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Research Article

PHARMAGONOSTICAL ANALYSIS AND CHROMOTOGRAPHICAL FINGERPRINTING OF *VAJRADANTI* (*POTENTILLA FULGENS* (WALL.) EX HOOK.)

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ABSTRACT

Poor oral hygiene or tooth disorders are very common and severely agonizing ailment leads to teeth loss or damaging of teeth, so it is the need of the hour to search for a potent drug which can fight this condition. *Vajradanti* is first described in Ayurvedic text *Arka Prakash* and *Dravyagunakosha.* Later in some Ethnomedicinal books *Vajradanti* is mentioned as potent medicine for *Dantvikar, Udarshoola* etc*. Potentilla fulgens* (Wall.) ex Hook. of Rosaceae family is an alpine medicinal plant that is found in temperate Himalaya between 2000-3000m. 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, pyorrhoea, cough, cold and also has anthelmintic property. A detailed pharmacognostical character of its root is not reported still yet. **Methods:** An attempt has been made to study the macroscopic and microscopic character, physicochemical analysis, phytochemical analysis, and chromatographic studies of its root following standard procedures. Two different extracts of *Vajradanti* root was used to access the physicochemical and HPTLC analysis. Research work was carried out as per standard operating procedures and specified protocols. **Result:** Results showed that in physicochemical evaluation aqueous extract 2.4%, hydro-ethanol extract 2.5%, total ash 8.6%, water soluble ash 1.38%, acid and insoluble ash 3.01%. HPTLC results for the quantification of epicatechin in EPF was analyzed for the first time by scanning at wavelength (λmax 366nm) and the quantity of epicatechin present in *Vajradanti* was estimated to be 0.032% w/w. In phytochemical analysis presence of carbohydrate, protein, glycosides, amino acid, protein in extract of *Vajradanti.* **Conclusion:** *Vajradanti* is a highly potent medicine for oral diseases due to presence of flavanols like epicatechin.

INTRODUCTION

Oral health is of prime importance to all individuals and oral hygiene habits are instilled in childhood itself irrespective of the nationality or geographic location of an individual. Oral diseases are perhaps, the most widespread of all diseases prevalent in the world. No population is free from caries and periodontal diseases – the most common of all such diseases and affecting almost 80% of the population. Poor oral hygiene or tooth disorders are very common and severely agonizing ailment leads to teeth loss or damaging of teeth, so it is the need of the hour to search for a potent drug which can fight this condition. Oral diseases are one of the most important problems in public health and burden in many developing countries around the globe. Among 10 people out of them 9 may experience pain and problems with eating, chewing, smiling, discolouration, damaged teeth on their daily basis. According to worldwide data prevalence of dental caries is about to be 99% and among them prevalence of periodontal disease reported more than 90% of worldwide population.

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Vajradanti is first described in Ayurvedic text *Arka Prakash* and *Dravyagunakosha.* Later in some Ethnomedinial books *Vajradanti* is mentioned as potent medicine for *Dantvikar, Udarshoola* etc. *Potentilla fulgens* (Wall.) ex Hook. of Rosaceae family is an alpine medicinal plant that is found in temperate Himalaya between 2000-3000m. Traditionally this plant is frequently used by tribals and their various products are also uses by common peoples for caring for various diseases especially in pyorrhea[1]. 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, pyorrhoea, cough, cold and also has anthelmintic property.[2]

The plant locally called under the English name 'Himalayan Cinquefoil', 'Bajradanti' (Assamese and Hindi), 'Ganephul' (Nepali) and 'Lynniangbru' (Meghalaya). The effective pharmacotherapeutic ingredients can be termed as the secondary metabolites or the active principle of floras[3]. The secondary metabolites comprise tannin, glycosides, alkaloids, saponin and enzymes or ferments. Specific odour, taste and colours of plants are resolute by them.[4]

MATERIAL AND METHODS

Plant Collection and Authentication

The plant fresh root was collected during the month of September from Dhanaulti regions, Tehri district of Uttarakhand state. The botanical authentication of the specimen was done by Dr.Gajendra Singh, Scientist/Engineer, Forestry and Climate Change Division, Uttarakhand Space Application Centre, Nalapani, Dehradun.

Pharmacognostical Studies

Morphological, Histological Evaluation

All the studies on morphological and histological were performed based on the standard method. For morphological study, the fresh roots were evaluated for the texture, size, shape, colour, odour and taste. The sections were dehydrated with varying strength of absolute alcohol and then stained with safranin and fast green solution. Finally the stained sections were mounted with glycerine for histological observation.

