Original article

Quality control standardization of the roots of *Potentilla fulgens* Wall.: A potent medicinal plant of the Western Himalayas and North-eastern India

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Background & aims: *Potentilla fulgens* Wall. ex Hook. (PF) (Family: Rosaceae) is a shrub growing at high altitudes (1800–4350 m ASL) of the Western Himalayas. Traditionally, the roots of the plant are used to treat diabetes, peptic ulcers, diarrhoea, anthelmintic and cancer. The objective of the present study is to scientifically develop a standard monograph for PF on the basis of the pharmacognostical and phytochemical aspects.

Methods: The quality control standardization was performed as per the standard methods provided in World Health Organization for standardization of medicinal plants and Indian Herbal Pharmacopoeia.

Results: Morphologically, the roots are cylindrical, elongated and tapering towards the end, dark brown and astringent to bitter in taste. Histologically, the matured root showed the formation of secondary growth with wood formation. Physicochemical standards quantified are foreign organic matter (1.50%), loss on drying (14.43%), total ash (8.60%), acid insoluble ash (3.02%), water soluble ash (1.37%), alcohol soluble extractive (30.80%), water soluble extractive (18.40%), foaming index (142.85), swelling index (5.5), haemolytic index (89.47) and pesticide residue content was also estimated. The powdered drug characterization showed the presence of lignified xylem vessels with annular and reticulate thickenings, bordered pit tracheids, simple, druse type calcium oxalate crystals, compound starch grains and slender shaped fibres. Phytochemical screening showed the presence of polyphenolics (phenolics, tannins, flavonoids), steroids, saponins, sugars, and amino acids. Quantification of phytoconstituents in the extract was done spectrophotometrically which includes total phenolics (177.4 mg/g tannic acid equivalent, TAE), tannins (115.3 mg/g TAE), flavonoids (21.4 mg/g rutin equivalent, RE), flavonols (5.7 mg/g RE), saponins (41.2 mg/g diosgenin equivalent, DE), sapogenins (20.3 mg/g DE) and carbohydrates (52.6 mg/g D-fructose equivalent). Quantification of epicatechin in the root extract by HPTLC analysis was also carried out and was found to be 9.52% (w/w).

Conclusion: In conclusion, the present study will provide necessary information for the proper identification of the crude drugs.

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**1. Introduction**

*Potentilla fulgens* Wall. ex Hook. (PF) of the Rosaceae family is a plant commonly found at temperate and higher altitudes (1800–4350 m ASL) of the Northern and North-eastern regions of India particularly in Jammu and Kashmir, Himachal Pradesh, Uttarakhand, West Bengal, Meghalaya, Assam, Nagaland, Arunachal Pradesh and Manipur. The root of the plant is of good medicinal value and has been used as folk remedy for treating ailments like diabetes mellitus, gastric problems, peptic ulcers, diarrhoea, cancer, toothache, pyorrhea, cough, cold and also has anthelmintic property. The plant is an edible short slender herb locally called under the English name ‘Himalayan Cinquefoil’, ‘Bajradanti’ (Assamese and Hindi), ‘Ganephul’ (Nepali) and ‘Lynnngbru’ (Meghalaya). Traditionally, in Meghalaya the pieces of the roots are eaten and chewed along with raw areca nut (*Areca catechu*) and Betel leaf (*Piper betel*). The roots are receiving much attention by the tribal people for its tremendous ethno-medicinal uses and is well domesticated and well commercialized. The root is also formulated and manufactured in India by Vicco Laboratories as Vicco Vajradanti tooth powder and tooth paste.

The survey of literature on this plant results in a very little information both in the field of phytochemistry and pharmacological activity. Pharmacologically, the aerial and root portion of the plant have been reported to possess antioxidant, anti-inflammatory and anti-inflammatory activities.
antihyperlipidemic, hypoglycaemic, anti-hyperglycaemic and gastroprotective activity. Phytochemically, compounds so far reported from the root portion of the plant are epicatechin and potifuglene (epiafzelchin-6–o-8 epiafzelchin) and from the aerial parts are potentene A, potentene B, afzelchin-4x–o-8 -catechin, epiafzelchin and rutin. The objective of the present study was aimed to perform for the first time the comprehensive pharmacognostical and phytochemical standardization of the roots of *P. fulgens*. The study will be helpful in identification as well as in preventing possible steps of adulteration which are considered to be the primary stage of quality control standardization of herbal drugs.

