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Prunella vulgaris: A comprehensive review of chemical constituents, pharmacological effects and clinical applications.

Prunella vulgaris: A comprehensive review of chemical constituents, pharmacological effects and clinical applications

Su-Juan Wang[1,2†], Xiao-He Wang[1†], Yuan-Yuan Dai[1], Ming-Hua Ma[3], Khalid Rahman[4], Hua Nian[1*], Hong Zhang[5*]

[1] Pharmaceutical Center of Yueyang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China

[2] Department of Drug Preparation, Hospital of TCM and Hui Nationality Medicine, Ningxia Medical University, Ningxia 751100, China

[3] Department of Pharmacy，Yangpu Hospital，Tongji University School of Medicine，Shanghai 200090, China

[4] School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, Liverpool L3 3AF, England, UK

[5] Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

[†] These authors contributed equally to this work.

Correspondence to:

[*] Hong Zhang and Hua Nian

E-mail: hqzhang51@126.com (HZ), jackynian@126.com (HN)

Abstract:

Prunella vulgaris (PV) is a perennial herb belonging to the Labiate family and is widely distributed in northeastern Asian countries such as Korea, Japan, and China. It is reported to display diverse biological activities including anti-microbial, anti-cancer, and anti-inflammation as determined by *in vitro* or *in vivo* studies. So far, about 200 compounds have been isolated from PV plant and majority of these have been characterized mainly as triterpenoids, sterols and flavonoids, followed by coumarins, phenylpropanoids, polysaccharides and volatile oils. This review summarizes and analyzes the current knowledge on the chemical constituents, pharmacological activities, mechanisms of action and clinical applications of the PV plant including its potential as a future medicinal plant. Although some of the chemical constituents of the PV plant and their mechanism of action have been investigated the biological activities of many of these remain unknown and further clinical trials are required to further enhance its reputation as a medicinal plant.

Keywords: *Prunella vulgaris*, constituent, activity, application, review

1. Introduction

In recent years, interest in the development of herbs and botanicals from plants for use as drugs has significantly increased [1]. Natural products such as microbial metabolites are a rich source for the development of new drugs as they display diverse chemical structures and biological activities [2]. *Prunella vulgaris* (PV), also known as self-heal, is a plant belonging to the Labiatae family and is traditionally used as folk medicine in Northeastern Asian countries such as Korea, Japan, and China [3]. The Chinese name for this plant is Xia-Ku-Cao which originates from a description that the herb is dried and withered after the summer solstice [4]. It is reported to be rich in bioactive chemicals, including polysaccharides, flavonoids, triterpenes and phenolic acids. Thus, PV has many notable pharmacological activities, such as anti-colitic, antioxidant, anti-inflammatory, anticancer, neuroprotective [5], antiestrogenic [6], and anti-metastatic effects [7]. In China, it is also extensively used as a health-promoting food or tea. For example, PV is a principle raw material of Guangdong Herbal Tea well known as Wanglaoji-Liang-Cha, which has been historically consumed as a healthy beverage in Southern China [8]. In Europe, this plant is also consumed widely as food or tea on a regular basis [9]. Although dietary supplement manufacturers cannot legally make disease claims without approval of a new drug application, the unsubstantiated medical uses for many botanical dietary supplements are well known and promoted in literature and news media or on the Internet although dietary supplement manufacturers cannot legally make such claims [10].

Plants have always been a very good source for research and development of new drugs and many beneficial uses of medicinal plants are extensively documented as traditional medicines in many cultures. The present review mainly focuses on the in vitro an in vivo studies involving PV. The compounds and extracts which display potential pharmacological properties are presented in Tables 1 and 2, respectively. Some of the major effective phytochemicals present in PV are listed in Table 3. It has also been reported that out of the 21 herbs screened for either estrogenic or antiestrogenic activitiy using the alkaline phosphatase assay only PV extract displayed the strongest biological activity [11]. Meanwhile, SKI 306X, a purified extract from a mixture of three oriental herbal medicines (*Clematis mandshurica*, *Trichosanthes kirilowii* and *Prunella vulgaris*), has been widely used for the treatment of inflammatory diseases such as lymphadenitis and arthritis in Far East Asia [12]. The current

state of research regarding the medicinal use of PV is summarised in this review.

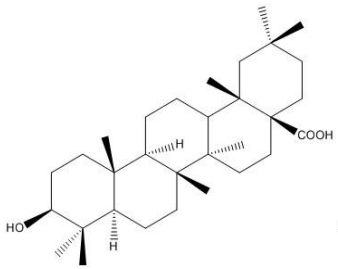
2. Phytochemistry

Natural products are compounds or substances produced by living organisms (or found in nature), which have pharmacological or biological activities often used for drug discovery and design [13]. These plant metabolites have noteworthy structural complexity and a variety of pharmacological and biological activities, making them effective nutritional compounds and pharmaceutical drugs. Many bioactive constituents from PV have been identified, including phenolic constituents, complex carbohydrates and hydrophobic metabolites such as triterpenes. The aqueous extract contains abundant polyphenols, rosmarinic acid and complex carbohydrates, whereas more hydrophobic metabolites, such as triterpenes and flavonoids along with some polysaccharides and polyphenols, are found in the ethanol extract [14, 15]. The abundant polysaccharides have a number of reported biological activities, such as antioxidant and immunomodulatory [9]whilst several of the triterpenes display significant anti-inflammatory activity. Rosmarinic acid has also shown to be an anti-inflammatory compound due to its specific inhibition of T cell signaling and its impact on glucose metabolism [15].

