Natural Product Sciences
13(3) : 214-219 (2007)

Pharmacognostical Evaluation of the Roots of *Pseudarthria viscida* (Linn.)

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Abstract – The roots of *Pseudarthria viscida* (L.) Wight and Arnott. (Leguminosae) are used against various disorders in Indian systems of medicine, namely as anthelmintic, anti-inflammatory, cardiotonic, aphrodisiac, rejuvenating, tonic and as a remedy for cough, asthma, tuberculosis, diarrhoea and alternate fever. The present communication deals with the detailed pharmacognostical evaluation of the root sample using light and confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *P. viscida* root and may possibly help to differentiate the drug from its other species.
Keywords – *Pseudarthria viscida,* Leguminosae, pharmacognosy, HPTLC, stem and root

Introduction

Pseudarthria viscida (L.) Wight and Arnott (Leguminosae) is a controversial plant commonly known as Salaparni in Sanskrit and is an essential component of many famous Ayurvedic formulations like Dashamoola, Mahanarayana taila and Dhantara taila (Deepa *et al.,* 2004). The plant is a perennial viscid pubescent semi-erect diffuse undershrub. The roots are used as astringent, sweet, bitter, emollient, digestive, anthelmintic, anti-inflammatory, diuretic, cardiotonic, aphrodisiac, febrifuge, rejuvenating and tonic. It has a curative effect on vitiated conditions of vata and pitta, cough, bronchitis, asthma, tuberculosis, dyspepsia, diarrhoea, alternate fever, food poisoning, vomiting and general debility. A decoction of the roots is given for treating rheumatism, asthma, heart diseases and piles. The root juice is given as a nasal drop in case of headache and hemicranias (Warrier *et al.,* 1996). The root of the plant has been reported to contain leucopelargonidin, flavonoids and proteins (Deepa *et al.,* 2004; Prasad and Nambisan 1976).

In view of its diverse medicinal applications and in order to ensure the quality of its supply, especially in times of adulteration and substitution prevailing on the crude drug markets, the present investigation deals with the pharmacognostical evaluation of the roots of *P. viscida.* The study includes morphological and anatomical evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the different extracts of *P. viscida.*.

Experimental

Plant material – The roots of *P. viscida* were collected from Erode, Tamil Nadu, India during the month of August, 2005. The botanical identity of the plant was confirmed by Dr. P. Jayaraman, Botanist, Medicinal Plant Research Unit, Chennai, Tamil Nadu. A voucher specimen (PP 546) has been deposited at the Museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal.

Chemicals and instruments – Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents *viz.* ethanol (95%), hexane, petroleum ether, diethyl ether, chloroform, acetone, n-butanol and reagents *viz.* phloroglucinol, glycerin, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and microscopic analysis – The macroscopy and microscopy of the root were studied according to the method of Brain and Turner (1975a). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (1940). The

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micropowder analysis was done according to the method of Brain and Turner (1975b) and Kokate (1986a).

Physico-chemical analysis – Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed (Indian Pharmacopoeia, 1966) and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992). Fluorescence analysis was carried out according to the method of Chase and Pratt, 1949 and Kokoski *et al*., 1958.

Preliminary phytochemical screening – Preliminary phytochemical screening was carried out by using standard procedures described by Kokate, 1986b and Harborne, 1998.

HPTLC studies – Qualitative densitometric HPTLC analysis was performed for the development of characteristic finger print profile for successive acetone and EtOH extracts of roots. 10 µL of the sample solutions were applied and the plates were developed in $CHCl_3$-EtOAc (7 : 3) and toluene: solvent ether (8.5 : 1.5) respectively for acetone and EtOH extracts. Developed plates were then scanned densitometrically at various wavelengths. R_f values, peak area and spectrum of each peak were determined for these extracts. The presence of Lupeol in petroleum ether extract was confirmed by co-chromatography with an authentic sample (Sigma Chemical Company, USA). Quantification of lupeol was carried out for the petroleum ether extract of the root. The linearity of the HPTLC method was investigated for lupeol in the range of 100 - 1000 ug/mL at five concentration levels using the Camag Linomat V applicator onto the precoated silica gel plate (Merck). The plate was then eluted with hexane-ethyl acetate (9 : 1). After elution the plate was sprayed with 10% methanolic H_2SO_4, heated at 105 °C for 5 mins and scanned densitometrically using Camag TLC scanner 3 at 460 nm. The percentage of lupeol in the petroleum ether extract was calculated by calibration using peak height and peak area ratio.

