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COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES OF THE RAW DRUG SOURCES OF PRASARINI USED IN AYURVEDA

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ABSTRACT

According to classical texts *Paederia foetida* is used as the genuine source plant of the drug *Prasarini,* it posses properties like astringent, aphrodisiac, laxative, bitter etc. In Ayurvedic practitioners *Merremia tridentata* ssp. *tridentata* and *Merremia tridentata* ssp. *hastata* are used as the source plant of this drug. However no conclusive pharmacognostic study of these plants has been performed yet. The present investigation deals with the comparative study of morphological, anatomical, histochemical and phytochemical characters of these three plants. Three plants show similarities and differences between them at morphological, anatomical, histochemical and phytochemical levels. Anatomically these three plants show differences in many characters. But phytochemical studies revealed that these plants show much similarity. β- sitosterol was present in all the three plants but their concentrations shows differences. The parameters used in this study will help to identify the genuine and substitute source plants of the drug *prasarini* for the preparation of ayurvedic medicines and to ensure the quality of formulations.

INTRODUCTION: The term *prasarini* indicates the spreading habit of the plant. It also indicates the property of the drug of stretching out parts of the body contracted by paralysis. It is an important ingredient of many Ayurvedic formulations like *prasaranyadi kasayam*, *prasaranyadi tailam*, *balaristam etc*. Several authors have equated this drug with *Paederia foetida* L. of Rubiaceae. This is probably the plant used as the drug source in North India.

According to Ayurvedic Pharmacopoeia of India this is the accepted source. In Kerala however the source of this drug is an altogether different plant *Merremia tridentata* of convolvulaceae. There are two subspecies of this species, viz. ssp. *tridentata* and ssp. *hastata*, which can easily be recognised by their foliar characters [1].

Comparative Pharmacognostic and Phytochemical studies are the reliable source to identify the genuine raw drug from their substitutes and adulterants.

MATERIALS AND METHODS:

Anatomical studies: Plant materials for the present study were collected from Herb Garden, Arya Vaidya Sala, Kottakkal. The materials for anatomical study were fixed in Formaldehyde- Acetic acid mixture.

High Pressure Liquid Chromatography: The Shimadzu HPLC system (Kyoto, Japan) consisting of LC - 10ATVP pump, a rheodyne injector, SPD M10AVP photodiode array detector and CLASS-VP 6.12 SP5 integration software was used for the analysis. Stock solutions of β-sitosterol (Sigma-Aldrich, Germany) were prepared

in methanol (analytical grade; Hayman Ltd, England) at 100µg/Ml is used as the standard solution. The stationary phase was Phenomenex Luna C 18 (2) (250 x 4.6mm) column with 5µ particle size with a C18 guard column (Phenomenex, 4mmX2.0mm ID). The mobile phase consisting of Methanol (HPLC grade, Merck) and water in the proportion (25:75 v/v) was used. The mobile phase was degassed by sonication before use. The column was equilibrated with the mobile phase for an hour and then pumped at the rate of 1.0 ml/min. Plant Histological and histochemical staining was carried out according to Johansen2. Photomicrographs were taken using Canon G3 camera attached to Zeiss microscope. For examining the cell structure in powder form, material were powdered and sieved and mounted under glycerol and safranin to study the nature and identification of particles.

Chemical Characterization:

Extraction: The dried samples were powdered and 5g each of *M. tridentata, M. hastata* and *P. foetida* was extracted with 50 ml methanol in a Soxhlet extractor for 12 hr. The extract was filtered and methanol removed by distillation under reduced pressure. The residue was subjected to TLC and HPLC profiling.

Thin Layer Chromatography: 1mg ml-1 solution of the whole plant extracts of *Merremia tridentata, Merremia hastata, Paederia foetida* (5mg ml-1) were spotted on pre-coated silica gel 60 F254 TLC plate. Developed the plate in Toluene: Ethyl acetate: Glacial acetic acid (5:5:0.2) solvent system. The plate was then dried and derivatised with Anisaldehyde-Sulphuric acid (AS) reagent and visualized in the visible light. Samples were assayed in triplicate and detection was done at 265 nm. The concentration of β- sitosterol was calculated using area under the curve method.

