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Phamacognostical Evaluation of *Phyllanthus reticulatus* Poir.

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ABSTRACT
This paper deals with the detailed pharmacognostical evaluation of the crude drug *Phyllanthus reticulatus* Poir. (Euphrobiaceae). Morphoanatomy of the entire plant have been studied with the aim to aid pharmacognostic and taxonomic species identification 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. reticulatus* and can possibly help to differentiate the drug from its other species.
Keywords – ß-sitosterol, Euphrobiaceae, HPTLC, Pharmacognosy, *Phyllanthus reticulatus*

INTRODUCTION

Phyllanthus reticulatus Poir. (Euphrobiaceae) commonly known as pancoli or karineli is a large glabrous or pubescent shrub with smooth or lenticellate branches; leaves elliptic to oblong or obovate, fruit a purplish black berry (1,2). The plant grows throughout India, in hedges or waste places. The plant is astringent, sweet, cooling, diuretic and constipating. It is useful in vitiated conditions of *pitta*, burning sensation, ophthalmodynia, diarrhoea, skin eruption and obesity (3). Decoction of bark is used as astringent, diuretic and alternative (4). The juice of leaves is used medicinally in the Konkan. It is made into a pill with camphor and cubebs, which is allowed to dissolve in the mouth as a remedy for bleeding gums (2). In Indo-china, the whole plant is used in the treatment of small pox and syphilis. The fruit is said to be eaten in times of scarcity in E. Africa. An ink is prepared from ripe fruits in the Philippines. The roots are used in Madras as a red dye (5).

In spite of the numerous medicinal uses attributed to this plant, pharmacognosy information about this plant has not been published. Hence, the present investigation is an attempt in this direction and includes morphological and anatomical evaluation, determination of physico-chemical constants and the preliminary phytochemical screening of the methanolic extract of *P. reticulatus*.

MATERIALS AND METHODS

Plant material – The plant *P. reticulatus* was collected from river side, in Udupi district, Karnataka, India during the month of August, 2007. The botanical identity of the plant was confirmed by Dr. Gopalakrishna Bhat, Botanist, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP-561) 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 camara. Solvents *viz.* petroleum ether, benzene, chloroform, acetone, ethanol (95%), n-butanol and reagents *viz.* phloroglucinol, glycerine, 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 different parts like leaf, stem and root were studied according to the method of Brain and turner (1975a) (6). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (1940) (7). The micropowder analysis was done according to the

method of Brain and Turner (1975b) (8) and Kokate (1986a) (9).

Physico-chemical analysis - Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed (10) and the WHO guidelines on the quality control methods for medicinal plant materials (11). Fluorescence analysis was carried out according to the method of Chase and Pratt (1994) (12) and Kokoski *et al*. (1958) (13).

Preliminary phytochemical screening - Preliminary phytochemical screening was carried out by using standard procedures described by Kokate (1986b) (14) and Harborne (1998) (15).

HPTLC studies - Qualitative densitometric HPTLC analysis was performed to confirm the presence of ß-sitosterol in methanolic extract of entire plant by co-chromatography with authentic sample (Sigma Chemicals, USA). Quantification of ß-sitosterol was carried out for the methanolic extract of whole plant. The linearity of the HPTLC method was investigated for ß-sitosterol in the range of 100-1000 µg/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 (8: 2). After elution the plate was sprayed with 10% methanolic H_2SO_4, heated at 105 °C for 5 min and scanned densitometrically using Camag TLC scanner 3 at 400 nm. The percentage of ß-sitosterol in the methanolic extract was calculated by calibration using peak height and peak area ratio.

