



RECENT ADVANCES AND SPORADIC PHYTOCHEMICAL AND
PHARMACOLOGICAL REVIEW ON POTENTIAL HERBS OF THE GENUS
“PULSATILLA”

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ABSTRACT

Horrid emergence of mental disorders has attracted the attention of researchers towards various pharmacotherapeutic approaches for the management of these ‘modernization borne diseases. Barbiturates, benzodiazepines (BZDs), azaspiroines, norepinephrine and serotonin-reuptake inhibitors, monoamine oxidase inhibitors and phenothiazines are some of the commonly used psychotropic drugs. Among these, BZDs are the most widely prescribed synthetic chemical drugs for the treatment of anxiety, insomnia, epilepsy, and stress. Regular use of BZDs causes deterioration of cognitive functioning, addiction, physical dependence and tolerance. Abrupt cessation of chronic treatment with BZDs causes the appearance of withdrawal effects comprising re-bound anxiety, restlessness, epilepsy, and motor agitation. In the light of adverse effects associated with the synthetic drugs, researchers have been exploring natural resources to find out safer and effective drugs. Investigating plants, based on their use in traditional systems of medicine, is a sound, viable and cost effective strategy to develop new drugs. Plants like *Valeriana officinalis*, *Nardostachys jatamansi*, *Withania somnifera* and *Panax ginseng* have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Inclusion of these well-established CNS affecting plants in the arsenal of modern therapeutics has revived the faith of researchers in the plants. The genus *Pulsatilla* belongs to family Ranunculaceae, also known as Buttercup family which comprises of nearly 70 species, mainly as herbs. Upon literature survey, it has been found to be very potential plant for alleviating various human diseases.

KEYWORDS: *Pulsatilla*, health benefits, anxiety, *Pulsatilla nigricans*.

INTRODUCTION

Plants have been used since centuries as part of our life as food, spices, building material, medicines for prevention and cure of various diseases etc. Plants as herbal medicines are the basis of Ayurveda, Sidha and Unani systems of medicine. These are all alternative systems of medicine. The interest in herbal medicines is increasing day by day due to reliability of folk medicine results and easy economical availability. Research interest in herbal medicines in crude form as well as extracted phytoconstituents is fetching young scientists in this natural arena.

Herbs have shown therapeutic as well as preventive effects in various health conditions of mankind.

Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or in a more conventional medical sense, to treat, cure or prevent disease in animals or humans¹. Various plant origin drugs which are used as synthetic agents are atropine from *Atropa belladonna*, digoxin from *Digitalis purpurea*, quinine from *Cinchona officinalis* and, vincristine and viblastine from *Catharanthus roseus* etc.². Humans use herbal medicines as home remedies and make a huge portion of global drug market share. According to Aggarwal and Raju³ (2006) the current size of worldwide market is between US \$ 80 to 100 billions and expect to be US \$ 2500 in 2010. There are 1500 identified indian medicinal plants and out of which 500 plants are commonly used in indian system of medicine. WHO reported that 70-80% population of world relies for primary healthcare needs on plant drugs as major part of traditional system of medicine^{3, 4}. So, this shift imparting risk of adverse effects/side effects or interactions for population due to lack of complete knowledge of plant drugs. Problem of using herbal medicines is its variability in efficacy and desired therapeutic effects along with adverse and side effects. This is all due to non-availability of standards for plant drugs i.e. standardization is necessary for herbal medicines. Even this is necessary for synthetic drugs. Standardization is a quality control process to setup standards or characteristics, constant parameters, quality and quantitative assurance for safety and effectiveness of particular herbal medicine or plant. All the processes should be reproducible. Many challenges hinder standardization of herbal drugs viz. mixture of constituents, unknown active principal, analytical methods, chemical and natural variability, source and quality of raw material etc⁵.

Mental Disorders and Herbal Medicines

WHO defines health as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”⁶. Mental disorders include anxiety, depression, alzheimers disease, migraine, attention deficit/ hyperactivity disorders etc⁷. According to WHO report (2001) about 450 million people suffer from mental disorders⁶. One person in four will develop one or more mental or behavioural disorders during their lifetime⁸. The economic impact of mental disorders is increasing day by day. In USA, US \$147 billion dollars have been reported to be the annual total costs related to mental disorders which is much more than the costs made to cancer, respiratory disease or AIDS⁹. Moreover in low income countries, the costs do not

reach these levels because of the low availability and coverage of mental health care services⁸. Hence, they use traditional medicines for a variety of health complications.

Major portion of traditional therapies contain plant extracts or their active principles¹⁰. Although a variety of pharmaceutical agents are available for treatment of mental disorders but physicians understand the patients do not tolerate the side effects and adverse effects of these drugs. Herbal medicines have lesser side effects as compared to allopathic drugs, rather wide availability and lesser cost make them to use herbal drugs. Lots of plant drugs have proved the improvement in mental disorders. In Alzheimer's disease, *ginkgo biloba* extract called EGb 761 has shown comparable effects as with conventional drugs like donepezil and rivastigmine^{11, 12}. St. John's Wort is therapeutically equivalent to imipramine in treatment of depression and the tolerability is better than imipramine^{13, 14}.

In case of anxiety, *passiflora incarnate* and kava has shown significant results as compared with standard drugs^{15, 16}. Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral components¹⁷. Anxiety Disorders Association of America (ADAA) described anxiety disorders as the most common mental illness in the US, that have affected 40 million adults of 18 years and older US population¹⁸.

Above all, the prevalence of mental disorders is increasing widely and patients shift towards herbal system of medicine for therapeutic and economical benefits. So, from above background, the present study was aimed to screen plant drug for mental disorders.

Management of Anxiety in Allopathic System of Medicine

Such a horrid emergence of mental disorders has attracted the attention of researchers towards various pharmacotherapeutic approaches for the management of these 'modernization borne diseases'¹⁹. Barbiturates, benzodiazepines (BZDs), azaspiroines, norepinephrine and serotonin-reuptake inhibitors, monoamine oxidase inhibitors and phenothiazines are some of the commonly used psychotropic drugs¹⁴. Among these, BZDs are the most widely prescribed synthetic chemical drugs for the treatment of anxiety, insomnia, epilepsy, and stress. Regular use of BZDs causes deterioration of cognitive functioning, addiction, physical dependence and tolerance¹⁹⁻²¹. Abrupt cessation of chronic treatment with BZDs causes the appearance of withdrawal effects comprising re-bound anxiety, restlessness, epilepsy, and motor agitation^{22,23}. In the light of adverse effects associated with the synthetic drugs, researchers have been exploring natural resources to find out safer and effective drugs. Investigating plants, based on their use in traditional systems of medicine, is a sound, viable and cost effective strategy to develop new drugs²⁴. Plants like *Valeriana officinalis*, *Nardostachys jatamansi*, *Withania somnifera* and

Panax ginseng have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Inclusion of these well-established CNS affecting plants in the arsenal of modern therapeutics has revived the faith of researchers in the plants²⁵.

The genus *Pulsatilla*

The literature review on the genus *Pulsatilla* covers the traditional claims and clinical efficacy of *Pulsatilla* species, especially *Pulsatilla nigricans*. The genus *Pulsatilla* belongs to family Ranunculaceae, also known as Buttercup family which comprises of nearly 70 species²⁶, mainly as herbs²⁷. *Pulsatilla* also known as pasque flower which is grown in Turkey, Russia, Germany, France, Denmark, Sweden, Southern England and Asia²⁸. Almost every plant of the genus *Pulsatilla* is covered with soft and silky white hairs providing them a lax, shaggy and wooly appearance. Leaves are generally immature at the early flowering period of the plant.

