REVIEW
Pharmacology Discovery 2024;4(4):21. https://doi.org/10.53388/PD202404021

Chemical constituents, pharmacology and safety of isoflavones in *Puerariae Lobatae Radix*

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Author contributions

The study was conceived by Han-Qing Pang and Yong-Juan Zhao, the review was designed by Xiao-Yu Shang, Jia-Lu Li, Tong-Yu Zhou, Hao-Feng Wang and Han-Qing Pang. Literature collection by Xiao-Yu Shang, Jia-Lu Li, Yan Zhu and Tong-Yu Zhou. The manuscript was drafted by Xiao-Yu Shang, Jia-Lu Li, Yan Zhu, Tong-Yu Zhou, Zhe Tao, and Hao-Feng Wang, with figures drawn by Tong-Yu Zhou, Hao-Feng Wang, Yang Zhou, Tong Su and Bin-Bin Zeng. Language polishing was performed by Han-Qing Pang, Yong-Juan Zhao and Yu Chen.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This study is funded by China Postdoctoral Science Foundation (2024M752727), the Natural Science Research of Jiangsu Higher education Institution of China (22KJB360018), and Jiangsu Province Graduate Research and Practical Innovation Plan (SJCX23_2042).

Peer review information

Pharmacology Discovery thanks all anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

PLR, *Pueraria Lobatae Radix*.

Citation

Shang XY, Li JL, Zhu Y, et al. Chemical constituents, pharmacology and safety of isoflavones in *Puerariae Lobatae Radix*. *Pharmacol Discov*. 2024;4(4):21. doi: 10.53388/PD202404021.

Executive editor: Xin-Yun Zhang.

Received: 19 August 2024; **Accepted:** 15 October 2024; **Available online:** 08 November 2024.

Abstract

Pueraria Lobatae Radix (PLR), also known as kudzu root, is abundant in various of active compounds. Among these compounds, isoflavones (puerarin, daidzein, genistein, etc.) is extensive studied due to their extensive pharmacological properties. This review focuses on the chemical compounds, pharmacological effects, action mechanisms and clinical studies of the isoflavone in PLR to offer new insights for prospective research of PLR. Isoflavones in PLR possessed multiple pharmacological effects, such as anti-inflammatory, anti-oxidation and neuroprotection. Studies have shown that isoflavones are expected to be applied in cardiovascular diseases, intestinal diseases, diabetes, liver disorders, and neurological conditions. Although isoflavones derived from PLR exhibit therapeutic potential for treating a variety of diseases, they may also lead to adverse reactions, such as gastrointestinal discomfort, estrogen-like effects, hepatotoxicity and nephrotoxicity. Therefore, clinical investigations should be carried out to ascertain the pharmacological actions of isoflavones obtained from the laboratory and animal studies, ensuring their safety and effectiveness in humans. More studies on developing advanced drug delivery approaches to improve the bioavailability of isoflavones and their effectiveness, as well as exploring their precise molecular and cellular mechanisms, will be useful in developing new drugs and novel therapies.

Keywords: *Pueraria Lobatae Radix*; isoflavones; pharmacological effects; side effects; safety and risks

Introduction

Pueraria Lobatae Radix (PLR), commonly known as kudzu, is a climbing plant native to East Asia and widely used in traditional Chinese medicine [1]. PLR contained isoflavones, flavonoids, triterpenoids and saponins, organic acids, polysaccharides and alkaloids, among which isoflavones are the highest and are also the active components of PLR. The isoflavones (puerarin, daidzein, genistein, and tectoridin, etc.) have been extensively studied for their potential benefits in cardiovascular diseases, diabetes, liver diseases, neurological disorders. Recent research suggested that these isoflavones executed their biological effects by influencing key cellular signaling pathways, such as reducing the generation of inflammatory mediators, protecting cells from oxidative damage, and regulating blood sugar levels [2], and endocrine regulation. The molecular structure of these isoflavones were similar with that of estrogen, which could affect estrogen-related receptors, thereby providing therapeutic potential for various diseases [3].

Despite isoflavones in PLR exerted various pharmacological activities, the development of these isoflavones in clinical applications also faced some challenges. The most significant ones included their low bioavailability and potential safety issues. The low absorption and metabolism efficiency of these isoflavones limited their effectiveness and application scope. Advanced drug delivery systems such as nanotechnology have been utilized to enhance the bioavailability of these compounds [4]. Moreover, due to their estrogen-like effects, there may be safety risks for specific populations, such as patients with hormone-sensitive diseases [5]. To reduce the potential risks caused by isoflavones from PLR, some clinical studies have been used to validate their safety and efficacy, especially their potential applications in human health and disease treatment [6].

The present review summarized the modern research progress on the chemical compounds, pharmacological actions, side effects, and clinical application of isoflavones from PLR. It offers overall knowledge of isoflavones from PLR and some deficiencies were also proposed, which could accelerate the further development of PLR isoflavones related products. The whole plant and Chinese herbal medicines of PLR were shown in Figure 1.

Materials and methods

The accessible literatures on isoflavones from PLR were obtained from published materials of electronic databases, such as SCI finder, PubMed, Wed of Science, Springer and Google Scholar. The relevant information was also acquired from *Chinese Pharmacopoeia* and Chinese herbal classic books. ChemDraw Ultra 20.0 software was used to draw the chemical structures. Figdraw was utilized to draw the action mechanisms of isoflavones in nervous system, cardiovascular system and intestinal tract diseases.

Ingredients of Pueraria mirifica isoflavones

Composition and contents

Currently, more than 100 types of isoflavones have been isolated in PLR, mainly including puerarin, daidzein, tectoridin, and irisolidone [7] (see Table 1 and Figure 2). The types and contents of isoflavones varied significantly among different plants parts of PLR. The roots and leaves are main sources of isoflavones, and the total contents of isoflavones decreased in the following order: roots > leaves > stems > flowers. The content of puerarin in roots was the highest, while the contents of irisolidone and daidzein were relatively high in leaves [8]. Moreover, the growth environment also significantly affected the contents and types of PLR isoflavones [9]. Some studies have indicated that puerarin, irisolidone, and daidzein and their derivatives were the main PLR isoflavones [9].

