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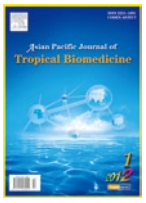

Document heading

Pharmacognostic investigations on the leaves of *Heterophragma quadriloculare* K. Schum.

Bhavik kumar H Satani*, Shri Hari Mishra

Herbal Drug Technology Laboratory, Shri G. H. Patel Pharmacy building, Donor's plaza, The M. S. University of Baroda, Vadodara-390001, Gujarat, India

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ABSTRACT

Objective: To generate pharmacognostic parameters of leaves of *Heterophragma quadriloculare* K. Schum since data on pharmacognostic and phytochemical characterization which provide means to authentic raw material or drug for medicinally important formulation are not available. **Methods:** The present study reports detailed pharmacognostic characters as identification parameters of the leaves which were subjected to morphological, histological, quantitative microscopic and chromatographic studies. Morphological, histological, quantitative microscopic were done using reported methods and chromatographic studies were performed using HPTLC. **Results:** Diagnostic features for identification of shape of leaves being compound, odd-pinnate, dorsiventral asymmetric at base with unicellular non-covering and glandular trichomes having unicellular stalk and bicellular head, actinocytic stomata and rhombus calcium oxalate crystals. Water soluble and alcohol soluble extractives were found to be (23.6±0.346)% w/w and (10.8±0.115)% w/w, respectively. **Conclusions:** The determinations from above studies establish the macro- and microscopic parameters which can be utilized for quick identification of the drug both as whole and in the powdered leaf materials and may helpful in laying down pharmacopoeial standards of this important member of traditional medicine.

1. Introduction

Heterophragma quadriloculare K. Schum. (HQ) is representative of genus *Heterophragma* belongs to family Bignoneaceae[1]. It is also known as *Heterophragma roxburghii* (Spreng.) DC, *Bignonia quadrilocularis* Roxb. and *Spathodea roxburghii* Spreng[2]. Commonly it is known as Warras, Varas, Pullung, Paatang, Kusaga, Bondugu, Barukoli-gottu, Kaligottu, Baro-kala-goru, Bechadi, Adwi-nuggi in different part of India. In India it founds in different regions of Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu[1,3-5]. Name of the plant gives idea about characteristic of flower and fruit - Heterophragma is correlates with fragrance of the flowers and quadriloculare is correlates with four lobes of the fruit. Various part of the plant is traditionally used by some practitioners in rural area for different purpose like anti-diabetic[6], menorrhoea[7], premature ejaculation[7], night emission[7], antidote[8], food[9], antimicrobial activity[3], antifungal activity[10], antiseptic activity[11] and skin disease[3,12]. The leaves of this plant are utilised for medicinal use, specifically for anti-diabetic, antifungal, and antiseptic activities along with in skin disease like toe sores and in chilblain. Leaves are reported to contain several types of alkaloids, terpenoids, steroid, flavonoids, tannins, phenolics[13,14]. Since data on pharmacognostic and phytochemical characterization which provide means to authentic raw material or drug for medicinally important formulation are not available for leaves of *Heterophragma quadriloculare* K. Schum. Hence the present studies were plant to generate pharmacognostic parameters like macroscopic, microscopic, quantitative microscopic values,

*Corresponding author: Bhavikkumar H Satani, Department of Pharmacognosy, Maliba Pharmacy College, Uka Tarsadia University, Tarsadi, Bardoli-Mahuva Road, Bardoli, Dis.-Surat 394350, Gujarat, India.
Tel: 09228506252
E-mail: satanib1231@gmail.com, satanib_mpc@yahoo.co.in

physicochemical beside chromatographic profile of the leaves. These parameters may serve as pharmacopoeial standards as identifying parameters for this species.

2. Materials and methods

2.1. Plant material

The fresh leaves of wildly growing *Heterophragma quadriloculare* K. Schum. were collected from the outskirts of village Dungaripada, near Silvasa, Dadaranagar Haveli, during April-May 2010 and authenticated in Botany Department, M.S.University of Baroda, Vadodara. A voucher specimen of sample (No. Pharmacy/HDT/HQ/09-10/01/BS) has been deposited in Herbal Drug Technology Laboratory Pharmacy Department. The M. S. University of Baroda, Gujarat, India.

