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Research Article

Pharmacognostical Evaluation of Leaves of *Parkia biglandulosa* Wight & Arn.: An Important Ethnomedicinal Plant

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ABSTRACT
The aim of the current study was to evaluate pharmacognostic characters of leaves of *Parkia biglandulosa* Wight & Arn. The evaluation was carried out in terms of macroscopic, microscopic, physicochemical, phytochemical and chromatographic analysis.Leaves of *P. biglandulosa* showed the presence of circular dark brown gland at its base on either side of rachis. Microscopically, transverse section of leaflet and rachis showed unicellular trichomes. The total ash, acid insoluble ash and water soluble ash content was found to be 5.158 %, 0.915 %, 4.689 % respectively. Impact of regional variation on the content of β-sitosterol in the samples collected from different regions was clearly evident using HPTLC and HPLC. Findings of the current study will aid in the correct identification and standardization of leaves of *P. biglandulosa*.

Key words: Chromatography, microscopy, *Parkia biglandulosa* Wight & Arn., physicochemical, phytochemical

INTRODUCTION

Pharmacognosy, in recent years has gained immense importance as it is an efficient tool for the authentication and identification of plant raw materials and therefore evaluation of pharmacognostic parameters is an indispensible step when dealing with herbal drugs [1]. Recently, many medicinal plants namely *Urtica dioca* [1], *Pongamia pinnata* [2], *Brunfelsia americana* [3], *Crotolaria burhia* [4], etc have been studied extensively for the development of their pharmacognostic profile. Inspite of this, there are many plants which have not gained the attention of scientists in terms of their standardization, phytoconstituents and pharmacological studies. *P. biglandulosa* is one such plant. As other species of *Parkia* viz. *P. biglobosa*, *P. bicolor* and *P. roxhburghii* are used traditionally as foods, medicinal agents and are of high commercial value in many countries [5], they have been studied to some extent, but so far, there are very few reports on *P. biglandulosa* in the literature. A thorough literature survey revealed that the stem bark [6,7], seeds [8, 9, 10, 11], flowers and pods [10] of this plant have been extensively explored in terms of the bioactive markers present as well as various therapeutic activities, but the leaves of this plant have not been evaluated in terms of their pharmacognostic profile.

Hence in this research work, an attempt has been made to standardize leaves of *P. biglandulosa* in order to assure its authenticity and purity for further exploitation of its safety and efficacy. Standardization of leaves of *P. biglandulosa* has been carried out in terms of macroscopy, microscopy, physicochemical, phytochemical and chromatographic analysis.

MATERIALS AND METHODS

Chemicals and reagents- β-sitosterol (98 % purity, Figure 1) was procured from Sigma Aldrich Chemical Company, (Steinheim, Germany). Chemicals of analytical grade were purchased from Merck Specialties Private Limited, Mumbai.

Plant samples- Fresh leaves of *P. biglandulosa* were collected from different geographical regions of India. The taxonomic identification of a representative sample was confirmed by Agharkar Research Institute, Pune (Voucher specimen no. Auth 11-124). The plant samples were shade dried for a week followed by their incubation at 45^0 C, powdered in a mixer grinder, sieved through 85 mesh (BSS) and stored in an air tight containers.

Macrosopy- Various characteristic features of fresh leaves such as type of leaf base, presence or absence of petiole and characters of lamina (venation, margin, apex, base, surface and texture) were studied.

Microscopy- Thin transverse sections of leaflets and rachis were taken, stained with dilute safranin and observed under 100x magnification using Leica light microscope conjugated with digital camera. Powder of the dried leaves was also evaluated microscopically.

Physicochemical evaluation- The quality of the leaves was assessed by determining the proximate parameters like foreign organic matter, ash content, extractive values and loss on drying using standard pharmacopoeial methods [12, 13, 14].