RESULTS AND DISCUSSION

Macroscopic characters of the plant - The plant is an erect or straggling shrub. The branches often slender and drooping, leaves are elliptic-orbicular, rounded at both ends, glabrescent and up to 3 cm long. Petioles are up to 3 mm long, stipules minute. Flowers are in axillary fascicles, pedicels filiform. Calyx lobes are 5 with inner 3 are often larger. Stamens are 5 and it arranged in 2 series, the outer short and free whle the inner 3 slightly larger and connate. Ovary is 5 - many-celled, ovules 2 in each cell and superposed. Fruits are baccate and 6 mm in diameter. It becomes purple-black after ripening. Root is cylindrical, slightly tapering, branched and shows fibrous fracture, 4-8 cm long and 3-7 mm in thickness. The inner wood is yellowish brown in color.

Microscopic characters

Transverse section of leaf - The leaf has thick lamina and prominent midrib. The lamina is uniformly smooth and even. Leaf is dorsi-ventral in nature showing three layers. The upper epidermis consists of a single layer of rectangular cells. Anisocytic type of stomata and uniseriate multi-cellular covering trichomes are seen in the epidermal layer. Below the upper epidermis, is a mesophyll tissue showing three layers of palisade cells and spongy parenchyma. Thin vascular strands of xylem elements are also seen in the section. The lower epidermis is similar to upper epidermis in its appearance.

The epidermal layers are continued even in the midrib. A patch of 5-7 layers of collenchyma are present below the upper epidermis and above the lower epidermis. The vascular bundles are collateral having xylem towards the ventral side and a pad of phloem on the dorsal side. Few layers of parenchyma encircle the vascular tissue are seen in the ground tissue region (Fig. 1A).

Transverse section of stem - The stem is circular in cross-sectional view consists of epidermis having a single layer of cells containing brownish matter. The epidermal layer also covered with full of covering trichomes. The cortex is filled with parenchyma cells whereas the cells are collenchymatous towards the periphery. The vascular cylinder composed of a thin patch of phloem covering the xylem elements. On the outer surface of the phloem cylinder is a thin discontinuous layer of bast fibres which crown the vascular bundles. The central pith if filled with thin walled parenchyma cells (Fig. 1B and 1C).

Transverse section of root - The root shows the presence of periderm which consists of 3-5 layers of phloem containing brownish matter. The phellogen shows 2 rows of cells encircling the single layer of phelloderm. The cortex consists of 4-6 layers of tangentially oblong and radially compressed parenchyma cells. Some of the parenchyma cells contain rhomboidal prism type of calcium oxalate crystals. The secondary phloem consists of small groups of sieve elements which are intervened by the medullary rays. The central cylindrical secondary xylem is lignified and consists of xylem elements such as parenchyma, vessels and fibres. Cambium separates the xylem and phloem region. Medullary rays in the phloem region are non-lignified whereas lignified in the xylem region (Fig. 1D, 1E).

Powder characters - The leaf powder is dark green in colour with an unpleasant odour. On microscopical examination the powder showed multi cellular (1-3 celled) covering trichomes (Fig. 2A). Anisocytic types of stomata (Fig. 2B) surrounded with irregularly shaped epidermal cells are seen. The stomata occur on the

Table 1: Preliminary phytochemical screening of the entire plant powder of P. reticulatus

Test	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	+	-
Carbohdrates	-	-	-	-	+	+
Phytosterols	+	+	+	-	-	-
Terpenes	+	+	+	-	-	-
Fixed oil and fats	-	-	-	-	-	-
Saponin	-	-	-	-	-	-
Phenolic compounds and tannins	-	-	-	-	+	+
Flavonoids	-	-	-	-	+	+
Gums and mucilage	-	-	-	-	-	+

+ Denotes the presence of the respective class of compounds

Table 2: Ash values of the entire plant powder of P. reticulatus

Parameters	Values %(w/w)
Total ash	3.86
Acid insoluble ash	1.42
Water soluble ash	3.12
Sulphated ash	3.0

Table 3: Extractive values of the entire plant powder of P. reticulatus

Parameters	Values %(w/w)
Water soluble extractive	7.89
Ethanol soluble extractive	4.56
Ether soluble extractive	2.37