Traditional claims

Bai Tou Weng, a traditional Chinese medicine containing *Pulsatilla* species such as *P. ambigua*, *P. chinensis*, *P. dahurica*, *P. koreana*, *P. turczaninovii*, has been used against bacteria, amoeba and vaginal trichomoniasis²⁹⁻³³. *P. Cernua* has been used traditionally in China as antitumor and antidiabetic³³ and its roots have been used as a home remedy for astringent and diuretic properties³⁴. The plant has also been used as antiphlogistic and hemostatic³⁵. *P. chinensis* has been used in the treatment of amoebiasis, fever, diarrhoea, hematochezia, trauma and lung tumour. In Korea, *P. koreana* roots have been used for the treatment of hematochezia due to intense evil heat, malaria, chills and fever, amoebic dysentery, epistaxis and internal hemorrhoids²⁵⁻³⁷. *P. nigricans* has been used in nervousness, sadness, mild restlessness and mental unrest²⁸. The plant has been used as a remedy for ovaritis, ovaralgia, pain associated with debility and due to acute inflammation, epididymitis, and orchitis. It increases sexual power, but lessens morbid sexual excitement. *P. nigricans* relieves urethral irritation, consequent spermatorrhoea and prostaticorrhoea, amaurosis, cataract and opacity of the cornea. *P. nigricans* has been used in uterine affections, dyspepsia, coryza, otitis, rhinitis, conjunctivitis, coughs, cutaneous affections, acute meningitis, and as taeniafuge³⁸. *P. nigricans* roots have been used for blood-cooling and detoxifying effects in traditional system of Chinese medicine³². *P. patens* var. *multifida* roots have been used as an antibacterial, antiamoebic and antitumor in China³⁹.

Reported Alternative medicinal uses of plants of the genus *Pulsatilla*

The pharmaceutical preparation used as hair tonic for the prevention of alopecia, depletion and cleaning of scalp contains *P. cernua* as one of the main ingredients⁴⁰. An effective and safe skin lightening cosmetic contains 0.001 to 20.0% w/w saponins extracted from *P. cernua* as one of

the ingredients⁴¹. *P. chinensis* is one of the ingredients in the colon targeting capsule used for treatment of ulcerative colitis⁴². A pharmaceutical preparation containing *P. chinensis* as one of the ingredients is used as oral cavity healthcare liquid⁴³. Ethanolic extract of *P. koreana* has been included in pharmaceutical preparations used for the treatment of diabetes⁴⁴⁻⁴⁵, and as antiplaque dentrifices in concentration ranging from 0.005-5%⁴⁶⁻⁴⁷. *P. nigricans* is given to produce sleep, when there is great exhaustion and opiates are inadmissible²⁸. *P. nigricans* frequently proves a useful remedy in headache of various types. Methanol extract of *P. nigricans* roots has been included in number of pharmaceutical formulations used for treatment of periodontal disease (antimicrobial effect), dysentery, and in cosmetic composition for skin fairness effect⁴⁸⁻⁵⁰. Formulations of *P. nigricans* have been used to alleviate the physical, physiological and psychological problems associated with normal and premature menopause, vaginal discharge, and its associated problems such as itching, redness and burning micturation⁵¹⁻⁵². Homeopathic medicines of *P. nigricans* have been used for the treatment of clinical cases of bovine-mastitis⁵³. *P. nigricans* 200 CH has been reported to decrease total sperm defects, increased sperm motility and number of doses of semen produced in infertile nelore bull⁵⁴. A homoeopathic complex containing *Calcarea phosphorica* 30C, *Aletris farinosa* 30C, *Pulsatilla* 30C, *Aurum muriaticum* natronatam 30C, *Sepia* 30C and phosphorus 30C (15 pills twice daily orally for 10 days) induced oestrus in anoestrus cows, and reported to increase serum estradiol concentration⁵⁵. *Pulsatilla* is one of the constituent of homeopathic remedies most frequently prescribed for ENT allergies⁵⁶. *Pulsatilla* as a homoeopathic medicine has been found to be effective in the treatment of acute otitis media in children⁵⁷⁻⁵⁸. Fluid extract (1/2-2 minims) or tincture (5-30 minims) of *P. nigricans* have been prescribed by physicians in various disorders of nervous and reproductive organ systems⁵⁹. It has also been prescribed in uterine disorders which induce melancholia and hysteria, general nervousness due to chronic uterine disorders, nervous exhaustion, nervous headaches, urinary irregularities during pregnancy, etc.

Pharmacognostic reports of the genus *Pulsatilla*

Morphological characters

P. nemorosa Schrank (Synonym *Anemone nemorosa* Linn.), is about 4 inches high; root slender, horizontal root-stalk; stems simple, slender, erect, leafless, at top it bears a whorl of three-petiole; flowers solitary, small, peduncled, white or purple in colour²⁸.

P. nigricans Stoerck (Synonym *P. pratensis* Mill.)²⁶ is a perennial plant; stem simple, erect, rounded, 3-5 inches high; leaves radical, pinnatifid, downy, the segments many-parted, with linear lobes; flowers solitary, terminal, pendulous, deep-purple or violet-brown, somewhat

narrow, pointed, reflected at the point, erect and converging at the base; sepals 6; stalked glands or sterile stamens are found between the fertile stamens and sepals, the proximity of the involucre is such that it has a calyx like appearance²⁷⁻²⁸. *P. patens* Mill. (Synonym *Anemone patens* Linn.), commonly known as American Pulsatilla, root perennial; stem simple, upright, naked except the floral leaf; flowers large, terminal, very conspicuous, in early spring; floral leaf cup-shaped, surrounding the stem about an inch below the flower, divided into 15 to 20 linear spreading divisions; calyx 6 petaloid, purplish or white, covered externally with silky hairs; petals represented by a few gland-like bodies, resembling stamens, but smaller; stamens numerous; pistil numerous in a head; fruit borne on an elongated stalk; achenes many, bearing slender silky tails, about 2 inches long. *P. vulgaris* Mill. (Synonym *Anemone Pulsatilla* Linn.) has involucre, hairy, scape curved and shaggy²¹.

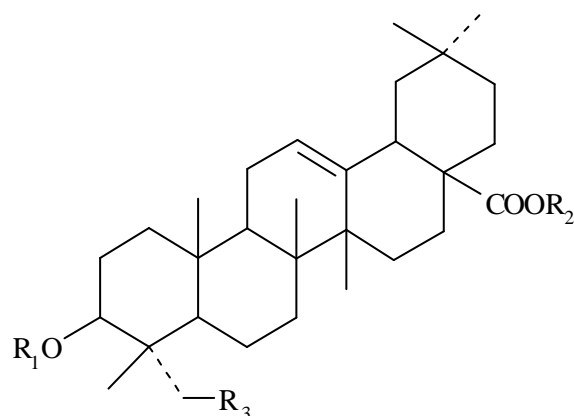
Phytochemical reports of the genus *Pulsatilla*

The available literature on phytochemical reports of *Pulsatilla* species suggests the presence of mainly triterpenoid saponins and flavonoids. Amongst various species, *P. chinensis* is rich in triterpenoid saponins. More than 20 triterpenoid saponins have been isolated from *P. chinensis*. Similarly, other species like *P. albana*, *P. apina*, *P. campanella*, *P. cerna*, *P. cernua* Thunb, *P. dahurica*, *P. oreana*, *P. montana*, *P. nigricans*, *P. patens* and *P. xkissi* have been discussed and the reported constituents have been listed as below:

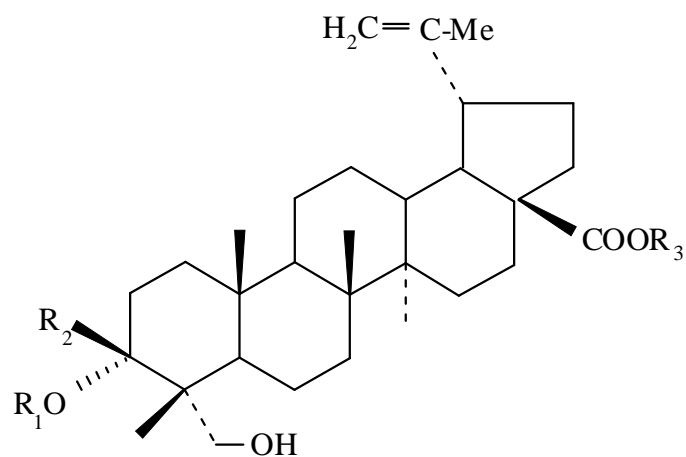
Table 1: Phytoconstituents of various species of *Pulsatilla*.