Physicochemical Evaluation[6]

The determination of various physicochemical constants such as foreign matter, total ash values, water soluble ash value, acid soluble ash value, different extractive values, was done as per the standard methods.

Phytochemical Evaluation

Preliminary Phytochemical Screening[7]

The preliminary phytochemical analysis of methanolic extract of *Vajradanti* root extract was evaluated to detect the presence of various classes of phytochemicals such as alkaloids, glycosides, flavonoids, steroids, tannins, saponins, protein, amino acids and carbohydrates.

Test for Carbohydrate

a) Molisch's Test: A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.

b) Fehling's Test: The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

c) Benedict's Test: The extract will be treated with Benedict's reagent; appearance of reddish orange colour precipitate indicates the presence of reducing sugar.

Mayer's Reagent Test: 2ml of test solution was taken in a test tube to which and 2ml of the Mayer's reagent (Potassium Mercury Iodide solution) was added. A white or pale yellow precipitate if formed indicated presence of alkaloids except with alkaloids of the purine groups and few others.

Dragondroff's Reagent Test: 2ml of test solution was taken in a test tube in which 2ml of the Dragon Droff's reagent (mixture of potassium iodide and bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of alkaloids

The ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

Millon's Test: The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

A small quantity of test sample was taken with 2ml of water and 0.5ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponins.

Borntragor's Test: 1ml of Benzene and 0.5ml of dilute ammonia solution was added to the ethanolic extract and was observed for the formation of reddish pink colour.

Molisch's Test: 2ml of test solution was taken in a test tube and 2ml of the Molisch's reagent was added and shaken carefully and then about 1ml of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for one 1 minute. A purple colour ring at the junction of the two layers if formed indicated the presence of glycosides.

A 5 percent solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deep blue colour indicates the presence of tannins.

A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins

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 (for amino acids). The plates were also visualized under ultra violet light (366nm) for the detection of different classes of UV active components containing active chromophore groups.

The quantification of catechin in coarse powder of *Vajradanti* sample was carried out by using HPTLC. Mobile phases of Toluene: Ethyl acetate: Acetone: Formic acid (6:6:6:1 v/v/v/v) were used for the identification of catechin11. Preparation of standard solution, 10mg of catechin was dissolved in 10ml methanol, sonicated for 5 minutes and diluted from 0.1ml to 1ml. For identification 20µl and for quantification different amounts (1.2, 1.4, 1.8, 2, and 3µl) were applied on TLC plate. The 500mg of sample was dissolved in 10ml methanol, sonicated for 20 minutes and centrifuged to obtain clear solution. For identification 20µl and for quantification 10µl was applied on TLC plate. Quantification was done by application of the spots on the HPTLC plate which were coated with silica gel (plate size of 6.0x10.0cm). The plates were developed at 60°F 254nm and 366nm under UV visible wavelength. The details of the instrument used in studies are CAMAG LINOMAT 5 with 5 application parameters, inert gas (nitrogen) as spray gas and methanol as sample solvent. The syringe size was 25µl with 3 tracks. The calibration parameter used was calibration mode multilevel, with CV statistics mode and the evaluation mode was based on peak areas.

The study was carried out at Drug Discovery and Drug Development, Patanjali Research Institute, Haridwar. Chemicals used; Standard biomarkers, Epicatechin is used from Sigma Aldrich/ WXBC6250V/ 98.8, Private Limited, Mumbai and all the chemicals used throughout the research were procured from institute.

RESULTS

Table 1: Pharmacognostical Studies of *Vajradanti* roots

S. No.	Character	*Vajradanti* root
1.	Shape	Cylindrical
2.	Size	10-15cm
3.	Odour	Odourless
4.	Colour	Blackish cover with orangish inside
5.	Taste	Bitter
6.	Surface	Rough

Morphological, Microscopical Drug Evaluation

Macroscopic Characters of Different Parts of *Potentilla fulgens* (Fig.1)

An erect, perennial, stout, ascending herb, 15-75cm tall with a thick rootstock.

Leaf: It possesses both radical and cauline leaves. Radical leaves are 4-30cm 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 × 0.8-1.5cm 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 but has less pairs of leaflets.

Flowers: Flowers 1-2cm in diameter are crowded in terminal corymbs. Floral pedicel is 2-4cm 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 subbasal and achenes glabrous.