RESULTS AND DISCUSSION:

Pharmacognostic Study:

Morphological Characters: Morphologically the plants show differences in leaf size, leaf shape, stem shape, stem thickness etc. *M. tridentata* ssp. tridentata is a small glabrous prostrate, herbaceous annual or perennial with thick small woody root stock from which arise numerous closely prostrate but or slender elongate angular branches bearing very short petioled, tridentate at base; small pale yellow flowered; fruit two celled, four ovuled; four seeded capsules (**Fig. 1 A-B**). *M. tridentata* ssp. hastata is a diffuse perennial or rarely annual herb with several elongate slender or angular smooth creeping branches.

They have short but thick rootstock and bearing simple alternate, sessile, linear hastate, acute leaves; long and small cream yellow flowers on long slender peduncles; fruits an ovoid, two celled capsule; seeds four glabrous (**Fig. 1C-D**). *P. foetida* is an extensive climber; leaves are ovate to lanceolate, entire, membranous with long petioles; flowers in scorpioid cymes, purple or violet; fruit ellipsoid, compressed, red or black (**Fig. 1E-F**).

FIG. 1: (A-B) *M. TRIDENTATA* SSP. TRIDENTATA. (A) PLANT (B) DRIED WHOLE PLANT, (C-D) *M. TRIDENTATA* SSP. HASTATA. (C) PLANT (D) DRIED WHOLE PLANT, (E-F) *PAEDERIA FOETIDA* L. (E) PLANT (D) DRIED WHOLE PLANT

Anatomical Characters: Anatomically and histochemically these three plants show variations in many characters. In stem they show variations in nature of epidermis, nature of cortex, nature of

inclusions in the cortex, nature of xylem, inclusions in the pith region etc. In *M. tridentata ssp. tridentata* TS of stem is angular in outline with 8 protruberances (**Fig. 2 A**), in *M. tridentata ssp.hastata* TS of stem is pentagonal in out line (**Fig. 2 F**). But in the case of *P. foetida* TS of stem is oval in outline (**Fig. 2 K**). Starch grains are present only in some cortical cells and pith cells in the case of both *Merremia* species (**Fig. 2 D, E, I & J**). In *P. foetida* starch grains are present in all the cells of the cortex and cells towards the periphery of the pith (**Fig. 2 N & O**). Details of anatomical characters of stem are given in **table 1 and Fig. 2**. Root anatomical characters are also shows differences in many characters like nature of vascular region, medullary rays, starch grains etc.

Figure 2. (A-E). TS of stem of *M. tridentata ssp. tridentata*. (A). Ground plan x 40, (B). One portion enlarged x 200, (C). Outer portion enlarged showing cortex, phloem fibres and xylem x 400, (D) Histochemical staining for starch x 400, (E). Pith cells showing rosette crystals of calcium oxalate. (F-J). TS of stem of *M. tridentata ssp. hastata*. (F). Ground plan x 40, (G). One portion enlarged x 200, (H). Outer portion enlarged showing cortex, phloem fibres and xylem x 400, (I) Histochemical staining for starch x 400, (J). Pith region x400. (K-O). TS of stem of *P. foetida*. (K). TS of stem x 40, (L). Outer portion enlarged x 400, (M). Outer portion enlarged showing cortex, phloem and xylem x 400, (N & O) Histochemical staining for starch x 400. ck, cork; ct, cortex; e, epidermis; ph, phloem; phf, phloem fibre; p, pith; rcr, rosette crystals; sg, starch grains; v, vessel; xy, xylem.

In *M. tridentata ssp. tridentata* and *M. tridentata ssp.hastata*, vessels are wide and many in number. But in the case of *P. foetida* vessels are less in number (**Fig. 3 C, H & M**). In *M. tridentata ssp. tridentate*, medullary rays are usually uniseriate but are multiseriate at four points which gives furrowed appearance (**Fig. 3A**). In *M. tridentata ssp.hastata* medullary rays are usually uniseriate, fully filled with starch grains (**Fig. 3 F**). Usually medullary rays are uniseriate but multi seriate medullary cells are present and xylem core become furrowed in the case of *P. foetida* (**Fig. 3 K**). Starch grains are lesser in amount in *M. tridentata ssp. tridentata* when compared to *M. tridentata ssp.hastata* and *P. foetida* (**Fig. 3 D, J, N & O**). Oil globules are more in *M. tridentata ssp.hastata* when compared to *M. tridentata ssp. tridentate* and *P. foetida* (**Fig. 3 E, J & P**). Details of anatomical characters root are given in **table 2 and Fig. 3**.