Phytochemical and heavy metal analysis- The powder of dried leaves of *P. biglandulosa* was subjected to phytochemical evaluation by successively soxhlet extraction with various organic solvents in order to analyze the percent extract of major class of compounds

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present in the plant raw material as per the method reported [15]. The leaf powder was also evaluated for the presence of four heavy metals namely Lead, Arsenic, Cadmium and Mercury (as recommended by AYUSH) using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) technique [16].

Figure 1: Structure of β-sitosterol

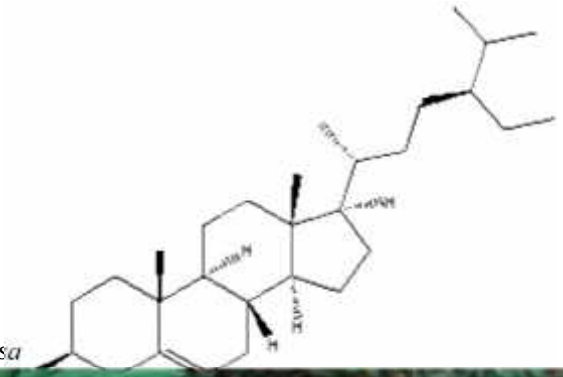
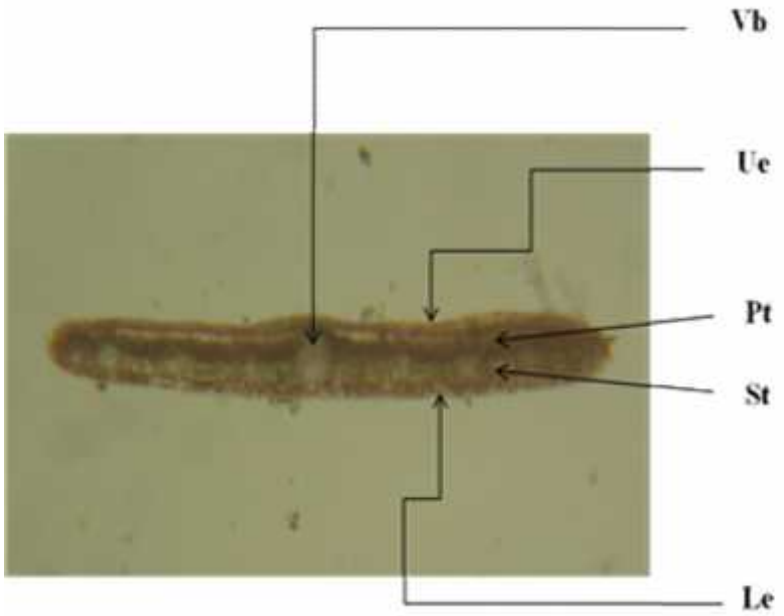


Figure 2: Leaves of *P. biglandulosa*

Figure 3: Transverse section of leaflet showing epidermis, mesophyll tissue and vascular bundles

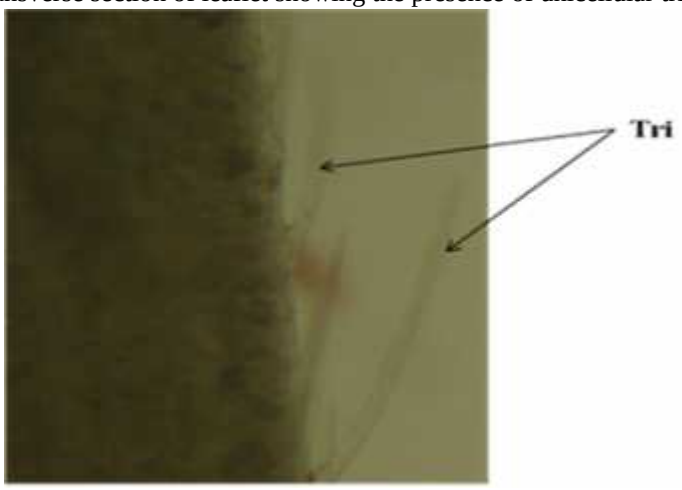


Abbreviations: Vb: *Vascular bundle;* ***Ue:*** *Upper epidermis;* ***Pt:*** *Palisade tissue;* ***St:*** *Spongy tissue;* ***Le:*** *Lower epidermis.*

Extraction of phytochemical constituents from P. biglandulosa - Extraction of phytochemical constituents from *P. biglandulosa* was carried out separately for HPTLC and HPLC analysis. For HPTLC, the powdered sample (0.2 g) was extracted with methanol (5 mL), vortex mixed for 1 min and kept standing overnight followed by filtration through Whatman filter paper No. 1. The filtrates were subjected to HPTLC analysis.

