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Review on pharmaceutical properties and conservation measures of *Potentilla fulgens* Wall. ex Hook. - A medicinal endangered herb of higher Himalaya

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Plenteous ethnotherapeutic properties and pharmacological actions have been attributed to *Potentilla fulgens* Wall. ex Hook. (Family- Rosaceae). It is one of the highly valued indigenous medicinal herbs of higher Himalaya. Biomedical reports have indicated presence of medicinally important chemical constituents represented by polyphenols, tannins, flavonoids and triterpenoids in the genus. Pharmacological studies report that *P. fulgens* possesses anti-hyperglycemic, hypoglycemic, anti-hyperlipidemic, antitumor, antioxidant, antiinflammatory and antiulcerogenic properties thus supporting its ethnotherapeutic use. In view of immense medicinal importance of the plant, this review aims to coherently discuss the results obtained from several studies on its chemical constituents, pharmacological use, cultivation and conservation strategies.

Keywords: *Potentilla fulgens*, Alpine, Antioxidant, Antitumor, Hypoglycemic, Polyphenols, Conservation.

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Introduction

Potentilla fulgens Wall. ex Hook. (Rosaceae) is an important medicinal plant of higher Himalaya known for its therapeutic and commercial importance. More than three hundred species of genus *Potentilla* Linn. are used in Ayurvedic, Unani, Siddha, Chinese and Tibetan systems of medicine[1-4] due to high content of polyphenols in their aerial and underground parts. Currently, medicinal plants rich in polyphenols are gaining significance in maintaining good health[5] due to their antioxidant and radical scavenging properties[6]. These polyphenols form stable complexes with metal ions, proteins and polysaccharides and help healing of wounds, burns and inflammations, hinder gut secretions and protect underlying mucosa from toxins and irritants, control dental caries and ameliorate degenerative diseases[7]. Biomedical reports have indicated presence of medicinally important chemical constituents represented by polyphenols, tannins, flavonoids and triterpenoids in the genus. Pharmacological studies report that *P. fulgens* possesses anti-hyperglycemic, hypoglycemic, anti-hyperlipidemic, antitumor, antioxidant, antinflammatory and antiulcerogenic properties thus supporting its ethnotherapeutic use. In view of immense medicinal importance of the plant, the present review is aimed at compiling currently available information on its chemical constituents, pharmacological use, cultivation and conservation strategies.

P. fulgens is commonly called Himalayan Cinquefoil in English, Bajradanti in Hindi[8], Bajradanti", Ganephul and Dentamanjari in Nepali[8,9], Bhuitara in Bengali, Akanada and Dentamanjari in Uttarakhand, San ge Zil pa in Tibetan[8,9] and Lyngniangbru in Meghalaya[8]. In India the species grows in Jammu and Kashmir, Himachal Pradesh, Uttarakhand, West Bengal, Sikkim, Assam, Meghalaya, Arunachal Pradesh, Manipur and Nagaland in temperate and higher Himalaya up to an altitude of 1800-4350 m asl and in Nepal, Bhutan and Tibet. The species finds habitat in open meadows and grassy slopes of oak and *Rhododendron campanulatum* D. Don forests, in temperate and alpine Himalaya. The plant is an erect perennial herb, 15-75 cm high, with a thick rootstock, pinnate leaves and yellow flowers[8]. It possesses both radical and cauline leaves. Radical leaves are 4-30 cm long, possessing 5-13 pairs of leaflets which are alternately large and small and diminish in size from uppermost downwards; terminal leaflet is oblong or broadly obovate, 1.5-4 × 0.8-1.5 cm in size, with closely and sharply toothed margins and silky tomentose abaxial surface. Cauline leaves are also abaxially white and sericeous; leaf blade resembles that of radical leaves