Structures

Isoflavones in PLR are a class of plant estrogens, most of which are glycosidic compounds with a 3-phenylchroman structure [10]. These isoflavones had a basic skeleton of C_6-C_3-C_6 combined with glycone or glycoside to form phenolic compounds. Isoflavone glycosides have a large π-π conjugated system [11, 12], forming an approximately planar structure in space. The types of isoflavones in PLR was extremely complex, containing many different isomers.

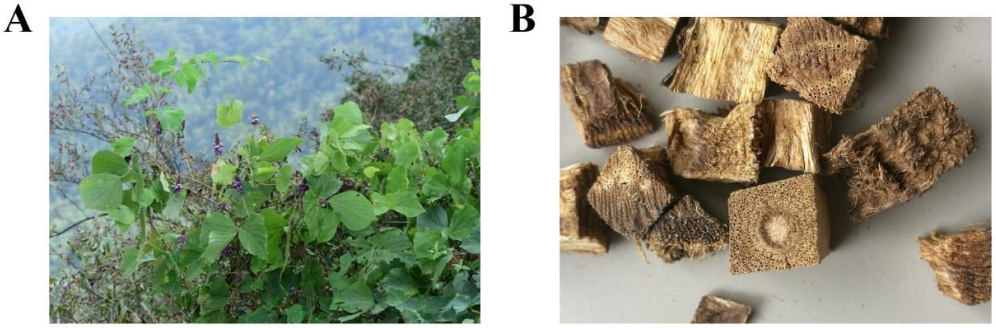


Figure 1 Whole plant and Chinese herbal medicines of *Pueraria lobata*. (A) Whole plant of *Pueraria lobata*. (B) Chinese herbal medicines of *Pueraria lobata.*

Table 1 Compound, molecular formula, CAS and logp of major isoflavones in PLR

NO	Compound	Molecular formula	CAS	Logp	Reference
1	Puerarin	$C_{21}H_{20}O_{10}$	3681-99-0	–0.67	[12]
2	Daidzein	$C_{15}H_{10}O_4$	486-66-8	2.78	[13]
3	Genistein	$C_{15}H_{10}O_5$	446-72-0	2.96	[14]
4	Prunetin	$C_{16}H_{12}O5$	552-59-0	3.53	[15]
5	Genistein-7-*O*-β-D-glucoside	$C_{21}H_{20}O_{10}$	529-59-9	0.79	[16]
6	Daidzin	$C_{21}H_{20}O_9$	552-66-9	0.45	[17]
7	Kakkalide	$C_{28}H_{32}O_{15}$	58274-56-9	1.62	[18]
8	Glycitein	$C_{16}H_{12}O_5$	40957-83-3	2.57	[19]
9	Glycitin	$C_{22}H_{22}O_{10}$	40246-10-4	0.16	[20]
10	Tectoridin	$C_{22}H_{22}O_{11}$	611-40-5	0.29	[21]

PLR, *Pueraria Lobatae Radix*.

Table 1 Compound, molecular formula, CAS and logp of major isoflavones in PLR (*Continued*)

NO	Compound	Molecular formula	CAS	Logp	Reference
11	Tectorigenin	$C_{16}H_{12}O_6$	548-77-6	2.54	[22]
12	Formononetin	$C_{16}H_{12}O_4$	485-72-3	2.96	[23]
13	3'-Hydroxyl daidzein	$C_{15}H_{10}O_5$	485-63-2	2.58	[24]
14	3'-Methoxy daidzein	$C_{16}H_{12}O_5$	21913-98-4	2.54	[15]
15	3'-Methoxypuerarin	$C_{22}H_{22}O_{10}$	117047-07-01	1.72	[25]
16	3'-Hydroxypuerarin	$C_{21}H_{20}O_{10}$	117060-54-5	1.75	[15]
17	Isoformononetin	$C_{16}H_{12}O_4$	486-63-5	3.17	[25]
18	Irisolidone	$C_{17}H_{14}O_6$	2345-17-7	2.88	[26]
19	4;-methoxypuerarin	$C_{22}H_{22}O_9$	92117-94-7	2.14	[27]
20	BiochaninA	$C_{16}H_{12}O_5$	491-80-5	3.14	[28]
21	Ononin	$C_{22}H_{22}O_9$	486-62-4	0.63	[29]
22	NeopuerarinA	$C_{21}H_{20}O_9$	1150314-34-3	0.90	[30]
23	NeopuerarinB	$C_{21}H_{20}O_9$	1150314-39-8	0.90	[30]
24	8-prenyldaidzein	$C_{20}H_{18}O_4$	135294-00-8	4.87	[28]
25	Lupiwighteone	$C_{20}H_{18}O_5$	104691-86-3	5.05	[31]
26	Wighteone	$C_{20}H_{18}O_5$	51225-30-0	5.05	[32]
27	6,7,4'-Trihydroxyisoflavone	$C_{15}H_{10}O_5$	17817-31-1	2.17	[33]
28	IristectorigeninA	$C_{17}H_{14}O_7$	39012-01-6	2.59	[34]
29	Puerarin-6"-*O*-xyloside	$C_{26}H_{28}O_{13}$	114240-18-5	1.48	[35]
30	Mirificin	$C_{26}H_{28}O_{13}$	103654-50-8	1.21	[36]
31	Mirificin-4'-*O*-glucoside	$C_{32}H_{38}O_{18}$	168035-01-6	–1.44	[37]
32	Puerarin-4'-*O*-glucoside	$C_{27}H_{30}O_{14}$	117047-08-2	–0.70	[38]
33	Genistein-8-*C*-glucoside	$C_{21}H_{20}O_{10}$	66026-80-0	0.09	[39]
34	6"-O-malonyl genistin	$C_{24}H_{22}O_{13}$	51011-05-3	2.22	[40]
35	Kakkalidone	$C_{23}H_{24}O_{11}$	6009-88-7	0.36	[41]
36	6"-*O*-xylosyl-glycitin	$C_{27}H_{30}O_{14}$	231288-18-9	1.37	[22]
37	Tectorigenin-7-*O*-xylosylglucoside	$C_{27}H_{30}O_{15}$	231288-19-0	1.44	[42]
38	Daidzein-4', 7-diglucoside	$C_{27}H_{30}O_{14}$	53681-67-7	–2.20	[43]
39	Ambocin	$C_{26}H_{28}O_{14}$	108044-05-9	1.47	[35]
40	8-O-methyl retusin	$C_{17}H_{14}O_5$	37816-20-9	2.32	[44]
41	Fujikinetin methyl ether	$C_{18}H_{14}O_6$	2746-85-2	3.44	[45]
42	Calycosin 7-*O*-glucoside	$C_{22}H_{22}O_9$	20633-67-4	0.09	[46]
43	Psi-Tectorigenin	$C_{16}H_{12}O_6$	13111-57-4	2.22	[47]
44	Pseudobaptigenin	$C_{16}H_{10}O_5$	90-29-9	3.06	[48]
45	5-Hydroxypseudobaptigenin	$C_{16}H_{10}O_6$	40624-03-1	3.24	[15]
46	4',7-Dimethoxyisoflavone	$C_{17}H_{14}O_4$	1157-39-7	3.43	[25]
47	Isoflavone	$C_{15}H_{10}O_2$	574-12-9	3.58	[49]
48	3'-methoxy-daidzin	$C_{22}H_{20}O_{10}$	200127-80-6	0.22	[25]
49	Neobavaisoflavone	$C_{20}H_{18}O_4$	41060-15-5	4.87	[50]
50	Corylin	$C_{20}H_{16}O_4$	53947-92-5	4.45	[51]
51	IristectorigeninB	$C_{17}H_{14}O_7$	86849-77-6	2.17	[52]
52	Puerarone	$C_{20}H_{16}O_5$	116107-15-4	5.09	[53]
53	Parvisoflavanone	$C_{17}H_{16}O_7$	49776-79-6	2.87	[54]
54	Calycosin	$C_{16}H_{12}O_5$	20575-57-9	2.41	[55]
55	Maximaisoflavone J	$C_{21}H_{20}O_4$	16277-87-5	5.32	[56]
56	Artocarpanone	$C_{16}H_{14}O_6$	520-25-2	2.65	[57]
57	Piscerythrone	$C_{21}H_{20}O_7$	6506-96-3	4.76	[58]
58	Dihydrodaidzein	$C_{15}H_{12}O_4$	17238-05-0	2.79	[59]