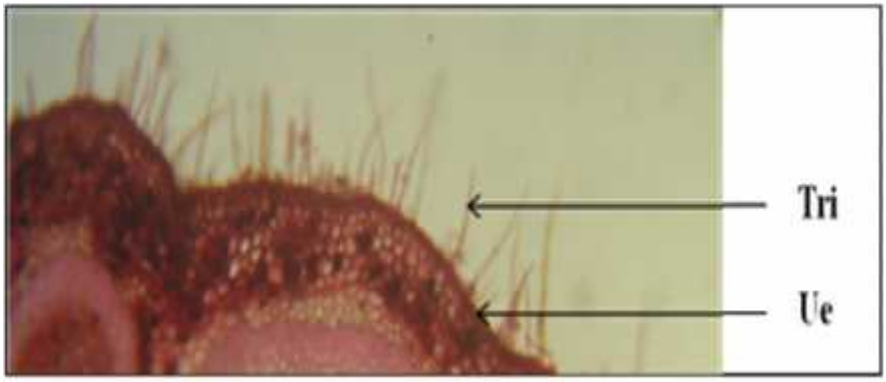
For HPLC, the powdered sample of same weight was

Figure 4: Transverse section of leaflet showing the presence of unicellular trichomes



Abbreviations: Tri: Trichome

Figure 5: Transverse section of rachis showing trichomes on upper epidermis



Abbreviations: Tri: Trichomes; Ue: Upper epidermis; Hyp: Hypodermis; Cor: Cortex.

extracted in petroleum ether (5 mL), vortex mixed for 1 min and kept standing overnight followed by filtration through Whatman filter paper No. 41. The filtrates obtained were evaporated to dryness, reconstituted in equal amount of methanol, vortex mixed for 1 min and filtered through nylon micro filter paper (0.45 µm) prior to their injection into HPLC system.

Chromatographic evaluation- Leaf powder of *P. biglandulosa* was also subjected to chromatographic evaluation using HPTLC and HPLC techniques wherein the content of pharmacologically active marker – β-sitosterol was evaluated for the samples collected from different regions using validated chromatographic methods [17].

Optimized instrumental and chromatographic conditions

HPTLC

- Stationary phase: Silica gel 60 F_{254} TLC pre-coated plates (Merck)
- Mobile phase: Toluene: methanol (8:1, v/v)
- Sample application: Samples (10 µL) were spotted using CAMAG Linomat IV equipped with Hamilton syringe.
- Detection: The plate was derivatized using 10 % methanolic sulphuric acid and scanned at 366 nm using CAMAG Scanner II equipped with winCATS 3 software.
- Documentation: CAMAG Reprostar 3