S.N	Species	Phytoconstituents
1.	<i>P. albana</i> (Stev.)	Essential Oils ⁶⁰ pulegone, piperitenone, menthone, 1, 8-cineoleand p-mentha-3,8-diene
2.	<i>P. alpine</i>	Lactones ⁶¹⁻⁶² protoanemonin, anemonin.
3.	<i>P. campanella</i>	Triterpenoid saponins ^{30,63} pulsatilosides A [1], B [2], C [3], D [4], leontosides A [5], B [6], D [7], caulosides D [8], F [9], calcoside D [10].
4.	<i>P. cerna</i>	Flavonoids ⁶⁴ quercetin, kaempferol.
5.	<i>P. cernua</i> Thunb.	Triterpene aglycones ⁶⁵ hederagenin, oleanolic acid; triterpenoid saponins ^{34,36,66,67} cernuaside A [11], B [12], C [13], D, pulsatilla saponin A [14], D [15], F [16], H [17], dipsacoside B [18], daucosterol; hederagenin saponins such as hederagenin-3-O-β-D-glucopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside; acylated pelargonidine-diglycoside ⁶⁸ ; cinnamic acids ⁶⁹ 4-hydroxy-3-methoxy cinnamic acid, 3, 4-dihydroxycinnamic acid; sterol β-sitosterol, 3-O-beta-D-glucopyranosyl-(1→4)-alpha-L-arabinopyranosyl-bayogenin-28-alpha-L-rhamnopyranosyl-(1→4)-beta-D-glucopyranosyl-(1→6)-beta-D-glucopyranosyl ester ⁷⁰ anemoside A3 [1]; anemoside B4 [2]; 23-hydroxybetulinic acid [3]; cirensenoside S [4]; pulsatilloside B [5]; pulsatilloside C [6]; oleanolic acid [7]; ajugasterone C [8] and ecdysterone [9] ⁷¹ .
6.	<i>P. chinensis</i> Bunge	Triterpenoid aglycone anemosapogenin [19] ⁷² ; triterpenoid saponins anemoside A3 [20], B4 [21], pulchinoside A, B [22], C ⁷³⁻⁷⁵ , ranunculin [23] ⁷⁶ , chinensiosides A, B, hederasaponin C ⁷⁷ ; lupane type triterpenoid saponins pulsatilloside A [24], B [25], C, D ⁷⁸⁻⁸⁰ ; bayogenin-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester ⁸¹ ; hederagenin saponins ^{34,31,81} such as hederagenin-3-O-{O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}; oleanolic acid saponins ³¹ such as oleanolic acid 3-O-{O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}; lupanoic acid saponins ⁸² , such as 3β-[O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] oxy] lup-20-(29)-en-28-oic acid 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester; 23-hydroxy betulinic acid [26] ⁸³ , pulsatillic acid [27] ⁸⁴ flavonoids ⁶⁴ quercetin, kaemferol; lignans ³¹ (+)-pinoresinol; β-peltatin; 2β, 3β, 14α, 20, 22R, 25-hexahydroxy-cholest-7-en-6-one ⁸⁵ Betulinic acid ⁸⁶ Pulchinoside B ₄ ⁸⁷ ; Hederagonic acid ⁸⁸ .
7.	<i>P. dahurica</i> Fischer	Hederagenin ⁸⁹ , hederagenin-3-O-α-L-arabinopyranoside, hederagenin-3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside, hederagenin-3-O-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside, β-sitosterol, daucosterol
8.	<i>P. koreana</i> Nakai	Triterpenoid saponins ³⁶ pulsatilla saponin A [14], B, D [15], F [16], H [17]; hederagenin saponins ⁴¹ ; lupane saponins ⁹⁷ ; cinnamic acids ⁹¹ 4-hydroxy-3-methoxy cinnamic acid, 3, 4-dihydroxycinnamic acid; resin

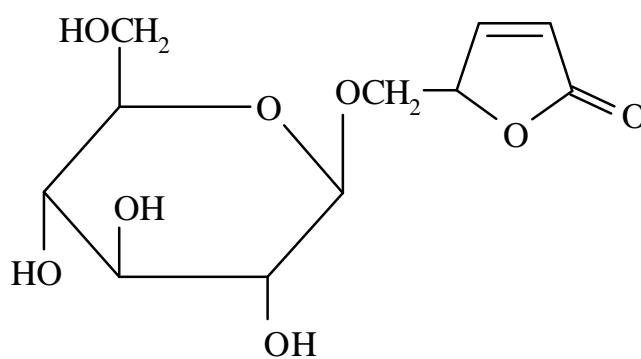
		deoxypodophyllotoxin ³⁷ ; ketone pulsaquinone [28] ⁹² oleanolic-glycoside saponin ⁹³ SK-PC-B70M; Triterpenoidal saponins ⁹⁴ 23-hydroxy-3 -[(O- -L-arabinopyranosyl)oxy] lup-20(29)-en-28-oic acid 28-O- -D-glucopyranosyl ester [1], 23-hydroxy-3 -[(O- -L-rhamnopyranosyl-(1 2)- -L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid 28-O- -D-glucopyranosyl ester [2], 3 -[(O- -L-rhamnopyranosyl-(1 2)- -L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid 28-O- -D-glucopyranosyl-(1 6)- -D-glucopyranosyl ester [3], and 3 -[(O- -L-rhamnopyranosyl-(1 2)-O-[-D-glucopyranosyl-(1 4)]- -L-arabinopyranosyl)oxy]lup-20(29)-en-28-oic acid 28-O- -L-rhamnopyranosyl-(1 4)-O- -D-glucopyranosyl-(1 6)- -D-glucopyranosyl ester [4]; lignans ⁹⁵ (2 R,3 R)-2 -(4"-hydroxy-3"-methoxybenzyl)-3 -(4'-hydroxy-3'-methoxybenzyl)- -butyrolactone 2-O-(-D-glucopyranoside) [1] and (1 S,2 R,3 S)-dimethyl-1,2,3,4-tetrahydro-3,6,7-trihydroxy-1-(3',4'-dihydroxyphenyl)naphthalene-2,3-dicarboxylate [2]; Pulsaquinone, hydropulsaquinone ⁹⁶ ; Hederacolchiside E ⁹⁷ .
9.	<i>P. montana</i> (Hoppe) Riechenb.	Quercetin-3'-methyl ether ⁹⁸
10.	<i>P. nigricans</i> Stoerck	Glucoside pulsatoside A ⁹⁹ hederagenin
11.	<i>P. patens</i> var. <i>multifida</i> Linn..	Triterpenoid saponin ^{100,32} patensin; hederagenin saponins such as 3-O-β-D-glucopyranosyl (1→2)-β-D-galactopyranosyl hederagenin 28-O-β-D-glucopyranosyl ester; oleanolic acid saponins ³² such as 3-O-β-D-glucopyranosyl (1→2)-β-D-galactopyranosyl oleanolic acid 28-O-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester.
12.	<i>P. xkissii</i> Linn.	Flavonoids ⁶⁴ quercetin, kaempferol.



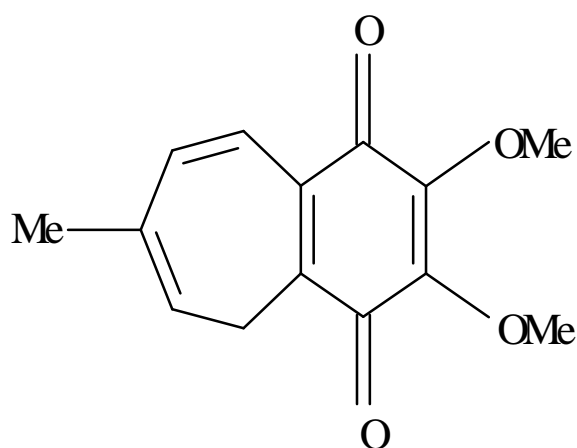
S. No	R1	R2	R3
1	ara(2→1)glc(4→1)glc	H	OH
2	ara(2→1)glc(4→1)glc	glc(6→1)glc(4→1)rha	OH
3	H	glc(6→1)glc(4→1)rha	OH
4	glc(2→1)glc	H	OH
5	Ara	H	OH
6	ara(4→1)glc	H	OH
7	ara(4→1)glc	glc(6→1)glc(4→1)rha	OH
8	Ara	glc(6→1)glc(4→1)rha	OH
9	ara(2→1)glc	glc(6→1)glc(4→1)rha	OH
10	ara(2→1)glc	H	OH
11	glu(1→3)rha(1→2) glu(1→4)ara	rha(1→4)glu(1→6)glu	H
12	glu(1→3)rha(1→2) glu(1→4)ara	rha(1→4)glu(1→6)glu	OH
13	rha(1→2)[glc(1→4)]ara	Glc	OH
14	rha(1→2)ara	H	OH
15	rha(1→2)[glc(1→4)]ara	H	OH
16	rha(1→2)ara	rha(1→4)glu(1→6)glu	OH
17	rha(1→2)glu(1→4)ara	rha(1→4)glu(1→6)glu	OH
18	rha(1→2)ara	glc(1→2)glc	OH