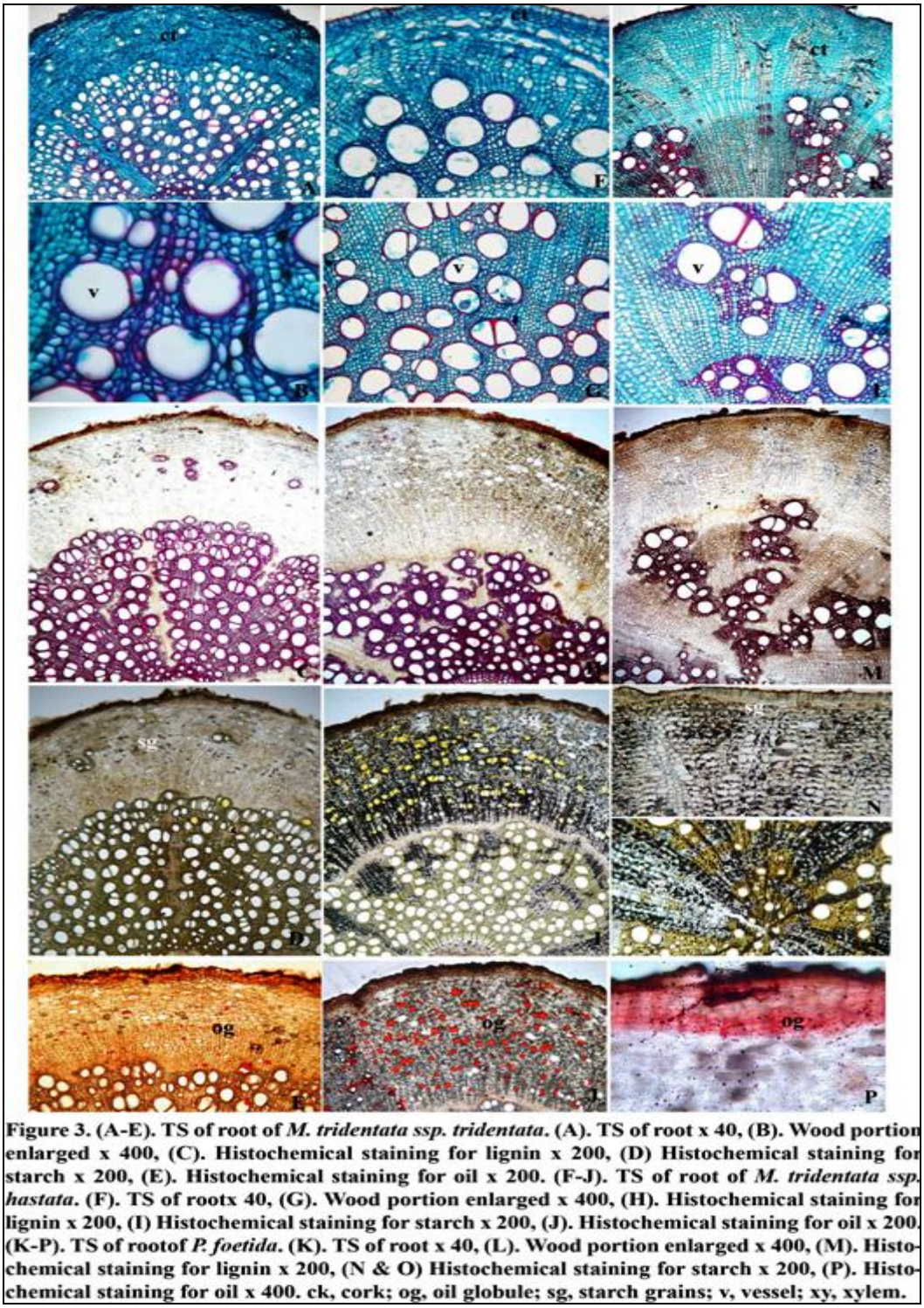

Figure 3. (A-E). TS of root of *M. tridentata ssp. tridentata*. (A). TS of root x 40, (B). Wood portion enlarged x 400, (C). Histochemical staining for lignin x 200, (D) Histochemical staining for starch x 200, (E). Histochemical staining for oil x 200. (F-J). TS of root of *M. tridentata ssp. hastata*. (F). TS of rootx 40, (G). Wood portion enlarged x 400, (H). Histochemical staining for lignin x 200, (I) Histochemical staining for starch x 200, (J). Histochemical staining for oil x 200. (K-P). TS of rootof *P. foetida*. (K). TS of root x 40, (L). Wood portion enlarged x 400, (M). Histochemical staining for lignin x 200, (N & O) Histochemical staining for starch x 200, (P). Histochemical staining for oil x 400. ck, cork; og, oil globule; sg, starch grains; v, vessel; xy, xylem.