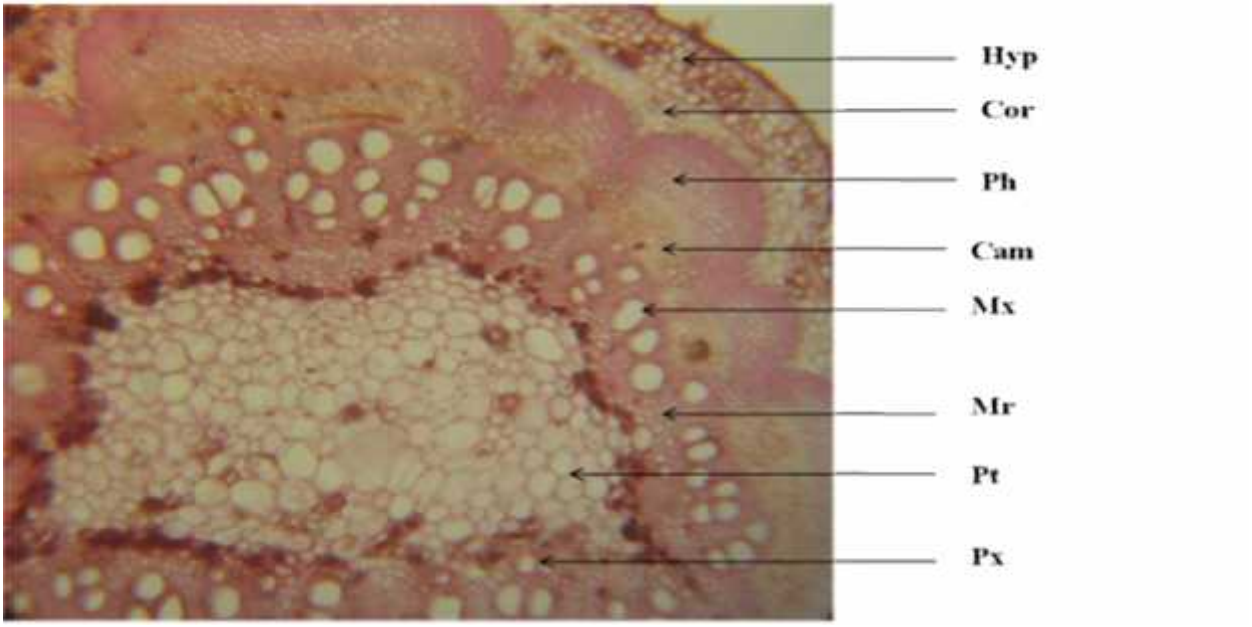
HPLC

- Stationary phase: Cosmosil C_{18}-column (150 mm x 4.6 mm, 5 µm)
- Mobile phase: Acetonitrile: ethanol (40:60, v/v)
- Instrument details: Jasco's HPLC system comprising of two PU- 1580 pumps (HG-1580-31), rheodyne injector (20 µL loop).
- Sample application: Samples (20 µL) were injected into HPLC system comprised of two pumps (Jasco PU-1580) with a solvent mixing module (Jasco HG-1580-31) and Rheodyne injector (20 µL loop).
- Detection: Samples were detected using Photo Diode Array detector (Jasco MD-1510) at 210 nm.
- Documentation: Chromatograms were recorded by means of Borwin chromatography software, version 1.50.

Statistical analysis- Microsoft Excel-2007 was used to determine mean, standard deviation (SD), relative standard deviation (RSD) and mean difference during the analysis.

RESULTS AND DISCUSSION

Figure 6: Transverse section of rachis showing hypodermis, cortex, cambium, vascular bundles and pith



Abbreviations: Hyp: Hypodermis; Cor: Cortex; Ph: Phloem; Cam: Cambium; Mx: Metaxylem; Mr: Medullary rays; Pt: Pith; Px: Protoxylem.

Figure 7: Powder microscopic observation of leaves showing trichomes, epidermal and parenchyamatous cells