Table 4: Fluorescence analysis of the entire plant powder of P. reticulatus

Treatment	Day light	UV light (254 nm)
Powder as such	Straw color	Light green
Powder + 1N NaOH (Aq.)	Brown	Dark brown
Powder + 1N NaOH (Alc.)	Yellowish brown	Light yellow
Powder + 1N HCL	Slight turbidity	Light green
Powder + Ammonia	Yellowish brown	Greenish yellow
Powder + Iodine	Dark brown	Brown
Powder + 5% $FeCl_3$	Dark yellowish brown	Dark brown
Powder + 1N H_2SO_4	Blackish brown	No color
Powder + Acetic acid	Light brown	Orange
Powder + 1N HNO_3	Light brown	No color

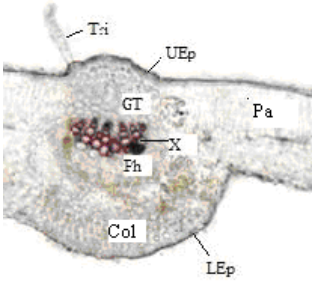

Fig. 1A

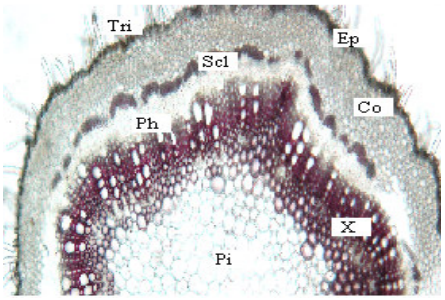

Fig. 1B

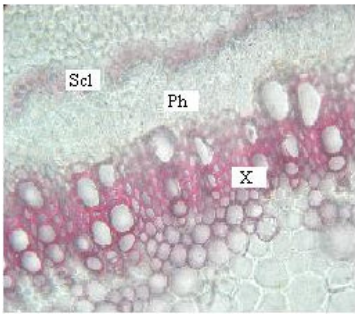

Fig. 1C

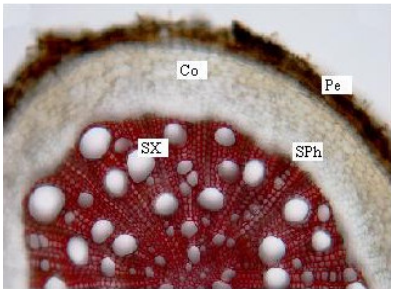

Fig 1D

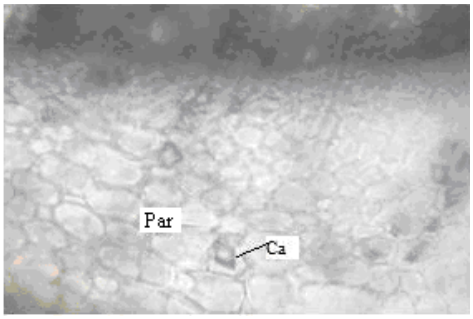

Fig. 1E

Fig 1.:Histology of leaf, stem, and root

Fig. 1A: T.S. of leaf through midrib with lamina (UEp-Upper epidermis; Tri-Trichom, GT-Ground tissue; X-Xylem; Ph-Phloem; Col- collenchyma; Pal-Palisade cell LEp-Lower epidermis)

Fig. 1B: T.S. of stem (Ep-Epidermis; Tri-Trichom; Co-Cortex; Scl-Sclrenchyma; X-Xylem; Ph-Phloem; Pi-Pith)

Fig. 1C: T.S of secondary phloem and secondary xylem in stem (Ph-Phloem; Scl-Sclrenchyma; X-Xylem)

Fig. 1D: T.S. of root (Pe-Periderm; Co-Cortex; SPh-Secondary phloem; SX-Secondary xylem)

Fig. 1E: T.S. of root showing Calcium oxalate crystals (Ca-Calcium oxalate crystals; Par- parenchyma cells)

