terminating the oxidation reaction of free radicals, and enhancing the total antioxidative capacity of organism.	(Liu et al., 2008)
Protecting kidney	windflower	Renal dysfunction in mice	Mice	300mg/kg	Pulsatilla chinensis treatment can improve renal dysfunction 72 hours after cisplatin administration. Compared with the control group, the level of pro-inflammatory cytokines in mice treated with Pulsatilla root decreased significantly. In addition, Pulsatilla chinensis does not interfere with the antitumor effect of cisplatin on tumor-bearing mice.	(Nho et al., 2013)

Table 9. Processing methods of PR

Name	Operate
Raw product	Clean and dry.
Fried yellow	Put the pieces of Pulsatilla chinensis in a pot, stir-fry with slow fire until they are fragrant, the surface color becomes dark and the cross section is light yellow, and take them out.
Chaojiao	Stir-fry Radix Pulsatillae decoction pieces with strong fire until they are brown on the outside, dark on the inside, and have a burnt smell. Take them out.
Fried charcoal	Stir-fry Pulsatilla chinensis slices with strong fire until the surface is dark and the inside is brown, and take out.
Wine roasting	Add Chinese Pulsatilla slices with yellow wine, mix well, soak thoroughly, put in a pot, stir-fry with slow fire until slightly dry, and take out.
Baked with vinegar	Add rice vinegar to Chinese Pulsatilla pieces, mix well, soak thoroughly, stir-fry with slow fire until slightly dry, take out and cool.
Salt roasting	Dissolve salt in proper amount of water, pour into Pulsatilla chinensis decoction pieces, mix well, soak thoroughly, stir-fry with slow fire until slightly dry, and take out.
Ginger roast	Add ginger juice to pieces of Pulsatilla chinensis, mix well, soak thoroughly, stir-fry with slow fire until slightly dry, and take out.
Honey roasting	Dilute honey with appropriate amount of water, pour into pieces of Pulsatilla chinensis, mix well, soak thoroughly, stir-fry with slow fire until it doesn't stick to hands, and take out.

However, the reports on the toxicity of PR are mainly about PRS, and there is little research on whether other components in PR cooperate or antagonize the toxicity of PRS. There are few studies on the toxicity of other components of PR, and the toxicity of these compounds is still unclear. This study summarized the toxicity of PR and provided a strong basis for rational drug use of PR.

Pharmacokinetics

As natural antitumor drugs, Saponins have unique advantages and great development prospects. Similar to other saponins, the application of PRS is severely limited due to its hemolysis, harmful gastrointestinal irritation, and low bioavailability. To avoid harmful irritation to the gastrointestinal tract, oral administration is usually used. Generally speaking, PRS-Na is a suitable oral preparation for saponin treatment of liver cancer. It is the potential target tissue of PRS-O/W in lung, while the liver is the potential target tissue of PRS- HPβCD and PRS- Na-HPβCD. Among all the designed preparations, PRS micronized silica is the least suitable for saponin treatment of liver cancer.¹¹

CONCLUSION AND PROSPECT

As a traditional Chinese medicine, PR has complex chemical components and a wide range of pharmacological effects. Scholars have enriched the research and development of PR by continuously studying new chemical components in PR and new clinical uses of PR. This paper summarizes the limited literature on PR and presents a more comprehensive

and in-depth summary analysis and outlook. Based on previous PR studies, this review is the most comprehensive, up-to-date, and in-depth summary of PR compared with previous PR reviews. This article summarizes the phytochemistry, pharmacological effects, traditional uses, and toxicity of PR and summarizes and analyzes the analytical methods, processing methods, and pharmacokinetics of PR. However, several research challenges still need to be addressed to accelerate the research.

First, most of the current studies on the active components of PR are saponins, but there are fewer studies on the active components other than saponins, so scholars can conduct more research on these components. The 2020 edition of the Chinese Pharmacopoeia stipulates that the medicinal part of PR is its root and research on its aerial parts is scarce. In order to use resources reasonably and enrich PR research, scholars can fully study the aerial part of PR.

Second, this article summarizes the pharmacokinetics of PR for the first time. It was found that there were few studies on the pharmacokinetics of PR, mainly focused on saponins and few studies on the pharmacokinetics of other active ingredients. Scholars can conduct more in-depth and comprehensive studies on the pharmacokinetics of PR.

Third, this article summarizes the treatment methods of PR for the first time. It was found that there were fewer comparative studies on the chemical composition, pharmacological effects, toxicity, related processing mechanisms, and different processing methods of PR. In the future, scholars can conduct related studies through these

aspects to provide more comprehensive and reasonable scientific evidence for the clinical application of PR.

Fourth, toxicity assessment is as important as efficacy assessment. The toxicity of PR is currently limited to the damage of PR to the liver, with fewer studies on the toxicity of other components in PR. Scholars can enrich the toxicity study of PR and provide a reasonable basis for the rational use of PR.

As a traditional Chinese medicine, PR plays an important role in maintaining human health. This paper systematically analyzes the botany, phytochemistry, traditional uses, pharmacological effects, processing methods, analytical methods, toxicity and pharmacokinetics of PR, aiming to further analyze PR and provide a theoretical basis and guidance for its research. We firmly believe that with continued research in PR, its application can be expanded from clinical medicine to other fields.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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