2.2. Reagents and chemicals

All solvents and chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany), SD Fine chemical (India), RFCL limited (India) and Loba Chemie Pvt. Ltd. (India).

2.3. Preparation of samples

The leaves and leaflets were separated from the branches of young stems of *Heterophragma quadriloculare* K. Schum. Some fresh leaves of these plants were preserved in formaldehyde-acetic acid and alcohol (FAA) as preservation medium for further use in anatomical studies. The remaining bulk was dried for two weeks in shade, then, powdered to 60 mesh sizes and packed separately in airtight containers for powder microscopy, physicochemical studies and chromatographic studies.

2.4. Morphologic and microscopic investigations

The morphological features were determined taking entire fresh leaves, while, sections and surface preparations were used for microscopy according to the methods reported earlier with minor modifications[15-23]. Photo micrographic images were taken using Zeiss microscope (10× and 40×) using MIPS Olympus camera. Different features of leaf were identified and reported.

2.5. Physico-chemical and chromatographic investigations

Physicochemical analysis i.e. foreign matter, loss on drying, ash value, extractive value, swelling index, foaming index and haemolytic index was done as per standard methods[16,17,23].

Chromatographic studies included generation of HPTLC fingerprinting of methanol extract of powdered leaves using Camag system equipped with Linomat V sample applicator, Camag TLC scanner 3 and CATS 4 software for interpretation of data. An aluminium plate (10 × 10 cm) precoated with silica gel 60F254 (E Merck) was used as an adsorbent. The plates were developed using toluene: ethyl acetate (9:1) as mobile phase in previously saturated twin trough chamber (CAMAG). Before derivatization plate was scanned at the wavelength 254 and 360 nm. The plate was derivatized by spraying with anisaldehyde- sulphuric acid (AS) reagent and after heating the plate at 110 ℃ for 10 min scanned at the wavelength 540 nm.

3. Results

3.1. Morphological characteristics

Heterophragma quadriloculare K. Schum. is a tree with height of 35-40 ft. Leaves are compound (odd pinnate) 15-30 cm long with 3-5 pairs of hairy leaflets, and a terminal one. Morphological characteristics of leaflets are described in Figure 1 and Table 1. Results recorded are average of six observations recorded for study.

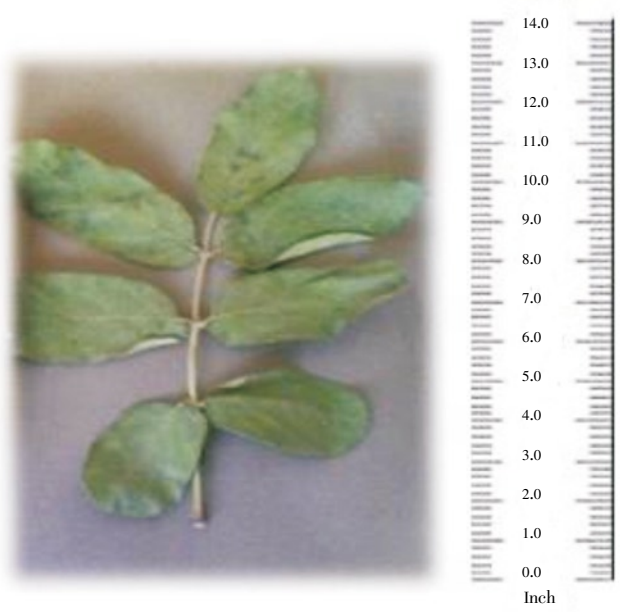

Figure 1. Macroscopy of *Heterophragma quadriloculare* K. Schum. leaf showing morphological arrangement and size of leaflets in leaf.