Fig. 2A

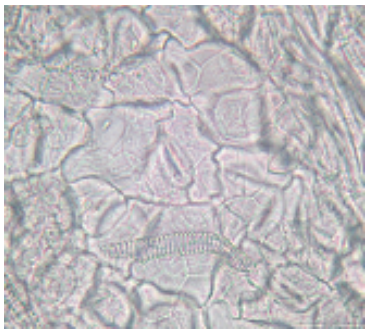

Fig. 2B

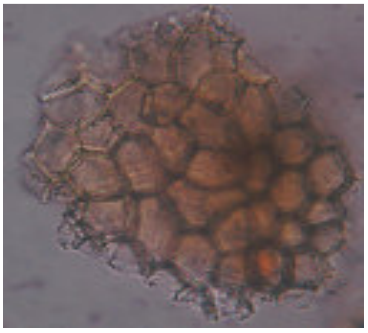

Fig. 2C

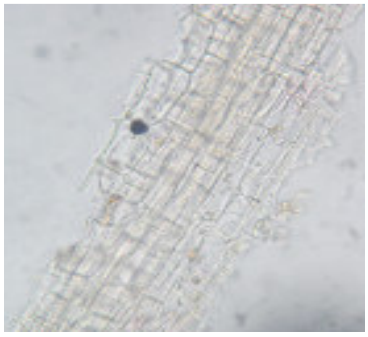

Fig. 2 D

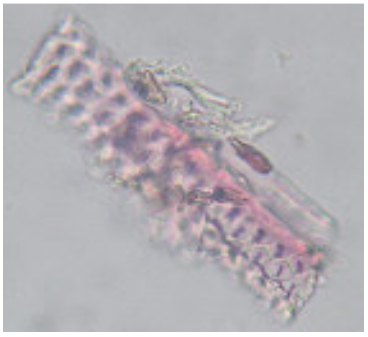

Fig. 2E

Fig. 2F

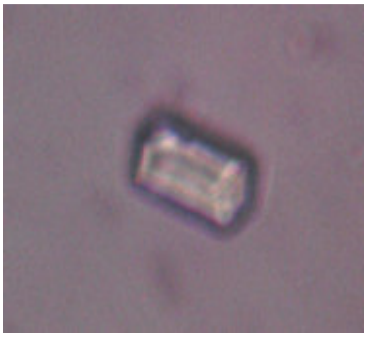

Fig. 2G

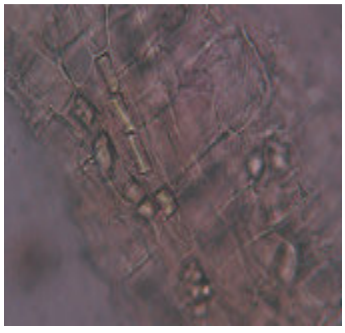

Fig. 2H

Fig. 2. Powder microscopy

A: Trichome, B: Stomata, C: Cork cells, D: Parenchymatous cells, E: Fibres and vessel, F: Lignified fibres, G and H: Prism ca. oxalate crystal

