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Prunella vulgaris L. : A Literature Review on its Therapeutic Potentials

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ABSTRACT

The phytotherapeutic approach is one of the ways for modern drug development and many synthetic drugs are being developed on the prototype analogues isolated from plant bioactive principles. One of such widely used plants is self-heal (*Prunella vulgaris L.*), an ancient therapy used for pain in the throat, fevers and accelerating wound healing. The different health benefits of *P. vulgaris* for a wide variety of diseases, including cancers, viral and inflammatory diseases, were recently reported. Many of these effects of *P. vulgaris* are related to the presence of rosamarinic acid, betulinic acid, prunellin and many other bioactive principles which make the plant interesting for antiviral, immunomodulatory and antiproliferative studies. This article provides a review of the data of this commonly used medicinal plant in number of countries. And in addition directs us for judicious use of such valuable species under scientific supervision so as to yield maximum health benefits.

Key words: Antioxidants, inflammation, medicinal plant, mutagens, virus

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INTRODUCTION

P. vulgaris L. (Labiatae), a rediscovered herb belonging to the mint family also known as self heal, was very popular in European, Asian and Chinese medicine and was used against fever, wounds and throat infections[1]. Its flowering season is from May to September[2]. For this reason the herb in Chinese is known as "Xia Ku Cao", meaning "Grass perished at the end of summer". The flowers are hermaphrodite[3]. This 1-2 feet high medicinal herb grows on grassland and usually prefers acidic, neutral and basic soils. It grows in semi shade or moist soil[4]. "*Prunella*" was derived from German word "Brunellen" which means "inflammation of mouth" as it was used by German military physicians for treatment of contagious fever characterized with sore throat and a brown-coated tongue among the troops in 1547 and 1566 (http://www.herbalextractsplus.com/wound-root.html). The epithet of the species "vulgaris" is from the Latin adjective "vulgar" meaning "common" as the plant is widespread. John Gerard's book "Herball" in 1597 mentioned that there was no "better wound herb" in the world than Self Heal". The great herbalist, Nicholas Culpepper, wrote that "Self Heal" if taken both "inwardly or outwardly for wounds and bleeding" would "cleanse the foulness of sores and heal them.

General health benefits of *Prunella vulgaris*: Dried fruit spikes with flowers are used for various pharmaceutical purposes, besides leaves and stems are used for olive green dye. Leaves are used as raw or cooked in salads and soups[5]. Fresh leaves and stem of herb are rich in protein, plant fat, carbohydrate, carotene, vitamin B and nicotinic acid[5]. The whole plant is considered as alterative, antibacterial, antipyretic, antiseptic, antispasmodic, astringent, carminative, diuretic, febrifuge, hypotensive, stomachic, styptic, tonic, vermifuge and vulnerary [6,7]. It was used to heal wounds, ulcers and sores[8]. It was used as a tea in treatment of fevers, diarrhoea, sore mouth and internal bleeding[9]. It is antibiotic and hypotensive[9].

Composition: The most active constituents reported in this herb were betulinic-acid, D-camphor, delphinidin, hyperoside, manganese, oleanolic-acid, rosmarinic-acid, rutin, ursolic-acid, tannins volatile oil, beta-carotene, sugar, cellulose, vitamins B-1, C and K[3]. A triterpene, 2α, 3α, 24-trihydroxyolean-12-en-28-oic acid was found in the leaves and stems of *P. vulgaris*[10]. Three pentacyclic triterpenes were isolated from the roots of *P. vulgaris*[11]. Again two hexacyclic triterpenoids (12R,13S)-2α, 3α, 24-trihydroxy-12,13-cyclotaraxer-14-en-28-oic acid and (13S,14R)-2α, 3α, 24-trihydroxy-13,14-cyclo-olean-11-en-28-oic acid as methyl esters were isolated from the roots of plant as well[12]. Ursolic acid and its derivatives were also reported in the herb[13]. Prunellin, an anti-HIV polysaccharide was isolated from aqueous extracts of *P. vulgaris*[14]. The molecular size of prunellin was about 10 kDa. Glucose, galactose, xylose, gluconic acid, galactonic acid and galactosamine were reported as the

