Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF LEAVES OF *TEPHROSIA VILLOSA* PERS.

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Received: 22 Feb 2013, Revised and Accepted: 12 Apr 2013

ABSTRACT

OBJECTIVE: An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of the plant both pharmacognostically as well as physiochemically. METHOD: Various parameters like macroscopy, microscopy, Physiochemical parameter and Preliminary phytochemical studies of the leaves powder were also carried out. RESULT: Physiochemical parameters like total ash content, water soluble ash, acid insoluble ash, sulphated ash, alcohol soluble extractive and water soluble extractive were found to 9.34%, 0.72%, 4.28%, 5.20%, 8% and 20% respectively. The qualitative parameters are reported. The plant is rich in carbohydrates, saponins, coumarin glycosides, flavanoids and phytosterol. CONCLUSION: The present study on Pharmacognostical investigation of *Tephrosia villosa* Pers. leaves might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs

Keywords: *Tephrosia villosa* Pers. leaves.

INTRODUCTION

Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [1-4]. There is a need for documentation of research work carried out on traditional medicines [5]. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [6]. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics [7]. These standards are of utmost importance not only in finding out gentry, but also in detection of adulterants in marketed drug [8]. *Tephrosia villosa* Pers. (Fabaceae) is a much-branched, perennial herb, up to 90cm. high, densely clothed with white, silky hair, found in Punjab, Rajasthan, Gujarat, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal. It is well known as Punaikkaivettali (Tamil), Nooguvempali (Telugu), Sroetokolothiya (Oriya) and Runchhalisarpankho (Gujarat). Its root and leaves are used as hypoglycemic agent and in dropsy [9, 10].The plant contains rotenoids, prenylated flavonone [11].

The objective of present study is to focus on Pharmacognostical and Preliminary Phytochemical characteristics of leaves of *Tephrosia villosa* Pers. [Figure 1].

Fig. 1: *Tephrosia villosa* Pers. Plant

MATERIAL AND METHOD

Plant material

The plant specimens for the proposed study were collected from Salem district (T.N.) in the month of June 2006 care was taken to select healthy plants and for normal organs. The plant was authenticated by P.Jayaraman, Plant Anatomy Research Center, West thambaram, Chennai, Tamil Nadu. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per method [12]. Infiltrations of the specimens were carried out by gradual addition of paraffin wax (melting point 58 – 60^{0}C) until TBA solution attained super-saturation. The specimens were casted into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary Microtome. The thicknesses of the sections were 10-12 μm. De waxing of the sections were done by customary procedure [13]. The sections were stained with Toluidine blue as per the method [14]. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some Cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to sub ring, violet to the mucilage, blue to the protein bodies etc.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab photo 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale – bars [15].

Physicochemical Parameters

Physicochemical parameter of leaves of *Tephrosia villosa* Pers. were determined such as Total ash, Acid insoluble ash, Water soluble ash, Sulphated ash, moisture content etc [1617].

Preliminary Phytochemical Parameters

Preliminary phytochemical test of *Tephrosia villosa* Pers. were performed and the chemical constituents were detected [18, 19].

RESULTS AND DISCUSSION

Macroscopy

Leaves nearly sessile, 5-7.5cm, 10ng; stipules linear – lanceolate,acute sometimes reaching km, long, deflexed or spreading (rarely ascending). Leaflets 11 – 19, Grey – green 1.2 xx– 2.2 cm by 4.5 – 8 mm, narrowly oblanceolate, usually emarginated and mucronate, nearly glabrous above, silky beneath, base cuneate; petioles of lateral leaflets 1.5mm, those of terminate 3mm long.

Microscopy

Midrib

The midrib is flat on the adaxial side and shallowly hemispherical on the abaxial side. The midrib is 250 μm in vertical plane and 250 μm in transverse plane. The adaxial epidermis is single layered, and the epidermal cells are cubical to squarish and thick walled. The adaxial palisade mesophyll tissue is partly horizontally transurrent on the adaxial part of the midrib. The hemispherical abaxial epidermal cells are small, cubical and single layered. The hemispherical abaxial part consists two to three layers of compact angular collenchymas cells and abaxial palisade tissue extending upto the shoulders of the hemispherical part. The ground tissue consist of few parenchyma cells which are angular compact [Figure 2.1].

The vascular tissue of the midrib occurs as an abaxial arc. The vascular bundle is collateral. The xylem elements are few and occur in radial sequence on the adaxial part of the midrib. The phloem elements are as a thin band beneath the xylem on the abaxial part. The vascular bundle consists of sclerenchymatous bundle cap on both abaxial and adaxial sides of the midrib [Figure 2.2].

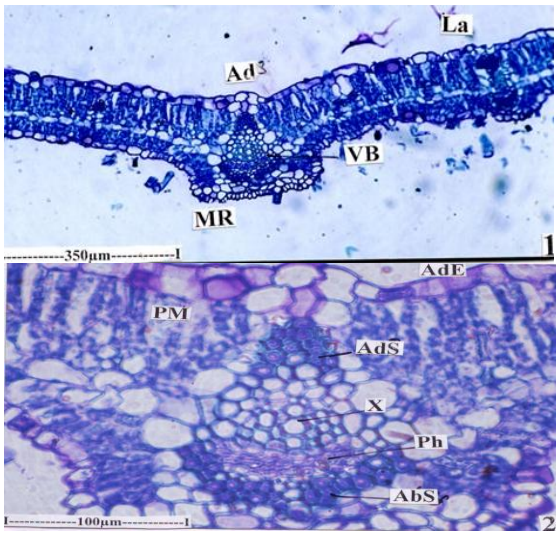

Fig. 2: T.S. of leaf, 2.1 T.S. of leaflet; 2.2 T.S. of Midrib of the leaflet (enlarged)

(La-Lamina, Ad- Adaxial side, VB- vascular Bundle, MR- Midrib AdE- Adaxial Epidermis, PM- Palisade Mesophyll, AdS- Adaxial Sclerenchyma Cap,X- Xylem, Ph- Phloem, AbS- Abaxial Sclerenchyma cap).