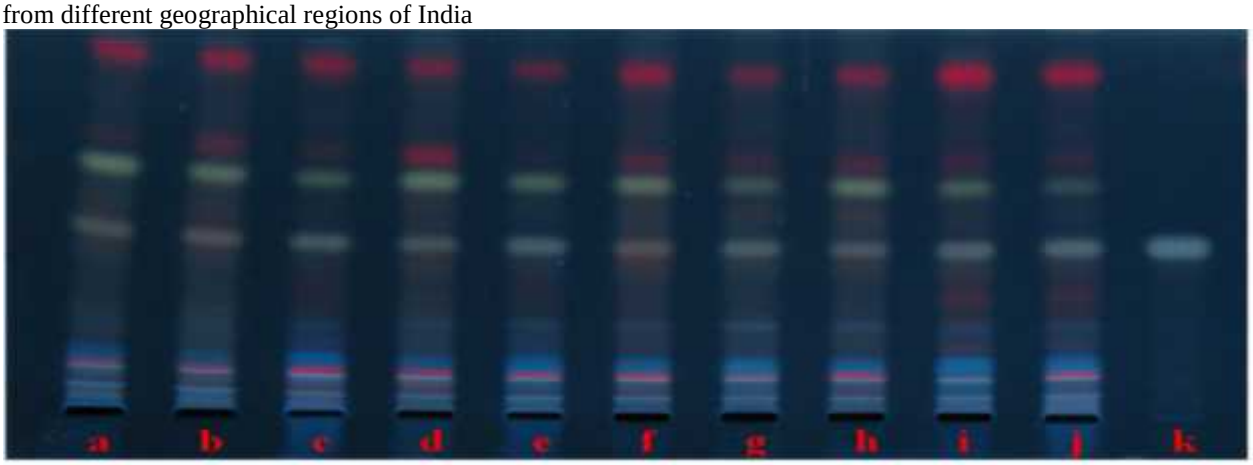
Macroscopy- Leaves of *P. biglandulosa* are compound, bipinnate, large and feathery. Each leaf consists of 40-50 pinnae and a pubescent rachis. Each pinnae has about 130-140 leaflets. Leaflets are small, sessile, linear, 0.8 cm long, opposite and dark green in colour. Leaflets have entire margin, obtuse apex and unicostate reticulate venation. At the base of each leaf on either side of the rachis, there is a presence of circular dark brown gland (Figure 2).

Microscopy

Transverse section of leaflet- Transverse section of the leaflet showed the presence of single layered upper and lower epidermis. The upper epidermal layer was followed by mesophyll layer which was further differentiated into single layered palisade tissue and 2-3 layered spongy tissue. Round or oval shaped vascular bundles with xylem and phloem were embedded linearly in the spongy tissue. The upper epidermis showed the presence of unicellular trichomes (Figure 3 and Figure 4).

Transverse section of rachis- Transverse section of the rachis showed the presence of single layered epidermis covered with unicellular trichomes. Below the epidermis, was the multilayered hypodermis followed by the

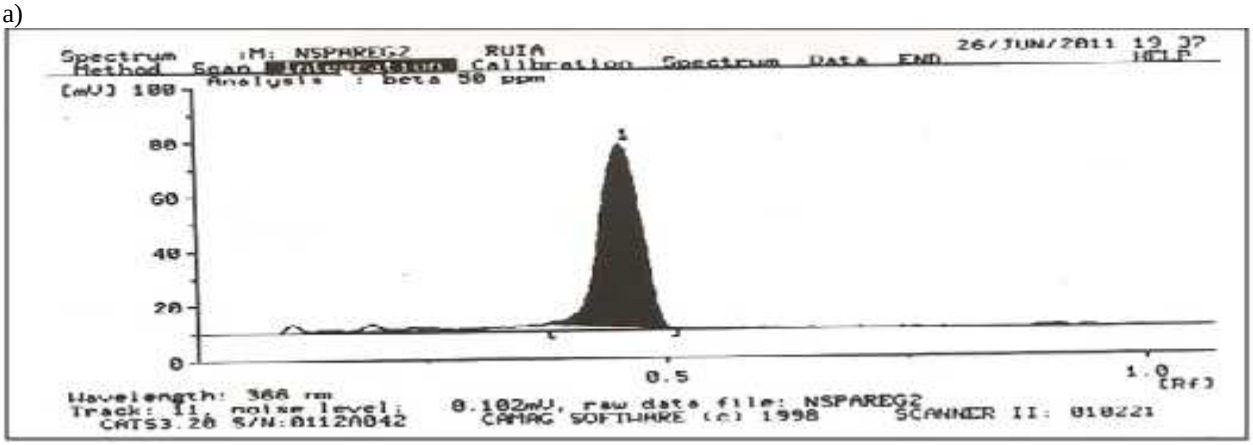
Figure 8: HPTLC plate photo (366 nm) showing presence of β-sitosterol in the leaves of *P. biglandulosa* collected from different geographical regions of India