Powder Microscopy: Powder microscopy of whole plant of *M. tridentata* ssp. *tridentata* shows epidermal cells in surface view; fragments of vessels and tracheids with pitted and spiral thickenings; fragments of fibres; lower epidermis with stomata; rosette crystals of calcium oxalate; fragments of trichome. Powder microscopy of *M. tridentata* ssp. *hastata* shows epidermal cells in surface view; fragments of vessels and tracheids with pitted and spiral thickenings; fragments of fibres; rosette crystals of calcium oxalate. Powder microscopy of *P. foetida* shows epidermal cells in surface view; fragments of vessels and tracheids with pitted and spiral thickenings; groups of fibres; fragments of trichomes; lower epidermis with stomata (**Fig. 4**).

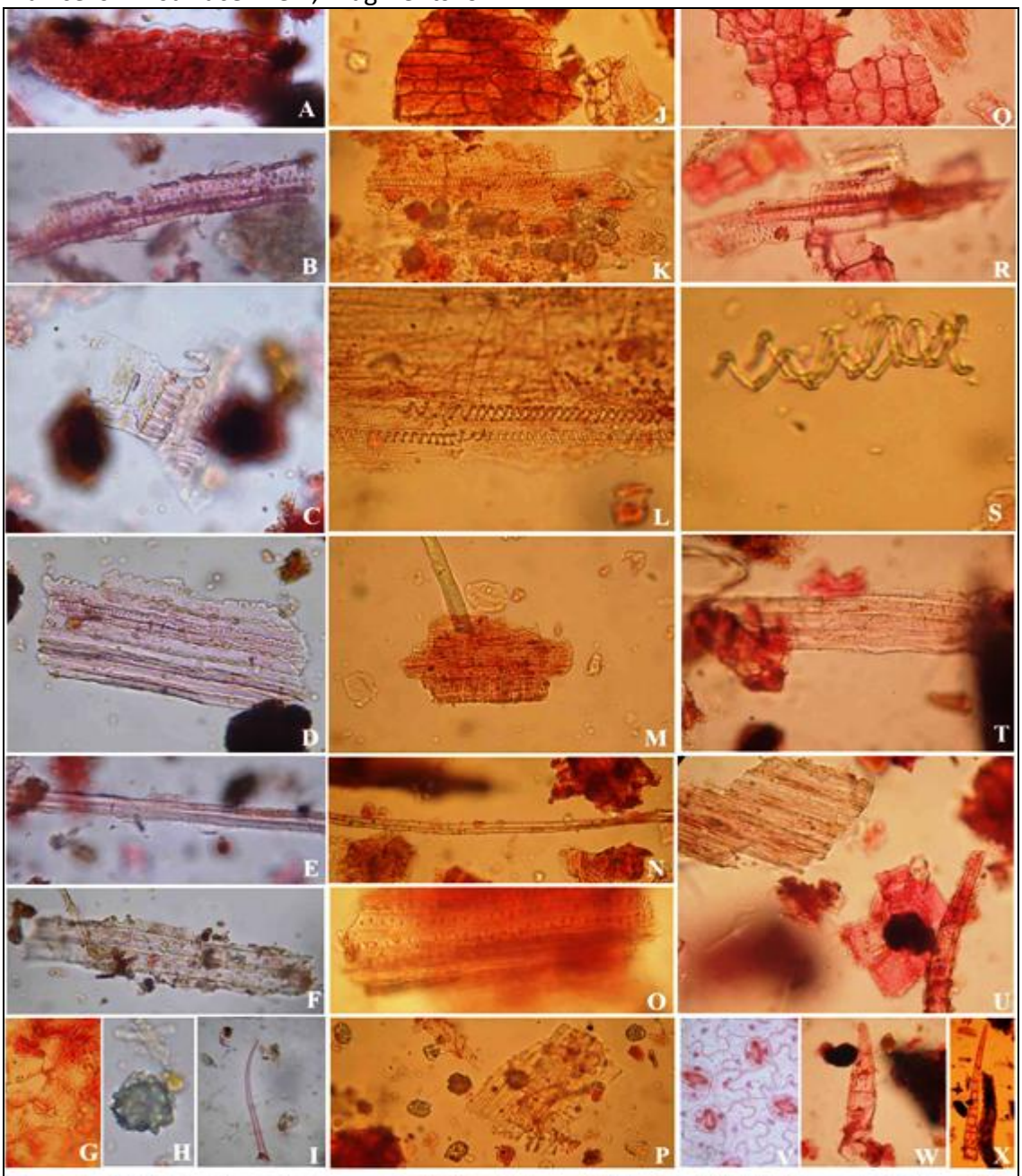

Figure 4. (A-I). Powder microscopy of *M. tridentata ssp. tridentata.* (A). Epidermal cells in surface view, (B-D). Fragments of vessels and tracheids with pitted and spiral thickenings, (E-F) Fragments of fibres, (G). Lower epidermis with stomata, (H). Rosette crystals of calcium oxalate, (I). Fragments of trichome. (J-P). Powder microscopy of *M. tridentata ssp. hastata.* (J). Epidermal cells in surface view, (K-M & O). Fragments of vessels and tracheids with pitted and spiral thickenings, (N) Fragments of fibres, (P). Rosette crystals of calcium oxalate. (Q-X). Powder microscopy of *P. foetida.* (Q). Epidermal cells in surface view, (R-S). Fragments of vessels and tracheids with pitted and spiral thickenings, (T). Groups of fibres, (U, W & X). Fragments of trichomes, (V). Lower epidermis with stomata.

TABLE 1: COMPARATIVE STEM ANATOMICAL CHARACTERS

Characters	*M. tridentata* ssp. tridentata	*M. tridentata* ssp. *hastata*	*Paederia foetida*
Shape in TS	TS of stem is angular in outline with 8 protruberances (Fig. 2 A).	TS of stem is pentagonal in out line (Fig. 2 F).	TS of stem is oval in outline (Fig. 2 K).