2. Experimental methods

2.1. Plant material and extraction process

The plant samples were collected during the monsoon period (May–July) from different regions of Meghalaya state such as East Khasi Hills District (Shillong) and Jaintia Hills District (Jowai), Northeast India. The botanical authentication of the specimen was done by Dr. N Odyuo (Scientist C in-charge), Botanical Survey of India, Shillong, Meghalaya and was identified as *P. fulgens* Wall. ex Hook. (Letter No.: BSI/ERC/2010/Plant identification/281) belonging to Rosaceae family. For future reference the voucher specimen (COG/PF/016/2010) has been deposited in Department of Pharmacognostics, Indian Institute of Technology, Banaras Hindu University, Varanasi- 221 005.

The fresh roots of *P. fulgens* were thoroughly washed and shade dried for one week at temperature not exceeding 60 °C to prevent the inactivation of unstable phytoconstituents particularly the polyphenolics components. The dried roots were coarsely ground into homogenous powder using mechanical grinder, passed through 60 mesh sieve and stored at room temperature until extraction. The homogenous powdered drug (1 kg) was exhaustively extracted with 95% ethanol (3.0 L) in a soxhlet apparatus for 72 h. The extract was filtered, concentrated under reduced pressure to generate the crude ethanolic extract of *P. fulgens* (EPF) and was finally stored in air tight container for further study. Preparation of various successive fractions from the ethanolic extract obtained by soxhlation was done by suspending the dried ethanolic extract in aqueous layer and then partitioned with solvents of increasing chemical polarity (hexane, chloroform, ethyl acetate and n-butanol).

2.2. Pharmacognostical studies

2.2.1. Morphological, histological and powder evaluation

All the studies on morphological, histological and powder evaluations were performed based on the standard method of Brain and Turner (1975) and Khandelwal (2007). For morphological study, the fresh roots were evaluated for the texture, size, shape, colour, odour and taste. For histological sectioning, the roots were preserved in n-butyl alcohol of varying proportional strength and the sections were taken with the help of rotary microtome (York Scientific Industries Pvt. Ltd.). The sections were dehydrated with varying strength of absolute alcohol and then stained with safranin and fast green solution. Finally the stained sections were permanently mounted with DPX for histological observation. For the study of macerated tissue and isolated cells, pieces of roots were macerated with the mixture of concentrated nitric acid and potassium chlorate, washed with distilled water and finally mounted in glycerine for observation. Photographs of different magnifications were taken with Nikon digital microscope (eclipse E200). For normal observations bright field was used whereas for the study of crystals, fibres and lignified cells, polarized light was employed. Since these structures have bi-refringent property under polarized light, hence they appear bright against dark background.

2.2.2. Physicochemical evaluation

The determination of various physicochemical constants such as foreign matter, loss on drying, ash values, extractive values, swelling index, foaming index, haemolytic index, volatile oil content and pesticide residue content was done as per the methods described in WHO 2002 guidelines, and Indian Herbal Pharmacopoeia.

2.2.3. Fluorescence analysis of powdered drug

Fluorescence analysis of the crude powdered drug was carried out as per the method of Chase and Pratt, 1949; and Kokoski et al. 1958. The powdered drug was made to react with various classes of chemical reagents bearing acidic or basic media and the fluorescence pattern of the solution was monitored both in day light as well as under ultra violet light (365 nm).

2.2.4. Quantification of starch grain and crude fibre content

The quantitative determination of starch grains was done by incorporating the Wallis’s Lycopodium spore method whereas the crude fibre content was quantified as per the Dutch method.

2.3. Phytochemical evaluation

2.3.1. Preliminary phytochemical screening

The preliminary phytochemical analysis of EPF and its sub-fraction was evaluated to detect the presence of various classes of phytochemicals such as alkaloids, glycosides, flavonoids, steroids/triterpenoids, phenolics, tannins, saponins, mucilages, protein, amino acids and carbohydrates.

2.3.2. Quantitative estimation of various classes of phytochemicals

Various phytochemicals present in the EPF was quantified for total phenolic, total tannin content, flavonoid and flavonol content, total saponin content, total sapogenin content and total carbohydrate content by anthrone methods. Standard curve was plotted using different classes of marker components such as Diosgenin (total saponin and sapogenin estimation), tannic acid (total phenolic and tannin estimation) and D-fructose.