S. No	R1	R2	R3
19	H	H	H
20	rha(1→2)ara	OH	H
21	rha(1→2)ara	H	rha(1→4)glu(1→6)glu
22	Glu	H	glc(6→1)glc(4→1)rha



23



28

ara – arabinopyranosyl

rha – rhamnopyranosyl

glc – galactopyranosyl

glu – glucopyranosyl

Me – Methyl

Pharmacological reports of the genus *Pulsatilla*

Essential oil obtained from hydrodistillation of the aerial flowering parts of *P. albana* showed antibacterial effect against certain Gram-positive and Gram-negative bacteria⁶⁰. Martin et al.¹⁰¹ reported that hexane and chloroform extracts of the flowering aerial parts of *P. alpina* exhibited sedative, hypothermic and antipyretic activities in rats. Anemonin and protoanemonin (10 or 20 mg/kg, i.p.), isolated from *P. alpina* aerial parts, exhibited sedative activity in mice using actophotometer apparatus while antipyretic activity was observed due to anemonin (20 or 40 mg/kg, i.p.) alone⁶¹. Protoanemonin also exhibited antifungal activity against *Candida albicans* and *Aspergillus niger* with the MIC 15 µg/ml using in vitro agar dilution method⁶². These reports reveal that anemonin and protoanemonin are bioactive constituents of *P. alpina*. Cinnamic acid derivatives such as 4-hydroxy-3-methoxy cinnamic acid and 3,4-dihydroxycinnamic acid, isolated from *P. cernua* and *P. koreana* roots, exhibited strong growth inhibiting activity against *Streptococcus mutans*, *Clostridium perfringens* and *Escherichia coli* using an impregnated paper disc method^{91,69}. Cernuosides A and B, isolated from *P. cernua* roots, displayed moderate inhibitory activity against the intestinal sucrase of rats with IC₅₀ values of 59.5 and 45.8 mM respectively, thereby, confirming its antidiabetic activity³³. 3, 4 dihydroxy cinnamic acid and 4

methoxy cinnamic acid isolated from *P. cernua* have been reported to possess antityrosinase activity¹⁰². Pulsatilllic acid, isolated from chloroform soluble part of the methanolic extract of *P. chinensis* roots, exhibited cytotoxic activities against P-388 (IC₅₀ 4.8 µg/ml), lewis lung carcinoma (IC₅₀ 5.9 µg/ml) and human large cell lung carcinoma (IC₅₀ 1.9µg/ml)⁸⁴. Triterpene saponins and lignan (-peltatin), isolated from methanolic extract of *P. chinensis* roots, have been reported to exhibit cytotoxic activity against HL-60 human leukemia cells (95.9% cell growth inhibition at a sample concentration of 10 µg/ml) with IC₅₀ value of 5.1 µg/ml and 0.0052 µg/ml respectively³¹. Anemosapogenin, isolated from *P. chinensis* roots, displayed antitumor activity against Hep-A liver carcinoma and Ehrlich ascites cancer in mice with transplantable tumors¹⁰³. Betulinic acid derivatives isolated from *P. chinensis* have been reported to exhibit cytotoxic (apoptotic) activity on murine melanoma B16 cells¹⁰⁴. A glycoprotein, isolated from the roots of *P. chinensis*, displayed immune-enhancing effect by enhancing immune function of macrophages¹⁰⁵. It has been reported that 2, 3, 14, 20, 22R, 25-hexahydroxy-cholest-7-en-6-one, isolated from ethylacetate extract of *P. chinensis* radix, exhibits a significant hypoglycaemic effect on alloxan diabetogenic mice⁸⁵. Anemonin isolated from *P. chinensis* prevented intestinal microvascular dysfunction by significantly inhibiting the production of NO and endothelin-I induced by lipopolysaccharides at a concentration of 5 g/ml in primary cultures of rat intestinal microvascular endothelial cells, thus, inferring its anti-inflammatory activity¹⁰⁶. *P. chinensis* prevented hepatitis B virus infection by specifically increasing superoxide release in the liver and increasing superoxide dismutase activity to minimize superoxide-mediated toxicity²⁹. The saponins (PCS) from the roots of *Pulsatilla chinensis* were evaluated for its haemolytic activity, acute toxicity and tested for potential adjuvant activity in mice immunized with ovalbumin (OVA) compared with that of Quil A saponin. The haemolytic activity of PCS was¹⁰⁷ determined using 0.5% rabbit red blood cell with values of 15.41 and 7.42% at concentrations of 500 and 250 µg/mL, respectively. The saponins were tested for their toxicity by lethality in mice and were found to be less toxic at the same dose than their counterpart Quil A. 23-Hydroxybetulinic acid showed a synergistic cytotoxic effect on multiple cancer cell lines by combined use with doxorubicin. *In vivo* studies further demonstrated that co-administration of 23-HBA significantly improved the sensitivity of the tumour to doxorubicin through increasing intra-tumor doxorubicin concentration and inhibiting doxorubicin-induced up-regulation of P-gp in tumor¹⁰⁸. Anemoside A3, isolated from *P. chinensis* produced concentration-dependent relaxation in precontracted aortas, mesenteric, left coronary and renal arteries. The release of CTX/apamin-sensitive endothelium-derived hyperpolarizing factor, stimulation of TEA (+)-

sensitive K⁺ channel, and inhibition of Ca⁺⁺ influx jointly contribute to the relaxation of rat renal arteries, showed the role of endothelium and Ca⁺⁺ inhibitory activity¹⁰⁹. The growth of various strains of bacteria viz. *S. aureus*, *E. coli*, *Salmonella* and *B. subtilis* were inhibited by different extracts of *P. chinensis*, but interestingly comparative lower effect on *B. subtilis*¹¹⁰. Aqueous extract of *P. koreana* roots exhibited anti-inflammatory and analgesic activities in mice at a dose of 349 mg/kg¹¹¹. *Pulsatilla* saponin D (64 mg/kg, i.p.) and Deoxypodophyllotoxin (20 mg/kg/day, i.p. for 14 days), isolated from *P. koreana* whole plant, exhibited antitumour activity in mice bearing lewis lung carcinoma cells (ED₅₀ 6-18ng/ml) with an inhibition ratio of 60%^{38,112}. A pregnane-type steroidal compound isolated from the methanol extract of the plant exhibited antitumour activity against cell lung cancer, ovarian cancer, melanoma, CNS cancer and coloncancer¹¹³. Oleanolic acid and hederagenin glycosides isolated from the roots of *P. koreana* have been reported to exhibit significant in vitro cytotoxic activity against the human solid cancer cell lines, A-549, SK-OV-3, Sk-MEI-2 and HCT-15 using the SRB assay method, and in vivo antitumour activity in BDF1 mice bearing lewis lung carcinoma¹⁰⁷. In vivo and in vitro activity-guided fractionation of root extract of *P. koreana* led to isolation of an oleanic glycoside, hederacolchiside E¹¹⁵. Hederacolchiside E (30 or 60 mg/kg, p.o.) increased the step through latency time in passive avoidance test in rats, and exhibited neuroprotective effect on SK-N-SH cells against the toxicity of amyloid-beta-peptide. Oral administration of oleanolic glycoside saponins enriched fraction impaired scopolamine-induced impairments in consolidation and spatial working memory in rats¹¹⁶. SK-PC-B70M, an oleanolic-glycoside saponins fraction extracted from the root of *Pulsatilla koreana*, alleviates neurologic symptoms in G93A-SOD1 amyotrophic lateral sclerosis in mice⁹³. Pulsaquinone, isolated from the methanol extract of *P. koreana* roots, has been reported to exhibit potent antimicrobial activity⁹². The plant exhibited in vitro antiprotozoal activity against *Toxoplasma gondii* and *Neospora caninum* at higher doses¹¹⁷. The saponins isolated from the methanolic extract of the roots of *P. patens* var. *multifida* inhibited the growth of human melanoma A 375 cells with IC₅₀ value of 21.4 µg/ml³⁹. *P. pratensis* exhibited anti-inflammatory activity by abolishing hydroxyl radical generated in a Fenton type reaction system and inhibiting paw swelling¹¹⁸. Lu *et al*¹¹⁹ reported that activation of TGF-beta1/Smad3 signaling pathway by *Pulsatilla* decoction, may be responsible for its anti-inflammatory effect of Inflammatory bowel disease. Euphorbium compositum, a homoeopathic combination preparation containing *P. pratensis* exhibited antiviral activity against respiratory syncytial virus, human rhinovirus, influenza A virus and herpes simplex virus¹²⁰. Aqueous extract of *Pulsatilla* exhibited spasmolytic activity on isolated tissues

of rabbit jejunum¹²¹. Pulsatilloside A and anemoside A3 isolated from *Pulsatilla* spp. have been reported to protect PC 12 cells from apoptosis at dosage ranging from 0.1, 1 and 10 g/ml determined by MTT, LDH release analysis, and flow cytometry measurement¹²².