PLR, *Pueraria Lobatae Radix*.

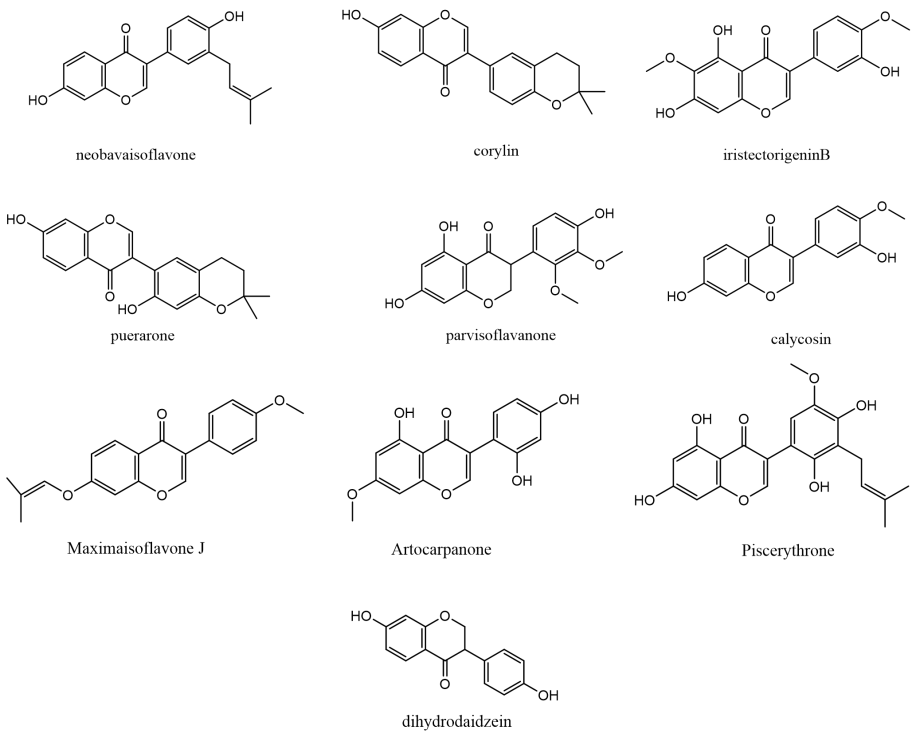


Figure 2 Chemical structures of major isoflavones in *Pueraria Lobatae Radix*

Quantitative analysis

Currently, high performance liquid chromatography and liquid chromatography coupled with mass spectrometry techniques have been utilized to determine the contents of isoflavones in PLR. Ultra-high performance liquid chromatography has been used for the simultaneous quantification of major isoflavones in PLR within 10 minutes [19]. To increase the extraction efficiency of isoflavones, subcritical water extraction has been used to extract the isoflavones from PLR, which was a greener and more sustainable extraction approach [60]. Moreover, proton nuclear magnetic resonance was used to effectively quantify seven isoflavones, providing a reliable method for the standardization of these isoflavones in PLR [61].

Biosynthesis

Roots and stems are the major sites of isoflavone biosynthesis in PLR [62]. Isoflavone biosynthesis is a branch of phenylpropanoid metabolism that occurs through the hybridization pathway of phenylpropanoid and isoflavones [63]. The 2-hydroxylation of the C ring of flavanones could be catalyzed by 2-HIS, and the products were then catalyzed by 2-HID to yield isoflavone products, such as daidzein or genistein [23]. 2-HIS is a membrane-associated cytochrome P450 enzyme belonging to the CYP93C subfamily, and it is the first key enzyme catalyzing the synthesis of isoflavones [24]. Puerarin is biosynthesized by hydroxylation at the C-2 position via the phenylpropanoid pathway, generating its isoflavone backbone [64]. Transcription factors play an important role in the biosynthesis of secondary metabolites [63]. The regulation of transcription factors of flavonoids has been extensively studied in many plant species, while the role of isoflavone biosynthesis in PLR is seldom reported [64]. Shen et al. found that the transcript levels of the PlMYB1, PlHLH3-4 and PlWD40-1 genes were closely related to the isoflavone biosynthesis in different tissues of PLR [24, 64]. The biosynthesis pathway of isoflavone in PLR was shown in Figure 3.