Results and Discussion

Macroscopic characters of the plant – The plant is perennial, diffuse and prostrate; stems are 60 - 120 cm long, slender, more or less clothed with soft whitish hairs. Leaves are 3-foliolate; petioles are 1.3 - 2.5 cm long and are densely hairy; stipules are 4.5 - 6 cm long, lanceolate-cuspidate and hairy. Flowers are small, numerous and in distant fascicles along the rachis of terminal and axillary racemes or panicles; peduncles are 1 - 3; pedicels are filiform and much longer than the calyx; bracts are lanceolate-subulate, shorter than the pedicels; seeds are 4 - 6, sub-reniform, compressed, brownish black; root is subcylindrical, slightly tapering, branched and posseses longitudinal wrinkles. It has a fibrous fracture and is 3 - 7 cm long and 4 - 7 mm in thickness. The inner wood is brown in colour and astringent to taste.

Microscopic characters of root – The anatomy of the young root shows distinct secondary characters with a circular outline and a fissured surface. The fissures are shallow and irregular. The periderm is narrow and superficial with no evident cortex; secondary phloem is more or less uniform around the xylem. The secondary xylem cylinder is solid, dense and is circular in its cross sectional view (Figs. 1A & 1B).

The periderm is about 80 mm thick and consists of 4 or 5 layers of tannin-filled tabular cells. Phelloderm is 2 or 3 layered and immediately followed by the secondary phloem. Cortex is not evident between the periderm and phloem zones. Secondary phloem consists of outer crushed and collapsed tissues and inner intact non-collapsed tissues. In the collapsed phloem, crushed sieve elements are seen in the form of dark tangential streaks, scattered masses of gelatinous fibres and large, conspicuous tannin-containing cells (Fig. 1C).

Secondary xylem consists of thick tangential bands of gelatinous fibres alternating regularly with thin bands of thick walled, lignified xylem parenchyma. The vessels are scanty, narrow and thick walled. Under the polarized light microscope a dense deposition of crystals of the prismatic type is seen in the cortex (Fig. 1D). The xylem ray cells and xylem parenchyma cells contain starch grains, which are fairly large with a concentric hilum.

Powder characters of root – The root powder is light brown in colour with a characteristic odour and slightly bitter and astringent taste. Thick walled brownish cork cells (Fig. 2A) and prism type calcium oxalate crystals are seen (Fig. 2B). Parenchymatous cells are rectangular to square in shape, thin walled and contain cellulose (Fig. 2C). Tannin containing cells are rectangular or polygonal with thick walls (Fig. 2D). The vessel elements are wide cylindrical cells with wide openings at the ends known as perforation plates and have lateral walls with reticulate thickenings. The vessel elements are 160 - 210 µm in length and 40 - 50 µm in width (Fig. 2E). Two types of fibres are seen i.e gelatinous fibres and fibri-form fibres. The fibri-form fibres are less lignified and appear in groups (Fig. 2F). The gelatinous fibres contain cellulose or mucilage, are strongly lignified and appear either singly (Fig. 2G) or occasionally in groups (Fig. 2 H). Both the fibres have tapering ends with secondary wall

