Natural Product Sciences
11(2) : 58-62 (2005)

Pharmacognostical Evaluation of Roots of *Pygmaeopremna herbacea* (Roxb.) Mold.

Subha Rastogi, Madan Mohan Pandey, Kaushal Kumar, Ajay Kumar Singh Rawat*, and Palpu Pushpangadan

Pharmacognosy & Ethnopharmacology Division,National Botanical Research Institute, Rana Pratap Marg, Lucknow-226 001, India

Abstract – *Pygmaeopremna herbacea* (Roxb.) Mold. (Verbenaceae) is a small herb or sometimes an undershrub arising from a perennial rootstock. The dry roots are dark muddy brown in colour having root nodules. Its extensively developed roots are widely used in tribal medicine. They are used as an aphrodisiac and for the treatment of gout, rheumatism and ulcers. This study deals with the detailed pharmacognostical evaluation of the dried roots and root nodules of *P. herbacea* which includes macro and microscopic studies, determination of physicochemical parameters and chemoprofiling of the extract using HPTLC fingerprint profiles. It was observed that the roots consist of a well developed cortical region consisting of tangentially elongated thin walled parenchymatous cells and contain polygonal stone cells as well as compound starch grains. Also the pith was parenchymatous. The HPTLC fingerprint profile of the methanolic extract showed the presence of seven major bands. Such an analysis may thus be utilized in identifying *P. herbacea* and in differenciating it from other species which are similar to it or are used as its adultrants/substitutes under the same vernacular name of Bharangi.
Keywords – *Pygmaeopremna herbacea*, Verbenaceae, Bharangi, Pharmacognosy, Root, HPTLC.

Introduction

Pygmaeopremna herbacea (Roxb.) Mold. (Verbenaceae) is a rare and potential ethnomedicinal plant widely used in tribal medicine (Prakash and Singh, 2000-2001, 2001). It is a small herb or sometimes an undershrub, arising from a perennial rootstock, found in the sub-tropical Himalayas from Kumaon to Bhutan and in Assam, extending southwards through W. Bengal, Bihar and Orissa into the Deccan Peninsula (Anonymous, 1950). Its extensively developed roots are used medicinally. Ethnomedicinally, the roots are used as an aphrodisiac (Saxena, 1986) and for the treatment of gout, rheumatism and ulcers (Goel *et al*, 1984; Maheshwari *et al.*, 1980). Fresh rootstocks and roots along with ginger are given in asthma, rheumatism and dropsy. The rootstocks are also used to cure toothache while the leaves are prescribed in fevers, cough and rheumatism and their poultices are applied to boils (Anonymous, 1986). The alcoholic extract of the roots of this plant has been reported to possess antipyretic, antinociceptive and anti-inflammatory activities (Narayanan *et al.*, 2000). Several diterpenoids viz. bharangin, isobharangin, pygmaeocin A, B, C and E, pygmacone, sirutekkone and pygmaeoherin have been reported from the roots and root nodules of *P. herbacea* (Qingchang *et al.*, 1988a, 1988b; Gopalan *et al.*, 1988; Weixin, 1989).

The roots of *P. herbacea* are usually confused with those of *Clerodendrum serratum*, which are also sold under the same vernacular name Bharangi (Anonymous, 1950). The anatomical characters of *C. serratum* have already been described in detail (Narayanan *et al.*, 2002) Certain diagnostic anatomical features that separate *C. serratum* from *P. herbacea* have also been described (Nayar *et al.*, 1976), although some of the observations are subject to verification (Narayanan *et al.*, 2002). Thus considering the medicinal applications of *P. herbacea* it was felt that a more thorough pharmacognostical analysis of the roots of *P. herbacea* would be worthwhile. Without proper identification the desired therapeutic effects of the drugs cannot be obtained. Therefore, in the present investigation the complete pharmacognostical study was carried out which includes determination of macro and microscopic features, physicochemical parameters and the HPTLC fingerprint profile. These characteristics would be useful in identifying and differenciating *P. herbacea* from its substitutes and adultrants.