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constituent monosaccharides[14]. Latter on four β-d-glucopyranosides (sitosterol, stigmasterol, stigmast-7-en-3β-ol and spinasterol) were identified[15]. Four novel triterpenes, i.e., betulinic acid, ursolic acid, 2α, 3α-dihydroxyurs-12-en-28-oic acid and 2α-hydroxyursolic acid were obtained by activity-guided fractionation of the *P. vulgaris* extract[16]. Fifteen triterpenoic acids, four flavonoids, four phenolics and a diterpene were isolated from MeOH extract of aerial parts of *P. vulgaris* var. Lilacina[17]. Oleanolic acid (OA) and Ursolic Acid (UA) in *P. vulgaris* were separated by modified HPLC[18]. Some novel depsides and two phenylpropanoids were isolated from the ethanol extract of the spikes of *P. vulgaris* i.e., Butyl rosmarinate, ethyl rosmarinate, methyl rosmarinate, rosmarinic acid, 3,4,α-trihydroxy-methyl phenylpropionate and p-coumaric acid[19]. Seven compounds from the spikes of *P. vulgaris* were isolated and their structures were established as autantiamide acetate, rhein, tanshinone I, danshensu, stigmast-7, 22-dien-3-one, 3, 4, alpha-trihydroxy-methyl phenylpropionate and butyl rosmarinate[20]. A novel triterpenoid saponin 16-oxo-17-demethyl-3beta, 24-dihydroxylolean-12-en-3-O-beta-D-glucuronoside (named as prunelloside A) and a flavones glycoside acacetin-7-O-beta-D-glucopyranoside were reported as constituents of *P. vulgaris*[21]. By using High Performance Liquid Chromatography (HPLC) and LC/MS analysis the main active compounds obtained were phenols, such as caffeic acid, rosmarinic acid, rutin and quercetin[22]. A new phenolic glycoside structurally elucidated as gentisic acid 5-O-beta-D-(6'-salicylyl)-glucopyranoside were obtained from the spikes of *P. vulgaris*[23]. Content of phenolic compounds in four species of Prunella (*P. vulgaris*, *P. laciniata*, *P. grandiflora* and *P. orientalis*) were reported as phenolic acids (rosmarinic acid, caffeic acid, ferulic acid, chlorogenic acid, protocatechuic acid), flavonoids (rutin, quercetin) in different quantitative proportions[24]. Again Oleanolic Acid (OA) was isolated from an ethanol extract of herb and its chemical structure was identified[25].

Effect on mutagens: P. vulgaris was found to be anti mutagenic in nature inhibiting mutagenicity of benzopyrene[26]. Similarly *P. vulgaris* spikes when tested against the environmental mutagens and carcinogens like benzopyrene, 1, 6-dinitropyrene and 3, 9-dinitrofluoranthene were reported effective[27].

Effect on different viral serotypes: Anti-HBsAg (Anti-Hepatitis B surface antigen) capability was reported in *P.vulgaris*[28]. The antiviral activity of this herb was screened qualitatively and quantitatively against *Herpes simplex* virus[29]. The crude extract of *P. vulgaris*, was able to effectively inhibit HIV-1 replication with relatively low cytotoxicity. The responsible active factor was purified and was anionic with a molecular weight of approximately 10 kDa[30]. Two triterpenes isolated from *P. vulgaris* as betulinic acid and 2α, 3α-dihydroxyurs-12-en-28-oic acid were reported with antiviral activity against *Herpes simplex* virus type 1. The antiviral activity was estimated as EC_{50}= 30 and 8 μg mL^{-1}, respectively by plaque reduction assay[31]. Further the anti-HIV-1 effects of the hot water extracts of *P. vulgaris* spikes was also reported. Their IC_{100} values were 16 μg mL^{-1} [32]. The ability of different dilutions of *P. vulgaris* extracts to inhibit replication of HIV strain H9/3B was monitored by inhibition of HIV-induced cytotoxicity in MT2 cells, measured by the MTT uptake assay[33]. The water soluble substance was isolated from *P. vulgaris*, by hot water extraction, ethanol precipitation and gel permeation column chromatography. Chemical tests showed that the component was an anionic polysaccharide. Using plaque reduction assay, the polysaccharide at 100 μg mL^{-1} was active against the *Herpes simplex* virus types 1 and 2 (HSV-1 and HSV-2), but was inactive against *cytomegalovirus*, the human *influenza virus* types A and B, the poliovirus type 1 or the *vesicular stomatitis* virus. The *Prunella* polysaccharide was not cytotoxic to mammalian cells up to the highest concentration tested, 0.5 mg mL^{-1} and has not showed any anti-coagulant activity[34]. The aqueous and methanol extracts were also reported for their *in vitro* inhibition on *human immunodeficiency* virus type-1 protease (HIV-1 PR). Among different herbal extracts examined, the aqueous extracts of *P. vulgaris* elicited inhibition (>90%) at a concentration of 200 μg mL^{-1} [35]. The extract of *P. vulgaris* spikes were again reported to inhibit HIV replication at reverse transcription *in vitro*[36]. Potent HIV-1 inhibitory activity was observed in aqueous extract of *P. vulgaris*[37]. A polysaccharide fraction was found in *P. vulgaris* that showed its effects on the expressions of HSV-1 and HSV-2 antigens. The effective concentrations of extract with 50% reductions of the HSV-1 and HSV-2 antigens were 20.6 and 20.1 μ mL^{-1}, respectively[38]. Multiple *P. vulgaris* constituents were indicated to have profound anti-viral activity against Equine Infectious Anaemia Virus (EIAV), providing evidence of the anti-viral abilities of its extracts. Aqueous extracts prevented entry of viral particles into permissive cells suggesting that these extracts may function as promising microbicides against *Lentiviruses*[39]. Aqueous extracts of *P. vulgaris* were reported to display potent antiviral activity against HIV-1 infection than ethanol extracts. Extract inhibited both virus/cell interactions and post- virion binding events[40]. In another study anti-HSV compound from *P. vulgaris* was identified as lignin-polysaccharide (PPS-2b) complex having a molecular weight of 8500 with strong activity against HSV-1 and HSV-2 and its mode of action is inhibiting viral binding and penetration into host cells[41].