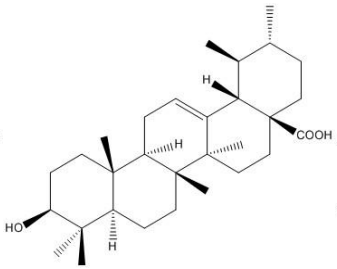
2.1 Triterpenoids and saponins

The triterpenoids isolated from PV are mainly oleanane, lupinane and ursane. At present, a total of 28 triterpenoids have been isolated: 20 triterpenoids (free state), 8 saponins (binding state); the two compounds with highest content, oleanolic acid (1) and ursolic acid (2), are mainly resoponsible for the pharmacological acticity of PV.

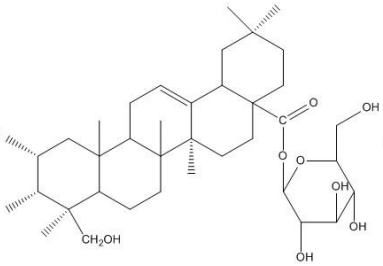
Many compounds have also been isolated from methylated PV extracts such as methyl oleanolate (3), methyl ursolate (4), methyl maslinate (5) and other related compounds [16-18]. Pravuloside A (6) and Pravuloside B (7) along with two ursane-type specific triterpenoid saponins have also been isolated from PV [19, 20]. Some other compounds present in PV, are vulgarsaponin A (8) [21] and vulgarsaponin B (9) [22], which is a newly identified glucopyranoside compound (**Fig.1**).



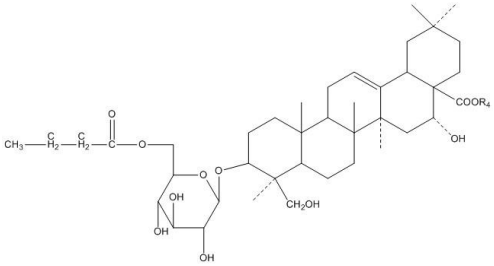
(1) Oleanolic acid



(2) Ursolic acid

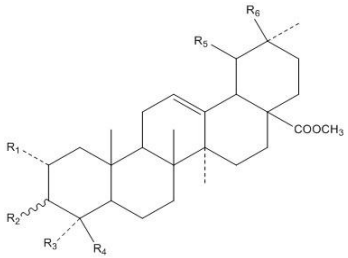


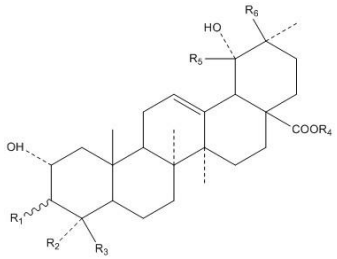
(8) Vulgarsaponin A



(9) Vulgarsaponin B

Structure



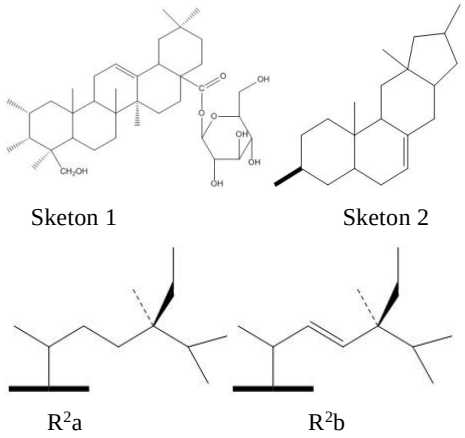


	Groups	R1	R2	R3	R4	R5	R6
(3)	Methyl oleanolate	H	β-OH	CH_3	CH_3	H	CH_3
(4)	Methyl ursolate	H	β-OH	CH_3	CH_3	CH_3	H
(5)	Methyl maslinate	OH	β-OH	CH_3	CH_3	H	CH_3
(6)	Pruvuloside A	α-OH	CH_3	CH_3	Glc2-glc	CH_3	H
(7)	Pruvuloside B	α-OH	CH_3	CH_3OH	Glc	CH_3	H

Fig 1. Structures of triterpenes

2.2 sterols

The main sterols present in in PV include: β-sitosterol (10), stigmasterol (11), α-spinolol (12), and stigmast-7-en-3β-ol (13) [23]. Eight sterol compounds have been isolated from the ear, stem, leaf and other parts of PV, of which four compounds are present in a free state: ducosterol (14), α-spinasterol, β-sitosterol, stigmasterol-7-olefinic alcohol; The other four compounds are glucose glucosides: stigmast-7-enyl-β-D-gluco-pyranoside (13), (22E,20S,24S)-stigmast-7,22-diene-3-ene (15), α-spinasterolyl-β-D-glucopyranose glucoside, stigmasterolyl-β-D-glucopyranose glucoside [24] (**Fig. 2**).