*Author for correspondence
Fax: +91-522-2205836; E-mail: pharmacognosy1@rediffmail.com

Experimental

The roots of *P. herbacea* were collected from Jharkhand, and deposited in the departmental herbarium of National Botanical Research Institute. For microscopic studies, the hand section was cut transversely and stained with safranin and fast green (Johanson, 1940). Histo-chemical studies were performed for the presence of lignin, suberin, mucilage, oil cells, starch grains and type of crystals present (Kokate, 1986). The quantitative analysis viz. total ash, acid insoluble ash, total alcohol and water soluble extractives and successive Soxhlet extraction were assayed according to the Indian Pharmacopoeia (Anonymous, 1966) methods.

For HPTLC fingerprinting, methanolic extract was prepared. For this 2 g. of the powdered roots of *P. herbacea* was extracted with methanol (3×10 ml) at room temp, the extracts combined, filtered and concentrated under vacuum below 50°C using rotatory evaporator and finally lyophilized. 10 mg of this dried extract was re-dissolved in 1 ml of methanol and 10 ul of it was applicated with Camag Linomet IV applicator on a precoated silica gel G 60 F_{254} TLC plate (Merck) of uniform thickness of 0.2 mm. The plate was developed in solvent system Chloroform : Methanol : Water (70 : 30 : 04) upto a height of 8 cm. The plate was visualized under visible light after derivatization with ceric sulphate spray reagent followed by heating at 110°C for 5-10 mins. in order to develop the chromatogram. The fingerprint profiles were documented with the help of Camag photo documentation unit Reprostar 3 and scanned densitometrically with Camag TLC Scanner3 equipped with winCats software.

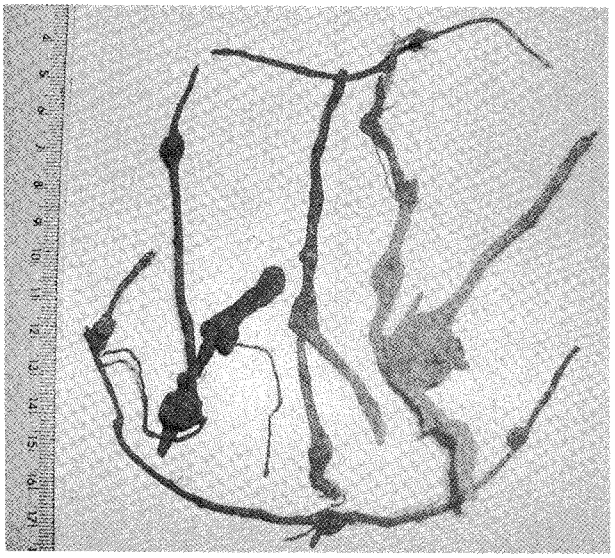


Fig. 1. Dried roots of *P. herbacea*

Results and Discussion

Macroscopic Characters (Fig. 1) – The dry roots are dark muddy brown in colour having root nodules. The circumference of the root nodules varying from 1-7 cm. Texture rough, fracture short and brittle, tasteless with no characteristic odour.

