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The Genus *Pulsatilla* : A Review

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ABSTRACT

The review includes 84 references on the genus *Pulsatilla*, and comprises ethnopharmacology, morphology, phytoconstituents, pharmacological reports, clinical study and adverse effects of the prominent species of *Pulsatilla*. Triterpenoid saponins and flavonoids constitute major classes of phytoconstituents of the genus. A few species of this genus have medicinal value, among these, *P. nigricans* Stoerck. (family Ranunculaceae) has been traditionally used in the treatment of nervous disorders, and as a remedy for ovaritis, ovaralgia and sexual debility. Despite a long tradition of use of some species, the genus has not been explored properly. In the concluding part, the future scope of *Pulsatilla* species, especially *P. nigricans*, has been emphasized with a view to isolate bioactive moieties which could be used for multifarious biological activities.

KEY WORDS: Pharmacology, *Pulsatilla*, *Pulsatilla nigricans*, Triterpenoid saponins

INTRODUCTION

This review emphasizes the traditional uses and clinical potential of *Pulsatilla* species. Additionally, it raises a question on traditional claims of *P. nigricans* which have not been proved scientifically. Through this review, authors hope to attract the attention of natural product researchers through out the world to focus on the unexplored potential of *Pulsatilla* species. This genus needs to be investigated systematically so that potential species can be exploited as therapeutic agents.This review has been compiled using references from major databases as Chemical Abstracts, Medicinal and Aromatic Plants Abstracts, Pubmed, King's American Dispensatory, Henriette's Herbal Homepage, Duke's Phytochemical and Ethnobotany. The available information on *Pulsatilla* has been divided into six sections, i.e., ethnopharmacology, morphology, phytoconstituents, pharmacological reports, clinical study and adverse effects. The ethnopharmacological section has been further subdivided into two sections, i.e., traditional uses, and alternative and complimentary uses. The reports in which *Pulsatilla* species have been used as a domestic remedy by common men without any prescription for the treatment of various ailments have been discussed under traditional uses. The subhead "Alternative and Complimentary medicinal uses" highlights *Pulsatilla* species as medicine prescribed by medical practitioners for the treatment of various ailments. It also mentions uses for which *Pulsatilla* species or their preparations available in the market. Under every section, *Pulsatilla* species have been arranged in alphabetical order.

The genus Pulsatilla

The genus *Pulsatilla* (Ranunculaceae, Buttercup family) comprises about 70 species (1), mainly as herbs (2). *Pulsatilla* (pasque flower) grows in Turkey, Russia, Germany, France, Demnark, Sweden, Southern England and Asia (3). The plants of the genus *Pulsatilla* are covered with soft, silky, white hairs, giving to them a lax, shaggy, wooly appearance. Leaves are generally not fully matured at the early flowering period.

Ethnopharmacology

Traditional uses

Bai Tou Weng, a traditional Chinese medicine containing *Pulsatilla species* such as *P. ambigua*, *P. chinensis*, *P. dahurica*, *P. koreana*, *P. turezaninovii*, has been used against bacteria, amoeba and vaginal trichomoniasis (4-7). *P. cernua* has been used traditionally in China as antitumor and antidiabetic (7). *P. cernua* roots have been used as a home remedy for astringent and diuretic properties (8). The plant has also been used as antiphlogistic and hemostatic (9). *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 hemorrhoids (9-11).

P. nigricans has been used in nervousness, sadness, mild restlessness and mental unrest (3). 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 prostatorrhoea, amaurosis, cataract and opacity of the cornea. *P. nigricans* has been used in uterine affections, dyspepsia, coryza, otitis, rhinitis, conjuctivitis, coughs, cutaneous affections, acute meningitis, and as taeniafuge (12). *P. nigricans* roots have been used for blood-cooling and detoxifying effects in traditional system of Chinese medicine (6). *P. patens* var. *multifida* roots have been used as an antibacterial, antiamoebic and antitumor in China (13).

Alternative and complimentary medicinal uses

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 ingredients (14). An effective and safe skin lightening cosmetic contains 0.001 to 20.0% w/w saponins extracted from *P. cernua* as one of the

ingredients (15). *P. chinensis* is one of the ingredients in the colon targeting capsule used for treatment of ulcerative colitis (16). A pharmaceutical preparation containing *P. chinensis* as one of the ingredients is used as oral cavity healthcare liquid (17). Ethanolic extract of *P. koreana* has been included in pharmaceutical preparations used for the treatment of diabetes (18, 19), and as antiplaque dentrifrices in concentration ranging from 0.005-5% (20, 21).