Effect on cell proliferation: The organic fraction (25.7% w/w of rosmarinic acid) of *P. vulgaris* displayed antiproliferative effects against HaCaT cells and mouse epidermal fibroblasts[42]. Rosmarinic acid was isolated from the methanolic extract of *P. vulgaris* which showed inhibitory activity against Lymphocyte Cell-specific Kinase (Lck) Src-Homology 2 (SH2) binding to a synthetic phosphotyrosine-containing peptide (phosphopeptide) of hamster polyomavirus middle-sized tumor (hmT pY324). The IC_{50} value for Lck SH2 binding to phosphopeptide was 7 μM[43]. *P. vulgaris* extracts was reported to suppress the proliferation in Raji cells and may be a new anti-lymphoma drug. Immunocytochemistry showed that after Raji cells were treated by the injection of *P. vulgaris* (50 mg mL^{-1}) for 48 h, the expression of bcl-1 was up-regulated and the expression of bax was down-regulated[44]. *P. vulgaris* displayed significant antiestrogenic activity against endometrial cancer cell line, ECC-1[45]. A phenolic components in *P. vulgaris* ethanolic extract was reported to significantly inhibit the tumor growth in C57BL/6 mice[22]. Chemoprevention by *P. vulgaris* 60% ethanol extract (P-60) was again reported against non-small cell lung cancer via promoting apoptosis in SPC-A-1 cells and regulating the cell cycle[46]. Two polysaccharides (P31 and P32) were isolated from the aqueous extract of herb. The main monosaccharide composition of polysaccharide P32 consisted of rhamnose, arabinose, xylose, mannose, glucose and galactose in a molar ratio of 3.46: 49.32: 58.91: 0.43: 2.64: 3.11, respectively. Polysaccharides showed anti-lung cancer activity in a C57BL/6 mouse-Lewis Lung cCarcinoma (LLC) model that increased the thymus index and the spleen index[47]. Oleanolic acid was isolated from *P. vulgaris* ethanolic extract that induced apoptosis in lung adenocarcinoma SPC-A-1 cell line through down-regulating Bcl-2 expression and up-regulating Bax and Bad expression. Oleanolic acid at 16 and 8 μM increased the apoptosis rate compared to normal[25]. Aqueous extract of *P. vulgaris* was reported to affect migration and invasion of human liver carcinoma cells by inhibiting activities of metalloproteases, MMP-2 and MMP-9, without affecting cell viabilities[48]. A strong inhibitory effect of *P. vulgaris* was observed on growth of two lung adenocarcinoma cell lines A549 and SPC-A-1. Inhibition rate of lung tumor deterioration by high dosage of extract was 3.56±6.79% and low dosage was 33.45±10.98%. Furthermore, it enhanced thymus index whereas no effect was observed on spleen index in tumor-bearing mice. The content of TNF-α in serum was increased in *P. vulgaris* group hence *P. vulgaris* was reported to possess the prevention effects on lung cancer[49]. Different concentrations of the extract from *P. vulgaris* were found to inhibit the proliferation of both Raji and Jurkat cells and with the increase of the concentration of the extract the early cell apoptosis rate was increased and the expression of BCL-2 protein was down-regulated and BAX up-regulated[50].