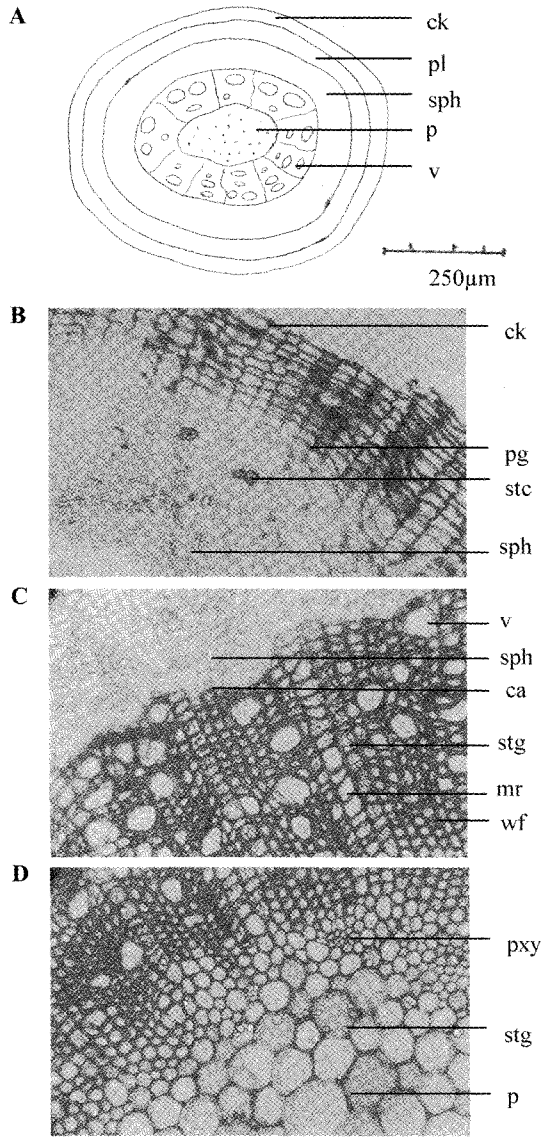


Fig. 2. Microscopy of *P. herbacea* roots (B-D 10×25x)
A- T.S. of root (Diagrammatic), **B-** T.S. of root showing cork, phellogen, stone cells and secondary phloem region, **C-** T.S. of root showing xylem vessels, cambium and medullary rays and wood fibres, **D-** T.S. of root showing protoxylem, starch grains and pith region.
Abbreviations: ca, cambium; ck, cork; mr, medullary rays; p, pith; pg, phellogen; pl, phellem; pxy, protoxylem; sph, secondary phloem; stc, stone cells; stg, starch grains; v, vessel; wf, wood fibre.

Microscopic Characters (Fig. 2) – The T.S. of the root is almost circular in outline. The outer 8-12 layers of phellem cells thick walled, tangentially elongated and brick shaped measuring about 40-60×20-30 μm. Phellogen consists of 2-3 layers of tangentially elongated cells. The cortical region is well developed and consists of 10-15 layers of tangentially elongated thin walled parenchymatous cells. The polygonal shaped stone cells, measuring about 90-260 μm in diameter, are present in groups of 2-4 or single in the cortical region. Although earlier Nayar *et al.* have reported the absence of stone cells in the cortex, our observations say otherwise. Compound starch grains are present in the cells of cortical region. The size of the starch grains is 10-50 μm in diameter. The endodermis is not distinct. Phloem is present in 4-8 layers, which contains phloem parenchyma, sieve tubes, companion cells and phloem fibres. The phloem cells are slightly isodiametric to polygonal, thin walled, compact, measuring about 20-80 μm containing starch grains in the cells.

The cambium is 2-4 layered consisting of elongated, thin walled cells. The vascular region is broad, the xylem consisting of vessels, fibres and xylem parenchyma. The vessels are various in size. The vessels are almost cylindrical with oblique pores. The walls of the vessels are simple pitted. The wood fibres are thick walled, long and pointed at their ends. The fibres become red or pink when treated with phloroglucinol and conc. HCl.

The medullary rays are 3-4 layers broad and 7-18 cells in height. The parenchymatous cells contain starch grains which become blue in colour when section mounted in iodine. The pith is present in the center portion, of which the cells are parenchymatous and measuring about 32-84 μm in diameter. Nayar *et al.* have reported the pith to be parenchymatous. However, according to Narayanan *et al.*, this needed verification. Our observations also show the pith to be constituted of parenchymatous cells, thus verifying what Nayar *et al.* had reported earlier.

It can thus be inferred that the characteristic microscopical features of the roots of *P. herbacea* are

a) presence of a well developed cortical region consisting of tangentially elongated thin walled parenchymatous cells

b) presence of polygonal stone cells in groups of 2-4 or single in the cortical region

c) presence of compound starch grains in the cortical and vascular region

d) presence of parenchymatous pith.

Study of Powdered Root (Fig. 3) – The powder of the roots is dark muddy brown in colour. Under microscopic observation the powder shows xylem vessels with reticulate secondary wall thickening, wood fibres, stone cells and compound starch grains. Patches of cork cells and parenchymatous cells are also observed.