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but has less pairs of leaflets. Flowers 1-2 cm in diam. are crowded in terminal corymbs. Floral pedicel is 2-4 cm long and bears gland-tipped, multicellular and unicellular hair. Sepals have entire margins, epicalyx segments are either entire or with 3-6 teeth, outer surface of calyx lobes is silvery and silky. Petals are yellow, obovate with rounded apex. Styles are sub-basal and achenes glabrous. In western Himalaya the plant growth initiates in May, followed by flowering in June-July, fruiting in July-August and senescence in September[10]. The plant reproduces both by seed and underground parts. In natural habitat seedlings are uncommon and vegetative reproduction predominates by underground parts[11]. In wild habitats of Meghalaya the plant is reported to have a symbiotic association with an endophytic fungus *Penicillium verruculosum* which may be contributing to the vigorous growth of the plant in extreme rainfall habitats. The fungal endophyte is being explored for early establishment of its seedlings and successful micropropagation[12]. *P. fulgens* also bears nodules on its root surface and thus it is a non-leguminous nitrogen fixing plant[13]. The whole plant is valued for its ethno-medicinal properties and is receiving much attention for its domestication and commercialization. The species is listed under endangered category due to over harvesting from natural habitats[14].

Therapeutic and commercial importance

P. fulgens root-stock and whole herb is utilized as astringent and tonic for curing gum and tooth ailments (pyorrhea, toothache and caries), diarrhoea, stomach problems, cough, cold, diabetes mellitus and cancer (Table 1). In medieval ages *Potentilla* extracts (water, milk, honey and alcoholic) were used for curing toothache, throat inflammations, wound-healing, jaundice, mouth ulcers, dysentery and as a homeostatic. In Nepal and Bhutan plant juice is taken for treatment of stomach problems, cough, cold and respiratory complaints[9, 15-23].

Root powder is an effective anthelmintic and is used for toothache and stomach disorders. Root juice is taken for treatment of peptic ulcer and disusia[9] and root paste is used for controlling tooth infections[24]. Leaves, when masticated are beneficial for pyorrhea[9, 25]. Twigs and leaves are used as tooth brush by Bhutias in Uttarakhand, India. In trans Himalayan region (Nubra valley, Ladakh) leaf paste is used for curing stomach pain, cough, cold, throat sore and ulcer[26]. In Garhwal Himalaya roots are used for treatment of wounds and tiger bites (Table 1). In Uttarakhand region whole plant is used for stomatitis and aphthae[27]. The species is utilized commercially by Vicco Laboratories in India for the manufacture of Vicco Vajradanti tooth powder and paste[8, 23, 28-29].

Phytochemistry

Phytochenmical investigation on its allied species, viz. *P. erecta* (Linn.) Rausch , *P. fruticosa* Linn., *P. discolor* Bunge, *P. multicaulis* Bunge, *P. chinensis* Ser., *P. multifida* Willd. ex Ledeb., etc. has led to the isolation of sterols, triterpenoids, hydrolysable tannins, proanthocyanidins and flavonoids[4,30]. Sixty eight constituents have been reported from *P. erecta* and thirty seven from *P. anserina* Linn.[31]. Novel triterpenoids have been isolated from *P. discolor*,

Table 1—Some ethnomedicinal uses of *P. fulgens*

Himalayan Region	Part(s) used	Ethnomedicinal uses	Reference(s)
Assam	Whole herb	Gum and tooth ailments (pyorrhea, toothache and caries), diarrhoea, stomach problems, cough and cold, diabetes mellitus, cancer	17, 18, 19
Nepal & Bhutan	Plant juice	Stomach problems, cough and cold	9, 18, 19, 21, 22, 23
	Root powder	Toothache, stomach disorders, anthelmintic	
	Fresh root	Cough and cold, diarrhoea, diabetes mellitus cancer and for strengthening gums. Tooth infections.	
	Root juice		
	Root paste	Peptic ulcer and disusia masticated for pyorrhoea	
	Leaves, whole plant	Respiratory complaints	
Uttarakhand	Twigs & leaves	Used as tooth brush	22, 27, 58, 59
	Whole plant,	Stomatitis and aphthae	
	Roots	Wounds and tiger bites mouth ulcer	
Ladakh	Leaf paste	Stomach pain, cough, cold, sore throat and ulcer	26
Sikkim	Plant juice	Stomach trouble, cough & cold,	28, 29
	Root powder	toothache, pyorrhea & gingivitis	