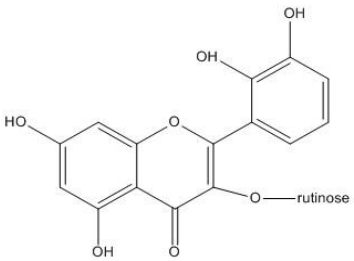


	R^1	Sketon+R^2
(10) Sitosterol	α-OH	1a
(11) Stigmasterol	α-OH	1b
(12) Spinasterol	α-OH	2b
(13) Stigmast-7-en-3β-ol	β-OH	2a
(14) Ducosterol	=O	
(15) (22E，20S，24S)-stigmast-7,22-diene-3-ene	β-OH	2a

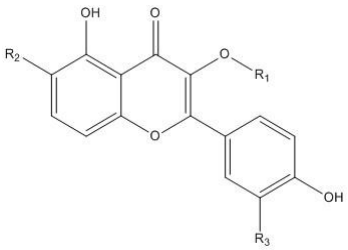
Fig 2. Structures of steroids and derivatives

2.3 Flavonoids

In addition to rutin (16) and hyperoside, three other flavonoids have been isolated and identified from PV, namely luteolin (17), homoorinetin (18) and cinaroside (19) [24]. Besides these compounds, PV also contains quercetin (20), quercetin-3-O-β-D -galactoside (21) [25], quercetin-3-O-β-D-glucoside (22), kaempferol-3-O-β-D-glucoside (23) [26] and other related components (**Fig. 3**).



(16) Rutin

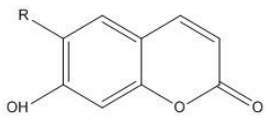


	R1	R2	R3
(17)Luteolin	H	H	OH
(18)Homoorinetin	H	Glc	OH
(19)Cinaroside	H	O-Glc	OH
(20)Quercetin	H	H	OH
(21)Quercetin-3-O-β-D-galactoside	Glc	H	OH
(22)Quercetin-3-O-β-D-glucoside	Glc	H	OH
(23)Kaempferol-3-O-β-D-glucoside	Glc	H	H

Fig. 3 Structure of steroids

2.4 Coumarins

Pv is reported to contain only small amounts of coumarin although Dmitruk [27] is reported to have isolated three coumarin compounds. The calculation of managerial constants and infrared spectroscopy identified these asc umbelliferone (24), scopoletin (25) and esculetin (26) (**Fig.4**).



(24)Umbelliferon H

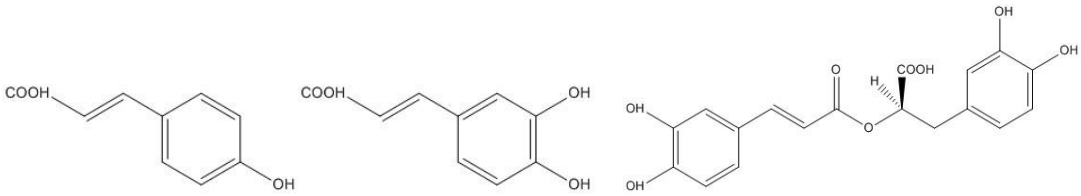
(25)Scopoletin OMe

(26)Esculetin OH

Fig. 4 Structure of Coumarins

2.4.1 Phenylpropanoids

Phenylpropanoids present in PV include: *P*-cumaric acid (27), cis-caffeic acid and trans-caffeic acid (28), rosmarinic acid (29), and others such as methyl rosmarine, ethyl rosmarine, *E*-butyl rosmarine, 3,4α-trihydroxy-methyl-phenyl propionate, 3,4α- trihydroxy-butyl-phenyl propionate [28] (**Fig. 5**).



(27)*P*-cumaric acid (28) caffeic acid (29) rosmarinic acid

Fig. 5 Structure of Phenylpropanoids

2.6 Long-chain fatty acids

PV contains long-chain fatty acids such as palmitic acid, ethyl palmitate, tetracosanoic acid, stearic acid, 6,9-octodecadienoic acid, 3,6,7-eicosatrienoic acid, oleic acid, peanut oleic acid, moringoic acid, lauric acid, myristic acid, linolenic acid, palmitic acid, myristic acid, and linoleic acid [25-29].

2.7 Volatile oils

The volatile components present in PV include 1,8-eucalyptol, β-pinene, myrcene, linalylacetate, α-phellandene, linalool, 1,6-cyclononone, palmitic acid and trihexadecane. Among these, the content of 1,8-anthracene and β-pinene is the highest, and accounts for more than 60% of the total volatile oils [30].

2.8 Sugar

PV contains free monosaccharides (mainly rhamnose, glucose, xylose, arabinose, mannose, galactose, etc [31], disaccharides (sucrose and fructose) and polysaccharides. In addition, a sulfur-containing polysaccharide has also been isolated from PV [32].

2.9 Other components

Alkaloids, inorganic salts, vitamins, resins, bitter taste, tannic acid, proteins and lipids are also present in PV.