Table 1
Morphological characteristics of *Heterophragma quadriloculare* K. Schum. leaflets

Parameters	Observations (*n*=6)
Size	Length: 5-10 cm (Avg.) Width: 3-6 cm (Avg.)
Shape	Elliptic
Apex	Acute
Base	Asymmetric
Venation	Reticulate
Surface	Pubescent
Margin	Entire
Petiole	Medium
Phyllotaxis	Whorled
Inflorescence	Panicle Umbel
Colour	Dull green (fresh), Dark green (dry)
Odour	Characteristic
Taste	Sour with light Sweet

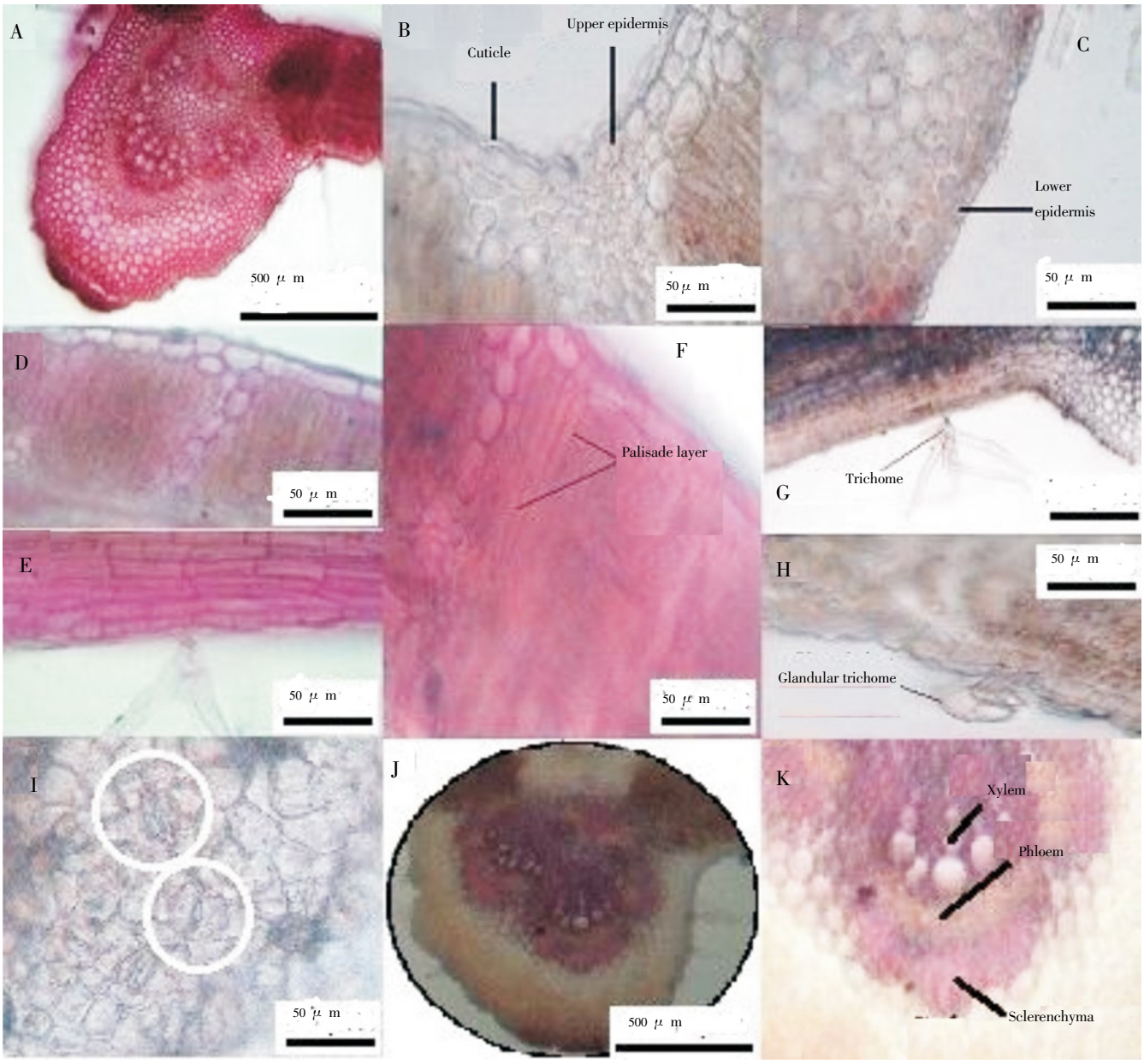

Figure 2. Microscopic characters of *Heterophragma quadriloculare* K. Schum.
Leaf showing A– Transverse section (TS) of HQ leaf stained in safranin; B– Upper epidermis at midrib portion; C– Lower epidermis at midrib portion and calcium oxalate crystals present in paranchymatous cells; D– Upper epidermis at lamina portion; E– Lower epidermis with trichomes at lamina portion; F– Double layered palisade cells underneath upper epidermis; G– Non–covering multiseriate unicellular trichomes (stellate trichomes) emerging from lower epidermis; H– Glandular trichomes; I– Circular (actinocytic) stomata; J– Midrib portion showing arrangement of (conjoint) vascular bundles; K– vascular bundle covered by sclerenchymatous cells