P. multicaulis and *P. freyniana* Bornm.[32-34]. A triterpenoid saponin (2α, 3β, 19α-trihydroxyurs-12-en-28-oic acid *β*-d-glucopyranosyl ester) from *P. anserina* has been reported to inhibit duck hepatitis B virus (DHBV) DNA replication[4]. Most of the triterpenes in this genus have urs-12-ene skeleton and hydroxyl substitutions at C-2, C-3 and C-19, α-hydroxyl group is usually attached at C-19. Among the flavonoids in this genus, flavonol is the main skeleton with mostly hydroxyl and methoxyl group substitutions[2]. Four flavones and four flavonols were identifed first time from *P. multifida*[3]. Ellagic acid derivatives have been isolated from *P. chinensis*[35,36] and *P. candicans* Fisch. ex Lehm.[35,37]. Such compounds have not been reported from other genera of *Rosaceae* and therefore, can be useful taxonomic markers for this genus. Hydrolysable tannins and proanthocyanidins are important constituents of genus *Potentilla* responsible for astringent effects[31]. Tannin content (total, condensed and hydrolysable) of *P. fulgens* roots from wild and cultivated source (1200 amsl, at Palampur, Himachal Pradesh, India) was estimated during dormancy, rosette, bolting and at flowering stages. It was observed as 13 and 14% in wild and cultivated plants, respectively during dormancy[38]. Phytochemical investigation of the root parts of *P. fulgens* led to the isolation of a novel bioflavonoid potifulgene (Epiafzelchin-6-o-8"epiafzelchin) along with epicatechin[39]. Investigation of its aerial parts yielded two new triterpenes, Potentene-A (30-methyl-17α-hopan-12-ene-3-one) and Potentene-B (3-O-β-D-gluconopyranosyl-(1,2)-β-D-glucuronopyranosyl hopan-12-eno-11-oxo-28oic acid)[40] along with three known compounds, afzelchin-4 α →8" catechin, epiafzelchin and rutin. Structures of these compounds were evaluated by extensive spectroscopic techniques and chemical evidences and they are given in Figures 1, 2 and 3.

Pharmacological activities

Modern pharmacological studies have confirmed traditional uses of *Potentilla* species and their extracts from aerial and underground parts. Therapeutic applications of *Potentilla* species are due to high amounts of condensed and hydrolysable tannins in their aerial and underground parts[31]. Some of the research findings are summarized below.

Anti-neoplastic activity

Methanolic root extract *of P. fulgens* was found active against certain tumors in a dose-dependent manner[19] showing high antitumor activity on Dalton's