Pharmacological studies

Nervous system disease

Isoflavones in PLR, especially puerarin, play a neuroprotective role by inhibiting oxidative stress, cytotoxicity, and apoptosis [65]. The neuroprotective effects of PLR isoflavones were shown in Table 2. Transient receptor potential melastatin-related 2 is an ion channel that regulates pyramidal neuron death in the CA1 region of the hippocampus. The TRPM2/NMDAR pathway was blocked in neurons of bilateral common carotid artery occlusion rats treated with puerarin, preventing the overproduction of reactive oxygen species [66]. Puerarin reduced the blood-brain barrier damage, and the possible mechanism was the inhibition of the NLRP3/Caspase-1/GSDMD-mediated classical pyroptosis pathway [67]. Moreover, rhodopsin in PLR could activate the BDNF-TRK pathway and increase neuronal cell viability and proliferation [30]. Isoflavones in PLR could exert antidepressant effects through increasing protein expression of AKT1 and FOS, and decreasing protein expression of CASP3, STAT3 and TNF-α [68]. The antidepressant effects observed in the treatment of ovariectomy mice with puerarin may be related to the inhibition of hypothalamic-pituitary-adrenal axis or the up-regulation of BDNF mRNA expression in the hippocampus [69]. Mechanisms of isoflavones in nervous system have been summarized in Figure 4.

Cardiovascular system

Numerous evidences indicated that isoflavones in PLR had excellent effects in cardiovascular system. Puerarin promoted cardiac function and prevented myocardial infarction by regulating the PPAR-γ/NF-κB and Akt/HO-1 pathways, thereby decreasing the cardiovascular risk [1, 3]. Furthermore, puerarin protected from ischemia/reperfusion-induced myocardial injury by enhancing VEGFA and Ang-1, so as to reverse cardiovascular fibrosis through the Nrf2/ROS pathway [70]. Iron death has been discovered as a new cell death mechanism of the failing heart, and some studies have demonstrated that puerarin could counter iron death caused by cardiovascular diseases [5]. Aside from puerarin, other isoflavones derived from PLR also played a crucial role in the cardiovascular system. Daidzein had anti-inflammatory and antioxidant properties, which could maintain the vascular homeostatic state [7]. Mirificin could diminish oxidative injury to cardiovascular tissues, it has potential to reduce blood pressure and enhance the heart's function

[71]. The cardiovascular effects of isoflavones in PLR were summarized in Table 3.

Isoflavone in PLR could exert anti-inflammation, anti-oxidation, anti-apoptotic, and inhibiting iron death effects through regulating a variety of important cardiac vasculature pathways, thus enhancing heart function. Mechanisms of PLR isoflavones in cardiovascular system were shown in Figure 5.

Intestinal tract diseases

Recent studies have shown that the isoflavones in PLR showed benefits for intestinal function and microecology. Isoflavones exerted their effects on the gut diseases through diverse mechanisms. The effects of isoflavones in PLR against intestinal tract diseases were listed in Table 4. A few PLR isoflavones exerted significant effects on gut metabolism, such as puerarin, genistein, and daidzin.

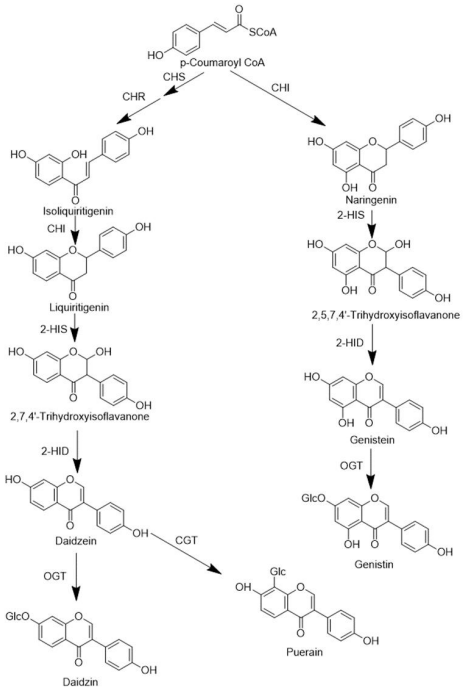


Figure 3 The biosynthesis pathway of isoflavone in PLR. PLR, *Pueraria Lobatae Radix*; CHS, Chalcone Synthase; CHR, Chalcone Reductase; CHI, Chalcone Isomerase; OGT, O-GlcNAc transferase.

Table 2 The neuroprotective effects of major isoflavones in *Pueraria Lobatae Radix*

Component	Dose	Animal models	Effects	Mechanism	Ref
Puerarin	20, 40 and 80 mg/kg	Early brain injury rat model	Reducing neurological dysfunction, and oxidative stress injury	Regulating AMPK/PGC1α/Nrf2-signaling pathway	[72]
Daidzein	50 mg/kg	High-fat diet rat model	Increasing SGZ cell proliferation and educing hippocampal apoptosis and gliosis	Regulating caspase 3, FosB, GFAP and Iba1	[73]
Daidzein	10 and 20 μM	LPS induced BV2 cells model	Exerting neuroprotective effects	Preventing against mitochondrial oxidative stress	[74]
Daidzein	200 mg/kg	High-fat diet rat model	Neuroprotective effects of DZ in the cerebellar layers	Activating TrkB signaling event	[75]
Formononetin	30 mg/kg	Male Sprague Dawley rats	Alleviating the neurological deficit and the pathological state of brain tissues and reducing the volume of cerebral infarction	Regulating JAK2/STAT3 signaling pathway	[76]
Formononetin	10, 30 mg/kg	Traumatic brain injury rat model	Exerting the neuroprotective and antioxidant effects against TBI	Regulating Nrf2-dependent antioxidant pathway	[77]
Formononetin	25 mg/kg	Chronic inflammatory pain mouse model	Anxiolytic effect	Inhibiting microglia activation by NF-κB p65 signaling pathway	[78]
Genistein	15 and 30g/kg	Ovariectomized rat model	Reducing the neural apoptosis	Attenuating oxidative stress, lipid peroxidation and the mitochondria-mediated apoptotic pathway	[79]
Daidzin	1, 5, 10 mg/kg	Pentylenetetrazole-induced mice model	Antioxidant and anti-epileptic properties	Inhibiting VEGF signaling pathway	[80]
Tectorigenin	5, 10 mg/kg	LPS induced BV2 cells model	Exerting anti-neuroinflammatory activity	Suppressing NF-κB/ERK/JNK-related signaling pathways	[81]
Tectorigenin	25, 50 and 100 μM	Rat C6 astroglioma cells	Inhibiting oxidative stress	Regulating HO-1/NQO1 signaling pathways	[82]

