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Pharmacognostical, phytochemical investigations and HPTLC fingerprinting of *Pentapetes phoenicea* L. leaves

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Pentapetes phoenicea L., commonly known as Dopa-hariya in Hindi, is used traditionally in the treatment of many diseases by different system of primitive medicine. The decoction of capsule is used as an emollient. The roots are said to be astringent and used as antibilious, antiphlegmonous, alleviates wind and fever, constipation diarrhoea, burning sensation, psychopathy, vitiated conditions of vata and pitta. Leaves boiled in water and juice of the leaves has been used traditionally for treatment of glandular inflammation, cold and cough. The fruits are mucilaginous and used in gastropathy, fever and vitiated conditions of vata and pitta. The plant has not been explored scientifically for its pharmacological or for pharmacognostical details. Therefore, the study of morpho-anatomical characters and physicochemical analysis of *P. phoenicea* was undertaken to establish the pharmacognostic and phytochemical details about the plant. Morpho-anatomical studies of leaf showed the presence of simple leaf, length 4 to 10 cm, linear to oblong in shape and anisocytic stomata, thin walled parenchymatous cells, scattered, sclerenchymatous vascular bundles as some of the diagnostic features in T.S. of leaf. Physicochemical standardization of leaf showed the presence of 0.1 % foreign matter, 10.2% loss on drying, 25.83% total ash, 12.35% alcohol and 21.26% water soluble extractives. Preliminary phytochemical screening of leaf extract confirmed the presence of tannins, flavonoids, saponins, sterol, carbohydrate and traces of alkaloids. HPTLC of hydro-alcoholic extract of plant leaves tried with solvent system chloroform and methanol (9:1) confirmed the presence of 07 spots with different R_f value under U.V. light 366λ. The results obtained from preliminary evaluation on the plant can be utilized as a basis for anatomical identification and preparation of monograph of the plant.

Keywords: *Pentapetes phoenicea*, Sterculiaceae, Morpho-anatomy, Transverse section, Physicochemical, Quantitative microscopy.

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Introduction

Pentapetes phoenicea L. (Family-Sterculiaceae), commonly known as Dopa-hariya in Hindi, an annual erect herb, up to 1.5 m tall, leaves are hastate, lanceolate or oblong. Flowers are red 1-2, axillary; Fruits are capsule, subglobose; seeds subglobose, dotted. The capsules are mucilaginous and used for treatment of diseases of bowels. The decoction is used as an emollient. The roots are said to be astringent and used as antibilious, antiphlegmonous and alleviates wind and fever[1]. Leaves boiled in water and juice of the leaves has been used traditionally for treatment of glandular inflammation, cold and cough[2].

The fruits are mucilaginous and used in gastropathy, fever and vitiated conditions of vata and pitta[3]. Root decoction is administered for treatment of burning micturition[4]. The plants may be cultivated in the rainy season, following flowering after a month and fruiting within ten days.

However, the available literature reveals that no anatomical and physicochemical studies have been carried out on *P. phoenicea.* Therefore, the present investigation was under taken with the aim to determine morpho-anatomical, physicochemical parameters such as foreign matter, loss on drying, total ash, acid insoluble ash, water and alcohol insoluble extractive along with phytochemical studies that would serve as few of the basic protocols for standardization of medicinal plants and it can also be helpful in preparation of monograph of the plant.

Materials and Methods

Plant material

P. phoenicea leaves were collected from the local areas of Kanpur, India, during the month of September, 2011. The plant was authenticated and

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identified by taxonomist & voucher specimens (UIOP/M-1101) were preserved at the herbarium section of departmental museum of C.S.J.M. University, Kanpur for future reference.

Pharmacognostic studies

Macromorphology

Macroscopical evaluation was carried out by using dissecting microscope. The shape, apex, base, margin, taste and odor of leaves were determined as per the reported methods[5].

Microscopy

Histological evaluation was done by preparing thin hand sections of midrib of leaves. Cleaning of sections were done with chloral hydrate solution, treated with phloroglucinol and hydrochloric acid, and mounted with glycerin and as per the standard procedures. To identify the starch grains a separate section was prepared by staining with iodine solution.

To study the powder characteristics, powdered drug was separately treated with phloroglucinol and hydrochloric acid solution, glycerin and iodine solution to determine the presence of lignified cells, trichomes, calcium oxalate crystals, and starch grains[6-7]. The results obtained were recorded by taking photos with Olympus digital microscope assisted with 1/3" CCD Sony camera.