Nature of cortex	Cortex is chlorenchymatous and slightly tangentially elongated parenchymatous cells. Large cavities are present in the cortex (Fig. 2 B).	Cortex is chlorenchymatous; many cells contain rosette crystals of calcium oxalate. Large cavities with oil globules are present in the cortical region (Fig. 2 G).	Innermost 2-3 layers of cork cells contain a brownish deposit. Phellogen1-2 layered. Phelloderm broad and some of them contain acicular crystals of calcium oxalate (Fig. 2 L).
Nature of phloem	Phloem is very narrow and composed of sieve tubes, companion cells and phloem parenchyma (Fig. 2 C).	Phloem region is comparatively broad. Number of large irregular cavities and rosette crystals of calcium oxalate are present in the phloem region (Fig. 2 H).	Phloem region is broad, three types of crystals ie, acicular, raphide and prismatic crystals are present in the phloem region (Fig. 2 M).
Nature of vessels	Xylem forms a continuous ring with a few xylem vessels and numerous primary xylem groups along the inner periphery (Fig. 2 A-C).	Secondary xylem forms continuous ring with certain projections and furrows at places. Vessels are wide, large in number and solitary or in groups of 2-3 (Fig. 2 F-H).	Xylem region is very wide and it is highly furrowed. Vessels are wide solitary and are scattered. Primary xylem elements form a continuous ring towards the pith (Fig. 2 M).
Occurrence of starch grains	Starch grains are present only in some cortical cells and pith cells (Fig. 2 D & E).	Starch grains are present only in some cortical cells and pith cells (Fig. 2 I & J).	Starch grains are present in all the cells of the cortex and cells towards the periphery of the pith (Fig. 2 N & O).
Nature of pith	Pith is very large, parenchymatous. Some of the pith cells contain rosette crystals of calcium oxalate (Fig. 2 E).	Pith large and composed of parenchymatous thick walled cells (Fig. 2 J).	The centre is occupied by wide pith which is oval in shape and the centre is occupied by a linear strip of collapsed cells (Fig. 2 O).