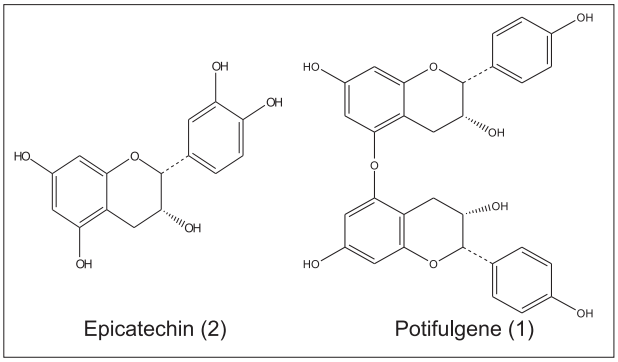

Fig. 1—Chemical structures of compounds isolated from *Potentilla fulgens* roots

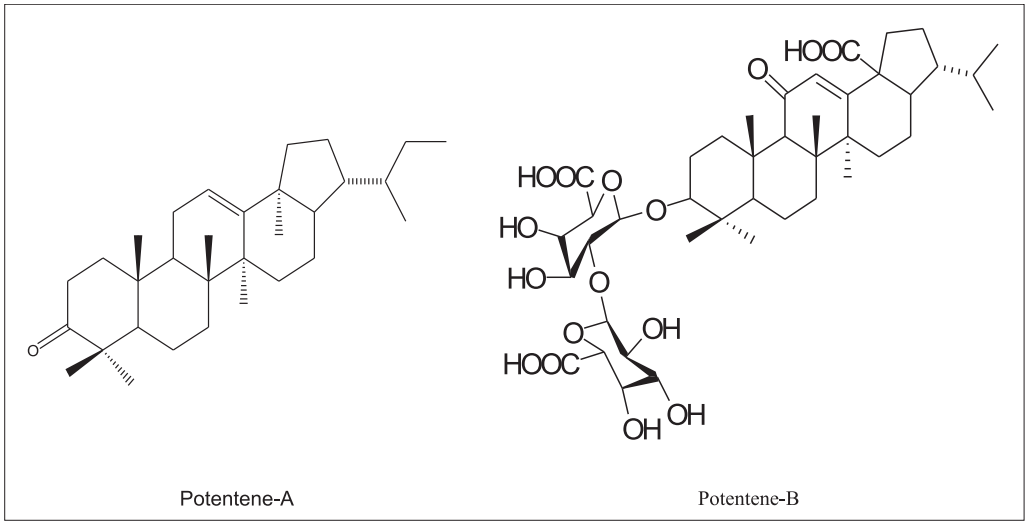

Fig. 2—Structures of new triterpenes isolated from *Potentilla fulgens*

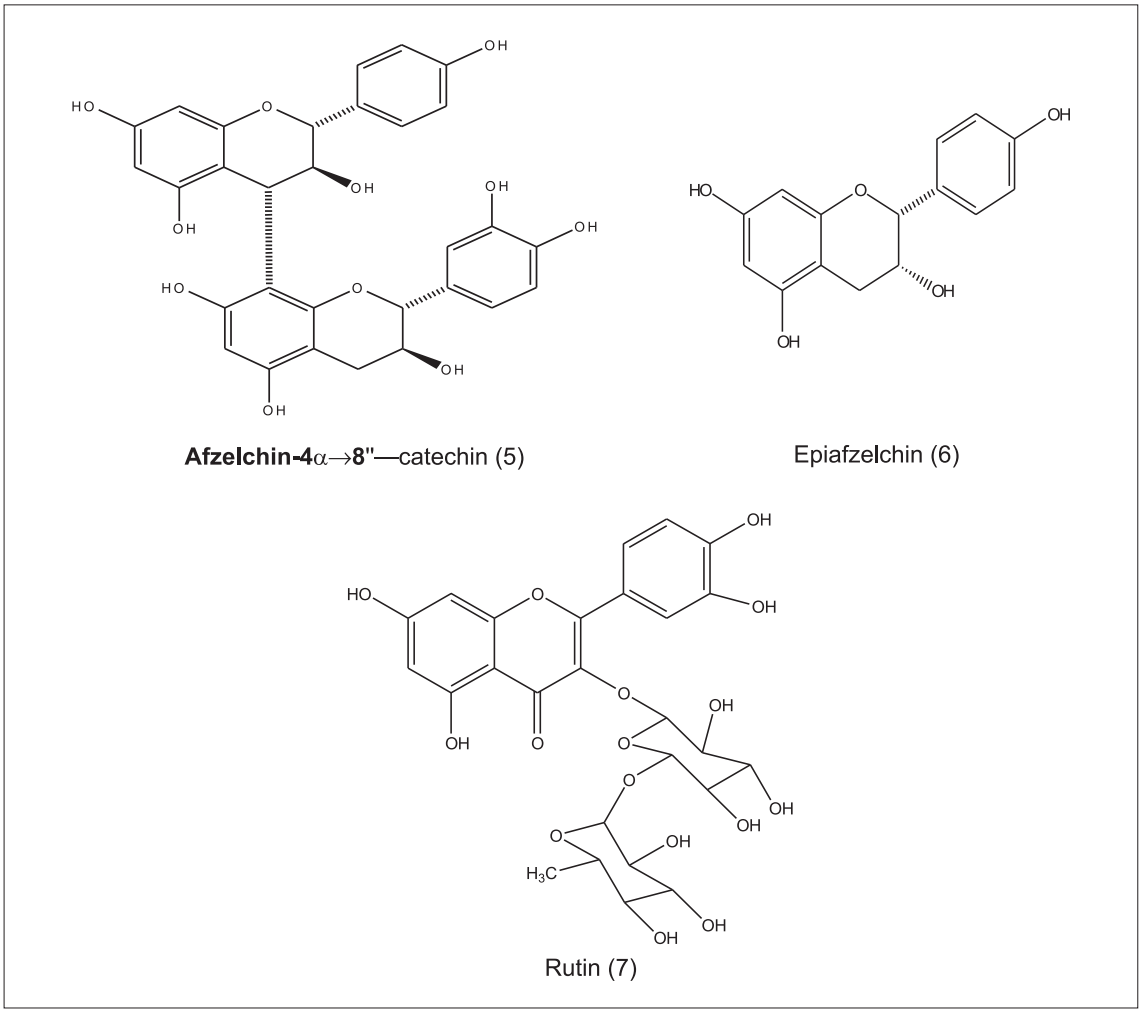

Fig. 3—Structures of isolated compounds from *Potentilla fulgens* aerial parts

lymphoma (DL) cells (1×10^6 approximately) when transplanted intraperitoneally into Swiss-albino mice. The treated/control value was 154% (250 mg/kg) when mice were treated during 1st, 3rd, 5th and 7th day after transplantation. The whole herb and underground parts of the plant are also reported to be active against neoplastic tumours murine ascites DL[41].

Hypoglycemic/antihyperglycemic activity

Methanol extract of *P. fulgens* roots was evaluated for hypoglycemic and antihyperglycemic activities in normal and alloxan-induced diabetic mice. Normal and diabetic mice were administered the extract intraperitoneally (i.p.) at varying doses (150-450 mg/kg b.w.) and blood glucose levels were measured at different time intervals up to a period of 5 days. Blood glucose level was reduced by 31% in normal and 63% in alloxan-induced mice, following administration of effective dose. A prolonged anti-hyperglycemic activity was observed in the diabetic mice and glucose level was found 79% low in comparison to control even on third day. The results were compared with that of insulin, glibenclamide and metformin, clearly indicating significant hypoglycemic and antihyperglycemic activities. Toxicity studies carried out on mice up to a dose of 450 mg/kg b.w. did not show any adverse effects during 4 weeks of observation[18].

Anti-hyperlipidemic activity