Quantitative microscopy

The fresh leaves of the plant were employed to determine stomatal number and index, vein islet termination number as per the reported procedures[5].

Physicochemical standardization

Physicochemical standardization of powdered material for various parameters such as foreign matter, loss on drying, total ash, acid insoluble ash, water soluble ash, water and alcohol soluble extractive, were carried and calculated as per the reported official methods and recommended procedures[7].

Preliminary phytochemical screening

Preliminary phytochemical screening of crude hydro-alcoholic extract of *P. phoenicea* was done by using the established procedures[5-6].

HPTLC fingerprint profile

To have quality standardization of material, HPTLC finger print profile of hydro-alcoholic extract of *P. phoenicea* leaves was developed. The HPTLC analysis was carried out on precoated Silica gel 60-F_{254} plate (Merck, India) by using Camag Linomat IV applicator. The plate was developed with mobile phase Chloroform: Methanol (9:1). The scanning of developed and dried plate was done densitometrically and peak area was recorded on a TLC scanner III at 366 nm using Wincat software (CAMAG, Switzerland).

Results

Macromorphological diagnosis

Macroscopically, *P. phoenicea* fresh leaf is green in color, simple, 4 to 15 cm long, hastate, lanceolate or oblong, with crenate margin, peltate; 1-2 flowers, umbrella shaped, 5 red petals, 5 sepals persistent, twisted, superior ovary, and raceme; Fruits are capsule, subglobose, seeds subglobose, dotted; Stem dark green, smooth, cylindrical, 2-4 mm in thickness; root light brown in color, conical to cylindrical, branched, about 2-3 mm in thickness.

Microscopical diagnosis

In microscopy of leaf lamina, single layer of palisade cells were observed below upper epidermis, confirms the dorsiventral type of leaf (Plate 1a).

Micro-morphological features revealed that the cells of the epidermis were slightly cuticularized. The upper epidermal cells are comparatively larger than lower one. The polygonal epidermal cells observed with slightly undulate anticlinal walls. The leaf showed the presence of anisocytic type of stomata (Plate 1b), abundant on the lower epidermis while upper epidermis showed comparatively less and mostly observed along the midrib region of the lamina.

The midrib (Plate 1a), in transverse section, is biconvex. Upper and lower epidermis layers continuous over the midrib, the shape of epidermal cells in the mid rib differs than that of in lamina region. Adjacent to the epidermis, angular collenchyma occur, comprising almost three to five rows on the ventral side and four to six on the dorsal side. The collateral vascular bundles arranged nearly as a closed arc showed lignified spiral xylem vessels and non lignified phloem as sieve tubes. Rhomboidal calcium oxalate crystals are found in the ground parenchymatous tissue (Plate 1c).

Powder microscopic characters

The leaf powder green in color; showing fragments of fibers, tracheids, with spiral reticulate and pitted vessels, calcium oxalate crystals, palisade cells, starch grains after treating with iodine, and epidermal cells with stomata in surface view (Plate 2).