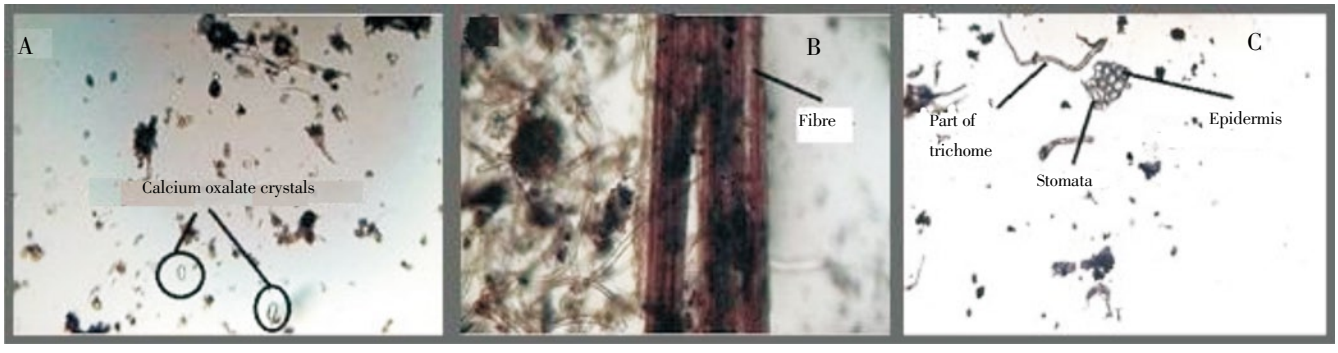

Figure 3. Microscopic characters of *Heterophragma quadriloculare* K. Schum.
Leaf powder showing A– Rhombus type of calcium oxalate crystal found during powder microscopy, B– Phloem fibre found during powder microscopy, C– Epidermis with stomata and part of trichomes found during powder microscopy

Table 2
Size measurement of cuticle, epidermis and palisade layer (mean±SEM) (*n*=6).

Parameters		Lamina		Midrib	
		Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis
Cuticle thickness (μm)		12.66 ± 0.60	4.78 ± 0.70	12.66 ± 0.60	4.78 ± 0.70
Epidermal cell size	Length(μm)	22.30 ± 1.04	17.91 ± 0.84	22.30 ± 1.04	10.85 ± 1.60
	Width(μm)	16.03 ± 1.00	10.85 ± 0.71	16.03 ± 1.00	9.46 ± 0.56
Palisade cell size	Length(μm)	53.07 ± 1.65	NA	53.07 ± 1.65	NA
	Width(μm)	10.15 ± 0.81	NA	10.15 ± 0.81	NA

Data in table shows comparative information on difference in epidermis of lamina and midrib portions. NA: Not applicable.

3.2. Microscopical characteristics

Transverse section of leaf shows it is a dorsiventral leaf (Figure 2A).