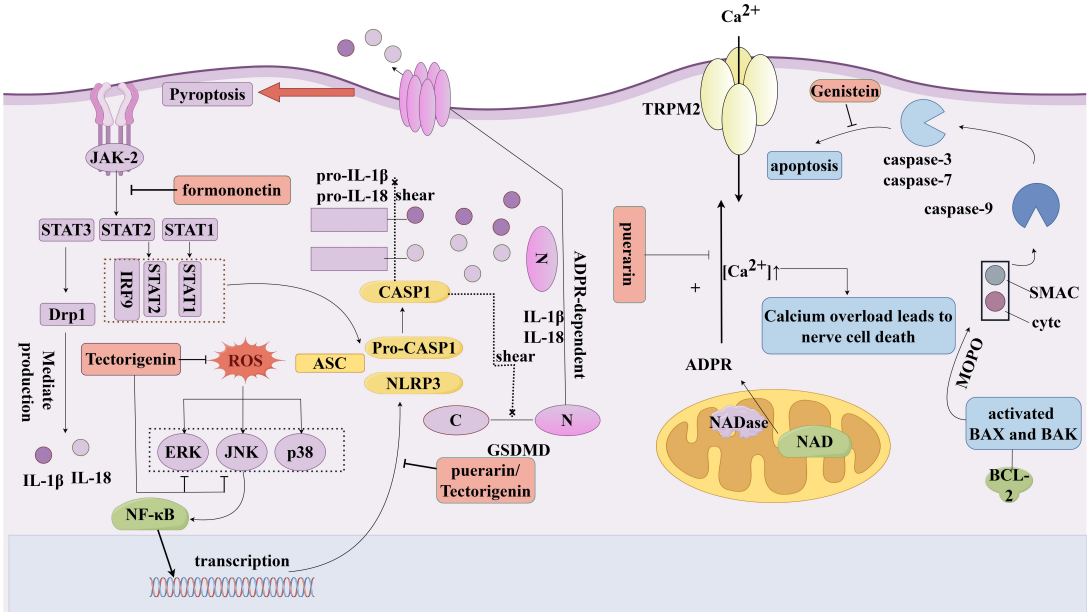
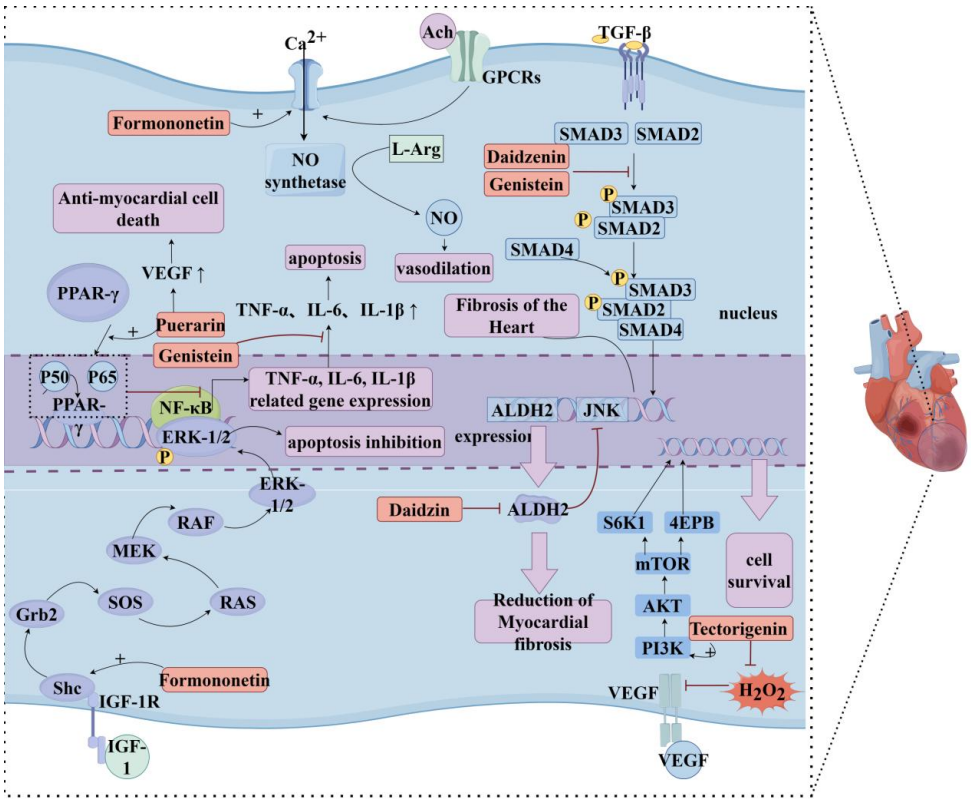


Figure 4 Mechanisms of major isoflavones actions in nervous system. JAK, janus kinase; STAT, recombinant signal transducer and activator of transcription 1; IRF, interferon regulatory factor; ERK, extracellular regulated protein kinases; JNK, c-Jun N-terminal kinase; p38, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-B; NLRP3, NOD-like receptor thermal protein domain associated protein 3; CASP1, caspase1; GSDMD, gasdermin D; HO-1, heme oxygenase-1; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid-2-related factor 2; Keap1, kelch-like ECH-associated protein 1; ER, estrogen receptor; ERE, estrogen response element; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia inducible factor-1; TRPM2, transient receptor potential melastatin 2; BCL-2, B-cell lymphoma-2; SMAC, second mitochondria-derived activator of caspases; BAK, recombinant Bcl2 antagonist/killer; ADPR, ADP-ribosylation; PRAP, proline-rich acidic protein; PARG, eukaryotic poly ADP ribose glycohydrolase. The figure was created by Figdraw (www.figdraw.com).