P. nigricans is given to produce sleep, when there is great exhaustion and opiates are inadmissible (3). *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 effect (22-24). 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 micturation (25, 26). Homeopathic medicines of *P. nigricans* have been used for the treatment of clinical cases of bovine-mastitis (27). *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 bull (28). Homoeopathic *P. nigricans* 200 CH decreased total sperm defects, increased sperm motility, and also increased impressive number of doses of semen production in a prize nelore bull (29). 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 concentration (30). *Pulsatilla* is one of the constituent of homeopathic remedies most frequently prescribed for ENT allergies (31). *Pulsatilla* as a homoeopathic medicine has been found to be effective in the treatment of acute otitis media in children (32, 33).

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 systems (34). 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.

Morphology

P. nemorosa Schrank (Synonym *Anemone nemorosa* Linn.), is about 4 inches high; root slender, horizontal root-stalk; stem simple, slender, erect, leafless, at top it bears a whorl of three-petiole; flowers solitary, small, peduncled, white or purple in colour (3).

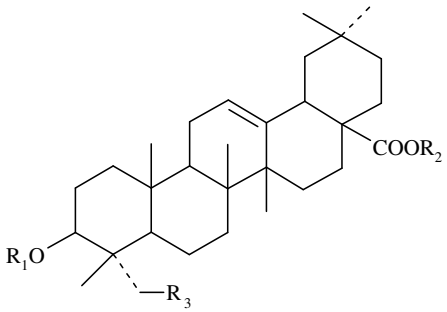
P.nigricans Stoerck (Synonym *P. pratensis* Mill.) (1) is a perennial plant; stem simple, erect, rounded, 3-5 inches high; leaves radical, pinnatifid, downy, the segments many-parted, with linear lobes; flowers solitary, terminal, pendulous, deep-purple or violet-brown, somewhat narrow, pointed, reflected 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 appearance (2, 3).

P. patens Mill. (Synonym *Anemone patens* Linn.), commonly known as American *Pulsatlla*, 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 (3).

P. vulgaris Mill. (Synonym *Anemone pulsatilla* Linn.) has involucre, hairy, scape curved and shaggy (3).

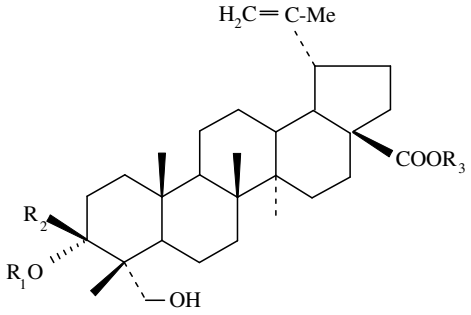
Phytoconstituents

The available literature on phytochemical reports of the genus *Pulsatilla* reveals that the *Pulsatilla* species comprise mainly triterpenoid saponins and flavonoids. Amongest various species, *P. chinensis* is rich in triterpenoid saponins. More than 20 triterpenoid saponins have been isolated from *P. chinensis*. Table 1 summarizes phytoconstituents reported from various species of *Pulsatilla*.

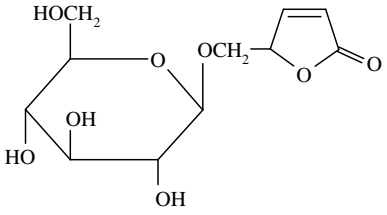


	R_1	R_2	R_3
1	ara(2→1)glc(4→1)glc	H	OH
2	ara(2→1)glc(4→1)glc	glc(6→1)glc(4→1)rha	OH
3	H	glc(6→1)glc(4→1)rha	OH

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



	R_1	R_2	R_3
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



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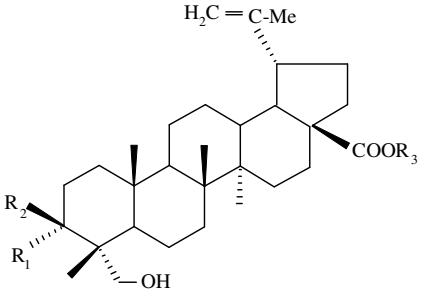
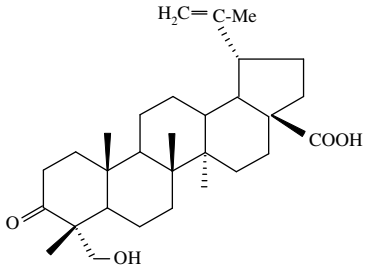