3. Pharmacology

3.1 Antitumor activity

Anti-tumor activities of extracts and monomeric compounds from PV plant have been investigated [33]. PV inhibits metastasis and promotes the apoptosis of many different kinds of tumor cells through different pathways. Triterpene compounds 2α-hydroxy ursolic acid and ursolic acid significantly inhibit breast cancer cells MCF-7, MDA-MB-231 and normal breast cells MCF-10A. Betulinic acid is reported to inhibit only breast cancer cells MCF-7 and MDA-MB-231, but has no effects on normal breast cells MCF-10A [34].

In addition, some studies have demonstrated that 19α-hydroxy ursolic acid and quercetin present in PV ethanol extract can inhibit the migration of tumor cells MDA-MB-231 in a dose-dependent manner, in which quercetin reduces the proliferation of tumor cells by inhibiting the PI3k/Akt pathway. IC_{50} of 19α-hydroxy ursolic acid and quercetin against MDA-MB-231 cells migration was 1.676 μmol • L^{-1} and 1.145 μmol • L^{-1}, respectively [35]. The effect of prunella polysaccharide-zinc complex (P1-Zn) on HepG2 liver cancer cells has been confirmed by morphological changes, chromatin agglutination and G_0/G_1 cell cycle arrest. P1-Zn complex increases the expression of caspase-3 and -9, excessive production of reactive oxygen species and destruction of mitochondrial function to achieve effective inhibition of HepG2 cell proliferation [36]. PV also alleviated thyroid cancer symptoms through increase of Bcl-2-related protein X and caspase-3 levels by inducing apoptosis of thyroid cancer TPC-1 and FTC-133 cell lines and down-regulating B-cell lymphoma-2 expression in TC-1 and FTC-133 expression [37]. Recent studies [38, 39] have also demonstrated that PV has significant inhibitory effects on lymphoma cell Jurkat, human lung cancer cell A-549, endometrial cancer cell Ishikawa, and cholangiocarcinoma cells QBC939 and RBE.

3.2 Anti-inflammation and immunoregulation

Inflammation is a potential risk factor in the development of many diseases and can create or exacerbate a range of diseases. Traditional Chinese medicines play a two-way immune regulatory role in the body, and the mechanisms of action is mainly related to immune organs, immune cells, immune molecules promotion, inflammatory response, hypersensitivity [40].

Many active components of PV such as rosmarinic acid and ursolic acid have significant anti-inflammatory and immunosuppressive effects. The water extracts of PV stems and leaves

markedly inhibited carrageenan induced rat paw swelling and xylene-induced mouse auricular swelling, reduced the content of tumor necrosis factor-α [41].

3.3 Antiviral effect

According to the lesions, the number of herpes zoster, the typical lesion score, and the expression levels of HSV-1 and HSV-2 in the lesion tissue, the guinea pig model of HSV-1 (skin) was established with HSV- Virus DNA copy number as an indicator. Prunella polysaccharide was found to not only inhibit the activity of the HSV-1 and HSV-2 *in vitro*, but also decrease HSV-1 and HSV-2 virus lesions in the guinea pig model, showing the better anti-herpes simplex virus activity [42].

3.4 Anti-oxidative effect

PV displays significant antioxidant activity [43]. High performance liquid chromatography (HPLC) and LC/MS analyses has shown that the main active compounds from 60% ethanol extract of PV (P-60) are phenols, such as caffeic acid, rosmarinic acid, rutin and quercetin. Total phenols are highly correlated with the antioxidant activity, and significantly inhibit tumor growth in C57BL/6 mice, increase superoxide dismutase (SOD) activity and decrease malondialdehyde (MDA) content in serum of tumor-bearing mice [44].

In addition, triterpenes, flavonoids and polysaccharides have the strongest antioxidant activity. The total reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) were measured in the shackle Balb/C mice model, and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were also analyzed, 2.50 $g \cdot kg^{-1}$ of PV reduced the levels of H_2O_2, ROS, protein peroxide protein carbonyl; 1.25, 2.50, 7.50 $g \cdot kg^{-1}$ of PV decreased the content of lipid peroxidation MDA and increased the activity of SOD. Besides, the water extract of PV can reduce the pyrazole-lipopolysaccharide-induced oxidative stress in mice liver tissue by inhibiting the protein expression of CYP2E1 and CYP2A5 [45].

3.5 Anti-osteoporosis

The flavonoid extract of PV shows anti-osteoporosis effect through promoting osteoblast function in ovariectomized rats, reducing bone resorption and bone metabolism, increasing bone formation and decreasing trabecular bone loss and bone mass loss.[46].

3.6 Anti-depression

The antidepressant effect of PV was investigated by use of mouse tail suspension, forced swimming and spontaneous activity tests with fluoxetine hydrochloride as a positive control drug. The water extract could increase the levels of 5-hydroxytryptamine, norepinephrine and dopamine in hippocampus of hippocampus, and decrease the content of cyclooxygenase -2,

prostaglandin E, interleukin-1β, interleukin-6, indicating that the anti-deprssion effect of PV aqueous extract may be achieved by increasing the content of monoamine neurotransmitter in hippocampal tissue and reducing the content of inflammatory cytokines Antidepressant effect [47].