3.2.1. Lamina

Upper and lower epidermis consists of single layer of polygonal cells; cells have wavy smooth walls covered with cuticle. Both non-covering and glandular trichomes emerge from the epidermal layer (Figure 2D & 2E). Non-covering trichomes are multiseriate, unicellular and sharp at the apex (Figure 2G). Glandular trichomes are made up of unicellular stalk and bicellular glandular head (Figure 2H). Number of trichomes in lower epidermis is higher than that in upper epidermis. Glandular trichomes are less in number as compare to non-covering trichomes. Lower epidermis resembles the upper epidermis but size of lower epidermis cell is smaller than size of upper epidermis (Table 2). Circular (actinocytic) stomata are present on both epidermis layer (Figure 2I). Mesophyll is differentiated in to palisade and quadrangular parenchymatous cells. Palisade cells are double layered, tightly packed, regularly arranged and does not form a continuous band throughout as it is absent above the vascular bundles of midrib (Figure 2F). Size of palisade cells is explained in Table 2. Multilayered, distinct quadrangular straight wall parenchymatous cells are present below palisade layer and continue till the lower epidermis. Layers of this type of cells are tightly arranged (Figure 2F).

3.2.2. Midrib

Epidermal layers of lamina are in continuity with that of midrib. But, size of the epidermal cell is smaller as compare to size of cells in lamina portion (Figure 2B & 2C). Conjoint vascular bundles are prominent occupying the central portion of the midrib (Figure 2J). Xylem is towards the centre of the leaf and covered by phloem. Vascular bundle is surrounded by distinct sclerenchymatous cells (Figure 2K). Calcium oxalate crystals and starch grains were also found in paranchymatous cells of midrib and lamina portion (Figure 2C). Stomatal index, stomatal number, palisade ratio, vein islet number and vein termination number were found to be 3-5 per sq mm, 2-4 per sq mm, 3-5, 19 per sq mm and 26 per sq mm respectively. Results of powder microscopy shown presence of rhombus type of calciumoxalate crystals, epidermal cells with stomata and portions of trichomes (Figure 3).

3.3. Physico-chemical characters

The results obtained from various determinations of physic-chemical analysis are compiled in Table 3. The values represented are average of three readings (n= 3).

Table 3
Physico-chemical determinations of Heterophragma quadriloculare K. Schum. leaf (mean±SEM) (*n*=6).

Parameters		Average values
Foreign organic matter		0.19±0.017 % W/W
Loss on drying (LOD)		9.79±0.026 % W/W
Ash value	Total ash	8.69±0.047 % W/W
	Water soluble ash	1.5±0.006 % W/W
	Acid insoluble ash	3.0±0.006 % W/W
Extractive value	Water soluble extractive	23.6±0.346 % W/W
	Alcohol soluble extractive	10.8±0.115 % W/W
Swelling index		No swelling
Foaming Index		< 100
Hemolytic index		No hemolysis

All parameters except foaming index and haemolytic index were performed on dry powdered leaves.

3.4. High performance thin-layer chromatography (HPTLC) profile

The methanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system Toluene : Ethyl acetate (9:1) was ideal and gave well resolved sample peaks. So, high performance thin layer chromatography of methanol extract was carried out using Toluene: Ethyl acetate (9:1) as mobile phase and R_f values were recorded. HPTLC fingerprinting studies on methanol extract showed presence of various phytoconstituents with their respective R_f values. Chromatogram with their densitogram at 254 and 366 nm before derivatization and at 540 nm after derivatization are shown in Figure 4-7.