Clinical reports

In a case report, homoeopathic therapy with *Pulsatilla* C200 cured a 44-years old patient with spontaneous bacterial peritonitis caused by *E. coli*¹²³.

Reported adverse effects

The anemones are listed as poisonous in many of the world publications on poisonous plants, but without clear-cut substantiation¹²⁴. They have been suspected of having caused livestock loss in the United States, but without proof. The fresh plant of *P. nigricans* is irritant upon topical application, and if kept long in contact with the skin, may produce vesication^{28,59}. When chewed, it produces a benumbing sensation and tingling formation, somewhat like that produced by aconite or prickly ash. In overdoses, it acts as a gastric irritant, producing a sensation of rawness, burning, pain in stomach, with endeavors to vomit, all accompanied with marked prostration. Further, large doses of *P. nigricans* can cause constriction and tightness of the chest, with chilliness, marked weakness congestion, lower arterial tension, and motor and sensory paralyses, while toxic doses may produce mydriasis, stupor, coma and convulsions.

Patented formulations of the genus *Pulsatilla*

Table 2: List of patented formulations of the genus *Pulsatilla*

Sr. No	Composition of patented formulations of the genus <i>Pulsatilla</i>	Description/ Activity	Ref.
1.	Traditional Chinese medicine decoction containing Flos Lonicerae, <i>Asparagus cochinchinensis</i> , <i>Codonopsis pilosula</i> , <i>Dioscorea opposita</i> , <i>Rehmannia glutinosa</i> , Semen Armeniacae Amarum, <i>Platycodon grandiflorum</i> , <i>Anemarrhena asphodeloides</i> , Semen Cassiae, <i>Forsythia suspensa</i> , Rhizoma Coptidis, <i>Fritillaria cirrhosa</i> , <i>Zingiber officinale</i> , <i>Stephania cepharantha</i> , <i>Pulsatilla chinensis</i> , <i>Morus alba</i> , and <i>Citrus reticulata</i>	Swine chronic schistosomiasis	125
2.	Chinese medical herb containing <i>Astragalus membranaceus</i> , Rheum, <i>Codonopsis pilosula</i> , alliin, <i>Aristolochia debilis</i> , <i>Isatis tinctoria</i> , <i>Arctium lappa</i> , <i>Citrus sinensis</i> dried peel, <i>Bupleurum chinensis</i> , <i>Lycium barbarum</i> , <i>Pulsatilla chinensis</i> , <i>Sophora flavescens</i> , <i>Phellodendron amurense</i> , <i>Isatis indigotica</i> , oxytetracycline, sulfamonomethoxine and florfenicol	Antibacterial agent	126
3.	Chinese medical composition containing <i>Coptis chinensis</i> , <i>Lonicera japonica</i> flower, <i>Phellodendron chinense</i> bark, <i>Scutellaria baicalensis</i> , <i>Morus alba</i> leaves, <i>Andrographis paniculata</i> , <i>Plantago asiatica</i> , <i>Cassia obtusifolia</i> seed,	Yellow diarrhea and white diarrhea	127

	<i>Sanguisorba officinalis</i> , <i>Pulsatilla chinensis</i> , <i>Gallus domesticus</i> gizzard membrane, and enrofloxacin raw powder		
4.	Traditional chinese preparation containing <i>Astragalus membranaceus</i> , <i>Scutellaria baicalensis</i> , Rhizoma Coptidis, Flos Lonicerae, Radix Paeoniae Rubra, Radix Glycyrrhizae, <i>Houttuynia cordata</i> , <i>Pulsatilla chinensis</i> , <i>Rehmannia glutinosa</i> , <i>Pinellia ternata</i> , Radix Bupleuri, <i>Eriobotrya japonica</i> , <i>Forsythia suspensa</i> , <i>Isatis indigotica</i> (leaf), <i>Isatis indigotica</i> (root), Flos Lonicerae, Semen Cassiae, <i>Carthamus tinctorius</i> , <i>Salvia miltiorrhiza</i> , and <i>Leonurus japonicus</i>	Avian influenza and newcastle disease	128
5.	Traditional Chinese medicine decoction containing <i>Sophora flavescens</i> , <i>Anemarrhena asphodeloides</i> , <i>Cornus officinalis</i> , <i>Morus alba</i> , Radix Paeoniae Rubra, <i>Aucklandia lappa</i> , <i>Schisandra chinensis</i> , <i>Pulsatilla chinensis</i> , Herba Taraxaci, Bulbus Lili, <i>Zingiber officinale</i> , <i>Illicium verum</i> , <i>Ziziphus jujuba</i> , <i>Viola yedoensis</i> , Semen Cassiae, Rhizoma Coptidis, and <i>Fritillaria cirrhosa</i>	Swine chronic superficial gastritis	129
6.	Traditional Chinese medicine containing <i>Pulsatilla chinensis</i> , Rhizoma Coptidis, <i>Portulaca oleracea</i> , <i>Lycium barbarum</i> , <i>Sophora flavescens</i> , <i>Saposhnikovia divaricata</i> , Radix Bupleuri, Rhizoma Atractylodis, <i>Atractylodes macrocephala</i> , Herba <i>Patriniae</i> , Semen Cassiae, <i>Cuscuta chinensis</i> , <i>Equisetum hiemale</i> , Cortex Fraxini, <i>Sargentodoxa cuneata</i> , <i>Ligustrum lucidum</i> , and Cortex phellodendri	Cancer of colon	130
7.	Traditional Chinese medicine antimicrobial lotion containing <i>Cnidium monnieri</i> fruit, <i>Sophora flavescens</i> , <i>Pulsatilla chinensis</i> , Radix Stemonae, <i>Dictamnus dasycarpus</i> bark, <i>Kochia scoparia</i> , <i>Pericarpium Zanthoxyli</i> , <i>Smilax glabra</i> , Rhizoma Atractylodis, <i>Psoralea corylifolia</i> fruit, Cortex Phellodendri, sulfur and borneol, by grinding borneol and sulfur	Vulval leukoplakia and vulval refractory pruritus	131
8.	Feed additive containing <i>Agastache rugosus</i> , Rhizoma Coptidis, Radix Gentianae, <i>Andrographis paniculata</i> , Radix et Rhizoma Rhei, <i>Pulsatilla chinensis</i> , spectinomycin, and lincomycin	Gill rot of aquatic animals	132
9.	Traditional Chinese medicine preparation containing <i>Portulaca oleracea</i> , <i>Pulsatilla chinensis</i> , <i>Rosa laevigata</i> and Fructus <i>Chebula</i>	Porcine diarrhea	133
10.	Feed additive of traditional Chinese medicine comprising <i>Crataegus pinnatifida</i> , <i>Triticum medicata</i> , <i>Atractylodes macrocephala</i> , <i>Isatis indigotica</i> , <i>Scutellaria baicalensis</i> , <i>Paeonia lactiflora</i> , <i>Pulsatilla chinensis</i> , Cortex Phellodendri, <i>Sophora flavescens</i> , <i>Areca Catechu</i> , <i>Punica granatum</i> , Cortex Fraxini, and <i>Astragalus membranaceus</i>	Porcine epidemic diarrhea	134
11.	Chinese medical compounded emulsion containing Fructus <i>Cnidii</i> , Radix <i>Scrophulariae</i> , Radix Glycyrrhizae, clove, areca, <i>Pulsatilla chinensis</i> , rhubarb, <i>Mahonia bealei</i> , loquat leaf, mint and eucalyptus leaf	Human demodicidosis	135
12.	Traditional Chinese medicine granules and premix containing <i>Pulsatilla chinensis</i> , Rhizoma Coptidis, Cortex Phellodendri, <i>Portulaca oleracea</i> , <i>Lysimachia christinae</i> , <i>Cirsium setosum</i> ,	Bacterial disease of livestock and	136