3.7 Hypoglycemic, hypotensive and hypolipemic effects

Hyperglycemia and glycosylated hemoglobin (HbA1c) are widely used as diagnostic markers for diabetes [48]. The proper management of diabetes includes blood glucose and HbA1c homeostasis, and improvement of antioxidant defenses by minimizing free radical adverse effects [49]. The indigenous hemoprotein antioxidant enzyme, catalase (CAT), directly scavenges the reactive oxygen species (ROS) and catalyzes the lessening and detoxification of hydrogen peroxides [50-52]. By using an alloxan-induced type 1 diabetes (T1D) mouse model, bio-guided fractionation, isolation, RP-HPLC, and 1H and 13C NMR identification, the active components of PV were determined: rosmarinic acid (RA), caffeic acid (CA, most active fraction) and p-coumaric acid(pCA), hence named, caffeic acid-rich fraction (CARF). CARF reduced blood glucose levels and improved in-vivo oxidative-stress. It also inhibited the carbohydrate-hydrolyzing enzymes (alpha-amylase and alpha-glucosidase) and reduced HbA1c levels more significantly than PV extract, CA or RA. In the longer time, CARF significantly increased serum-insulin, ameliorated thermal hyperalgesia and tactile allodynia more significantly than PV extract, CA or RA. Moreover, the tested compounds showed potential restoration of the lipid peroxide levels. CARF and PV extract were observed to increase serum-insulin and attenuate alpha-amylase and alpha-glucosidase, whose antioxidant potentials might be responsible for their antidiabetogenic and antinociceptive properties [53].

The stems, leaves, spikes, and the whole grass of PV have antihypertensive effect. The main active component is the total saponin. The water, 30% alcohol and ethanol - water extracts of PV exert antihypertensive effect on anesthetized animals. The water extract can reduce blood pressure by reducing the systolic pressure and diastolic pressure of SHR rats, significantly inhibit the alpha-amylase, alpha-glucosidase, and reduce the postprandial blood glucose levels of normal mice and alloxan diabetic mice, improve starch tolerance, promote liver glycogen synthesis and lower blood sugar. PV can also inhibit atherosclerosis, hyperlipidemia to some extent [54].

Table 1. The compounds with pharmacological activities from PV

Compound	Model	Observation	Dosage	Activity	Mechanism of action	Refs.
2,3-dihydroxyurs-12-en-28-oic acid (DHURS)	human acute leukemia Jurkat T cells	*in vitro*	20–25 μg/ml	Induces apoptosis	Mitochondria-dependent activation of caspase cascades	[55]
Ursolic acid and betulinic acid	MCF7 human breast cancer cell line and LNCaP (CRL-1740) human prostate cancer cell line	*in vitro*	20,50 μM	Anti-estrogenic	Inhibited estrogen signaling by suppressing the expression of estrogen receptor α (ERα) and enhanced prostate-specific antigen promoter activity	[56]
Rosmarinic acid (RA)	UVA-induced changes in a human keratinocyte cell line (HaCaT)	*in vitro*	25–50 mg/l	Photoprotection	Suppressed UVA-induced ROS production， reduced DNA damage，and inhibited UVA-induced caspase-3 activation	[57]

Table 2. The extracts with pharmacological effects from PV

Extract	Model /Cell type	Observation	Dosage	Activity	Mechanism of action	Refs.
Aqueous extract	Carbon tetrachloride-induced hepatic fibrosis	*In vivo*	50, 100, and 200 mg/kg	Anti-fibrosis	Inhibited the activation of hepatic stellate cells, promoted collagenolysis and regulated fibrosis-related microRNAs	[3]
60% Ethanol extract	Tumor-bearing C57BL/6 Mice	*In vitro,* and *in vivo*	5 and 10 g crude drug/kg	Antioxidant	Antioxidant and inhibition of the tumor growth	[58]
Methanol extract	Mouse Xenograft	*In vitro,* and *in vivo*	50 μg/ml	Antiestrogen	Inhibited estrogen responses	[6]
Ethylacetate extract	Doxorubicin-induced oxidative stress	*In vitro*	0.005 to 0.05 mg/ml	Cardioprotect	Antioxidant capacity	[59]
Water extraction followed by alcohol precipitation	Female BALB/c mice	*in vivo*	0.90 g/kg	Anti-inflammation and anti-tumor	Enhanced the activity of T lymphocytes in general and its subgroup Th cells and No significant changes in B lymphocytes	[60]
Ethanol extract	LPS-activated RAW 264.7	*In vitro* and *in*	10, 50 and 100	Treatment of sepsis	Induced heme oxygenase-1 (HO-1) expression through	[61]