The effect of *P. fulgens* root extract on lipid profiles was evaluated in alloxan–induced diabetic mice[42]. Methanol extract (250mg/kg b.w.) was administered to diabetic mice on alternate days for a period of one week. On 8th day blood samples were collected for the estimation of cholesterol, triglyceride and HDL cholesterol levels. Glycolytic enzymes – glycokinase and hexokinase were also assayed. The

activities were compared against standard drugs–metformin, glibenclamide and insulin. *P. fulgens* treatment was found successful in reducing the serum cholesterol (72%) triglyceride levels (81%) and improved HDL cholesterol to normal level using interperitonial mode. Methanol extract selectively enhanced hepatic hexokinase activity. Liver and skeletal glucokinase (GK) and hexokinase (HK) activities were reduced in alloxan induced diabetic mice compared to normal mice as alloxan is reported to inhibit GK and HK activity. Diabetic mice treated with the extract resulted in selective response in GK and HK activities in liver, HK activity got moderately activated. Skeletal GK activity was not altered[42].

Methanol extract of *P. fulgens* is reported to inhibit sorbital dehydrogenase, the second enzyme in the polyol pathway, responsible for conversion of sorbitol to fructose[43]. Polyol pathway is one of the intracellular events that occur in the presence of high glucose ambience and results in cellular abnormalities due to altered NADH/NAD^+ ratio. *P. fulgens* methanolic extract caused no mortality up to 350mg/kg b.w. At 350 mg/kg inhibition of SDH (Succinate dehydrogenase) activity was 37% (i.p.) and 33% (oral), respectively. The kidney SDH was reduced to 61% (i.p.) and 51% (oral), respectively. The eye SDH was reduced to 40% (i.p.) and 33% (oral), respectively from that of control.