Phytochemical Studies – The percentage of total ash, acid insoluble ash, alcohol soluble and water soluble extractives successive Soxhlet extraction values (in hexane, acetone, methanol and water) were determined. Experiments were carried out in triplicate and their mean values ± SD calculated. The results are tabulated in Table 1. The methanolic extract was subjected to qualitative phytochemical screening for the presence of phenolics, flavonoids, terpenoids, steroids, saponins, alkaloids and free sugars. It was observed that mainly terpenoids, phenolics and saponins were present in the extract (Table 2). The TLC fingerprint profile was documented under visible light and the R_f values, colour of the bands and relative percentage of the different bands were recorded by scanning the chromatogram at 400nm after post chromatographic derivatization with ceric sulphate (Table 3, Fig. 4). A total of 10 bands were obtained out of which 7 were major.

From the ongoing studies, it can thus be concluded that the above pharmacognostical characteristics *viz.* physiochemical parameters, HPTLC fingerprint profiles and the microscopic characters, together, may be utilized in identifying *P. herbacea* and in differenciating it from other species which are similar to it or are used as its adulterants/ substitutes under the same common name of Bharangi.

Acknowledgements

The authors are thankful to Ministry of Environment, Government of India for funding this research work. They

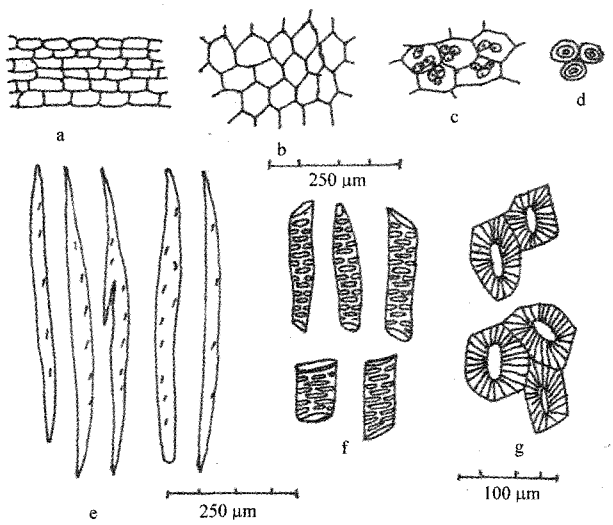


Fig. 3. Microscopic examination of powdered root of *P. herbacea.* **Abbreviations:** a-cork cells, b-parenchymatous cells, c and d-starch grains, e-fibres, f-vessels, g-stone cells

Table 1. – Quantitative standards for *P. herbacea*

S. No	Parameter		Values of 3 Replicates (%)	Mean ± SD
1.	Ash Values	Total ash	7.75	7.88 ± 0.175
			8.10	
			7.95	
		Acid insoluble ash	2.30	2.25 ± 0.233
			2.00	
			2.46	
2.	Extractive values	Alcohol soluble	19.00	18.91 ± 0.381
			18.50	
			19.25	
		Water soluble	23.50	23.76 ± 0.461
			23.50	
			24.30	
3.	Successive	*n*-Hexane soluble	8.44	7.69 ± 0.657
	Soxhlet		7.20	
	Extraction		7.44	
		Acetone soluble	4.68	4.63 ± 0.117
			4.72	
			4.50	
		Methanol soluble	14.02	13.81 ± 0.200
			13.62	
			13.80	
		Water soluble	4.56	4.77 ± 0.220
			5.00	
			4.76	

Table 2. Phytochemical screening of methanolic extract of *P. herbacea*

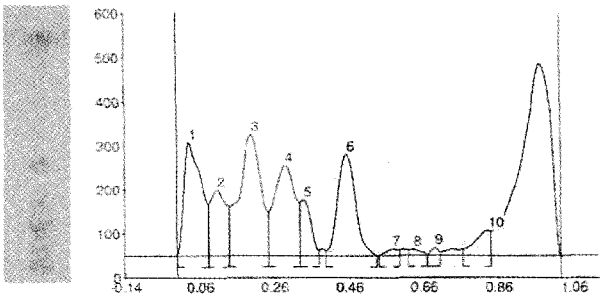
Group	Containing
Phenolics	+
Flavonoids	–
Terpenoids	+
Steroids	–
Saponins	+
Alkaloids	–
Sugars	–

++ present ; – absent

are also thankful to Mr. Sanjay Srivastava for technical assistance.