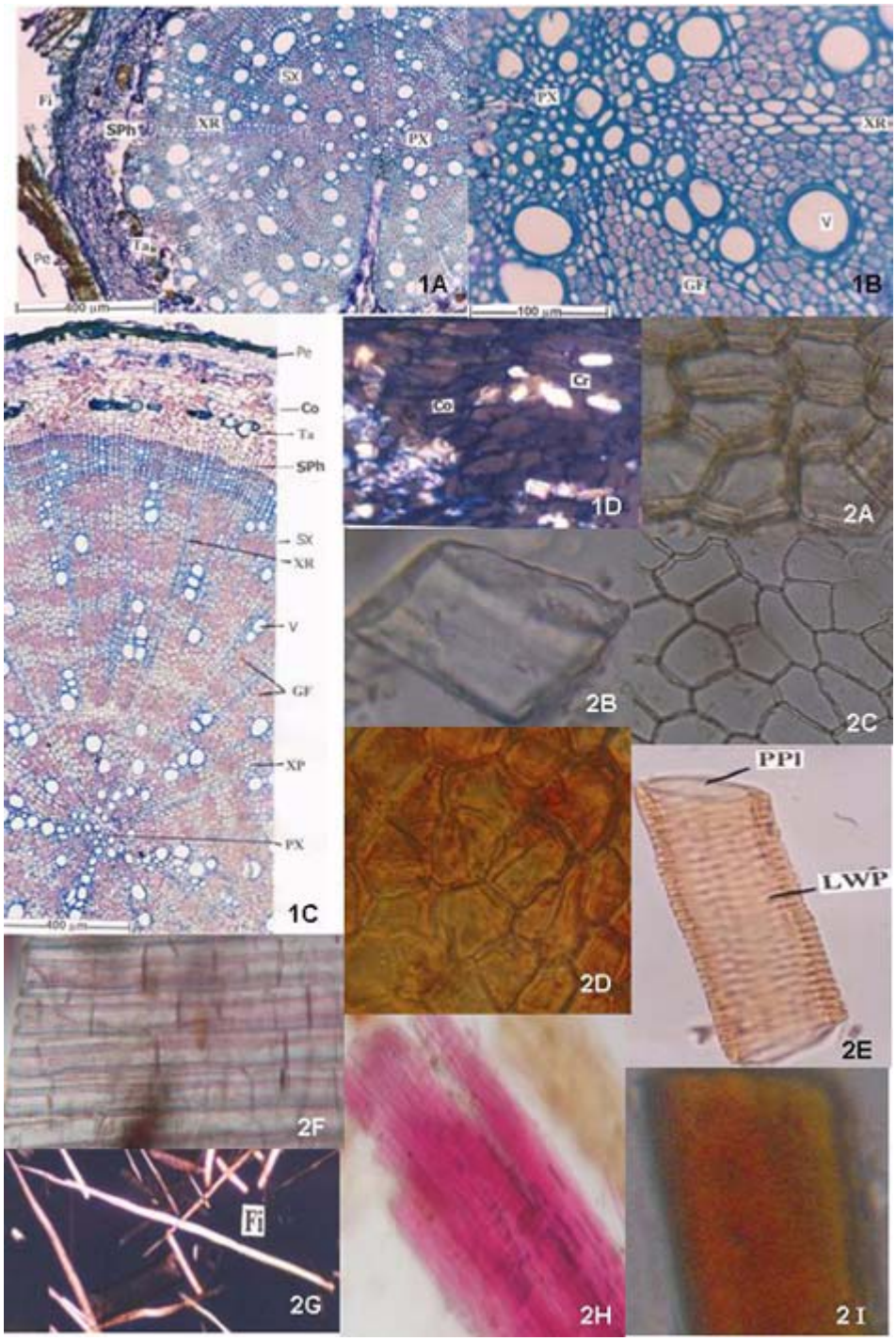

Fig. 1. **A**: T.S. of young root-a sector enlarged (**Fi**-Fibre; **Pe**-Periderm; **Px**-Primary xylem; **SPh**-Secondary phloem; **SX**-Secondary xylem; **Ta**-Tannin; **XR**-Xylem ray), **B**: T.S of root-xylem vessels and fibres enlarged (**GF**-Gelatinous fibre; **PX**-Primary xylem; **V**-Vessel; **XR**-Xylem ray), **C**: T.S. of the matured root-a sector enlarged (**Pe**-Periderm; **Co**-Cortex; **Ta**-Tannin; **SPh**-Secondary phloem; **SX**-Secondary xylem; **XR**-Xylem ray; **V**-Vessel; **GF**-Gelatinous fibre; **XP**-Xylem parenchyma; **PX**-Primary xylem), **D**: Crystal distribution in the root as seen under polarized light microscope (**Co**-Cortex; **Cr**-Crystals).

Fig. 2. Powder microscopy of the root.