Effect on oxidative stress: Significant antioxidative activitiy was found in hydroalcholic extract of *P. vulgaris*[51]. The antioxidative activity was partly with regard to the rosmarinic acid content. Antioxidative activity against superoxide, hydroxyl radicals and pro-oxidants were reported[52]. Furthermore it was reported that *P. vulgaris* extract exhibited scavenging activity on diphenylpicrylhydrazyl radical (DPPH), inhibited *in vitro* human LDL Cu (II)-mediated oxidation, protected rat mitochondria and rat hepatocytes exposed to either tert-butyl hydroperoxide, or to Cu(II) and Fe(III) ions. Extract also inhibited rat erythrocyte haemolysis and reduced the production of LTB in bovine PMNL generated by the 5-lipoxygenase pathway[42]. The positive effect of phenolics-rich extract of *P. vulgaris* was observed on blood, liver antioxidant status and lipoprotein metabolism. It affected plasma lipoprotein profile in an experimental animal model with induced dietary hypertriglyceridemia[53]. The *P. vulgaris* extract and its main phenolic acid component, rosmarinic acid significantly suppressed UVA-induced ROS production in a human keratinocyte cell line (HaCaT), which indicated a decrease in intracellular lipid peroxidation[54]. *P. vulgaris* aqueous extract was evidenced to inhibit mast cell-derived immediate-type allergic reactions, proinflammatory cytokines and nuclear factor-kappa B. Extract (0.001-0.1 g kg^{-1}) dose dependently inhibited compound I nduced systemic anaphylaxis and serum histamine release in mice. It also decreased the IgE-mediated local allergic reaction, passive cutaneous anaphylaxis. In addition, extract attenuated phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187-stimulated TNF-alpha, IL-6 and IL-8 secretion in human mast cells[55]. Ethanolic extract of the spikes of *P. vulgaris* was reported to yield two ursane-type triterpenes 3beta,23-dihydroxyurs-12-en-28-oic acid (23-hydroxyursolic acid) and 3beta-hydroxyurs-12-en-28-oic acid (ursolic acid) which protected cells against oxidant-mediated injury. Treatment with these compounds increased the expression of inducible heme oxygenase (HO)-1 enzyme in human liver-derived HepG2 cells[56].

Effect on inflammation: SKI 306X was extracted from a mixture of three herbal medicines *Clematis mandshurica*, *Trichosanthes kirilowii* and *P. vulgaris* that have been widely used for the treatment of inflammatory diseases such as lymphadenitis and arthritis[57]. Anti-inflammatory activity in one of the polysaccharide fraction PV21V of herb was reported[58]. Water extracts of *P. vulgaris* was reported for

anti-inflammatory activity. Extract stimulated the proliferation of T-lymphocytes and suppressed NO production in lipopolysaccharide-stimulated macrophages dose dependently without any cytotoxicity[59]. The anti-inflammatory effect was also observed in aqueous extract of *P. vulgaris* via inhibition of ROS/NF-κB pathway by inducing HO-1 and eNOS expression mediated by Nrf2, thereby suggesting that herb may be a possible remedy for inhibition of diabetic vascular diseases[60]. Anti-inflammatory activity of ethanol extract by inducing heme oxygenase-1 (HO-1) expression through PI3K/Nrf2 signal pathways was reported, which may be good for the treatment of sepsis because of decrease in high mobility group box?1(HMGB1) release, a cytokine released in late phase in sepsis[61].

Antimicrobial activity: *P. vulgaris* decoction showed strong inhibition and control on *diarrhea bacilli, salmonella typhi, Vibrio cholerae, E.coli, Proteus vulgaris, Staphylococcus aureus and Mycobacterium tuberculosis.* Morever alcoholic decoction of *P. vulgaris* showed inhibition on *Pseudomonas aeruginosa*, water decoction showed inhibition on fungi. It also inhibits *Pseudomonas, Bacillus typhi, E.coli, Mycobacterium tuberculosis*[6]. The rosmarinic acid of *P. vulgaris* was found to exhibit a moderate antimicrobial activity on gram-positive bacteria[42]. The antimicrobial effects exhibited by plant polysaccharide was also reported[58]. Antibacterial activity of the methanolic extract of *P. vulgaris* extracts was reported against *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Kleibsella pneumonae*[62]. Two polyacetylenic acids were isolated from *P. vulgaris* methanolic extract as active principles and were identified as octadeca-9,11,13-triynoic acid and trans-octadec-13-ene-9,11-diynoic acid. These two compounds inhibited the growth of fungal pathogens *Magnaporthe oryzae, Rhizoctonia solani, Phytophthora infestans, Sclerotinia sclerotiorum, Fusarium oxysporum f.* sp. *Raphani and Phytophthora capsici.* The n-hexane fraction of *P. vulgaris* significantly suppressed the development of rice blast, tomato late blight, wheat leaf rust and red pepper anthracnose[63]. The effect of the extract of *P. vulgaris* on Multiple Drugs Resistant Bacillus Tuberculosis (MDR-TB) was reported and the extract could enhance the cellular immunological function in rats by up-regulating level of genetic transcription which provided the basis of healing of MDR-TB with it[64]. Purified homodimer of lectin showed significant antimicrobial activity against *Salmonella typhi, Klebseilla pnuemonea* and *Escherhia coli*[65].