Table 3 The cardiovascular effects of major isoflavones in *Pueraria Lobatae Radix*

Component	Dose	Effects	Mechanism	Ref
Puerarin	100 mg/kg	Protecting against sepsis-induced myocardial injury	Exerting cardioprotective effects by attenuating inflammation and oxidative damage	[83]
Puerarin	60 mg/kg	Having a promising potential for treating chronic heart failure	Increasing the expression of PPARα and its downstream target genes GLUT4 and CD36	[84]
Puerarin	50, 100 and 150 mg/kg	Reducing myocardial fibrosis, inhibiting mitochondrial damage and improving myocardial contractile function	Inhibiting the activation of p38MAPK and its downstream activation by Na^+/H^+ exchange isoform 1	[85]
Daidzein	5, 10 mg/kg	Attenuating endothelium-intact aortas	Regarding oxidative stress markers, daidzein treatment attenuated the increased malondialdehyde content and reduced activity of superoxide dismutase	[86]
Daidzein	10 mg/kg	Treating doxorubicin-induced heart failure	Ameliorating cardiac inflammation and fibrosis, cardiac apoptosis, oxidative stress and cardiac energy imbalance	[87]
Daidzein	10 μM to 100 μM	Improving myocardial infarction-induced cardiac dysfunction and cardiac fibrosis	Reducing TGF-β1-induced cardiac fibroblast activation by regulating the TGF-β1/SMAD2/3 signaling pathway	[88]
Formononetin	10 mg/kg	Attenuating the development of atherosclerosis	Regulating the interplay between KLF4 and SRA	[89]
Formononetin	10, 30 and 100 μmol	Decreasing the arterial pressure	Regulating NO release and Ca^{2+} channels	[90]
Formononetin	0.5, 1, 2, 5, 8 and 10 μM	Exhibiting a protective effect on HUVECs	Increasing vascular endothelial growth factor and p-ERK1/2 expression levels	[91]
Genistein	1 mg/kg	Rescuing pulmonary vascular remodeling	Downregulating the estrogen receptor-β expression	[92]
Genistein	5 and 25 mg/kg	Attenuating myocardial fibrosis in type 1 diabetic rats	Inhibiting the TGF-β1/Smad3 signaling pathway and regulating collagen expression in dilated cardiomyopathy	[93]
Daidzin	60 μM	High glucose-induced cardiomyocyte injury	Inhibiting the activities of ALDH2	[94]
Tectorigenin	0.1, 0.2, 0.5, 1 and 10 μmol/L,	Protecting HUVECs from H_2O_2-induced oxidative stress injury	Activating the PI3K/Akt pathway	[95]
Tectorigenin	50 mg/kg	Improving cardiac fibrosis and cardiac function in diabetic cardiomyopathy mice	Upregulating the phosphorylation of adenosine 5'-monophosphate-AMPK by preventing the ubiquitination of AdipoR1	[96]



Figture 5 Mechanisms of PLR isoflavones in cardiovascular system. PPAR, peroxisome proliferator-activated receptors; IGF, insulin-like growth factor; Shc, Src homology 2 domain containing; Grb2, growth factor receptor-bound protein 2; SOS, son of sevenless; RAS, rat sarcoma; MEK, mitogen-activated protein kinase; RAF, raf protein kinase; ERK-1/2, mitogen-activated protein kinase; NF-B, nuclear factor kappa-B; VEGF, vascular endothelial growth factor; GPCRs, G protein-coupled receptors; L-Arg, arginine; SMAD, mothers against DPP homolog; JNK, c-Jun N-terminal kinase; ALDH2, aldehyde dehydrogenase; TGF-β, transforming growth factor-β; AKT, proteinkinase B; mTOR, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase beta-1; 4EPB, 4E-bind-ing protein 1; IL-1, interleukin-1β; IL-6, interleukin-6; TNF-α, transforming growth factor-α; PI3K, phosphatidylinositol 3-kinase. The figure was created by Figdraw (www.figdraw.com).

Table 4 The intestinal effects of major isoflavones in *Pueraria Lobatae Radix*

Component	Dose	Effects	Mechanism	Reference
Puerarin	10, 50 mg/kg	Preventing the breakdown of barrier integrity	Increasing levels of tight junction proteins	[97]
Puerarin	200 mg/kg	Relieving ulcerative colitis	Increasing the levels of SCFAs	[98]
Puerarin	100 mg/kg	Reversing the impairment of the intestinal caused by influenza virus infection	Reducing the levels of TLRs and inflammatory factors in the intestines and attenuating inflammatory damage	[99]
Daidzein	12.5–200 μM	Resisting intestinal epithelial barrier injury	Suppressing the PI3K/AKT and P38 pathways	[100]
Daidzein	50 mg/kg	Protecting from intestinal ischemia–reperfusion injury	Reducing caspase-6 expression	[101]
Daidzein	5 and 10 mg/kg	Inhibiting colon cancer	Inhibiting the p-ERK/ERK and p-AKT/AKT pathways	[102]
Formononetin	25, 50 and 100 mg/kg	Suppressing gastric ulcer	Inhibiting NF-κB signaling pathway	[103]
Formononetin	50, 250, and 500 mg/kg	Exerting gastroprotective effect	Decreasing gastric secretion volumes and increasing mucus production	[104]
Formononetin	10, 30, 50, 80, and 100 μM	Inhibiting the growth and aggressiveness of GC cells	Reducing the levels of miR-542-5p	[105]
Genistein	140 mg /kg	Preventing colon cancer	Reducing the mRNA expression of COX2, TNF, and FRAT-1	[106]
Genistein	0.01–50.00 μmol/L	Inhibiting the contractile activity of gastrointestinal smooth muscle	Activating α-adrenergic receptors, NO and cAMP pathways, and KATP channels	[107]
Genistein	100 mg/kg	Protecting against acetic acid-induced ulcerative colitis	Upregulating the INF-γ/JAK1/STAT1 and INF-γ /TLR-4/NF-κB signaling pathways	[108]
Irisolidone	20 or 50 mg/kg	Attenuating ethanol-induced gastritis	Inhibiting IL-8 secretion and NF-κB activation	[109]

Puerarin played significant roles in the gut diseases mainly through three probable mechanisms. Firstly, puerarin significantly altered the gut flora by increasing the Akkermansia muciniphila, so as to achieve anti-inflammatory and anti-obese benefits [110]. Puerarin also strengthened the intestinal barrier functions by increasing the overexpression of Muc2 and ZO-1 [111]. Furthermore, puerarin could inhibit the expression of various inflammatory cytokines in the gut such as TNF-α and IL-6, thus further strengthening the gut [112]. These findings make puerarin a potential natural adjuvant for gut pathologies prevention.

Genistein could undergo methylation and hydroxylation reactions to produce multiple active metabolites [64]. These metabolites have obvious physiological activities, such as antithrombotic and antiallergic effects. For example, genistein metabolites played essential roles in platelet aggregation and immune response [113]. Genistein-related metabolites could also influence intestine cell proliferation to improve the intestinal health via activating the estrogen-related pathway [114]. These findings have shown that the metabolic transformation of Genistein could maintaining gut health.