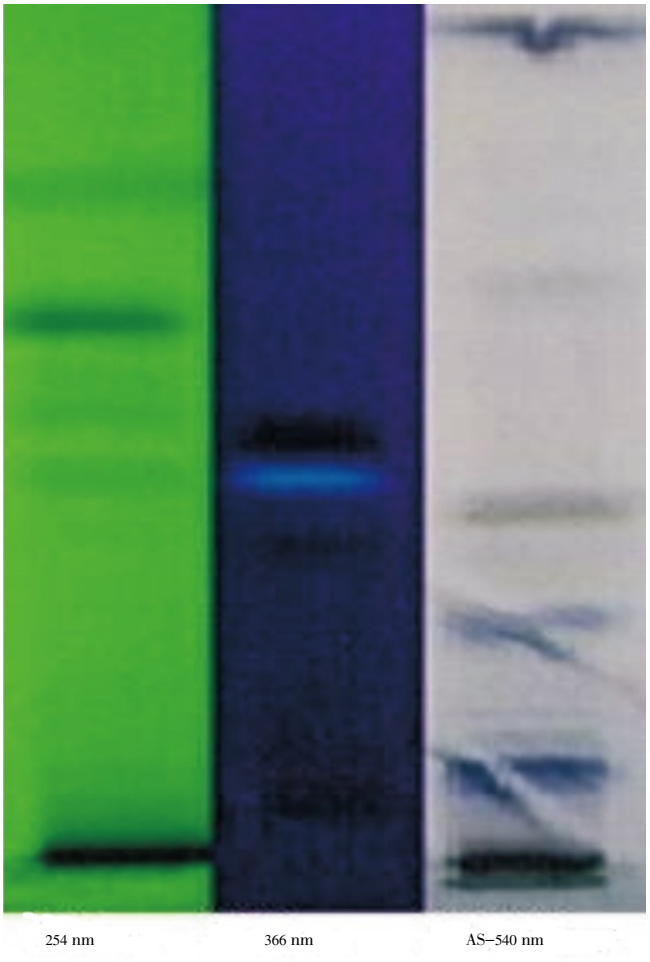

Figure 4. HPTLC plates visualized at 254, 366 and 540 nm showing TLC pattern of methanol extract of *Heterophragma quadriloculare* K. Schum. leaf powder in Toluene: Ethyl acetate (9:1) mobile phase. Pattern shown by 254 nm and 366 nm were recorded before derivatization while pattern shown by AS-540 nm was recorded at 540 nm after derivatization with anisaldehyde sulphuric acid (AS).

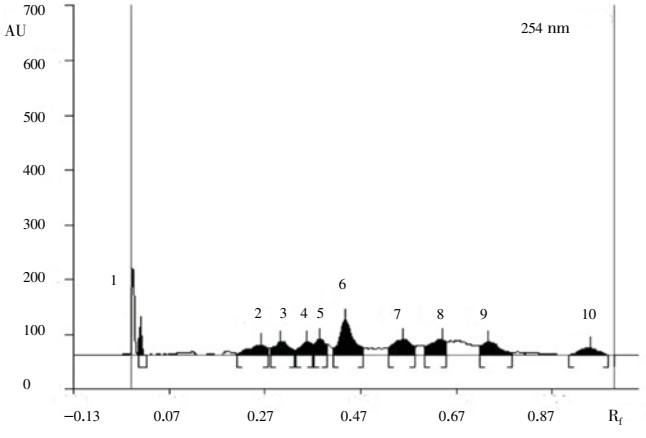

Figure 5. HPTLC densitogram of methanol extract at 254 nm showing HPTLC densitogram of methanolic extract of Heterophragma quadriloculare K. Schum. leaf powder in Toluene: Ethyl acetate (9:1) mobile phase at 254 nm before derivatization with anisaldehyde sulphuric acid (AS).

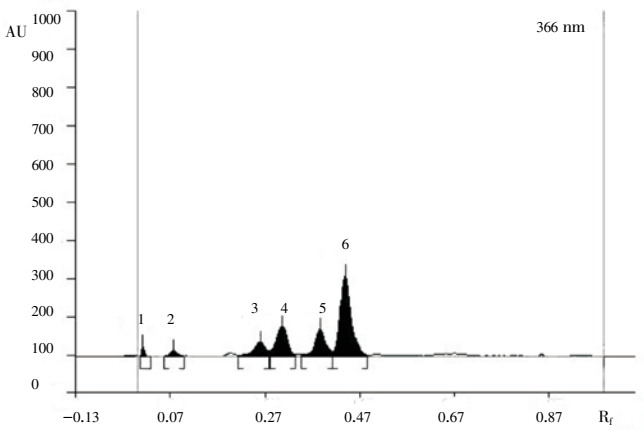

Figure 6. HPTLC densitogram of methanol extract at 366 nm showing HPTLC densitogram of methanolic extract of *Heterophragma quadriloculare* K. Schum. leaf powder in Toluene: Ethyl acetate (9:1) mobile phase at 366 nm before derivatization with anisaldehyde sulphuric acid (AS).