	Cortex Fraxini and <i>Ailanthus altissima</i>	poultry	
13.	Traditional Chinese medicine preparation containing <i>Pulsatilla chinensis</i> , <i>Dichroa febrifuga</i> , <i>Cinnamomum cassia</i> , <i>Polygonum multiflorum</i> , <i>Agrimonia pilosa</i> , Flos Lonicerae, and <i>Paeonia suffruticosa</i>	Chicken coccidiosis	137
14.	Medicinal composition containing extracts of overground part of <i>Pulsatilla koreana</i>	Analgesic	138
15.	Detoxifying Chinese medicine effervescent granules from <i>Scutellaria baicalensis</i> , <i>Polygonum cuspidatum</i> , <i>Pulsatilla chinensis</i> , <i>Sophora flavescens</i> , <i>Isatis tinctoria</i> , Rhizoma Dryopteris Crassirhizomae, <i>Isatis indigotica</i> , sodium hydrogen carbonate, fumaric acid	Effervescent granules	139
16.	Veterinary drug containing Sulfonamide, trimethoprim (TMP), polyvinyl pyrrolidone (PVP), sodium thiosulfate, Et lactate, -pyrrolidone, water for injection, <i>Angelica sinensis</i> , <i>Ligusticum chuanxiong</i> , processed <i>Polygonum multiflorum</i> , <i>Scrophularia ningpoensis</i> , <i>Sophora tonkinensis</i> root, <i>Cuscuta chinensis</i> seed, <i>Cnidium monnieri</i> fruit, actinolite, Herba Epimedii, Chinese liquor-processed <i>Paeonia lactiflora</i> (Radix Paeoniae Alba), Et acetate, lanoline, vaseline, <i>Pulsatilla chinensis</i> , <i>Melia toosendan</i> fruit, and <i>Sophora flavescens</i>	Livestock diseases	140
17.	Medicine composition containing <i>Codonopsis pilosula</i> , Cortex Phellodendri, <i>Atractylodes macrocephala</i> , <i>Poria cocos</i> peel, <i>Alisma orientalis</i> , <i>Lysimachia christinae</i> , <i>Pulsatilla chinensis</i> , <i>Zingiber officinale</i> , Folium Pyrrosiae, <i>Stephania tetrandra</i> , <i>Coix lacryma-jobi</i> , and Rhizoma Coptidis	Diarrhea of pig	141
18.	Feed additive containing sulfur, <i>Astragalus membranaceus</i> , Herba Taraxaci, <i>Portulaca oleracea</i> , Semen Plantaginis, gypsum, <i>Pulsatilla chinensis</i> , <i>Schisandra chinensis</i> , and Radix Glycyrrhizae	Feed additive for Rabbits	142
19.	Traditional Chinese medicine composition containing <i>Dichroa febrifuga</i> , <i>Bupleurum chinense</i> , <i>Pulsatilla chinensis</i> , <i>Coptis chinensis</i> , <i>Gentiana scabra</i> , and <i>Artemisia scoparia</i>	Coccidiosis and enteritis	143
20.	Traditional Chinese medicine preparation containing Puff-ball, Rhizoma Polygonati, Cortex Phellodendri, <i>Carthamus tinctorius</i> , <i>Curcuma longa</i> , akebia stem, Semen Persicae, <i>Pulsatilla chinensis</i> , <i>Morinda officinalis</i> , Cortex Phellodendri, <i>Arnebia euchroma</i> , <i>Polygonum cuspidatum</i> , <i>Hemerocallis fulva</i> , <i>Piper longum</i> , and <i>Brucea javanica</i>	External application for Scar elimination	144
21.	Traditional Chinese medicine bait containing <i>Isatis indigotica</i> , <i>Schisandra chinensis</i> , <i>Isatis indigotica</i> , <i>Galla chinensis</i> , <i>Houttuynia cordata</i> , <i>Pulsatilla chinensis</i> , <i>Andrographis paniculata</i> , <i>Hedyotis diffusa</i> , <i>Bletilla striata</i> , <i>Sanguisorba officinalis</i> , <i>Astragalus membranaceus</i> , <i>Angelica sinensis</i> , Cortex Phellodendri, <i>Sophora flavescens</i> , <i>Salvia miltiorrhiza</i> , Radix Puerariae, Pheretima, Radix Glycyrrhizae and Herba Inulae	Spring viremia of carp, septicemia and syndrome of liver and gall	145
22.	Traditional Chinese medicine containing <i>Coptis chinensis</i> , Cortex Phellodendri, Cortex Fraxini, Radix Glycyrrhizae, <i>Aucklandia lappa</i> , <i>Pulsatilla chinensis</i> , <i>Coix lacryma-jobi</i> ,	Damp-heat enteritis	146

	<i>Poria cocos</i> , Cortex Magnoliae Officinalis, <i>Codonopsis pilosula</i> , <i>Dioscorea opposita</i> , <i>Portulaca oleracea</i> , and cuttlefish bone		
23.	Preparation of health auxiliary food containing <i>Pulsatilla Radix</i> , Glycyrrhizae Radix, Atractylodis Rhizoma alba, and glutinous rice	Method of manufacturing Health auxiliary food	147
24.	Traditional Chinese medicine tablets from <i>Scutellaria barbata</i> , <i>Solanum lyratum</i> , <i>Paris polyphylla</i> , <i>Sophora tonkinensis</i> , natural indigo, bezoar, <i>Sophora flavescens</i> , <i>Pulsatilla chinensis</i> , <i>Dysosma versipellis</i> , <i>Panax ginseng</i> , <i>Cordyceps sinensis</i> , Ganoderma, batrycated silkworm, <i>Impatiens balsamina</i> , Rhizoma Arisaematis, <i>Stephania tetrandra</i> , Radix Clematidis, <i>Hibiscus mutabilis</i> and <i>Hemsleya szechuanensis</i>	Late malignant tumor	148
25.	Traditional Chinese medicine composition of <i>Melia toosendan</i> , <i>Poria cocos</i> , Cortex Phellodendri, <i>Lepidium apetalum</i> , <i>Angelica dahurica</i> , <i>Morus alba</i> , <i>Platycladus orientalis</i> , <i>Psoralea corylifolia</i> , <i>Polygonum multiflorum</i> , <i>Ligusticum chuanxiong</i> , <i>Rumex japonicus</i> , <i>Pulsatilla chinensis</i> , <i>Cnidium monnieri</i> , natural indigo, and <i>Euphorbia lunulata</i>	External application for treating tinea capitis	149
26.	Traditional Chinese medicine preparation containing <i>Astragalus membranaceus</i> , <i>Dioscorea opposita</i> , <i>Corydalis yanhusuo</i> , Cortex Magnoliae Officinalis, <i>Acanthopanax senticosus</i> , <i>Pulsatilla chinensis</i> , <i>Portulaca oleracea</i> , Cortex Phellodendri, Concha Ostreae, cuttlefish bone, Acacia catechu, <i>Bletilla striata</i> , and Radix Glycyrrhizae	Chronic nonspecific ulcerative colitis, chronic colitis, chronic proctitis, as well as celiodynia, abdominal distention and diarrhea caused by intestinal dysfunction	150
27.	Traditional Chinese medicine composition containing <i>Ajuga forrestii</i> , <i>Pulsatilla chinensis</i> , <i>Pteris multifida</i> , Rhizoma Coptidis, <i>Toxicodendron vernicifluum</i> , <i>Polygonum chinense</i> , <i>Portulaca oleracea</i> , Cortex Phellodendri, <i>Acalypha australis</i> , <i>Agrimonia pilodsa</i> , Cortex Fraxini, and <i>Rostellularia procumbens</i>	Dysentery	151
28.	Chinese medicinal decoction containing <i>Pulsatilla chinensis</i> , Cortex Fraxini, Rhizoma Coptidis, <i>Phellodendron amurense</i> , red halloysite, <i>Papaver somniferum</i> pericarp, <i>Syzygium jambos</i> , <i>Adina rubella</i> , <i>Lysimachia fortunei</i> , <i>Alternanthera sessilis</i> , <i>Fuligo Plantae</i> , <i>Celosia cristata</i> flower, and water	Dysentery	152
29.	Traditional Chinese medicine decoction containing Radix Puerariae, <i>Scutellaria baicalensis</i> , Rhizoma Coptidis, Flos	Acute diarrhea	153