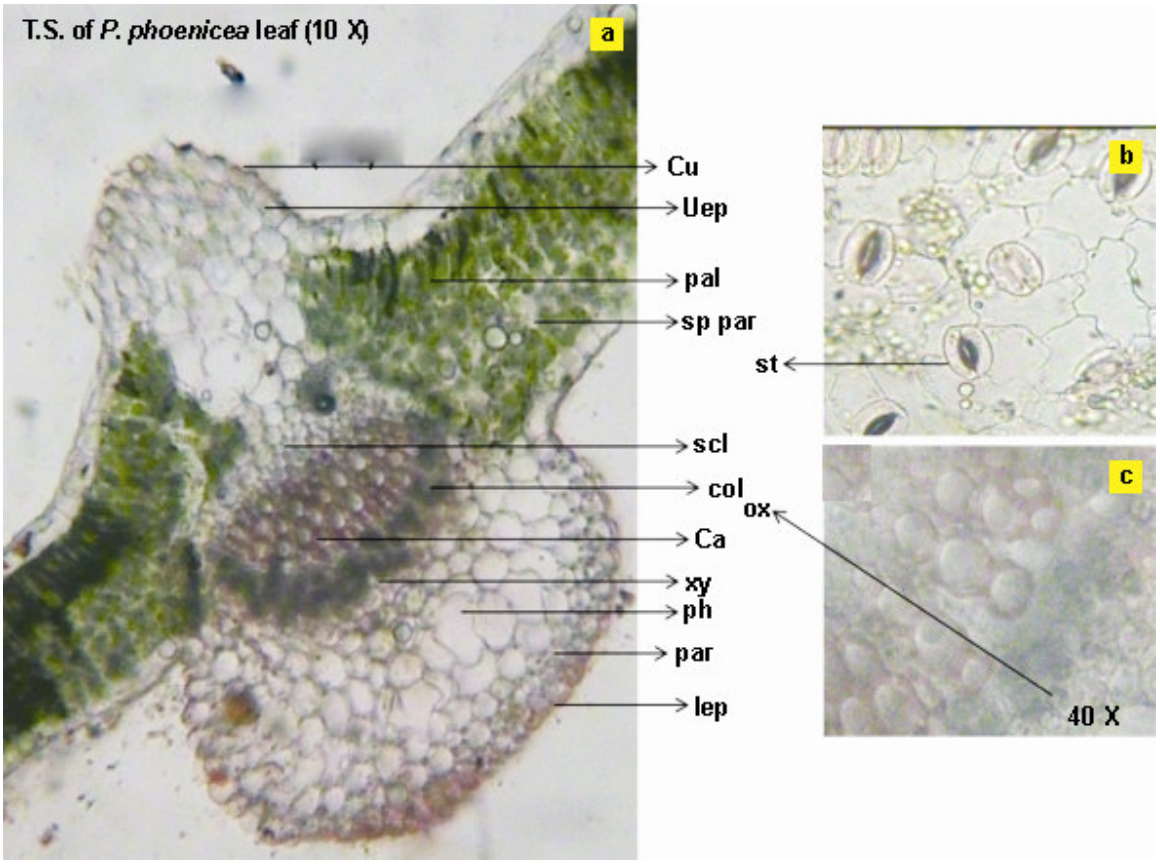

Plate 1-T.S. of *P. phoenicea* leaf (cu- cuticle, uep- upper epidermis, pal- palisade cells, sp par- spongy parenchyma, scl- schlerenchyma, xy- xylem, ph- phloem, par- parenchyma, col- collenchymatous cells, lep- lower epidermis, st- stomata cal ox- calcium oxalate)

Plate 2- Powder characters of *P. phoenicea* leaf (xy trach- xylem tracheids, cal ox- calcium oxalate, ep with st- fragments of epidermis with stomata)

Quantitative microscopy

The results of quantitative microscopy showed stomatal number 18 and 46 and stomatal index 18.18 and 21.43 for upper and lower epidermis, respectively. Palisade cells 10 cells per epidermis; Vein islet and vein termination number 6 and 4, respectively.

Physicochemical parameter

The results of physicochemical parameters such as percentage of foreign matter, loss on drying, ash values, and extractive values are shown in Table 1.

Preliminary phytochemical screening

Crude hydro-alcoholic extract and various fractions of *P. phoenicea* leaves were qualitatively examined for the major phytoconstituents confirmed the presence of tannin, flavonoid, saponin, sterol, carbohydrate and traces of alkaloid). Results are shown in Table 2.

Table 1— Physicochemical analysis of *P. phoenicea* leaf

Ash Values/Extractive Values	% w/w
Ash Values	
Total Ash	25.83
Acid insoluble Ash	5.016
Water soluble Ash	10.32
Extractive Values	
Petroleum Ether 60-80°C	0.5436
Chloroform	2.9549
Ethyl acetate	1.865
Alcohol	12.35
Water soluble	21.26

HPTLC Fingerprint Profile

A densitometric HPTLC analysis carried for the development of specific finger print profile for hydro-alcohol extract of leaves exhibited seven bands in the sample at R_f 0.02, 0.21, 0.47, 0.52, 0.56, 0.59 and 0.78, (Plate 3) with most prominent spot of maximum area at R_f 0.47, which can be used as identifying marker.

Sample preparation-10 mg/ml; Application-Linomat 5 Applicator (Camag); Solvent System-Chloroform: Methanol (9:1); TLC plate Development-Presaturated Camag Twin Trough Chamber

Table 2— Phytochemical analysis of *P. phoenicea* leaf extract

Phytochemical group	Chloroform fraction	Methanol fraction	Ethyl acetate fraction	Hexane fraction	Aqueous fraction
Alkaloids	++	+	-	-	- -
Carbohydrates	+	+++	+++	-	++
Glycosides	-	++	+	-	-
Flavonoids	-	++	++	-	-
Saponins	-	+++	-	-	+++
Tannins	-	+++	+++	-	++
Proteins and amino acids	-	-	-	-	-
Steroids	++	+	++	+++	-
Triterpenoids	++	+	++	+++	-
Fats and fixed oil	-	-	-	+	-