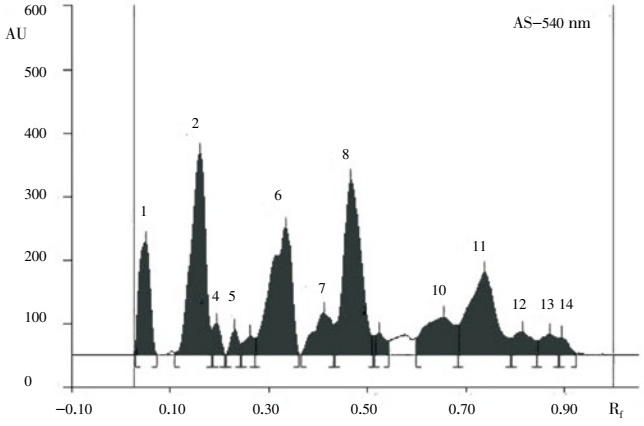

Figure 7. HPTLC densitogram of methanol extract at 540 nm after derivatization with AS showing HPTLC densitogram of methanolic extract of *Heterophragma quadriloculare* K. Schum. leaf powder in Toluene: Ethyl acetate (9:1) mobile phase at 540 nm after derivatization with anisaldehyde sulphuric acid (AS).

4. Discussion

As a part of generation of parameters for standardization, the morphological examination of leaf was carried out. Morphological evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs[16,17]. The morphological characters of leaf can serve as diagnostic parameter[16]. Plants belongs to Bignoniaceae family are characterized by oppositely paired, usually compound leaves[24] and results of the study also confirm this statement. This is the first report on pharmacognostic studies of leaf of *Heterophragma quadriloculare* K. Schum. The dorsiventral leaves, have tightly arranged double layered palisade cells, conjoint vascular bundles, sclerenchymatous sheath covering vascular bundles, rhombus type of calcium oxalate crystals and actinocytic (circular) stomata being characteristic features. Data available on histological architecture of leaf of bignoniaceous trees also shows that leaves are dorsiventral and heterogeneous mesophyll[25]. Presence of multiseriate, non-covering trichomes which are sharp at the apex and