TABLE 2: COMPARATIVE ROOT ANATOMICAL CHARACTERS

Characters	*M. tridentata* ssp. *tridentata*	*M. tridentata* ssp. *hastata*	*Paederia foetida*
Nature of cork	Cork is composed of 3-4 rows of thin walled polygonal cells.	Cork is 4-5 layered thin walled rectangular and tangentially elongated.	Cork consists of 8-10 layered narrow rectangular cells, innermost cells contains masses of fatty crystals and oil globules.
Nature of cortex	Cortex consists of 8-12 layers of polygonal and tangentially elongated cells. Most of the cortical cells consist of rosette crystals of calcium oxalate. A few solitary oil-containing cells are present. Starch grains are also seen (Fig. 3 A).	Cortex is broad, which are rectangular, tangentially elongated. Crystals of calcium oxalate and large number of oil containing cells are scattered throughout the cortical region. Almost all the cells contain simple rounded or oval starch grains (Fig. 3 F).	Cortex is comparatively broad, which are rectangular and compactly arranged and slightly tangentially elongated. Many of the cells containing acicular or raphide crystals of calcium oxalate. Oil cells are absent. Most cells consist of compound starch (Fig. 3 K).
Nature of phloem	Phloem is comparatively narrow; also contain oil containing cells and very few rosette crystals. Phloem is traversed by many uniseriate medullary rays (Fig. 3 E).	Phloem is comparatively narrow, all the phloem cells are fully filled with starch grains, many oil cells are scattered. Some cells containing rosette crystals of calcium oxalate (Fig. 3 J).	Phloem seen as radially elongated conical patches. Between the phloem patches medullary rays are broad and multiseriate. Starch grains very few Compressed cells are also seen (Fig. 3 P).
Nature of wood	Comparatively broad, 2/3rd portion of the root is occupied by wood. Wood region is characterized by interxylary phloem. Some of the interxylary phloem cells contain crystals of calcium oxalate. Wood is furrowed by broad medullary rays (Fig. 3 C &D).	Comparatively broad, 2/3rd portion of the root is occupied by wood. Wood region is characterized by interxylary phloem, but their number is more than *M. tridentata*, fully filled with starch grains. Some of the interxylary phloem cells contain crystals of calcium oxalate also (Fig. 3 H &I).	Wood is occupied by 3/4th portion, consists of xylem vessels, tracheids and parenchyma, it is widely deeply furrowed at four regions. All the parenchyma cells in the wood region fully filled with small compound starch grains. Some of the cells contain raphide crystals of calcium oxalate (Fig. 3 M, N &O).
Nature of vessels	Vessels are very wide and many. Tyloses present. Some of the vessels contain oil globules (Fig. 3 B & E).	Vessels are many in number; most of the vessels are solitary. Tyloses present. (Fig. 3 G).	Vessels are less in number (Fig. 3 L).
Nature of medullary rays	Medullary rays are usually uniseriate but are multiseriate at four points which gives furrowed appearance. Rosette crystals of calcium oxalate are present in some of the cells (Fig. 3 A).	Medullary rays are usually uniseriate, fully filled with starch grains. Rosette crystals are also present (Fig. 3 F).	Usually medullary rays are uniseriate but multi seriate medullary cells are seen at the furrowed region (Fig. 3 K).
Nature of primary xylem	Primary xylem groups are limited in number.	Primary xylem groups are seen in the form of a continuous ring and many in number.	Four primary xylem groups are seen in the center.

Phytochemical study: In TLC profiles of methanol extract of *M. tridentata* ssp*. tridentata M. tridentata* ssp*. hastata and Paederia foetida*, Rf values of the prominent bands were determined and observed that the TLC profiles of the three plants were almost identical except one additional band which is present in *P. foetida* (**Table 3 & Fig. 5**).

TABLE 3: TLC DETAILS OF METHANOL EXTRACTS

R_f value of bands in			
M. tridentata	***M. hastata***	***P. foetida***	**Colour of the band**
0.09	0.09	0.09	Violet
0.29	0.29	0.29	Dark violet
0.56	0.56	0.56	Light violet
0.61	0.61	0.61	Greyish blue
_	_	0.78	Light violet
0.87	0.87	0.87	Dark violet
0.92	0.92	0.92	Violet
0.96	0.96	0.96	Grayish violet