(+) Present (-) Absent

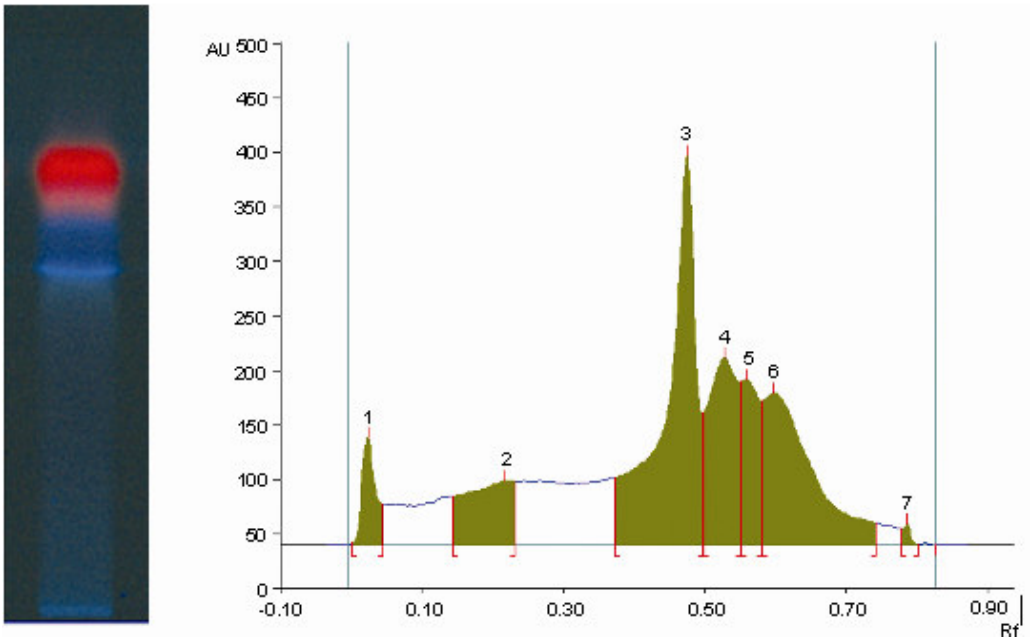

Plate 3- Qualitative analysis of hydro-alcoholic extract of leaves extract

Discussion

This is the first report on pharmacognostical studies of plant *P. phoenicea*. Standardization is an important tool in identifying crude drug correctly. For establishing the correct identity of source materials, microscopic method is one of the simplest and cheapest methods to start with[8-10]. Therefore, the results of present study, may serve as a basis for identification, collection and standardization of the plant. Microscopical study of leaf showed the presence of abundant rhomboidal calcium oxalate crystals, anisocytic stomata, schelernchymatous cells, cuticle, collenchymatous cells. Evaluating the physicochemical parameters like ash values of drug, gives an idea about the earthy matter and other impurities which might be present along with drug. Determination of Extractive values may primarily be useful for the identification of exhausted and adulterated drugs[11-12].

Physico-chemical parameters of leaves showed loss on drying 10.2 %, total ash 25.83%, acid insoluble ash 5.016%, and water soluble ash 10.32%. During the last two decades developing the HPTLC fingerprinting profile of herbal drugs, has gained much importance for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations[13]. Developing HPTLC fingerprint profile along with their R_f values, could serve as a reference standard for the researchers engaged in investigation of medicinal properties of plants. Qualitative tests carried on leaf extract and its various fractions confirmed the presence of various pharmacologically important plant constituents like tannins, flavonoids, steroids, terpenoids, phenols, saponins, carbohydrates and trace alkaloids. Saponins have been reported to exhibit wide-range of cytostatic effects against cancerous cells[14]. The saponins have the capacity to lower the serum cholesterol level of animals[15-16]. Alkaloids rich fractions show hypoglycemic effect[17], antitussive, expectorant, anti-inflammatory[18], antiprotozoal, antimicrobial, and antitumor[19]. Tannins have shown antiulcer[20], vasorelaxant, hypotensive[21], antioxidant[22], antimicrobial and antiviral[23] effects.

Conclusion

The *P. phoenicea* leaves possess constituents of tremendous potential for the prevention and treatment of various ailments that are yet to be explored. Various morpho-anatomical, physicochemical studies, reported for the first time in this paper may serve as diagnostic tool for identification and could be used in the preparation of a monograph on this plant.

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