	Cells and and CLP-induced Septic Mice	*vivo*	µg/ml		PI3K/Nrf2 signal pathways and a reduction of high mobility group box 1(HMGB1)	
Aqueous extract	Human T cell lymphotropic virus type 1-immortalized T cell lines MT-2 and MT-4 cells	*In vitro*	10, 50 µM	Anti-HIV activity	Inhibited syncytium formation and resultant cell death	[62]
Aqueous extract	murine macrophage RAW 264.7 cells	*In vitro*	10, 50, 100 µg/ml	Immunostimulatory and anti-tumor activity	Stimulated macrophage activation via NF-κB transactivation and MAP kinase activation	[63]
70% Ethanol extract	The mouse macrophage cell line RAW264.7, human liver cancer cell line HepG2, human colon cancer cell line HT29, human lung cancer cell line A549, human stomach cancer cell line MKN-45 and human cervical cancer cell line HeLa	*In vitro*	10, 50, 100 µg/ml	Anti-oxidant and anti-cancer	Increased the expression of p53, Bax and Fas	[64]
Aqueous extract	HeLa37 cells	*In vitro*	0.1, 1, 10 µg/ml	Anti-viral	Inhibited HIV-1 infectivity	[14]
Aqueous extract	male Sprague-Dawley rats and rat peritoneal mast cells (RPMC)	*In vivo*	0.005 to 1 g/kg	Inhibition of immediate-type allergic reactions	Inhibited the passive cutaneous anaphylaxis activated by anti-dinitrophenyl (DNP) IgE antibody dose dependently and suppressed the histamine release induced by compound 48/80 or anti-DNP IgE	[65]
Aqueous extract	murine peritoneal macrophages	*In vitro*	50, 100, 200, 400 µg/ml	Immunomodulatory and antiinflammatory activities	Stimulated the proliferation of T-lymphocytes and suppressed NO production in lipopolysaccharide-stimulated macrophages	[66]

Aqueous extract	Equine dermis cells	*In vitro*	66 µg/mL or 62.4 µg/mL	Inhibition of equine infectious anemia virus (EIAV) replication	Prevented viral particles from binding to the surface of permissive cells	[15]
Ethanolic extract	mdr1a-/- or wild type FVBWT mice	*In vivo*	2.4 mg/d	Anti-inflammation	Maintained mucosal homeostasis in mdr1a/ mice by regulating gene expression associated with innate inflammatory responses and attenuating the activation of the adaptive immune response	[67]
Aqueous extract	INS-1 cells	*In vitro*	100 µg/ml	Anti-inflammation	Signifcantly prevented IL-1β-increased INS-1 cell death and LDH activity and attenuated IL-1β-increased caspase-3 activity	[68]
Aqueous ethanol extract (30% v/v)	lipopolysaccharide (LPS) - induced oxidative damage and inflammation in human gingival fibroblasts	*In vitro*	5, 10, 25µg/ml	Anti-inflammation	Reduced reactive oxygen species (ROS) production, intracellular glutathione (GSH) depletion as well as lipid peroxidation, inhibited LPS-induced up-regulation of interleukin 1b (IL-1b), interleukin 6 (IL-6), tumor necrosis factor-a (TNF-a), and suppressed expression of inducible nitric oxide synthase (iNOS)	[69]
80% Ethanol extract	alloxan-induced diabetic mice	*In vivo*	50, 100 and 150 µg/ml	Hypoglycemic and antinociceptive effects	Increased serum-insulin and attenuated alpha-amylase and alpha-glucosidase	[70]
50% Ethanol extract	UVB-aged normal human dermal fibroblasts	*In vitro*	10, 100 µg/mL	Protection of normal human dermal fibroblasts (NHDFs) from UVB‐induced inflammatory and	Inhibited MAPKs, AP-1, and NF-κB signaling and promoted the TGF‐β1/Smad pathway	[71]

				photo aging damage		
Aqueous extract	Human umbilical vein endothelial cells (HUVEC)	*In vitro*	stock solution corresponding to 5 g raw PVL per ml	Upregulation of endothelial NO synthase (eNOS)	Increased Enos promoter activity, eNOS mRNA and protein expressions, as well as NO production.	[72]
Ethyl acetate parts of aqueous extract	scopolamine (SCOP)-induced aging rats	*In vitro* and *in vivo*	100 mg/kg	Anti-dementia	Attenuated SCOP-induced brain senescence in rats by improving behavioral performance and decreasing brain cell damage, reduced AChE activity and MDA level, increased SOD and GPx activities, and inhibited the expression of NF-κB and GFAP.	[73]
Aqueous extract	human umbilical vein endothelial cells	*In vitro*	10, 30, 50 μg/mL	Anti-inflammation	Inhibited ROS/NF-κB pathway by inducing HO-1 and eNOS expression mediated by Nrf2	[74]
Ethanol extract	RAW 264.7 Mouse Macrophages	*In vitro*	30 μg/mL	Anti-inflammation	inhibited lipopolysaccharide (LPS)-stimulated prostaglandin E2 (PGE2) and nitric oxide (NO) production.	[75]
Ethanolic extract	normal naive mice	*In vivo*	25 or 50 mg/kg	Enhances cognitive performance	Increased neural cell proliferation and the number of immature neurons, enhanced ERK, Akt and GSK-3β phosphorylation levels and up-regulated adult hippocampal neurogenesis.	[76]
Aqueous extract	Human fibrosarcoma HT-1080, mouse melanoma B16-F1 and B16-F10 cells	*In vitro* and *In vivo*	10,50,100,200 μg/ml	Anti-invasion and anti-metastasis	Suppression of MMP-9 expression by the inhibition of NF-κB via ERK1/2 signaling pathway as well as MMP-9 activity	[7]
Ethanolic extract	Male ICR mice	*In vivo*	25 or 50 mg/kg	Anti-amnesia	Ameliorated scopolamine-induced cognitive impairments.	[77]
Aqueous extract	Human liver carcinoma HepG2, Huh-7 and Hep3B cells	*In vitro*	1, 5 and 10 mg/ml	Anti-invasion and anti-metastasis	Inhibited activities of metalloproteases, MMP-2 and MMP-9, without affecting cell viabilities, and suppressed migration through attenuation of enzymatic activities of	[78]