Daidzein enhanced the integrity of the intestinal epithelial barrier by upregulating the expression of tight junction proteins like ZO-1, occluding, and claudin-1, while also dampened the inflammatory response through inhibiting PI3K/AKT and P38 MAPK pathways [100]. Moreover, daidzein have been discovered to enhance intestinal mucosal barrier function by boosting the expression of antioxidant and anti-inflammatory factors [115]. Mechanisms of PLR isoflavones in intestinal diseases were summarized in Figure 6.

Antitumor

Various biological pathways are involved in the mechanisms of PLR isoflavones for the treatment of tumors. Isoflavones in PLR significantly suppressed the proliferation of human breast cancer cells by enhancing mitochondria-dependent and non-dependent apoptotic pathways [6]. The isoflavones in PLR also possessed potential anti-tumor activity by downregulating the levels of TNF-α and IL-6 [9]. Puerarin obviously reduced the oxidative and inflammatory processes, thereby protecting from liver tumors [116]. 3'-methoxyneopuerarin A and 3'-methoxyneopuerarin B, two new isoflavones in PLR, exerted their antitumor effects through activating the apoptotic pathway, suppressing the inflammatory responses and oxidative stress [31]. Thus, these studies indicated that isoflavones in PLR had the potential application in cancer treatment.

Diabetes

The therapeutic effects of PLR isoflavones on diabetes were involved with several molecular mechanisms, including the promotion of pancreatic β-cell division and the reduction of cell apoptosis, leading to the normal insulin secretion. The treatment effects were closely related to the activation of cAMP/PKA signaling pathway, which could increase cellular response to insulin [117]. Isoflavones in PLR also prevented diabetes-induced inflammation and oxidative stress through anti-inflammatory and antioxidant activities, thereby protecting the pancreas islet cells [118]. Moreover, these isoflavones could enhance gut health and relieve from diabetes through the modulation of gut microbial composition [119]. These multi-mechanisms were geared towards the potential value of PLR isoflavones in diabetes.

Liver diseases

In recent times, isoflavones in PLR have exhibited favorable pharmacological effects in liver ailments by influencing metabolic and inflammatory pathways. Isoflavones in PLR, such as puerarin and daidzin, could effectively regulate lipid and alcohol metabolism, diminish lipid buildup and inflammation by activating AMP/AMPK pathways, thereby mitigating liver damage [120]. The anti-diabetic properties of these isoflavones also contributed to their efficacy in treating non-alcoholic fatty liver disease. Lots of isoflavones in PLR (puerarin, daidzein, genistein, and daidzein, etc.) exerted the obvious liver-protection effects by inhibiting oxidative stress and inflammatory response, as well as regulating liver function and lipid metabolism [121]. These findings heightened the potential of PLR isoflavones for the developments of novel hepatoprotective drug.

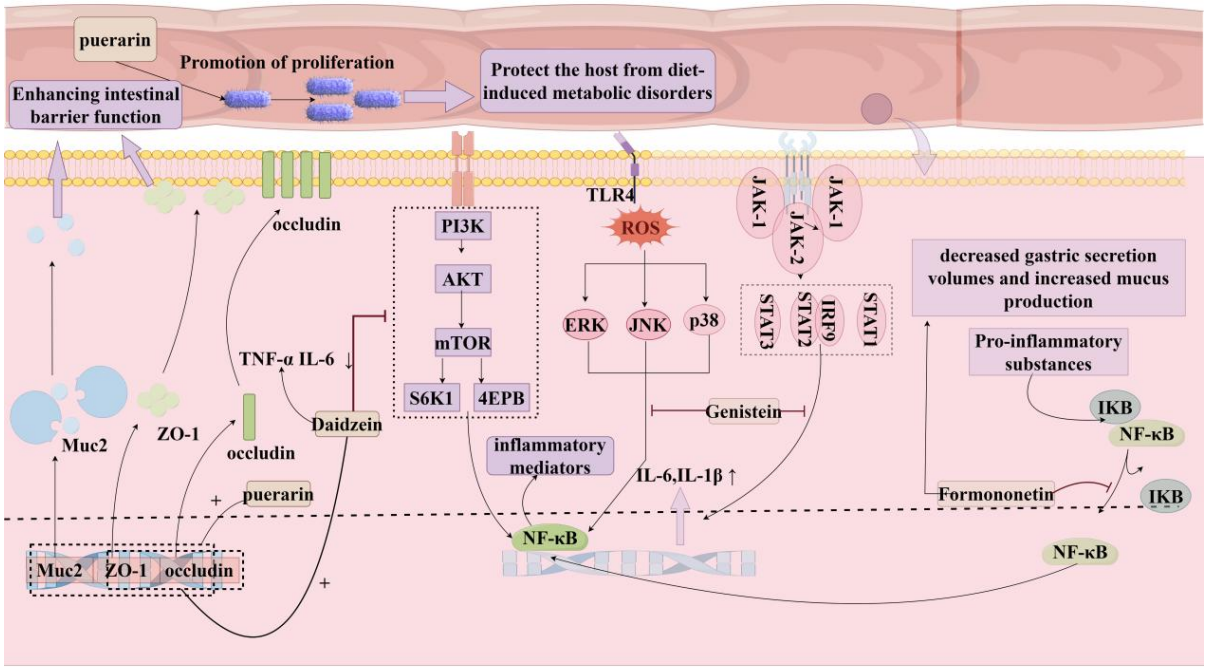


Figure 6 Mechanisms of major isoflavones actions in intestinal tract. Muc2, Mucin 2; ZO-1, zonula occludens-1; TNF-α, transforming growth factor-α; IL-6, interleukin-6; PI3K, phosphatidylinositol 3-kinase; AKT, proteinkinase B; mTOR, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase beta-1; 4EPB, 4E-bind-ing protein 1; NF-κB, nuclear factor kappa-B; IL-1β, interleukin-1β; ERK, extracellular regulated protein kinases; JNK, c-Jun N-terminal kinase; p38, mitogen-activated protein kinase; IKB, recombinant inhibitory subunit of NF Kappa B Delta; JAK, januskinase; STAT, signal transducer and activator of transcription; IRF-9, recombinant interferon regulatory factor 9; ROS, reactive oxygen species; TLR4, toll-like receptor 4. The figure was created by Figdraw (www.figdraw.com).