Fig. 1. *Potentilla fulgens* Wall. ex Hook. plant [a] and root [b].
carbohydrate estimation); whereas, for total flavonoid and flavonol content standard rutin was used as external standard. All the results were carried out in triplicates and were expressed as the mean ± S.E.M using statistical linear regression method.

2.3.3. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC)

Preparation of extracts, thin layer chromatography and development of chromatogram was done as per the standard methods. Normal TLC silica gel G was used as the stationary phase for TLC and solvent system for developing of chromatogram were composed of solvent mixtures of varying chemical polarity. Spraying reagent used for detecting the phytochemicals are Fast blue salt reagent followed by 10% KOH (for polyphenolics classes), Liebermann Burchard reagent (saponins and steroidal components), and ninhydrin reagent (for amino acids). The plates were also visualized under Ultra violet light (365 nm) for the detection of different classes of UV active components containing active chromophore groups.

The ethanolic extract of *P. fulgens* (EPF) was also standardized with epicatechin using high performance thin layer chromatography (HPTLC). A stock solution of EPF and standard epicatechin in methanol was prepared in concentration of 5 mg/mL and 0.2 mg/mL respectively. Mobile phase for developing the chromatogram were composed of chloroform: acetone and formic acid mixture in the ratio 130:53:17 (v/v/v). The study was carried out using Camag-HPTLC instrumentation equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and WINCATS 4 software for data interpretation. The *R*<sub>f</sub> value was recorded and the developed plate was screened and photo-documented at ultra violet range with wavelength (λ<sub>max</sub>) of 366 nm.

Fig. 2. Histological study of *P. fulgens* root. [a]: Transverse section of root (10×); [b–e]: Longitudinal section of root. [d–g]: TS showing vascular bundle with lignified xylem vessels (LXV) both in normal and polarized light. [f–g]: TS showing tannin cells (TC) and starch grains (SG). [h–i]: TS showing starch grains (iodine stained) and calcium oxalate crystals (under polarized light) [CC – cork cell layer; SCX – secondary cortex; SPL – secondary phloem; CB – cambium; MR – medullary rays; SXV – secondary xylem vessel; PXV – primary xylem vessel; C.Ox – calcium oxalate crystals].
3. Results

3.1. Pharmacognostical studies

3.1.1. Morphological, microscopical and powdered drug evaluation

Fresh roots are moderately soft, cylindrical with slender and tapering towards the tip, 10–15 cm long and 1–3 cm in width. Outer surface are externally dark brown and internally buff to light brown in colour. The root bark is 1–1.5 mm thick, externally corky and friable and internally smooth. The roots are odourless having strong astringent and bitter taste (Fig. 1).

The histological characteristics (Fig. 2) of the roots showed the presence of the outermost layer of bark which is made up of 4–6 layers of brick shaped cork cells filled with tannins. Tannin was also found to be well distributed throughout the whole section of the roots and produce blue stains with ferric chloride solution (5%). Lying next to the cork cell layer is the cork cambium. The formation of cork cambium is normal. Cork is followed by secondary cortex and is made up of thin walled parenchymatous cells which are round to oval or tangentially compressed in shape. Cortex is followed by the formation of secondary phloem and consists of 10–15 layers of thin parenchymatous cells followed by

![Fig. 3. Powder characteristics (a–l) and maceration (m) study of P. fulgens root.](image)
formed on the transverse section of the root (Figs. 2 and 3). It was matured root, but in the younger roots the calcium oxalate crystals cortical parenchymatous cells throughout the whole section in polarised light) were also found to be present extended from the with ruthenium red).

Fluorescence powdered drug analysis of Potentilla fulgens roots

Table 1 represent the results of the fluorescence characteristics of P. fulgens powdered drug both in day light as well as under long UV light (365 nm). The identification and comparison of the colours was done using the standard colour index chart (http://en.wikipedia.org/wiki/Web_colors).

The macerated powdered characteristics of P. fulgens roots were expressed in terms of μm (minimum-mean-maximum; length × width) and showed the presence of a large number of fibres having slender shape and tapering ends (242.07–580.07–970.11 × 9.6–14.05–22.71 μm), tracheids with bordered pits and scalariform thickenings measuring 142.11–203.24–302.69 × 12.04–18.12–27.92 μm, xylem vessels of varying size and shape measuring 111.15–243.84–521.11 × 12.04–27.92–57.97 μm and have spiral and annular thickenings. Parenchymatous cells were also visible which are round, oval and elongate in structure having size varying between 34.21–66.01–105.27 × 19.11–37.67–59.87 μm.