					MMP-9 and MMP-2 at transcriptional levels	

Table 3. The phytochemicals categories with pharmacological properties from PV

Effective phytochemicals	**Model /Cell type**	**dosage**	**Observation**	**Activity**	**Mechanism of action**	**Refs.**
Polysaccharides	Murine macrophage RAW 264.7 cells	125, 250, 500, 1000 μg/mL	*In vitro*	Antioxidation and immunomodulation	Stimulated the production of pro-inflammatory cytokines, including nitric oxide (NO), tumor necrosis factor- (TNF-), and interleukin-6 (IL-6)	[9]
Lignin–polysaccharide complex	BALB/c mice	10,20,30,40, 50 μg/mL	*In vivo*	Anti-herpes	Inhibited viral binding and penetration into host cells	[79]
Polysaccharide	HSV-1 and HSV-2	100 μg/mL	*In vitro*	Anti-HSV	Inhibited HSV by competing for cell receptors	[80]
Phenolics-rich	Hereditary hypertriglyceridemic rats or high-sucrose diet (HSD, 70 cal% of sucrose) for two weeks	1% w/w	*In vivo*	Antioxidation	Suppressed a high-sucrose diet induced oxidative stress and positively modified lipoprotein cholesterol profile in plasma of hereditary hypertriglyceridemic insulin-resistant rats	[81]
Polysaccharide-zinc	HepG2 cells	500 μg/mL	*In vitro*	Antiproliferation	Activated caspase-3 and -9, reactive oxygen species (ROS) overproduction and mitochondrial dysfunction	[82]
Polysaccharide	Human breast carcinoma-associated fibroblasts	5 μg/mL, 8 μg/mL	*In vitro*	Anti-migration and anti-apoptosis	Inhibited basic fibroblast growth factor (bFGF) expression, and suppressed the growth of breast cancer SKBr-3 cells.	[83]

4. Clinical efficacy of *Prunella vulgaris* preparation

In recent years, a variety of (PV) preparations have been widely used in clinical practice. Many of diseases treated by PV alone or in combination with other medicinal plants have given receive satisfactory results. Although most traditional Chinese medicines have only moderate therapeutic effects on diseases, they have few side effects compared with Western medicines. Furthermore, a large number of Chinese medicines can alleviate or eliminate the adverse reactions caused by western medicine and help the body to recover, reducing the recurrence rate. Currently PV is clinically applied for production of various preparations, such as injections, oral liquid, and ointment. Although PV is the main herb medicine, the efficacy of preparations varies with the addition of excipients, the extraction methods of medicinal substances, and the administration routes in the preparation process.

4.1 Granules

Rosmarinic acid, a major pharmacological agent of PV [84], has a number of activities, such as anti-inflammation, elimination of swelling, immune regulation and anti-thyroid, and is obtained from the water extract of PV and excipients processing, which is prepared as granular. Clinical research [85] founded that 356 cases of menopausal women were randomly divided into both the control group (181 cases) and the observation group (175 cases), and the control group were given cyclosporine eye drops and the observation group additional Prunella granules. After treatment, the amount of tear fluid and the time of tear film rupture in the observation group were significantly higher than those in the control group. The corneal topographic map corneal surface regular index, corneal surface irregular index, conjunctival epithelial cells TNF-α, IL-1β, ICAM -1 significantly reduced, indicating that PV granules has a significant efficacy in the treatment of menopausal women with dry eye.

When hyperthyroidism is treated with Western drug methimazole tablets, the long period of treatment is necessary due to the slow improvement of thyroid function. However, long-term use of methimazole tablets alone will inevitably lead to many side effects. Therefore the treatment with Western medicine alone is not considered ideal. Given the treatment with PV granules combined with methimazole tablets, the granules can enhance the function of methimazole and relieve its side responses owing to the efficacy of PV with clearing away heat and toxic material. A clinical study [86] showed that in the combination therapy group, the efficiency (91.84%) was significantly higher than that of methimazole alone (75.51%), and the difference was statistically significant ($P < 0.05$).

4.2 Tablets

PV extract is dried and pressed into tablets. The preparation has anti-tumor, antibacterial,

anti-inflammatory, liver protective effects, etc. In one study for observing the clinical efficacy of PV tablets on benign prostatic hyperplasia [87], the control group was given finasteride and the observation group given additional PV tablets. Prostate volume and prostate specific antigen in the observation group were improved compared to the control group, and the adverse reaction rate (5.0%) in the observation group was significantly lower than that in the control group (32.5%), these differences were statistically significant ($P<0.05$). A recent study [88] disclosed that PV tablets had better clinical effects, fewer adverse reactions, and shorter course of treatment in the treatment of acute thyroiditis when combined with glucocorticoid. In another clinical study on acute mastitis, PV tablets combined with antibacterial penicillin could evidently improve the symptoms, restore the normal breastfeeding and reduce the patient's resistance to antibacterials. PV tablets combined with adapalene gel also showed a significant clinical effect in the treatment of acne vulgaris [89].