	R_1	R_2	R_3
24	H	O-α-L-arabinopyranosyl	H
25	H	OH	glu(1→6)glu
26	H	OH	H



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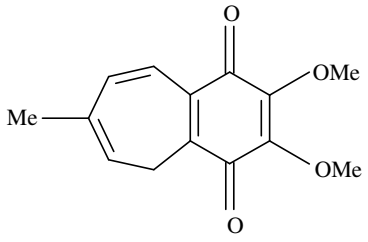

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ara – arabinopyranosyl
rha – rhamnopyranosyl
glc – galactopyranosyl
glu – glucopyranosyl
Me – Methyl

Structures of various phytoconstituents of Pulsatilla species

Table 1: Phytoconstituents of various species of Pulsatilla.

Species	Phytoconstituents
P. alpina	Lactones (35, 36) protoanemonin, anemonin.
P. campanella Fischer ex regel.	Triterpenoid saponins (4, 37) pulsatilosides A [1], B [2], C [3], D [4], leontosides A [5], B [6], D [7], caulosides D [8], F [9], calcoside D [10].
P. cerna Thumb.	Flavonoids (38) quercetin, kaempferol.
P. cernua Thumb.	Triterpene aglycones (39) hederagenin, oleanolic acid; triterpenoid saponins (8, 10, 40, 41) 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 (42); cinnamic acids (43) 4-hydroxy-3-methoxy cinnamic acid, 3, 4-dihydroxycinnamic acid; sterol β-sitosterol (39).

P. chinensis Bunge.	Triterpinoid aglycone anemosapogenin [19] (44); triterpenoid saponins anemoside A3 [20], B4 [21], pulchinenoside A, B [22], C (45-47), ranunculin [23] (48), chinensiosides A, B, hederasaponin C (49); lupane type triterpenoid saponins pulsatilloside A [24], B [25], C, D (50-52); bayogenin-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester (53); hederagenin saponins (5, 8, 53) such as hederagenin-3-O-{O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}; oleanolic acid saponins (5) such as oleanolic acid 3-O-{O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}; lupanoic acid saponins (54), 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] (55), pulsatillic acid [27] (56); flavonoids (38) quercetin, kaemferol; lignans (5) (+)-pinoresinol; β-peltatin; 2β, 3β, 14α, 20, 22R, 25-hexahydroxy-cholest-7-en-6-one (57).
P. dahurica Fischer.	Hederagenin (58), 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
P. koreana Nakai.	Triterpenoid saponins (10) pulsatilla saponin A [14], B, D [15], F [16], H [17]; hederagenin saponins (8); lupane saponins (59); cinnamic acids (60) 4-hydroxy-3-methoxy cinnamic acid, 3, 4-dihydroxycinnamic acid; resin deoxypodophyllotoxin (11); ketone pulsaquinone [28] (61).
P. montana (Hoppe) Riechenb.	Quercetin-3'-methyl ether (62)
P. nigricans Stoerck.	Glucoside pulsatoside A (63).
P. patens var. multifida Linn.	Triterpenoid saponin (6, 64) patensin; hederagenin saponins (6) such as 3-O-β-D-glucopyranosyl (1→2)-β-D-galactopyranosyl hederagenin 28-O-β-D-glucopyranosyl ester; oleanolic acid saponins (6) 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.
P. xkissii Linn.	Flavonoids (38) quercetin, kaempferol.

Pharmacological reports

Martin et al. (65) reported that hexane and chloroform extracts of the flowering aerial parts of *P. alpina* exhibit 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.) alone (35). Protoanemonin also exhibited antifungal activity against *Candida albicans* and *Aspergillus niger* with the MIC 15 μg/ml using *in vitro* agar dilution method (36). 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 disk method (43, 60). Cernuosides A and B, isolated from *P. cernua* roots, displayed moderate inhibitory activity against the intestinal sucrase of rats with IC_{50} values of 59.5 and 45.8 mM respectively, thereby, confirming its antidiabetic activity (7). 3, 4 dihydroxy cinnamic acid and 4 methoxy cinnamic acid isolated from *P. cernua* have been reported to possess antityrosinase activity (66).