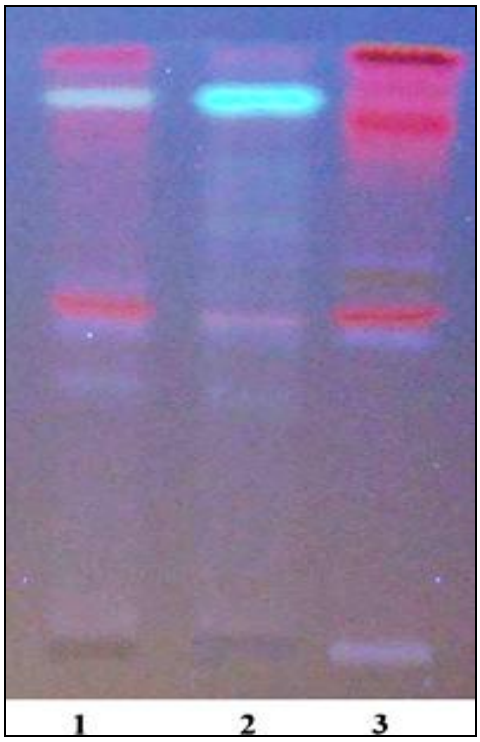

FIG. 5: TLC PROFILE OF METHANOL EXTRACT OF 1. *MERREMIA TRIDENTATA* SSP. *TRIDENTATE,* 2. *MERREMIA TRIDENTATA* SSP. *HASTATE, 3. PAEDERIA FOETIDA* (UV- 366nm)

HPLC method has been developed to distinguish the three plants have almost same chemical profiles with little difference in their quantity. Quantification of β-sitosterol was carried out using the area under the curve method. The concentrations β- sitosterol in *M. tridentata* ssp*. tridentata*, *M. tridentata* ssp*. hastata* and *P. foetida* were 0.099 %, 0.134 %, and 0.008 % respectively (**Table 4 & Fig. 6**).

TABLE 4: CONCENTRATIONS OF β- SITOSTEROL

Sample	Concentrations of β- sitosterol (%)			Average conc. (%) W/W
	1	2	3	
M. tridentata	0.096	0.097	0.104	0.099
M. hastata	0.132	0.130	0.140	0.134
P. foetida	0.0085	0.0084	0.0079	0.008

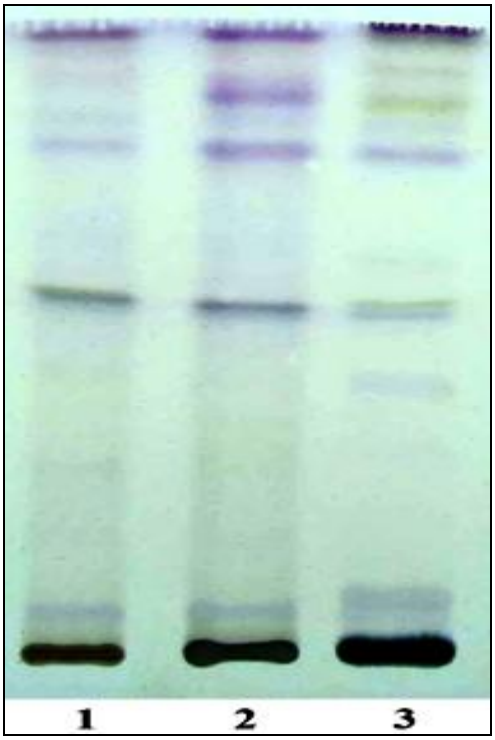

FIG. 6: TLC PROFILE OF METHANOL EXTRACT OF 1. *MERREMIA TRIDENTATA* SSP. *TRIDENTATE,* 2. *MERREMIA TRIDENTATA* SSP. *HASTATE, 3. PAEDERIA FOETIDA* (DERIVATIZED WITH AS REAGENT VIS.)

According to Ayurvedic Pharmacopoeia of India [3] the source plant of the drug *Prasarini* is *Paederia foetida*. In Kerala *Merremia tridentata* ssp. *tridentata* and *Merremia tridentata* ssp. *hastata* are used as the source plant of this drug [1].

Preliminary pharmacognostic studies of *Merremia tridentata* ssp. *tridentata* and *Merremia tridentata* ssp. *hastata* was carried out by Aiyer and Kolammal [4]. Preliminary pharmacognostic studies of *Paederia foetida* was carried out by Gupta *et al.*, [5] and Prasad *et al*., [6]. Chemical studies of *Paederia foetida* was carried out by Bose *et al*., [7].

There are no reports regarding the comparative study of these three plants. This paper deals with the comparative study of morphological, anatomical, histochemical and phytochemical characters of these three plants. Anatomically these three plants show differences in many characters, but phytochemical

studies revealed that these plants show much similarity. β- Sitosterol was present in all the three plants but their concentrations shows differences.