Effect on cardiovascular diseases: The cardioprotective effect of ethylacetate fraction and its constituent rosamarinic acid of *P. vulgaris* were reported on rats induced with oxidative stress[66]. Positive control used were less effective. The effect on eNOS gene expression of *P. vulgaris* was observed with favourable effects on the vasculature and could have therapeutic potential against cardiovascular diseases by acting as potent eNOS-upregulating agent [67].

Cosmetics: The plant extract or its saponin was reported to be used for the preparation of a cosmetic or pharmaceutical and dermatological composition. Composition of this herb helped to regulate the renewal and differentiation of the keratinocytes and has an anti-ageing activity, especially in inflammations caused by ultraviolet radiations[68]. The potency of *P. vulgaris* extract and its main phenolic acid component, Rosmarinic Acid (RA), was found to suppress UVB-induced (295 to 315 nm) skin damage to human keratinocytes HaCaT cells. Extract (5 to 50 mg L^{-1}) and RA (0.18 to 1.8 mg L^{-1}) reduced breakage together with the apoptotic process in cells. Extract and RA also eliminated ROS production and diminished IL-6 release[69].

Effect on teeth: Herbal-based dentifrice i.e., *P. vulgaris* extract was reported to be effective in reducing symptoms of gingivitis[70]. *P. vulgaris* extract and rosamarnic acid was able to suppress LPS-induced biological changes in gingival fibroblasts by modulating the inflammation process in periodontal disease[71].

Effect on immune system: The immonosupressive activity of the ethanol extract of spica prunellae on the immune response in mice was studied[72]. One of Polysaccharide fraction PV21V up-regulated the immune response of monocytes[58]. Again investigation showed that water extracts of *P. vulgaris* extract stimulated the proliferation of T-lymphocytes and suppressed NO production in lipopolysaccharide-stimulated[59]. The immunostimulatory and antitumor activity of *P. vulgaris* in murine macrophage RAW 264.7 cells was reported. Plants extract stimulated macrophage phagocytic activity, Nitric Oxide (NO) production and cytostatic activity. In addition induced gene expression and production of macrophage-related cytokines such as TNF-α, IL-1 β and IL-6[73]. Rosmarinic acid in ethanol extract of plant inhibited lipopolysaccharide-induced prostaglandin E2 and nitric oxide in RAW 264.7 mouse macrophages[74]. 0.1 and 1.0% doses of *P. vulgaris* extracts augmented diets increased the non-specific immune response and disease resistance of *P. olivaceus* against *U. Marinum*[75].

Anti-allergic: The effect of aqueous extract of herb on immediate-type allergic reactions was studied which showed that extract (0.005 to 1 g kg^{-1}) inhibited systemic anaphylactic shock in rats. When extract was given at

concentrations ranging from 0.005 to 1 g kg^{-1}, the serum histamine levels were also reduced[76]. Again the effect of aqueous extract of *P. vulgaris* on the mast cell-mediated allergy model was investigated and it was found that extract (0.001 to 0.1 g kg^{-1}) dose dependently inhibited systemic anaphylaxis and serum histamine release in mice[55].

Anti-diabetic: P. vulgaris extract at dose of 100 mg kg^{-1} significantly suppressed the rise in blood glucose after 30 min in the acute glucose tolerance test. It enhanced the antihyperglycemic effects of exogenous insulin without stimulating insulin secretion in streptozotocin-induced diabetic mice[77]. Extract also has a protective effect on IL-1β-induced INS-1 cell apoptosis. It attenuates IL-1β-increased NF-κB binding activity and inflammatory cytokine expression in INS-1 pancreatic β-cells. PVAE may have a benefit for type I diabetic patients[78].

Antistress: The ability of ethanolic extract of leaves of *P. vulgaris* to prolong the swimming time and ameliorate the stress induced changes in animal stress models was reported, therefore suggested its adaptogenic property[79].

CONCLUSION

Various laboratory *in vitro* and *in vivo* studies have shown the health effects of this herb, but the human clinical evidence is still limited, future research is needed to actually define the different benefits particularly for the ailments like viral diseases and cancers where synthetic drugs are not effective because of lesser safety margin and higher cost. Health benefits of the different bioactive principles need to be evaluated more.

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