glandular trichomes having unicellular stalk and bicellular head become identifying features of leaf of *Heterophragma quadriloculare* K. Schum. Here it is noticeable that glandular trichomes are very few in number as compared to non-covering trichomes. Significant variations were also observed in size of upper epidermal cell and lower epidermal cells particularly at midrib portion of the leaf. Epidermal cells of lower epidermis are comparatively smaller than that of epidermis cells. However it becomes very difficult when the drug is in dried powdered form. Therefore, some diagnostic characters have been evolved in order to differentiate this drug from other drugs[16,17]. In this context, some reliable characters like quantitative microscopy, type of stomata, trichomes and calcium oxalate crystals, physic-chemical parameters and chromatographic profile will be helpful. Foreign matter, LOD, ash value and extractive values were calculated with reference to dried powder. Percentage extractives in different solvents indicated the nature and quantity of constituents in the extracts. From the results of physic-chemical results it is clear that leaf of this plant contains high amount of polar compounds. Absence of saponins and gums can be postulated from foaming index, haemolytic index and swelling index respectively. The chromatographic profile may serve as a characteristic fingerprinting for qualitative evaluation of leaf samples in powdered form. The determinations from above studies establish the macro- and microscopic parameters for the characterization of correct source of *Heterophragma quadriloculare* K. Schum. These parameters can be utilized for quick identification of the drug both as whole and in the powdered leaf materials. The pharmacognostic and chromatographic fingerprinting details so obtained from these studies may be helpful in laying down pharmacopoeial standards of this important member of traditional medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Sandwith NY. What Is Heterophragma sulfureum Kurz? *Kew Bulletin* 1967; **21**(1): 21-30 & 38.
[2] USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Online Database]. Maryland: National Germplasm Resources Laboratory. Retrieved from: http://www.ars-grin.gov/cgi-bin/npgs/html/exsplist.pl (on14th June 2010).
[3] Khare CK. *Indian medicinal plants*. New Delhi: Springer publication; 2007, p. 308.
[4] Andhra Pradesh forest department. Retrieved from: http://forest.ap.nic.in/Forest%20Flora%20of%20Andhra%20Pradesh/Family/Bignoniaceae.htm [on14th June 2010].
[5] Zhi-Yun Zhang, Thawatchai Santisuk. Bignoniaceae. In: *Flora of China. Vol. 18*. Published by Science Press (Beijing) and Missouri Botanical Garden Press, 213. Retrieved from: http://zipcodezoo.com/Plants/H/Heterophragma%5Fquadriloculare/ [on14th June 2010].
[6] Amla soumyanath. *Traditional medicines for modern times: antidiabetic plants*. USA: CRC Taylor & Francis publication; 2006, p. 19.
[7] Pullaiah T (a). *Biodiversity in India. Vol. 4*. New Delhi: Regency publications; 2006, p. 328.
[8] Pandey CN, Raval BR, Mali Seema, Salvi Harsha. *Medicinal plants of Gujarat*. Gandhinagar: Gujarat Ecological Education and Research (GEER) Foundation; 2005, p. 196.
[9] Punekar Sachin A. Some food plants of human langur Semnopithecus entellus (DUFRESNE) in the western ghats of Maharastra, India. *Zoos' print journal* 2002; **17**(6): 797-801.
[10] Sahai A, Shrivastava A. *Indian-Perfumer*. Kanpur: Essential Oil Association of India; July/Dec 1984, 28 (3/4): 175-176.
[11] Jog Suneeti K, Sahyadris - flora and ethnobotany, Report of William L. Brown Fellowship 2007-2008, Department of Biology, The University of Texas at Tyler, August 2009.
[12] Pullaiah T (b). *Encyclopedia of world medicinal plants*. Vol.3, New Delhi: Regency publication; 2006, p. 1082.
[13] Zirvi Karimullah A, Miana GA, Jehangir S, Ghazanoi I. Constituents of H. quadriloculare. *Phytochemistry* 1972; **11**(7): 2349.
[14] Mukherjee K, Ray LN. Phytochemical screening of some Indian medicinal plant species, Part II. *Int J Crude Drug Res* 1986; **24**(4): 187-205.
[15] Evans WC. *Trease and Evans㊣ Pharmacognosy. 14th ed*. London: W. B. Sounders company limited; 1996, p. 545-546.
[16] Anonymous, *General guidelines for methodologies on research and evaluation of traditional medicine. WHO/EDM/TRM/2000*. Geneva: World Health Organization; 2000.
[17] Anonymous. *Analysis of questionnaire on traditional medicine. WHO DOC. 5183A/REV. DOC. 6163A*. Geneva: World Health Organization;1992.
[18] Brain KR, Turner TD. *The practical evaluation of phytopharmaceuticals*. Bristol: Wright Sciencetechnica; 1975, p. 36-45 & 81-82.
[19] Padashetty SA, Mishra SH. Phytochemical and pharmacognostical parameters for standardization of *Tricholepis glaberrima*: A medicinal herb. *J Med Aromatic Pl Sci* 2008; **30**(4): 381-388.
[20] Somashekar AP, Mishra SH. Pharmacognostic parameters for evaluation of the roots of Echinops echinatus marketed as Brahmandi. *Phcog Mag* 2007; **3**: 196-202.
[21] Sethiya NK, Trivedi A, Patel MB, Mishra SH. Comparative pharmacognostical investigation on four ethanobotanicals traditionally used as Shankhpushpi in India. *J Adv Pharm Tech Res* 2010; **1**: 388-395.
[22] Wallis TE. *Text book of pharmacognosy*. New Delhi: CBS Publisher & Distributer; 1985, p. 104.
[23] Khandelwal KR. *Practical Pharmacognosy: Techniques and Experiments. 8th ed*. Pune: Nirali Prakashan; 2001, p. 31-40 & 146-161.
[24] Bignoniaceae. In: Nicholas C. Encyclopedia Britannica. Chicago: encyclopedia britannica's international publishing; 2011. Retrieved from http://www.britannica.com/ EBchecked/topic/65081/Bignoniaceae.
[25] Flavia Aparecida Ortolani, José Roberto Moro, Fabíola Vitti Moro. Morfo-anatomia, Citogenética e palinologia em espécies de ipês (bignoniaceae). Jaboticabal - SP 2007; 39-69.