Other diseases

Isoflavones in PLR, such as calycosin and daidzein, could suppress the activity of tyrosinase (a key enzyme in the synthesis of melanin), thus reducing skin aging and pigmentation [56]. These isoflavones prevented ocular diseases (particularly macular degeneration) through protecting retinal cells [122]. Puerarin has been demonstrated to prevent osteoarthritis development through anti-inflammatory effects [123].

Negative reaction/evaluation of safety

Although isoflavones in PLR showed potential in treating various diseases, they also caused some adverse reactions, especially for specific populations. These isoflavones in PLR may cause gastrointestinal discomfort [61], liver toxicity [116], and drugs interactions [124], affecting the efficacy and safety of medications. Moreover, due to the phytoestrogen properties, PLR isoflavones at high doses might influence hormonal balance and reproductive health [125]. For individuals with kidney issues, high doses of these isoflavones may exacerbate renal stress. Therefore, it was important to consider these potential risks when using PLR isoflavones, and they should be used under professional medical guidance.

Drug delivery systems

The drug delivery system for PLR isoflavones has significantly promoted their bioavailability and therapeutic efficacy. The glycosylation modification was critical for improving the solubility and absorption of the isoflavones [126]. It also ensured the easier solubility of the isoflavones and more efficient intestinal uptake [127]. Furthermore, the role of the gut microbiota in the system is significant. The isoflavones could be metabolized into more active or easier to absorb forms, thus enhancing pharmacological effects [60]. The above modifications could enhance bioavailability and reduce toxicity of PLR isoflavones.

Pharmacokinetics in isoflavones

The PLR isoflavones, especially puerarin, exhibit widespread tissue distribution. After intravenous injection of puerarin, it has been detected in multiple organs, including the brain, heart, stomach, lungs, intestines, and spleen [128]. Puerarin can cross the blood-brain barrier and distribute in various regions of the brain, such as the hippocampus, cerebral cortex, and striatum, where it exerts neuroprotective effects [129]. The brain penetration index (AUC_{brain}/AUC_{plasma}) for puerarin, 3'-Methoxypuerarin, daidzein, and daidzein-8-C-apiosyl-glycoside were 9.29%, 7.25%, 11.96%, and 4.21%, respectively [130]. Compared to other isoflavones, puerarin can relatively quickly cross the brain and exert its effects. Moreover, puerarin can also cross the placental barrier and maintain high concentrations in fetal plasma [131]. After oral administration, puerarin reached a peak concentration (C_{max}) in the blood of approximately 140–230 μg/L within 1 hour, with an absolute oral bioavailability of 7% [132]. Glucuronidation related metabolites (puerarin-7-*O*-glucuronide, puerarin-4'-*O*-glucuronide, etc.) are the major metabolites of puerarin,), and these metabolites could be easily excreted through urine and feces [133]. Although these isoflavones can cross the blood-brain barrier, their distribution in the brain is quite limited.

Discussion and future perspectives

The isoflavones in PLR have been widely studied for their potential benefits in treating cardiovascular diseases, diabetes, liver diseases, neurological disorders. Up to date, more than 100 isoflavones have been identified from PLR. Among the various parts of PLR, the roots contained the highest concentration of puerarin, while the leaves had relatively high levels of irisolidone and daidzein. Moreover, the isoflavones contents in PLR was significantly influenced by its growing environment. Isoflavones are a type of plant estrogen, primarily composed of glycosidic compounds with a 3-phenylchroman structure. This structure possessed a fundamental C_6-C_3-C_6 skeleton that combined with glycone or glycoside to form phenolic compounds. PLR contained a variety of active ingredients, brought great challenges for the qualitative and quantitative analysis of isoflavones in PLR. Modern chromatographic techniques (ultra-high performance liquid chromatography, ultra-high performance liquid chromatography-mass spectrometry, etc.) have significantly improved the quantitative analysis of isoflavones, providing greater accuracy and efficiency in measuring the concentration of these bioactive compounds.

Isoflavones in PLR had multiple effects (neuroprotection, anti-tumor, heart-protection, etc.) by inhibiting oxidative stress, inflammation and apoptosis. Puerarin prevented myocardial infarction by regulating the PPAR-γ/NF-κB and Akt/HO-1 pathways. Daidzein played an essential role in reducing blood pressure and protecting heart. Daidzein was considered a powerful isoflavone due to its excellent anti-inflammatory and antioxidant properties, which played a crucial role in cardiovascular protection by reducing oxidative stress and inflammation. Isoflavones also influenced the gut through various mechanisms, such as promoting β-cell neogenesis and regulating the short-chain fatty acid metabolism. Taken daidzein as an example, it could upregulate the expression of tight junction proteins to mitigate intestinal dysfunction and improve intestinal epithelial cells.

Remarkably, the isoflavones in PLR have notable pharmacological effects on liver diseases, offering good therapeutic prospects. Puerarin and other isoflavones could upregulate AMP-activated protein to reduce liver damage and protect liver from alcohol-induced damage. Isoflavones like puerarin, daidzein, genistein, and daidzein played a crucial role in protecting the liver by regulating liver function and lipid metabolism. As isoflavones in PLR had the similar structure of estrogen, they may lead to some adverse reactions (especially at the high dose of isoflavones in PLR), such as gastrointestinal discomfort, liver toxicity and kidney toxicity. Thus, further in vivo and in vitro experiments should be conducted to reduce the side effects from PLR isoflavones. Research is needed to explore the most effective drug formulations through developing new drug delivery systems.

Most PLR isoflavones studies focused on puerarin, daidzein, and genistein, the other isoflavones' pharmacological potential should also be explored. Through these studies, the researchers could discover new isoflavones for the treatment of diseases. The pharmacological activity of isoflavones in PLR varied in different labs. PLR isoflavones possessed considerable pharmacological actions in fundamental researches, randomized, double-blind, placebo-controlled studies of these active isoflavones should be further explored.

Conclusion

Recently, more than 200 compounds have been identified from PLR, including isoflavones, terpenes, steroids, and coumarins, among which isoflavones are the predominant type. The metabolism of phenylalanine affected the formation of isoflavones. These isoflavones possess a wide range of pharmacological activities and had potential therapeutic effects on diseases in the nervous system, circulatory system, digestive system, endocrine system, and others. Further studies should focus on the medicine modifications and safety evaluation of PLR isoflavones to promote the drug development of PLR isoflavones related products.

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