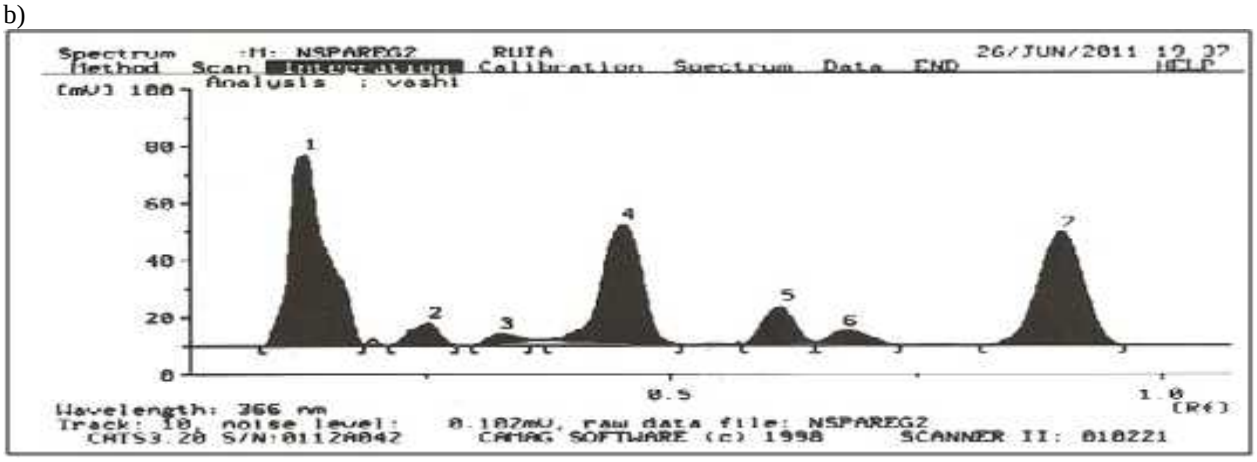
Track details: a-Ahmedabad; b-Bengaluru; c-Borivali; d-Kalyan; e-Sion; f-Mahabaleshwar; g-Nashik; h-Pune; i-Shahpur; j-Vashi; k-β-sitosterol (50 µg/mL)

Figure 9: Representative HPTLC chromatogram of a) β-sitosterol standard and b) *Parkia biglandulosa*

a)



b)

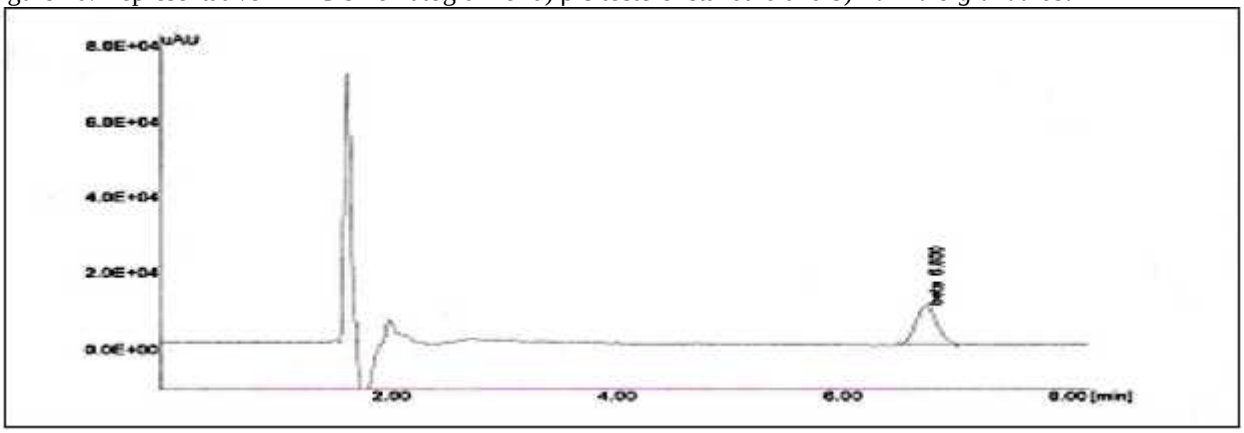


parenchymatous cortex. Next to the cortex, was the phloem followed by the cambial ring. The metaxylem and protoxylem were embedded within the parenchymatous medullary rays. Centrally placed large pith formed of parenchymatous cells was observed below this layer (Figure 5 and Figure 6).