3.1.2. Physicochemical evaluation

Air dried material was used for the quantitative determination of different physicochemical constant which are represented in Table 1. The powdered root of P. fulgens was observed to swell in the presence of aqueous medium thereby making the solution viscous and that led to the conclusion of its higher swelling index of 5.5 (expressed in terms of mL/g). In many medicinal plants swelling index is an indicative of therapeutic or pharmaceutical value based upon the presence of gums, mucilage, pectin or hemicelluloses. Foaming index indicates the presence of certain phytoconstituents such as saponins which readily produce foam in the presence of aqueous medium. The root powder of the plant was also observed to produce a slight haemolysis of the tested blood in in vitro studies which led to the conclusion for the reason of the presence of saponin. Pesticide residue estimated in the root was found to be within the permissible range of WHO standards which stated that phosphated pesticide present in any plant material should contain not more than 0.05–0.1 mg phosphorus per kilogram of plant material.13 Hence the overall evaluation of physicochemical constants can serve as a valuable source of information for the identification of this plant material from other closely related Potentilla species. It also provides suitable standards to determine the quality and purity of the plant.

3.1.3. Fluorescence analysis of powdered drug

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Powder as such</th>
<th>Fluorescence in day light</th>
<th>Fluorescence (254 nm)</th>
<th>Fluorescence (365 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Brown</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1 N NaOH in methanol</td>
<td>Dark red</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>3</td>
<td>Powder + 1 N NaOH in water</td>
<td>Dark red</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 1 N HCl in methanol</td>
<td>Maroon</td>
<td>Sea green</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Powder + 1 N HCl in water</td>
<td>Gold</td>
<td>Wheat</td>
<td>Spring green</td>
</tr>
<tr>
<td>6</td>
<td>Powder + 1 N HNO3 in methanol</td>
<td>Dark red</td>
<td>Lime green</td>
<td>Yellow green</td>
</tr>
<tr>
<td>7</td>
<td>Powder + 1 N HNO3 in water</td>
<td>Orange</td>
<td>Golden rod</td>
<td>Sea green</td>
</tr>
<tr>
<td>8</td>
<td>Powder + Iodine (5%)</td>
<td>Orange red</td>
<td>NF</td>
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</tr>
<tr>
<td>9</td>
<td>Powder + FeCl3 (5%)</td>
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</tr>
<tr>
<td>10</td>
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<td>11</td>
<td>Powder + ammonium (25%)</td>
<td>Dark red</td>
<td>NF</td>
<td>Sea green</td>
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<tr>
<td>12</td>
<td>Powder + saturated picric acid</td>
<td>Yellow</td>
<td>NF</td>
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<tr>
<td>13</td>
<td>Powder + acetic acid</td>
<td>Orange</td>
<td>Aquamarine</td>
<td>Spring green</td>
</tr>
</tbody>
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NF: no fluorescence.

distinct cambium. The central region is represented by a wide zone of lignified xylem. The xylem becomes more dissected due to parenchymatisation and shows a number of lobes radiating more or less from the central cylinder of xylem. At one or two places, due to delignification the lobes appear to be separated from the solid centre of xylem. The xylem becomes more dissected due to delignification of this plant material from other closely related Potentilla species. It also provides suitable standards to determine the quality and purity of the plant.

Table 2

Fluorescence powdered drug analysis of Potentilla fulgens roots.

<table>
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NF: no fluorescence.
contain 19% w/w plant material. The quantification of both the starch grain and crude fibre content in the root of *P. fulgens* are important characters and can serve as diagnostic features of multiple applications according to the peculiarities and origin of the plant materials to be determined.

3.2. Phytochemical evaluation

3.2.1. Yield of subfractions and preliminary phytochemical screening

The percentage yield of all the EPF subfractions obtained successively after fractionation from the dried ethanolic extract was found to be hexane (1.24%), chloroform (6.03%), ethyl acetate (37.56%), n-butanol (45.11%) and aqueous fraction (9.32%). The results for the preliminary phytochemical screening of EPF and its subfraction were represented in Table 3. The phytochemicals present in EPF and its subfractions were found to be polyphenolics (tannins, phenolics and flavonoids), saponins, steroids, proteins, amino acids and carbohydrates. Whereas, glycosides, alkaloids and mucilage components seem to be absent. Flavonoid was also found to be present only in the ethanolic extract, ethyl acetate and butanol subfraction but was absent in other subfractions. Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytoconstituents present in the root extract.