Fruit: The accessory fruits are usually dry, but may be fleshy and strawberry-like, while the actual seeds– each one technically a single fruit – are tiny nuts

Root: Fresh roots are moderately soft, cylindrical with slender and tapering towards the tip, 10-15cm 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 1-1.5mm thick, externally corky and friable and internally smooth. The roots are odourless having strong astringent and bitter taste.

Fig.1 Morphological characters of *Vajradanti*

A. Flower

B. Abaxial surface of leaf

C. Adaxial surface of leaf

D. Size measurement of root

E. Fresh root

F. Dried root

Microscopic Study

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.

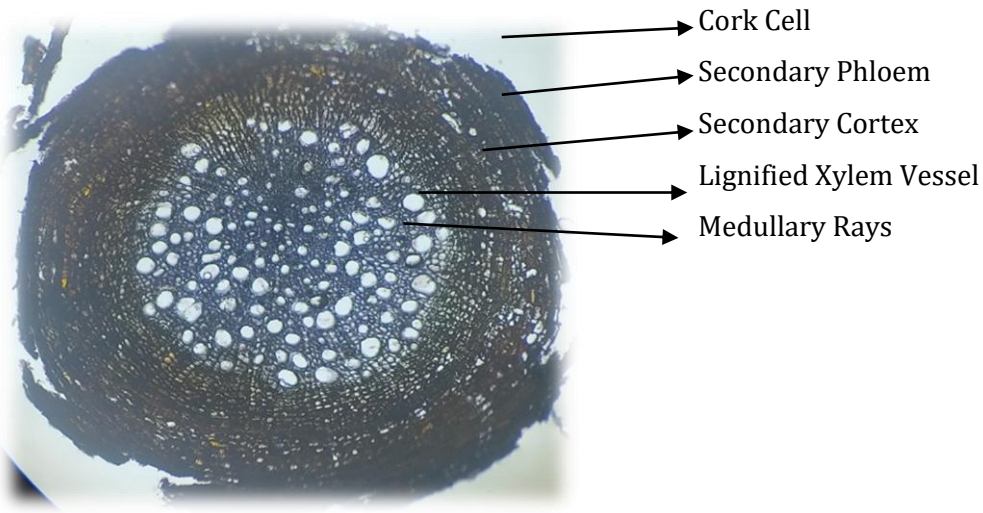
Cork is followed by secondary cortex and is made up of thin walled parenchymatous cells which are round to oval or tangentially compressed in shape.

Cortex is followed by the formation of secondary phloem and consists of 10-15 layers of thin parenchymatous cells followed by 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 medullary rays due to delignification become more deep and wider in the section. Phloem is represented by soft sieve elements whereas xylem is represented by vessels, tracheid, fibres and xylem parenchyma all showing strong lignifications on their walls.

Fig.2 Microscopic Characters of *Vajradanti*



Physicochemical and Phytochemical Study

Physicochemical parameters such as water-soluble extractives, alcohol-soluble extractive, n-hexane soluble extractive, chloroform-soluble extractive, ether soluble extractive, total ash value and moisture content of the samples were calculated and depicted (Table 2).

S. No.	Test	*Vajradanti* Root
1.	Foreign Matter	0
2	Aqueous soluble extract (%)	2.4
3.	Hydro-ethanol soluble extract (%)	2.5
4.	Total ash (%)	8.60%
5.	Water soluble ash (%)	1.38%
6.	Acid insoluble ash (%)	3.01%

Preliminary phytochemical analysis of alcoholic extract of *Vajradanti* was done. Phytochemical parameters such as test for carbohydrates, tannins, flavonoids, alkaloids, carbohydrate etc were calculated and depicted (Table 3).