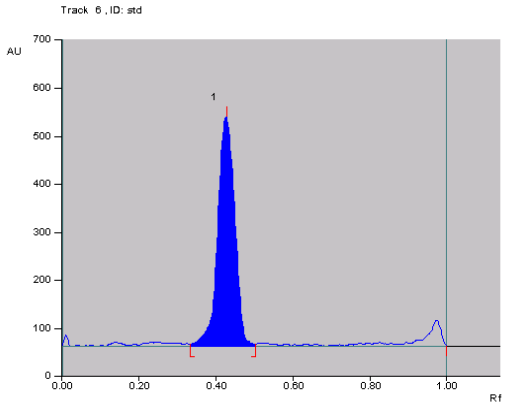

Fig. 3A

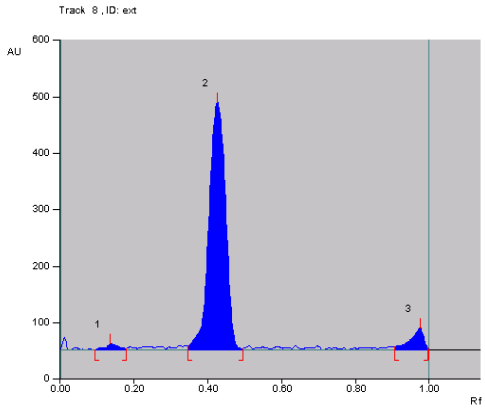

Fig. 3B

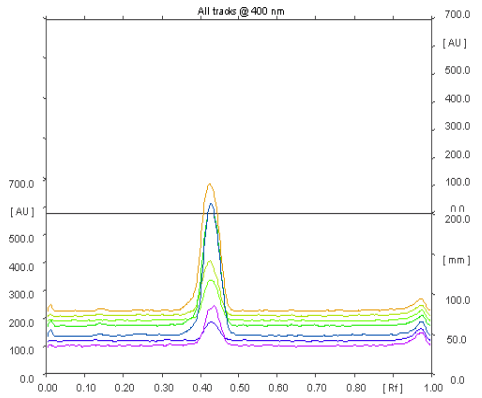

Fig. 3C

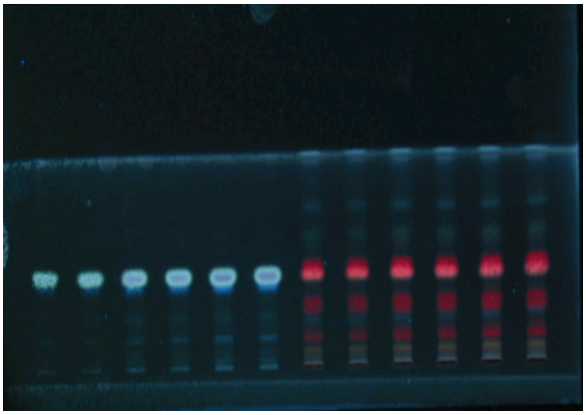

Fig. 3D

Fig. 3. HPTLC profile of methanolic extract of P. reticulatus and ß-sitosterol reference
A: ß-sitosterol reference, B: methanolic extract, C: 3D graph, D: TLC plate

lower epidermis, the common wall of the two bigger subsidiary cells and one small cell are seen. The guard cells are ellipsoidal in shape.

The stem powder is light greenish brown in colour with a characteristic odour. Thick walled brownish cork cells (Fig. 2C) are seen. The parenchymatous cells (Fig. 2D) are globular or rectangular in shape and thin walled.

The root powder is slightly yellowish brown in colour with a characteristic odour. The vascular elements with bordered pits are seen separately or found in association with lignified fibres (Fig. 2E). The fibres (Fig. 2F) measure 420-720 µm in length and 35-50 µm in thickness. Prism calcium oxalate (Fig. 2G and 2H) is observed 20-30 µm in diameter.

Preliminary phytochemical screening - Preliminary phytochemical screening revealed the presence of terpenes, phytosterols, phenolic compounds, carbohydrates, flavonoids and minute amount of alkaloids (Table 1).

Physico-chemical studies - Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash, sulphated ash and water soluble ash are carried out (Table 2). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble, alcohol soluble and ether soluble extractive values have been tabulated in Table 3. The results of fluorescence analysis of the drug powder are presented in Table 4.

HPTLC studies - A qualitative densitometric HPTLC analysis was performed to confirm the presence of ß-sitosterol in methanolic extract of entire plant. ß-sitosterol (R_f – 0.43) content in the entire plant drug (Fig. 3) was found to be 0.47% w/w.

CONCLUSION

The present study on pharmacognostical evaluation of *P. reticulatus* will provide useful information for its identification. Macro, micro and physiochemical standards discussed here can be considered as the

identifying parameters to substantiate and authenticate the drug.

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