	Lonicerae, <i>Polygonum aviculare</i> , <i>Agastache rugosus</i> , <i>Perilla frutescens</i> , <i>Pinellia ternata</i> , Cortex Magnoliae Officinalis, Cortex Fraxini, <i>Poria cocos</i> , and <i>Pulsatilla chinensis</i>		
30.	Preparation method for traditional Chinese medicine composition containing <i>Pulsatilla chinensis</i> , <i>Astragalus membranaceus</i> , <i>Atractylodes macrocephala</i> , <i>Isatis indigotica</i> , <i>Scutellaria baicalensis</i> , Radix Bupleuri and Radix et Rhizoma Rhei	Enhancing nonspecific immunity of animals	154-155
31.	Traditional Chinese medicine enema decoction containing <i>Bletilla striata</i> , <i>Pulsatilla chinensis</i> , <i>Sargentodoxa cuneata</i> , Cortex Phellodendri, <i>Sophora flavescens</i> , <i>Galla Chinensis</i> , <i>Sanguisorba officinalis</i> , <i>Chrysanthemum morifolium</i> , <i>Sanguis Draconis</i> , <i>Panax notoginseng</i> powder, Rhizoma Coptidis, and white alum	Enema decoction for Colitis	156
32.	Traditional Chinese medicine preparation containing <i>Polygonum perfoliatum</i> , <i>Cynanchum paniculatum</i> , <i>Citrus paradisi</i> , <i>Polygonum chinense</i> , <i>Solidago decurens</i> , <i>Artemisia argyi</i> , <i>Sophora flavescens</i> , Cortex Phellodendri, <i>Baeckea frutescens</i> , <i>Eucalyptus robusta</i> , <i>Senecio scandens</i> , <i>Stemona japonica</i> , Radix Gentianae, <i>Dictamnus dasycarpus</i> , <i>Saposhnikovia divaricata</i> , <i>Schizonepeta tenuifolia</i> , <i>Angelica pubescens</i> , <i>Scutellaria baicalensis</i> , <i>Pseudolarix kaempferi</i> , <i>Cnidium monnieri</i> , <i>Kochia scoparia</i> , <i>Mentha haplocalyx</i> , <i>Eclipta prostrata</i> , <i>Sargentodoxa cuneata</i> , <i>Lycopodium japonicum</i> , <i>Cinnamomum cassia</i> , <i>Cinnamomum cassia</i> , <i>Zingiber officinale</i> , <i>Alpinia officinarum</i> , <i>Pulsatilla chinensis</i> , <i>Portulaca oleracea</i> , Herba Artemisiae Scopariae, Herba Taraxaci, Flos Lonicerae, Cortex Fraxini, <i>Acorus tatarinowii</i> , Rhizoma <i>Atractylodis</i> , <i>Gardenia jasminoides</i> , and <i>Murraya paniculata</i>	Chronic cervicitis	157
33.	Chinese medicinal composition containing <i>Cordyceps sinensis</i> solid culture, culture of Ganoderma (<i>Ganoderma lucidum</i> and/or <i>Ganoderma sinense</i>), <i>Panax ginseng</i> (Radix Ginseng), <i>Cinnamomum cassia</i> twig, Radix Paeoniae Rubra (<i>Paeonia lactiflora</i> and/or <i>Paeonia veitchii</i>) or <i>Paeonia lactiflora</i> (Radix Paeoniae Alba), Radix Glycyrrhizae, Radix Bupleuri (<i>Bupleurum chinense</i> and/or <i>Bupleurum scorzonerifolium</i>), <i>Alpinia officinarum</i> rhizome, Rhizoma Pinelliae (processed with ginger juice), <i>Astragalus membranaceus</i> , <i>Saposhnikovia divaricata</i> , <i>Scutellaria baicalensis</i> , Cortex Phellodendri (<i>Phellodendron chinense</i> and/or <i>Phellodendron amurense</i>), Rhizoma Coptidis, <i>Artemisia annua</i> , Flos Lonicerae, <i>Forsythia suspensa</i> , gypsum, <i>Paris polyphylla</i> , <i>Magnolia officinalis</i> , <i>Citrus aurantium</i> (<i>Fructus Aurantii</i>), <i>Atractylodes macrocephala</i> , <i>Poria coco</i> , <i>Crataegus pinnatifida</i> , <i>Polygonum multiflorum</i> , Radix Gentianae, <i>Angelica sinensis</i> , Radix Puerariae (<i>Pueraria lobata</i> and/or <i>Pueraria thomsonii</i>), <i>Pulsatilla chinensis</i> , <i>Platycodon grandiflorum</i> , and <i>Bulbus Fritillariae Cirrhosae</i>	swine high fever	158
34.	Traditional Chinese medicine (TCM) composition <i>Xanthium sibiricum</i> , Flos Magnoliae, <i>Eucalyptus robusta</i> , <i>Solidago</i>	Accelerate the	159

	<i>decurens</i> , <i>Commelina communis</i> , <i>Celosia argentea</i> , Radix Gentianae, <i>Dictamnus dasycarpus</i> , Cortex Fraxini, <i>Paris polyphylla</i> , <i>Juncus effusus</i> , <i>Brucea javanica</i> , <i>Pulsatilla chinensis</i> , <i>Nelumbo nucifera</i> , Pollen Typhae, <i>Rubia cordifolia</i> , <i>Dalbergia odorifera</i> , carbonized human hair, <i>Sanguisorba officinalis</i> , <i>Bletilla striata</i> , <i>Ligusticum chuanxiong</i> , <i>Corydalis yanhusuo</i> , Radix Curcumae, <i>Curcuma longa</i> , frankincense, myrrh, <i>Salvia miltiorrhiza</i> , Semen Persicae, <i>Carthamus tinctorius</i> , <i>Panax notoginseng</i> , and ground beetles	microcirculation, reduce the peripheral vascular resistance and heart burden, as well as treat myocardial infarction	
35.	Oral liquid of TCM containing Rhizoma Coptidis, <i>Scutellaria baicalensis</i> , <i>Gardenia jasminoides</i> , <i>Andrographis paniculata</i> , <i>Pulsatilla chinensis</i> , and Radix Glycyrrhizae	Colibacillosis	160
36.	TCM enema manufactured from <i>Pulsatilla chinensis</i> , <i>Portulaca oleracea</i> , <i>Scutellaria baicalensis</i> , Rhizoma Coptidis, <i>Angelica sinensis</i> (carbonized), <i>Sanguisorba officinalis</i> (carbonized), Radix et Rhizoma Rhei (carbonized), <i>Cinnamomum cassia</i> , donkey-hide glue, <i>Paeonia lactiflora</i> , Radix Glycyrrhizae, and <i>Bletilla striata</i>	ulcerative colitis, bellyache and hemafecia	161
37.	TCM containing arsenic sulfide, <i>Agastache rugosa</i> , <i>Pulsatilla chinensis</i> , talc powder, Radix et Rhizoma Thalictri, Fructus Chebulae, <i>Portulaca oleracea</i> , Cortex Phellodendri, effervescent agent, and lubricant	Dysentery	162
38.	TCM containing <i>Saposhnikovia divaricata</i> , Saiga Tatarica (horn), <i>Acanthopanax gracilistylus</i> , <i>Xanthium sibiricum</i> , <i>Angelica pubescens</i> , <i>Asparagus cochinchinensis</i> , <i>Akebia quinata</i> , <i>Photinia serrulata</i> , <i>Pulsatilla chinensis</i> , <i>Rubia cordifolia</i> , <i>Gastrodia elata</i> , Semen Pharbitidis, Nodus Pini, <i>Aconitum carmichaeli</i> , <i>Ligusticum sinensis</i> , Folium Pyrrosiae, Radix Clematidis, <i>Smilax glabra</i> , <i>Bungarus multicinctus</i> , <i>Salvia miltiorrhiza</i> , myrrh, <i>Buthus martensii</i> , Radix Gentianae Macrophyllae, Rhizoma et Radix Notopterygii, <i>Drynaria fortunei</i> , <i>Ligusticum chuanxiong</i> , <i>Orobanchae coerulea</i> , <i>Trachelospermum jasminoides</i> , <i>Polygonum cuspidatum</i> , hairy antler, <i>Corydalis yanhusuo</i> , <i>Achyranthes bidentata</i> , <i>Lamium barbatum</i> , <i>Coix lacryma-jobi</i> , <i>Stephania tetrandra</i> , <i>Cnidium monnieri</i> , <i>Salix babylonica</i> , <i>Damnacanthus indicus</i> , <i>Gekko chinensis</i> , <i>Actinidia polygama</i> , <i>Rehmannia glutinosa</i> , Rhizoma Polygonati, and <i>Spatholobus suberectus</i>	Rheumatoid Disease	163
39.	TCM containing <i>Schizonepeta tenuifolia</i> , <i>Saposhnikovia divaricata</i> , <i>Forsythia suspensa</i> , Herba Taraxaci, Mentha haplocalyx, seed of <i>Arctium lappa</i> , <i>Cryptotympana pustulata</i> , <i>Glycine max</i> , Radix Puerariae, Radix Bupleuri, leaf of <i>Morus alba</i> , <i>Chrysanthemum morifolium</i> , crude gypsum, <i>Anemarrhena asphodeloides</i> , Radix Trichosanthis, <i>Lophatherum gracile</i> , <i>Scutellaria baicalensis</i> , <i>Dictamnus dasycarpus</i> , <i>Sophora flavescens</i> , Cortex Fraxini, <i>Rehmannia glutinosa</i> , <i>Scrophularia ningpoensis</i> , <i>Paeonia suffruticosa</i> , <i>Arnebia euchroma</i> , Flos Lonicerae, <i>Viola yedoensis</i> , natural indigo, <i>Houttuynia cordata</i> ,	Skin rashes in case of rubella, measles, eczema, scabies and local wind sore	164