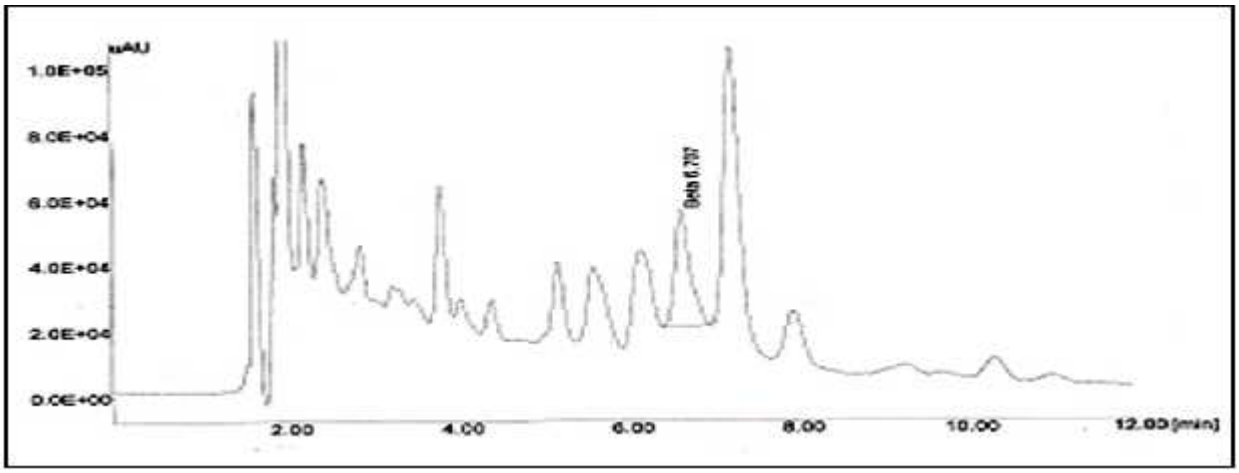
Powder microscopy of leaves- Microscopically, the leaf powder of *P. biglandulosa* showed the presence of unicellular trichomes of different sizes, multiseriate trichomes, leaf cover, epidermal and parenchymatous cells (Figure 7).

Physicochemical, phytochemical and heavy metal analysis- The values obtained for foreign matter, ash

Figure 10: Representative HPLC chromatogram of a) β-sitosterol standard and b) *Parkia biglandulosa*



a)



b)

Table 1: Results of physicochemical parameters of *P. biglandulosa*

Parameters		Observed Values
Foreign matter		0.252 ± 0.014
Total ash		5.158 ± 0.048
Acid insoluble ash		0.915 ± 0.138
Water soluble ash		4.689 ± 0.384
Loss on drying		7.442 ± 0.432
Extractive value	Water	12.579 ± 0.035
	Methanol	10.243 ± 0.079
	Petroleum ether	8.341 ± 0.052
	Ethyl acetate	5.806 ± 0.161
	Toluene	4.063 ± 0.051
	n- Hexane	3.161 ± 0.011

content (total ash, acid insoluble, water soluble ash), loss on drying and extractive values for leaves are summarized in Table 1. Extractive value of water for the leaves of *P. biglandulosa* was found maximum compared to other solvents. Impact of solvent volume and extraction period on the extractive values was clearly seen during the study. Amongst all the phytochemical fractions extracted, leaves of *P. biglandulosa* were found to be rich in quaternary alkaloids and n-oxides fraction. On the contrary, the fraction of fats and waxes i.e. the neutral extract was least (Table 2). None of the heavy metals analyzed were detected in leaves of *P. biglandulosa* which may reduce the possible risk of using it as a phytomedicine. *Chromatographic evaluation*- For HPTLC, mobile phase composition of toluene: methanol (8:1, v/v) showed a good resolution of β-sitosterol from other phytoconstituents (Figure 8). The R_f value of β-sitosterol was found to be 0.46. In case of HPLC, β-sitosterol eluted at the R_t of 6.8 min using the mobile phase of acetonitrile: ethanol (40:60, v/v). Both the