3.2.2. Quantitative estimation of various classes of phytochemicals

From the quantitative estimations, it was observed that EPF contains maximum content of phenolics (17.7%) and tannins (11.5%). The estimated values of other polyphenolics constituents like flavonoids and flavonols was found to be 2.14% and 0.5% respectively. The root extract was also found to contain other polar constituents like saponin (4.12%), sapogenins (2.03%) and carbohydrates (5.26%) which were also quantified spectrophotometrically. Estimation of phytoconstituents in the extract/plant material is an important step prior to conducting any isolation of active biochemical markers since it gives a brief idea about different classes of chemical constituent’s present in the extract.\(^2\)

3.2.3. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC)

The results from TLC analysis conducted on EPF extract and its subfractions revealed the presence of polyphenolics in EPF extract, ethyl acetate, butanol and water fractions \((R_f = 0.1-0.38)\), whereas steroidal/terpenoids \((R_f = 0.58)\) was found to be present in EPF, hexane and chloroform fractions. However, amino acids \((R_f = 0.6-0.87)\) were found only in EPF and water fraction.

HPTLC results for the quantification of epicatechin in EPF was analyzed for the first time by scanning at wavelength \((\lambda_{\text{max}} 366 \text{ nm})\) and the quantity of epicatechin present in EPF was estimated to be 9.52% w/w.

4. Discussion and conclusion

According to WHO, botanical standards should be proposed as a protocol for the diagnosis of any herbal drugs. It is well assumed that pharmacognostical studies on the basis of morphological and histological evaluation are still considered to be the primary steps in establishing the quality control profile of any crude drug.\(^{27,28}\)

Hence, the evaluation of any crude drug on the basis of morphological and histological aspect will probably help in the detection of adulteration and substitution, thus is therefore considered to be a preferred technique in availing the proper taxonomical nature of the drug.

In the present study, we have standardized the roots of *P. fulgens* both pharmacognostically and phytochemically. The roots are proven to be of potent and good medicinal value by various tribal of the Northern and North-eastern states of India, hence there is a need to scientifically develop a standard monograph for the plant which will serve as a good source of information to the consumers, manufacturers and researchers. From the research point of view and from the pharmacognostical studies, it was observed that the roots showed the presence of various unique diagnostic characters which are the druse type calcium oxalate crystals, simple and compound starch with central hilum and concentric lamellae. Phytochemically, majority of the phytoconstituents present in EPF are polyphenolics components with phenolics and tannins as the most dominant phytochemicals. Several literatures have supported the beneficial role of polyphenolics in maintaining good health care due to their antioxidant and free radical scavenging nature.\(^2\)

Recently a report was also documented that *P. fulgens* root extract possess a good antioxidant activity which might be due to the diversity in polyphenolics components particularly epicatechin and other reported bioflavonoids.\(^4\) Moreover, chemical standardization of the ethanolic root extract with the help of HPTLC was also ascertained and the amount of epicatechin was quantified as a chemical marker.

The process of standardization of medicinal plants is still regarded as the foremost steps to achieve the proper authenticity and genuine nature of the crude drug. The world health organization (WHO) suggests that most of the people still rely on the plant based medicine for their health care benefits and most of the plant materials are taken either as food supplementary diet or as vegetables or medicine. But a query might arise that not all plant materials are always safe to consume since there are a wide number of harmful organic and inorganic phytoconstituents present in some plants that are toxic to the human body like for examples pesticides (causing Parkinson disease and cancer risk),\(^{30,31}\) saponins (causing haemolysis),\(^32\) calcium oxalate crystals (causing urolithiasis)\(^33\) and many others. All these plant based constituents should be well standardized and documented and their limits present in the plants should be estimated. Hence, to avoid the misuse of harmful plant material it
is necessary to scientifically develop a pharmacognostical and physicochemical standards of a particular plant material which may ensure and maintain its quality, efficacy and safety profile.

Conflicts of interest
All authors have none to declare.

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