4.3 Ointment

PV ointment is composed of PV, licorice, Scrophulariaceae and 14 other herbs. These herbs are decocted with water, concentrated into a clear paste, and appropriate amount of refined honey or sucrose is added and heated which turns this into a concentrated into dark brown semi-fluid formulations. PV ointment also has the efficacy of clearing fire, loosing knot and eliminating swelling. For 50 cases with thyroid nodules [90], the total effective rate was 76.00% when treated with PV ointment combined with thyroxine tablets, while the total effective rate was only 42.00% in the patients treated with thyroid hormone tablets alone. The difference was statistically significant ($P <0.05$). Therefore the combination therapy has more obvious treatment effect, and can be widely used in clinic.

4.4 Oral solution

The preparation of PV oral solution is obtained by decoction with water, filtration, and concentration, followed by addition of sodium benzoate and sucrose which is then dissolved by heating the mixture. PV oral solution possesses several effects, such as anti-bacteria, anti-inflammation, dissipating binds and dispersing swelling, clearing heat and purging fire. The effect of PV oral liquid combined with betamethasone in the treatment of patients with subacute thyroiditis was more significant than that of betamethasone alone. The total effective rate (81.40%) was significantly higher in the combination group than in the betamethasone alone group (81.40%) [91]. A clinical study [92] showed that the total effective rate was 75.68% and the recurrence rate in one year was 36.84% when the chronic breast cancer patients were treated with the combination of PV with oral antibiotics (cefdinir dispersible tablets). The rates were significantly lower than those of patients treated with antibiotics alone.

4.5 Capsules

PV capsules are made by concentration of PV water extract plus brown sugar filled in empty capsules or soft capsules. The preparation possesses anti-tumor, immune regulative and anti-inflammatory analgesic effects. In a clinical study on Hashimoto's thyroiditis, PV capsules combined with levothyroxine sodium tablets apparently enhanced thyroid hormone levels in patients when compared with patients given L-thyroxine alone. The levels of thyroid peroxidase antibody and thyroglobulin antibody were significantly lower in the combination administration group than in the group given levothyroxine alone. This indicates that PV capsules have a significant effect on improving Hashimoto's thyroid function, lowering thyroid antibody levels, etc. [93].

5. Conclusions and prospects

In summary, PV is a widely distributed plant and is a very popular and commonly used traditional medicine for the treatment of clinical disorders. It is a widely investigated plant worldwide due to its promising therapeutic properties. In China, this plant has been used historically as a health food and traditional Chinese medicine (TCM) for the treatment of jaundice, hepatitis, gonorrhea, tuberculosis and diabetes mellitus [94]. *In vivo* and *in vitro* studies have provided the evidence of its various ethnomedical and potential pharmacological activities, indicating its effectiveness against many diseases. PV plant has rich chemical composition, and the major chemical components include triterpenoids and their glycosides, flavonoids, phenolic acids and their glycosides, organic acids, sterols, essential oils and saccharides. Of these, tannins and polysaccharides exhibit very good antiviral activities. The triterpenoids show anti-tumor activity, and the fractions enriched in phenolic acids, phenol components and polysaccharides have antioxidation activity whilst both rosmarinic acids and its derivatives possess anti-inflammatory activity. Polysaccharides, the main compounds isolated from *Prunella vulgaris,* are considered as biological response modifiers (BRMs) for their antioxidant, anticancer, and immune-modulating activities. These biological activities are affected by their unique structural characteristics of polysaccharides. The antioxidant activities are related to their compositions of proteins and uronic acid and structural features of molecular weights, monosaccharide composition and types of glycosidic.

In recent years, some investigations on biological activities of PV have been carried out on animal and at cell levels. PV single formulations and compound prescriptions have been been used in clinical practice with encouraging results. PV prapartions can be used alone in clinic or be combined with western medicines for the treatment of thyroiditis, breast

hyperplasia, cancer and other diseases. Owing to the efficacy for clearing away liver-fire, dissipating binding depression and so on, the "edible" PV gradually integrates with the "Chinese medicine" boom, and a series of PV life products are available for health protection, such as herbal tea (withered tea), ingredients (cold Prunella, Prunella porridge), and daily necessities (dried tangerine peel).

However, further research is needed to clarify the targets of the active compounds from PV, understand the mechanisms involved, and characterize the metabolites responsible for these activities. In addition, these promising compounds or their class should be extracted and purified by using more advanced technologies to treat increasing health issues including cancer, diabetes, tuberculosis and other diseases. Furthermore, the relationship between the biological properties and traditional uses should be clearly verified through valid studies. In conclusion, PV is a medicinal resource with great potential for development and should be further studied in all aspects, so as to extract, purify and synthesize active compounds under the premise of quality standard determination, and provide series of daily necessities ingredients and medicinal substances with highly effective and low toxic properties.

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