Table 2: Content of phytochemical fractions obtained from *P. biglandulosa*

Phytochemical fractions	% Content (Mean ± S.D., n=3)
Fats and waxes	0.256 ± 0.045
Terpenoids and phenolics	1.737 ± 0.149
Alkaloids	1.651 ± 0.051
Quaternary alkaloids and n-oxides	27.566 ± 0.066
Fibres	68.244 ± 0.070

Table 3: Results of method validation of β-sitosterol using HPTLC and HPLC

Parameters		Observed values	
		HPTLC	HPLC
LOD (µg/mL)		1.0	0.5
LOQ (µg/mL)		3.0	1.0
LWR (µg/mL)		5.0 - 60.0	5.0 -200.0
Regression equation		y = 39.76 x + 194	y = 3570x – 7704
Coefficient of determination (r^2)		0.993	0.996
System suitability (% CV)		Response 0.831 R_f 1.375	Response 0.114 R_t 0.152
Specificity		Specific	Specific
Precision (% nominal)	Intra-day precision	98.172 - 110.160	88.346 - 104.870
	Inter-day precision	99.028 - 108.400	89.330 -103.091
Stability (% Mean difference)	Long term stock solution stability (0 day and 31 day)	- 1.214	- 2.157
	Bench top stability (0 h and 6 h)	- 1.091	- 0.110
Ruggedness		Rugged	Rugged
Recovery		96.521 ± 5.187	99.492 ± 1.598

Table 4: β-sitosterol content in the leaves of *P. biglandulosa* collected from different provinces of India

Place of collection	β-sitosterol content in mg/g (Mean ± S.D., n=3)	
	HPTLC	HPLC
Ahmedabad	0.290 ± 0.003	0.131 ± 0.001
Bengaluru	0.291 ± 0.004	0.264 ± 0.001
Borivali	0.550 ± 0.005	0.429 ± 0.001
Kalyan	0.301 ± 0.005	0.268 ± 0.002
Sion	0.632 ± 0.005	0.618 ± 0.001
Mahabaleshwar	0.299 ± 0.005	0.242 ± 0.002
Nashik	0.456 ± 0.008	0.399 ± 0.002
Pune	0.343 ± 0.005	0.349 ± 0.003
Shahpur	0.650 ± 0.005	0.622 ± 0.001
Vashi	0.787 ± 0.004	0.881 ± 0.002

methods developed were validated as per ICH guidelines. The HPLC method was found more sensitive compared to HPTLC in estimating β-sitosterol (Table 3).

Both the methods were applied to evaluate the impact of regional variation on the content of β-sitosterol in the leaves of *P. biglandulosa* collected form different provinces of India. It was observed that β-sitosterol content was maximum in the leaves collected from Vashi (Maharashtra) whereas sample from Ahmedabad (Gujarat) showed minimum content (Table 4). The impact of regional variation on the β-sitosterol content was clearly evident from HPTLC (Figure 8, Figure 9 and Figure 10) and HPLC (Figure 11 and Figure 12) analysis.

CONCLUSION

Plants serve as a classic source of various phytoconstituents that exhibit varied pharmacological action. Thus, proper identification and authentication of the plant raw material becomes a necessity before its intended use as a drug, individually or as an ingredient in formulation. In the same context, the present study involves the evaluation of pharmacognostic parameters of leaves of *P. biglandulosa* which may ensure its quality, purity and authenticity. Thus, findings of this study could be useful for the compilation of a monograph in suitable pharmacopoeia for its proper identification and quality control.

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