Antioxidant activity

A number of antioxidant constituents from aerial parts and roots of *Potentilla* species have been reported[18, 44-49]. These constituents have wide nutritional and medicinal applications, besides having protective effects on human health against cardiovascular, neoplastic and blood clotting diseases. For antioxidant activity aqueous methanol extract of roots and its ethyl acetate, butanol and water fractions and isolated constituents were evaluated by DPPH assay[40]. The antioxidant activity was compared with known standards - quercetin, vitamin C and pyrogallol. Butanol fraction exhibited good scavenging response measured by TEAC (mM Trolox equivalent/mg extract). A significant correlation was observed between total polyphenols and antioxidant activity, indicating antioxidant activities of extract and fractions due to presence of polyphenols. The antioxidant activity of new bioflavonoid potifulgene was found higher, i.e. 6.85±0.38, 4.24±0.41, 5.35±0.53 than that of epicatechin, 2.13±0.05, 1.50±0.02, 1.57±0.03 in DPPH assays. Phytochemical investigation of the aerial parts of *P. fulgens* led to the isolation of two new triterpenes, potentene-A(3) and potentene-B(4). In addition, three known compounds afzelchin–4α→8′′–catechin (5), epiafzelchin (6) and rutin (7) were also isolated. The structures of all these compounds were elucidated by extensive spectroscopic and chemical evidences. Compounds 5, 6, and 7 exhibited significant 1, 1, diphenyl-2-picrylhydrazyl radical scavenging activity, with IC_{50} values of 1.21, 2.88 and 5.20 mg/mL, respectively; the known standard antioxidant, vitamin C, had a value of 0.44 mg/ml, Fractions and the isolated compounds exhibited significant antioxidant activities with IC_{50} values 4.90, 1.61, 1.21, 2.5 and 5.20 mg/ml, respectively in comparison to known standard L ascorbic acid. IC_{50} of the fractions and the standards decreased in the order rutin>ethyl acetatefraction>epiafzalchin>butanol fraction>afzelchin 4→8'>L-ascorbic acid. Maximum antioxidant activity was observed in afzelchin- 4'→8" catechin.

Cultivation and conservation strategies

P. fulgens was cultivated first time outside its natural habitat at Palampur (32º 06' 20N" latitude, 76º 33' 29" longitude, 1200 m asl), Himachal Pradesh, India[38]. Efficient sexual (seed) and asexual (rootstock segments) propagation methods were standardized for its cultivation, domestication and mass multiplication. Seed germination was studied both under laboratory and field conditions. In laboratory germination was carried out under continuous illumination, 18 h light/6h dark cycles and complete darkness at 25°C. Maximum seed germination was recorded under continuous illumination (52%) followed by 18h light/6h dark cycles (36%) and least germination was noticed in complete darkness (11%). Under field conditions seeds were sown at 0.5 cm. depth in a mixture of soil: sand: FYM @ 1:1:1 in raised nursery beds during October. Germination was observed in February 3 months after sowing and seedlings were ready for transplantation in May (Plate 1). Before transplantation soil was prepared by mixing 20 t/ha FYM. Seedlings were planted in ridges at a plant to plant spacing of 45 × 45 cm and row to row spacing of 60 × 60 cm, watered fortnightly; 2-3 hand weeding and hoeing was applied to manage the crop. Seed raised plants remained at rosette stage during first growing season and flowered in second growing season (August to October). Seed setting was observed from October to November. Asexual

Plate 1—Cultivation of *Potentilla fulgens* using seed and rootstock segments as propagating material under ex-situ conditions; a. Seed from wild source; b. Seedlings ready for transplantation; c. Plant at flowering stage; d. Root of two year old seed raised plant; e. Four month old seedling; f. Sprouted and rooted root–segment 30 days after treatment; g. Nursery raised from root-segments planted in rows; h. Plants raised from sub apical and basal segments; i. Plants propagated from apical root –segments.

propagation protocols were standardized for its mass multiplication[38]. Rootstock segments 2-2.5 cm long and 1.0-1.5 cm thick were treated with IBA (100 mg/l) for 30 seconds and sown in nursery beds at a depth of 0.5 cm. Leaf sprouts were observed 20 days after sowing and complete plantlets were ready for transplantation after two months. Basal, middle, sub apical and apical rootstock segments resulted in 50, 60, 75 and 95% plant establishment, respectively. Plants raised by this method were more healthy and sturdier than seed raised plants (Plate 1). Most of the plants raised from apical rootstock segments flowered during first growing season. Propagation through rootstock segments was suitable for mass multiplication and establishment of nurseries.