S.No.	Phytochemical Testing	Name of Test	AEV	HEEV
1.	Test for Carbohydrate	Molisch's test	+	+
		Fehling's test	+	+++
		Benedict's test	++	++
2.	Test for Alkaloids	Mayer's reagent test	+	-
		Dragondroff's reagent test	-	+
3.	Test for Amino acids	Ninhydrin test	+	+
4.	Test for Proteins	Millon's test	-	+
		Xanthoprotic test	+	+
5.	Test for Saponin	Foam test	+++	++
6.	Test for Glycosides	Borntragor's Test	-	+
		Molisch's Test	+	+
7.	Test for Tannin	Ferric chloride solution	+	+
		Lead acetate	+	+

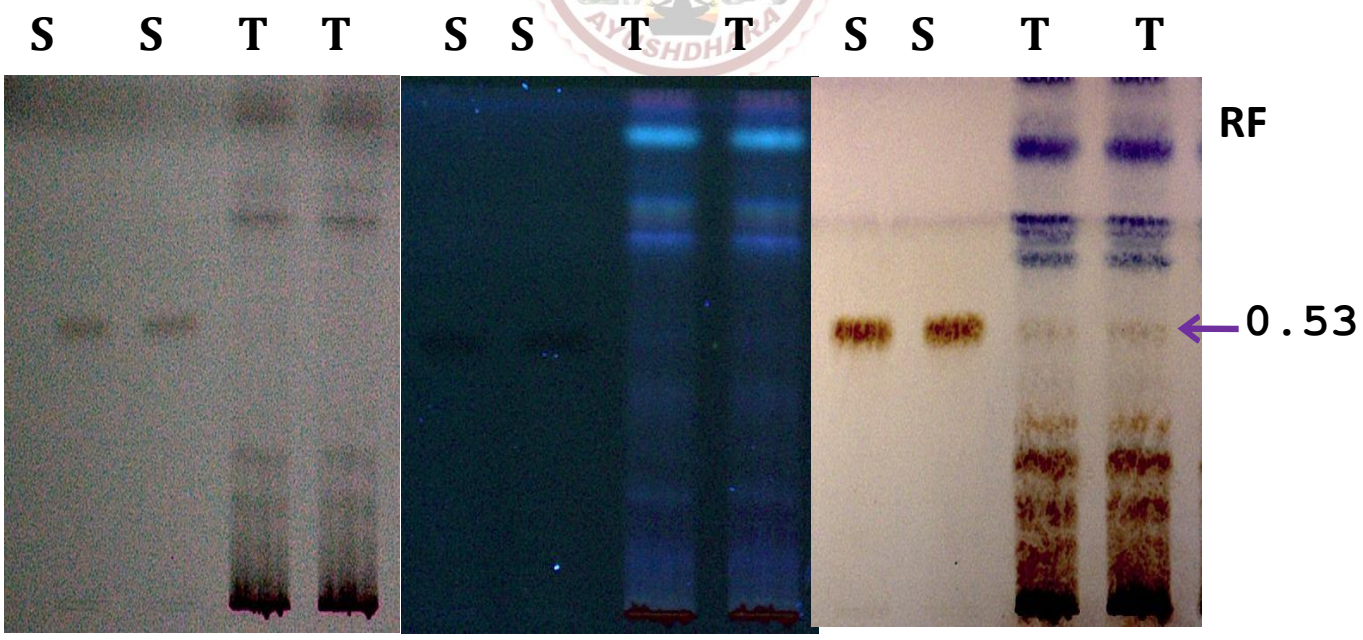
Chromatography Study

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

The results from TLC analysis depicted in Table no.4

	***Vajradanti* Sample**
TLC plate	
Rf value	0.51, 0.53, 0.57, 0.78, 0.82, 0.89, 0.95, 0.97
No. of spots	8

HPTLC results for the quantification of epicatechin in EPF was analyzed for the first time by scanning at wavelength (λmax 366nm) and the quantity of epicatechin present in *Vajradanti* was estimated to be 0.032% w/w.



254nm 366nm white light after derivation

CONCLUSION

Due to the less literature available on *Vajradanti* as it is very highly potent medicine for maintaining good oral health due to presence of phenols and flavonoids. *Potentilla fulgens* contains flavonoids like catechin and epicatechin, lupeol, stigmasterol, an isoflavone glycoside, an alkaloid, and small quantities of uncharacterized bases. *Vajradanti* in traditional Ayurvedic medicine used for the treatment of toothache, stomach pain, tooth and digestive disorders. Various studies show that showed

that catechins rich in antimicrobial and antioxidant properties, can prevent streptococcus mutants from adhering to tooth surfaces[8]. The catechins with concentration of 100mg/l and their effects on bacterial adhesion to salivary coated were observed. The observed parameters of the root of *Potentilla fulgens*, like morphology physicochemical parameters and TLC, HPTLC profile were always constant. It may be useful to establish certain botanical standards for standardization of *Potentilla fulgens* for the further studies. This paper explains the evidence-based information regarding the pharmacological activity of this plant.

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