	<i>Portulaca oleracea</i> , and <i>Pulsatilla chinensis</i>		
40.	Chinese medicine preparation containing <i>Angelica sinensis</i> , <i>Salvia miltiorrhiza</i> , <i>Drynaria fortunei</i> , <i>Pinellia ternata</i> , <i>Bletilla striata</i> , <i>Angelica dahurica</i> , Radix et Rhizoma Rhei, Rhizoma Coptidis, Herba Taraxaci, Flos Lonicerae, <i>Sanguis Draconis</i> , <i>Gardenia jasminoides</i> , <i>Ligusticum chuanxiong</i> , borneol, frankincense, myrrh, Caulis Akebiae, Semen Plantaginis, <i>Paeonia suffruticosa</i> , <i>Polygonum aviculare</i> , <i>Cnidium monnieri</i> , <i>Polygonum cuspidatum</i> , <i>Agrimonia pilosa</i> , <i>Pulsatilla chinensis</i> , <i>Viola yedoensis</i> , Herba Patriniae, <i>Panax ginseng</i> .	Recovery of cervicitis and cervical hypertrophy	165
41.	Essence contains linalool, -amyl cinnamaldehyde Schiff base, linalyl acetate, benzyl acetate, benzyl alc., <i>Michelia alba</i> ext., allyl hexanoate, <i>Cananga odorata</i> essence, -hexyl cinnamaldehyde, <i>Aglaia odorata</i> oil, phenylethanol, lylal, sage clary oil, p-tolyl acetate, benzyl salicylate, celestolide, musk, ketone musk, rockrose ext., coumarin, piperonal, Et vanillin, civet essence, <i>Citrus aurantium</i> leaf oil, -ionone, Me ionone, vetiver oil, <i>Cupressus funebris</i> oil, <i>Santalum album</i> , cinnamic alc., <i>Pulsatilla chinensis</i> oil, oak moss conc., and storax resin	<i>Michelia alba</i> -beaver essence for cosmetic purpose	166
42.	Injecta contains Chinese pulsatilla extn., cortex fraxini extn., radix scutellariae extn., phellodendron extn. and gentamicin sulfate and excipients.	Bacillary dysentery	167
43.	TCM contains <i>Sophora flavescens</i> and <i>Pulsatilla chinensis</i>	in vitro spermicidal and bactericidal functions	168
44.	natural plant preparation consists of <i>Astragalus membranaceus</i> extract, <i>Portulaca oleracea</i> extract, Herba Taraxaci extract, <i>Verbena officinalis</i> extract, <i>Crataegus pinnatifida</i> extract, <i>Pulsatilla chinensis</i> extract, pine extract, and a carrier	Diarrhea of animals	169
45.	TCM containing <i>Astragalus membranaceus</i> , Flos Lonicerae, <i>Forsythia suspensa</i> , <i>Pulsatilla chinensis</i> , Rhizoma Coptidis, Rhizoma Atractylodis, <i>Scutellaria baicalensis</i> , Poria cocos, and Radix Glycyrrhizae	Improvement of amunity of rabbits	170
46.	TCM composition containing <i>Pogostemon cablin</i> , <i>Polyporus umbellatus</i> , <i>Perilla frutescens</i> , Fructus Crataegi, <i>Raphanus sativus</i> , <i>Dioscorea opposita</i> , <i>Angelica dahurica</i> , <i>Platycodon grandiflorum</i> , Radix Bupleuri, Cortex Magnoliae Officinalis, Rhizoma Atractylodis, <i>Cinnamomum cassia</i> , <i>Pulsatilla chinensis</i> , <i>Portulaca oleracea</i> , <i>Ervatamia divaricata</i> , <i>Artemisia argyi</i> , <i>Foeniculum vulgare</i> , <i>Sophora flavescens</i> , Radix Puerariae, <i>Myristica fragrans</i> , <i>Cynomorium songaricum</i> , <i>Alpinia oxyphylla</i> , <i>Dryopteris crassirhizoma</i> , <i>Sanguisorba officinalis</i> , <i>Alisma orientalis</i> , <i>Citrus reticulata</i> , <i>Citrus aurantium</i> , and <i>Zingiber officinale</i>	Diarrhea of infants	171
47.	TCM composition manufactured from <i>Citrus reticulata</i> , Radix Curcumae, <i>Citrus aurantium</i> , <i>Raphanus sativus</i> , <i>Aristolochia debilis</i> , <i>Isatis indigotica</i> , <i>Cyperus rotundus</i> , <i>Melia toosendan</i> ,	Late hepatitis	172

	Lindera aggregata, medicated leaven, Areca catechu, <i>Scutellaria baicalensis</i> , Rhizoma Coptidis, <i>Gardenia jasminoides</i> , <i>Rehmannia glutinosa</i> , <i>Chrysanthemum indicum</i> , Herba Taraxaci, Radix Bupleuri, Houttuynia cordata, <i>Scutellaria barbata</i> , bezoar, <i>Arnebia euchroma</i> , Radix Puerariae, gypsum, <i>Anemarrhena asphodeloides</i> , natural indigo, Herba Patriniae, <i>Canarium album</i> , <i>Smilax glabra</i> , <i>Forsythia suspensa</i> , Flos Lonicerae, <i>Paris polyphylla</i> , and <i>Pulsatilla chinensis</i>		
48.	Traditional Chinese medicine decoction containing <i>Pulsatilla chinensis</i> , <i>Portulaca oleracea</i> , <i>Imperata cylindrica</i> , <i>Lysimachia christinae</i> , talcum, <i>Polygonum aviculare</i> , Herba Dianthi, <i>Rehmannia glutinosa</i> , <i>Gardenia jasminoides</i> , <i>Kochia scoparia</i> , and Semen Plantaginis	urinary tract infections	173
49.	Homeopathic composition containing <i>Allium cepa</i> , <i>Echineacea angustifolia</i> , <i>Euphrasia officinalis</i> (eyebright), <i>Pulsatilla nigricans</i> , <i>Pothos foetidus</i> , <i>Ignatia amara</i> (St Ignatius's bean), and <i>Thuja occidentalis</i> (tree of Life)	Alleviate symptoms of allergy	174
50.	Pelvorin composition containing Alumina, Borax, Sepia, Kreosolum and <i>Pulsatilla nigricans</i>	Wider spectrum of symptoms	175
51.	Synergistic medicinal composition containing Kreosolum (Bark), <i>Pulsatilla nigricans</i> , Sepia, Alumina, Borax and lactose	Vaginal discharge and associated problems	52

CONCLUSION

A close scrutiny of literature review on the genus "*Pulsatilla*" reveals that *P. nigricans* is widely known for its medicinal properties. The plant has a long history of use, in various traditional as well as alternative and complementary systems of therapeutics, in treatment of rheumatism, inflammation, and in various other ailments. *P. nigricans* has also been included in number of herbal formulations for the treatment of various ailments. Mother tincture (a homeopathic formulation) of *P. nigricans* roots is available in Indian market and frequently prescribed to the patients suffering from nervous disorders such as anxiety, depression and oxidative stress.

Despite a strong ethnopharmacological record of its medicinal use, no systematic work has been carried out on *P. nigricans* as is evident from the sporadic phytochemical and pharmacological reports on this potential plant.

Keeping in mind the traditional/alternative and complementary medicinal uses, sporadic phytochemical and pharmacological reports, *P. nigricans* seems to hold great potential for in depth phytochemical and pharmacological investigations.

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