Table 3. TLC details of *P. herbacea* by scan ning after derivatization with ceric sulphate (400 nm)

R_f(Colour)	Relative Percentage
0.03 (blackish)	18.77
0.11 (blackish)	9.66
0.20 (blackish)	25.31
0.29 (blackish)	17.65
0.34 (blackish)	5.56
0.45 (blackish)	17.21
0.58 (blackish)	0.78
0.64 (blackish)	0.69
0.69 (blackish)	0.54
0.84 (blackish)	3.83



A **B**

Fig. 4. HPTLC finger print profile of methanolic extract of *P. herbacea*
A. Under visible light after derivatization
B. Densitometric scanning at 400 nm after derivatization

References

Anonymous, Indian Pharmacopoeia, 2nd Edn. Government of India, New Delhi. (1966).

Anonymous, *The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products* (CSIR, New Delhi) **8**, 239 (1950).

Anonymous, The Useful Plants of India, NISCOM (CSIR, New Delhi), 489 (1986).

Goel, A.K., Sahoo, A.K., and Mudgal, V.A., Contribution to the ethnobotany of Santhal Pargana. *Bihar Bot. Surv. India*, Howrah (1984).

Gopalan, S., Krishnamurthy, R., and Narayanan, C., Structure of sirutekkone, a diterpenoid from *Premna herbacea*. *Phytochem.* **27**, 2249-2250 (1988).

Johansen, D.A., *Plant Microtechnique,* 1st Edn. Mc Graw Hill Book Co. Inc, New York and London, 1940.

Kokate, C.K., *Practical Pharmacognosy.* Vallabh Prakashan, Delhi, India, pp. 15-29 (1986).

Maheshwari, J.K., Singh, K.K., and Saha, S., Ethnomedicinal uses of plants by Tharus in Kheri district U. P. *Bull. Medico-*

ethnobot. Res. **1**, 318-337 (1980).

Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Reddy, M.K., Vijayasekaran, V., and Sukumar, E., Antipyretic, antinociceptive and anti-inflammatory activity of *Premna herbacea* roots. *Fitoterapia* **71**, 147-153 (2000).

Narayanan, N., Thirugnanasambantham, P., Viswanathan, S., Sukumar, E., and Jeyaraman, P., Pharmacognostical studies on the roots of *Clerodendrum serratum*. *Pharmaceutical Biol.* **40**, 362-368 (2002).

Nayar, R.C., Yoganarasimhan, S.N., and Subramanyam, K., Pharmacognosy of a local market sample of Bharangi- *Pygmaeopremna herbacea* (Roxb.) Moldenke. *Indian J. Pharm.* **38**, 39-42 (1976).

Prakash, A. and Singh, K.K., Observations on some high valued ethnomedicinal plants among the tribals of Uttar Pradesh. *J. Med. Arom. Plant Sci.*, **22-23**, 519-521 (2000-2001).

Prakash, A. and Singh, K.K., Observations on some threatened plants and their conservation in Rajaji National Park in Uttaranchal, India. *J. Eco. Tax. Bot.*, **25**, 363-366 (2001).

Qingchang, M., Naijue, Z., and Weixin, C.A diterpenoid with a new carbon skeleton from *Pygmaeopremna herbacea*. *Phytochem.* **27**, 1151-1152 (1988a).

Qingchang, M. and Weixin, C., Pygmaeoherin: novel coumarin from *Pygmaeopremna herbacea*. *Planta Med.* **54**, 48-49 (1988b).

Saxena, H.O., Observations on the ethnobotany of Madhya Pradesh. *Bull. Bot. Surv. India*, **28**, 149-156 (1986).

Weixin, C., Two novel diterpenoids from *Pygmaeopremna herbacea*. *J. Nat. Prod.* **52**, 581-587 (1989).

(Accepted April 28, 2005)