In-vitro Propagation

In vitro regeneration protocols have been standardized for *P. fulgens* through axillary shoot proliferation[50] and adventitious shoot bud proliferation from leaf explants[14]. Axillary shoot proliferation through shoot tip culture has been achieved on Murashige and Skoog (MS) medium containing 1mg/l 6-benzylaminopurine (BAP) and 1mg/l indole-3-acetic acid (IAA). Continuous production of plantlets with better rate of shoot multiplication and elongation was obtained on MS medium supplemented with 1mg/ l kinetin (Kin) alone or combined with 1mg/l α-napthalene acetic acid (NAA). Established plantlets were successfully transferred to soil in a green house. The procedure ensures 12-fold plantlet production every 6 weeks.

Adventitious bud differentiation and shoot regeneration from leaf explants of *P. fulgens* was observed on modified Murashige and Skoog's (MMS) agar medium supplemented with growth regulators BAP (6-benzylaminopurine) and NAA (α-naphthalene acetic acid). The most effective treatment was MMS with 0.1 mg/l BAP and 0.1 mg/l NAA, which gave 80% bud induction frequency with 38.4 BFC (Bud Forming Capacity) index and 48 shoots per explants of 3.5 cm length. Rooting was induced on MS basal medium. The regenerated plants had 70% survival rate.

Present status of conservation and cultivation strategies for *P. fulgens*

Commercial demand for herbal drugs and dependence on wild source has led to depletion of medicinal plants from their natural habitat. Over harvesting from natural habitat for medicinal and commercial use has posed major threat to *P. fulgens* and is placed in endangered category. In Meghalaya alone 9900 kg raw drug (medicinal plant parts) of *P. fulgens* is consumed annually[51-55]. Domestication and cultivation of medicinal plants is one of the viable options to meet the growing demands from industries and reduce the extraction pressure from natural habitats. Cultivation of medicinal plants can improve the economy of people residing in high elevation zones of the Himalaya and can be economically more profitable to the traditional crops being grown in such environments[56]. At present cultivation of medicinal plants have various limitations owing to availability of material at low prices from the wild with only collectors' labour as input, lack of appropriate agro-technology, shortage of desired planting materials, long gestation periods and lack of assured marketing opportunity in remote areas. Since majority of world's population still depend on medicinal plants as the exclusive source of drugs[57], appropriate policies must be framed out for economical and ecologically sustainable medicinal plant cultivation. *P. fulgens* is used as traditional medicine since ancient times. Propagation and multiplication protocols reviewed above can thus be utilized for conservation, domestication and extension of *P. fulgens* both under *in-situ* and *ex-situ* conditions. This will reduce dependence on its natural habitat and ensure sustainable supply of quality and characterized raw material for traditional, commercial utilization and will result in upliftment of local people by additional income generation[56].

Conclusion

P. fulgens, a potential medicinal plant of higher Himalaya has become endangered in its natural habitat. Therefore, efforts are required for its *in-situ* and *ex-situ* conservation, cultivation leading to sustainable supply of raw material to pharmaceutical industry. Modern pharmacological studies have confirmed traditional use of various *Potentilla* species and extracts from their aerial and underground parts. However, studies on individual phytoconstituents, *in vitro* and *in vivo* pharmacological profiles and clinical trials on *P. fulgens* needs to be further investigated and compared with other traditionally important *Potentilla* species. Recently *Potentilla erecta* Linn. rhizome extracts have been tested in clinical trials for the treatment of *Rotavirus*-induced diarrhoea and colitis ulcerosa for which only a limited number of medications exist. Therefore, it remains a challenge for scientists to provide efficient, safe and

cheap medications especially for rural masses. It is also needed to frame appropriate policies that must integrate the cultivation of medicinal plants with socio-economic development of local people.

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