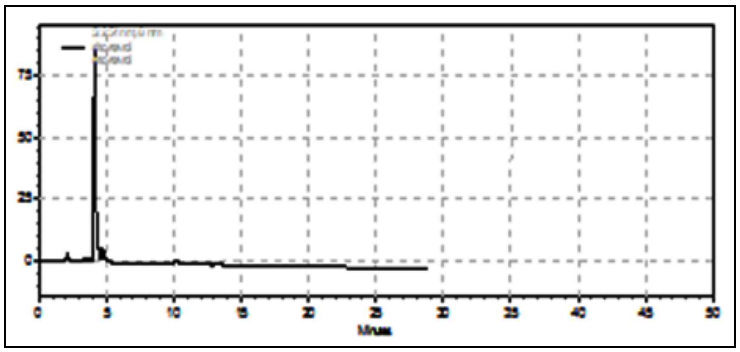

FIG. 7: HPLC CHROMATOGRAM OF STANDARD β- SITOSTEROL

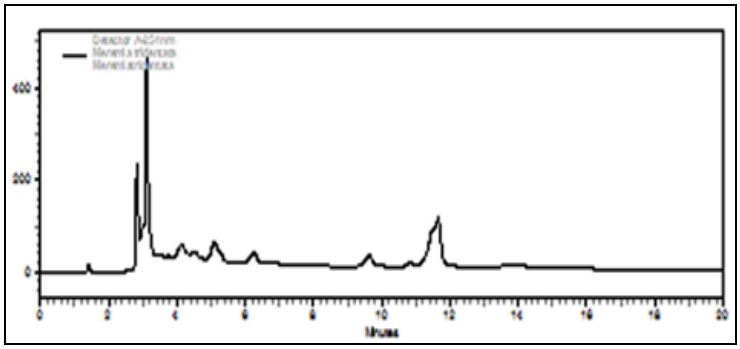

FIG. 8: HPLC CHROMATOGRAM OF *MERREMIA TRIDENTATA* SSP. *TRIDENTATA*

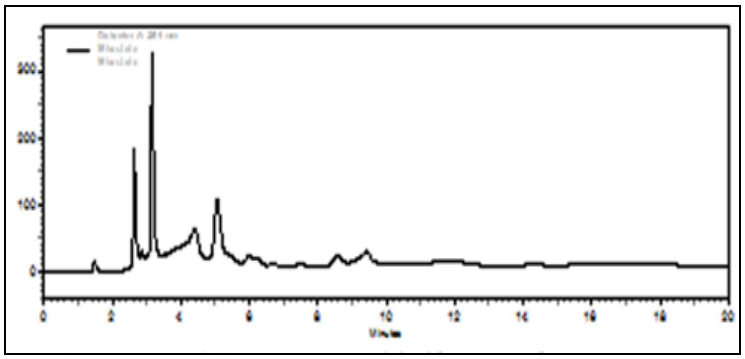

FIG. 9: HPLC CHROMATOGRAM OF *MERREMIA TRIDENTATA SSP. HASTATA*

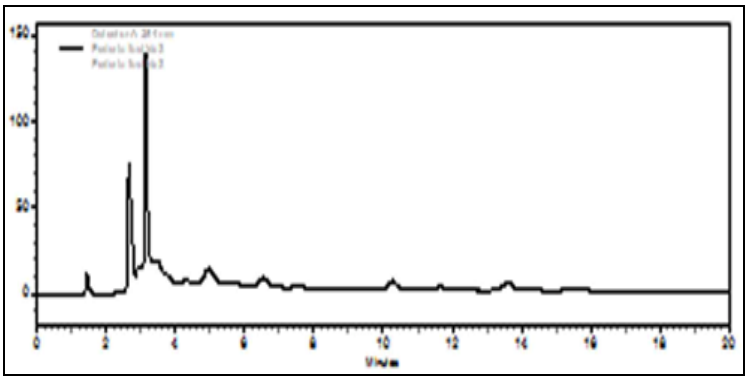

FIG. 10: HPLC CHROMATOGRAM OF *PAEDERIA FOETIDA*

CONCLUSION: The present study helps to distinguish them conveniently and coming to a conclusion that the drug *Prasarini* is equated with *Paederia foetida* as the source plant and the two sub species of *Merremia tridentata* are used as the substitutes. For further confirmation pharmacological studies are to be carried out.

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