A: Cork cells, **B**: Prism ca. oxalate crystal, **C**: Parenchyma, **D**: Tannin containing cells, **E**: Vessel element showing perforation plate and reticulate lateral wall pits (**PPI**-Perforation plate; **LWP**-Lateral wall pits), **F**: Less lignified fibri form fibres, **G**: Xylem fibres under polarized light microscope (**Fi**-Fibre), **H**: Strongly lignified gelatinous fibres, **I**: Brownish matter.

Table 1. Preliminary phytochemical screening of the root powder of *P. viscida*

Test	Hexane	Benzene	Chloroform	Acetone	Ethanol	Water
Carbohydrates	–	–	–	–	+	+
Phytosterols	+	–	–	–	–	–
Terpenes	+	+	–	–	–	–
Fixed oils and fats	+	+	–	–	–	–
Saponins	–	–	–	–	–	–
Phenolic compounds and tannins	–	–	–	+	+	+
Flavonoids	–	–	–	+	+	–

+ Denotes the presence of the respective class of compounds

Table 2. Ash values of the root powder of *P. viscida*

Parameters	Values % (w/w)
Total ash	7.55
Acid insoluble ash	0.86
Water soluble ash	1.24
Sulphated ash	8.51

Table 3. Extractive values of the root powder of *P. viscida*

Parameters	Values % (w/w)
Water soluble extractive	3.51
Ethanol soluble extractive	2.42
Ether soluble extractive	0.51

thickening especially in the pits. The fibres measure 450 - 700 μm in length and 40 - 60 μm in thickness. Brownish matter is also present throughout the tissue (Fig. 2I).

Preliminary phytochemical screening – Preliminary phytochemical screening revealed the presence of phytosterols, lipids, phenolic compounds, carbohydrates, flavonoids and tannins (Table 1).

Physico-chemical constants – Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 2) of the powdered *P. viscida* root revealed a high concentration of sulphated ash. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble extractive (Table 3) was high in *P. viscida*. The results of fluorescence analysis of the drug powder are presented in Table 4.

HPTLC studies – A densitometric HPTLC analysis was performed for the development of characteristic finger print profile for acetone and EtOH extracts of roots (Fig. 3), which may be used as markers for quality evaluation and standardization of the drug. Most of the compounds have shown maximum absorbance at 350 nm and 300 nm respectively for acetone and EtOH extracts. The bands in the sample were obtained at R_fs, 0.11, 0.19, 0.37, 0.51, 0.58, 0.94 (acetone extract) and 0.18, 0.30, 0.45, 0.56, 0.75, 0.88 (EtOH extract), which can be used as identifying markers. Lupeol (R_f – 0.31) content in the root (Fig. 4) drug was found to be 0.36% w/w.

Conclusion

As there is no pharmacognostic/anatomical work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. While discussing the purpose of systematic anatomy, Metcalfe and Chalk (1981) pointed

Table 4. Fluorescence analysis of the root powder of *P. viscida*

Treatment	Day light	UV light (254 nm)
Powder as such	Light brown	Light brown
Powder + 1N NaOH (Aqueous)	Slightly yellowish brown	Slightly yellowish brown
Powder + 1N NaOH (Alcoholic)	Yellowish brown	Yellowish brown
Powder + 1N HCl	Slightly reddish brown	Fluorescent green
Powder + Ammonia	Dark brown	Fluorescent greenish brown
Powder + Iodine	Dark brown	Greenish brown
Powder + $FeCl_2$	Slightly greenish brown	Dark greenish brown
Powder + 1N H_2SO_4	Brown	Dark brown
Powder + Acetic acid	Light brown	Dark brown
Powder + 1N HNo_3	Slightly reddish brown	Reddish brown