Pulsatillic acid, isolated from chloroform soluble part of the methanolic extract of *P. chinensis* roots, exhibited cytotoxic activities against P-388 (IC_{50} 4.8 μg/ml), lewis lung carcinoma (IC_{50} 5.9 μg/ml) and human large cell lung carcinoma (IC_{50} 1.9 μg/ml) (56). Triterpene saponins and lignan (β-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 IC_{50} value of 5.1 μg/ml and 0.0052 μg/ml respectively (5). Anemosapogenin, isolated from *P. chinensis* roots, displayed antitumor activity against Hep-A liver carcinoma and Ehrlich ascites cancer in mice with transplantable tumors (67). Betulinic acid derivatives isolated from *P. chinensis* have been reported to exhibit cytotoxic (apoptotic) activity on murine melanoma B_{16} cells (68). A glycoprotein, isolated from the roots of *P. chinensis*, displayed immune-enhancing effect by enhancing immune function of macrophages (69). It has been reported that 2β, 3β, 14β, 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 mice (57). 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 μg/ml in primary cultures of rat intestinal microvascular endothelial cells, thus, inferring its anti-inflammatory activity (70). *P. chinensis* prevented hepatitis B virus infection by specifically increasing superoxide release in the liver and increasing superoxide dismutase activity to minimize superoxide-mediated toxicity (71).

Aqueous extract of *P. koreana* roots exhibited anti-inflammatory and analgesic activities in mice at a dose of 349 mg/kg (72). 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 (ED_{50} 6-18

ng/ml) with an inhibition ratio of 60% (11, 73). 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 colon cancer (74). 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 carcinoma (75). *In vivo* and *in vitro* activity-guided fractionation of root extract of *P. koreana* led to isolation of an oleanic glycoside, hederacolchiside E (76). 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 rats (77). Pulsaquinone, isolated from the methanol extract of *P. koreana* roots, has been reported to exhibit potent antimicrobial activity (61). The plant exhibited *in vitro* antiprotozoal activity against *Toxoplasma gondii* and *Neospora caninum* at higher doses (78).

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 IC_{50} value of 21.4 µg/ml (13). *P. pratensis* exhibited anti-inflammatory activity by abolishing hydroxyl radical generated in a Fenton type reaction system and inhibiting paw swelling (79). Euphorbium compositum, a homoeopathic combination preparation containing *P. pratensis* exhibited antiviral activity against respiratory syncytial virus, human rhinovirus, influenza A virus and herpes simplex virus (80). Aqueous extract of *Pulsatilla* exhibited spasmolytic activity on isolated tissues of rabbit jejunum (81).

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 µg/ml determined by MTT, LDH release analysis, and flow cytometry measurement (82).

Clinical study

In a case report, homoeopathic therapy with Pulsatilla C200 cured a 44-years old patient with spontaneous bacterial peritonitis caused by *E. coli* (83).

Adverse effects

The anemones are listed as poisonous in many of the world publications on poisonous plants, but without clear-cut substantiation (84). 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 (3, 34). 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 accompained 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.

CONCLUSION

About 70 species of the genus *Pulsatilla* have been reported in various floras. An exhaustive survey of literature revealed that sporadic information is available only on 15 species. Among these 15 species, most of ethnopharmacological reports are available on *P. nigricans*. Further, only 11 species of *Pulsatilla* (Table 1) have been partially investigated for their phytoconstituents.

A close scrutiny of literature on *Pulsatilla* reveals that 5 species have been investigated pharmacologically. Among these, *P. chinensis* and *P. koreana* have been exhaustively explored for their antitumour activity. Pharmacological studies infer that *P. alpina* has sedative, hypothermic, antipyretic and antifungal properties due to presence of anemonin and protoanemonin; *P. cernua* exhibits antibacterial and antidiabetic activities due to cinnamic acid derivatives and cernuosides A, B respectively; *P. patens* possesses antitumour activity due to saponins; *P. chinensis* possesses antitumour and anti-inflammatory activities due to pulsatillic acid and anemone respectively; *P. koreana* possesses antitumour activity due to presence of various constituents such as oleanolic glycosides, hederagenin glycosides, Pulsatilla D, podophyllotoxin and hederacolchiside E.

Despite a long tradition of use of *P. nigricans* for treatment of various ailments, no pharmacological work has ever been carried out to prove its traditional claims. Additionally, the plant has been included in number of herbal and homoeopathic formulations, which are in clinical use for the treatment of various ailments. Mother tinctures of the plant are available in Indian market, and is frequently used for the treatment of CNS disorders.

Keeping in view the traditional, alternative and complimentary medicinal uses, sporadic phytochemical and pharmacological reports, low toxicity, and frequency of use in homoeopathic formulations, *P. nigricans* seems to hold great potential for in depth investigation for various biological activities, especially its effect on the reproductive and central nervous systems. The authors are involved in bioactivity-directed-fractionation of this plant with a view to isolate bioactive fraction / constituent(s).

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