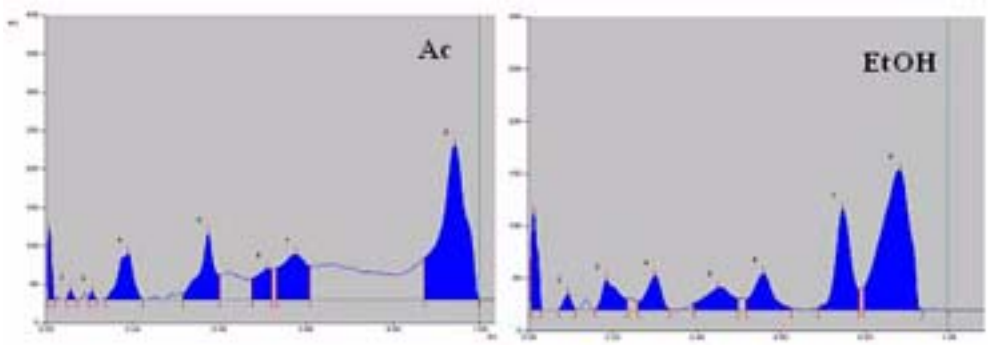

Fig. 3. HPTLC chromatogram of Acetone and EtOH extracts of *P. visicda* root (**Ac**-Acetone extract; **EtOH**-Ethanol extract).

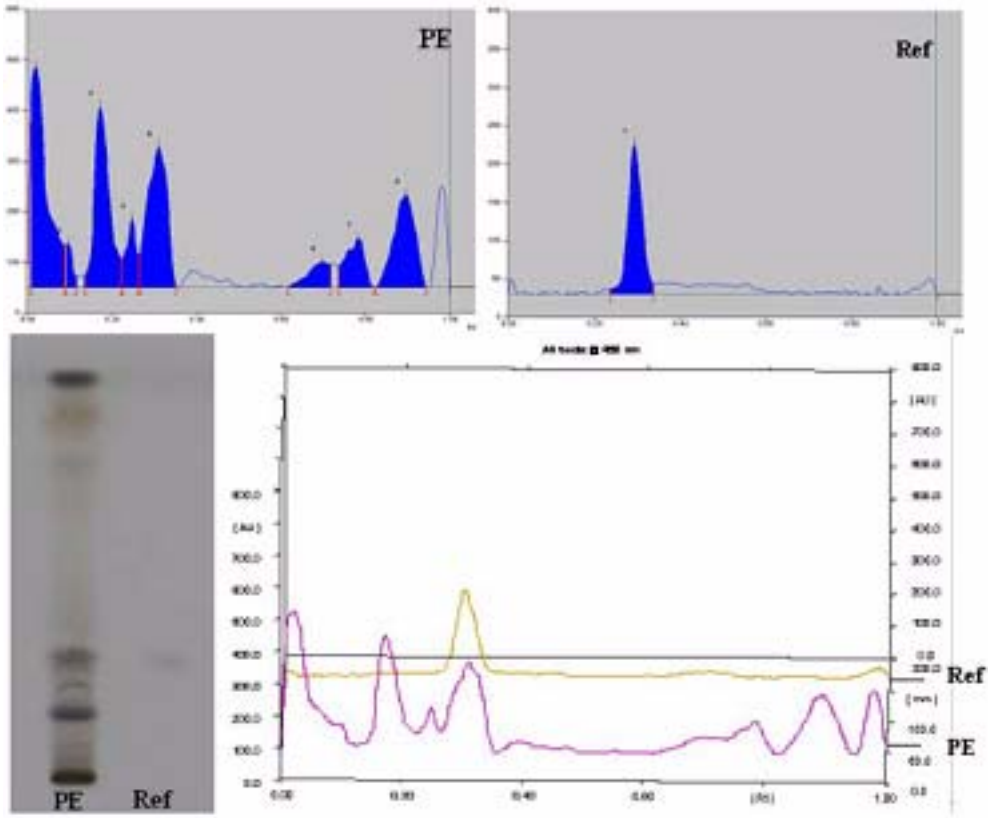

Fig. 4. HPTLC profile of PE extract of *P. visicda* root and Lupeol reference (**PE**- Pet. ether extract; **Ref**- Lupeol reference).

out that any exercise that involves the identification of plant material when it is in a fragmentary or partly decomposed condition can be achieved by the method of comparative histology. As regards, the subterranean plant parts, the correct identification of roots can often be achieved only by microscopical investigation.

Acknowledgements

The authors are thankful to Manipal Academy of Higher Education, Manipal for providing the facilities to carry out the study and Manipal Foundation for awarding the Faculty Study Fellowship Grant.

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(Accepted July 16, 2007)