Lamina

The lamina is 450 μm thick**.** The leaf is dorsiventral, mesomorphic, amphistomatic, glabrescent and even. The adaxial epidermal cells are rectangular, thick walled, single layered, and the cuticle is thick, the abaxial epidermal cells are small, cubical to squarish and single layered. Palisade mesophyll tissue is present on both abaxial and adaxial sides of the lamina. The palisade mesophyll tissue on the adaxial side is cylindrical, bi-layered measuring 200 μm in height, occupies two third thickness of the lamina ; abaxial palisade is single layered , cylindrical and 100 μm in height. Spongy mesophyll tissue is inconspicuous, single layered and occur in between the adaxial and abaxial palisade tissue. Lateral vein bundles are prominent and consist of few vascular tissue. Lateral vein bundles have sclerenchymatous bundle cap, on both the abaxial and adaxial part of the lamina. These sclerenchymatous bundle cap cells about the epidermal cells of lamina. Spherical globular trichomes are evident on the surface of lamina.

The marginal part of the lamina is semi circular. It is as thick as the middle lamina. The epidermal cells of the margin are slightly dilated and have prominent cuticle. There is a circular marginal vein, the mesophyll tissue are along the lateral side of the vascular showed [Figure 3.1, 2].

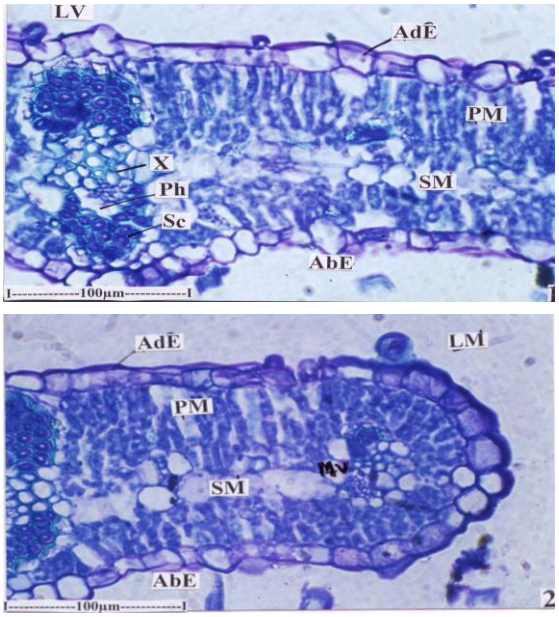

Fig. 3: T.S. of Lamina.; 3.1 T.S. of Lamina and lateral vein; 3.2 T.S. of Lamina through leaf margin

(LV- Lateral Vein, AdE- Adaxial Epidermis, PM- Palisade Mesophyll, SM- Spongy Mesophyll, X- Xylem, Ph- Phloem, Sc- Sclerenchyma AbE- Abaxial Epidermis LM- Leaf Margin, MV- Marginal Vein)

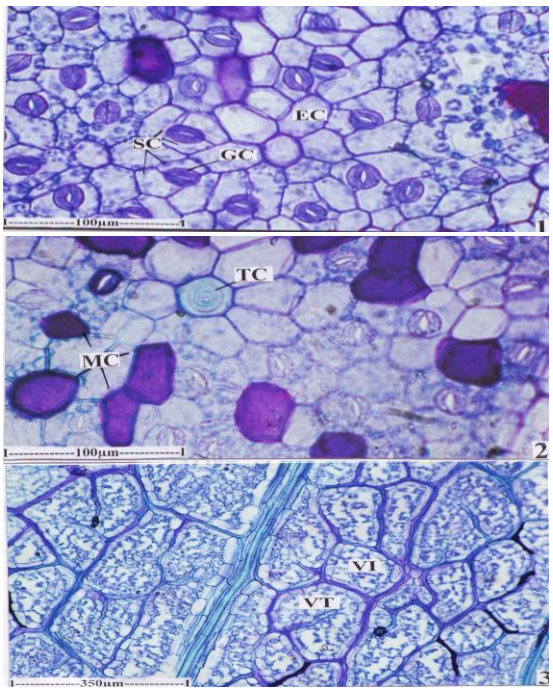

Fig. 4: lamina showing Stomatal type.; 4.1 Paradermal Section of the lamina showing Stomatal type; 4.2 Paradermal Section of the lamina showing mucilage epidermal cells; 4.3 Venation patern of the lamina

(VI- Vein Islet, VT- Vein Termination,EC- Epidermal cells, SC- Subsidary Cells, GC- Guard Cells, TC- Trichome Cell, MC- Mucilage Cells).

Stomatal Morphology

Both abaxial and adaxial epidermis is stomatiferous, the adaxial epidermal cells are thick walled and polyhedral, abaxial epidermal cells are, thin walled and polyhedral, stomata are Anisocytic i.e. The three subsidiary cells that surround the guard cells are unequal in size [Figure 4.1]**.** Mucilage cavities are abundant in the abaxial epidermal region. The trichome bearing epidermal cells is circular and thick walled [Figure 4.2].

Venation pattern

The lateral veins and vein lets are thick and form prominent islets. The vein lets form wide rectangular or polyhedral vein islets [Figure 4.3]. Vein terminations are fairly distinct, thick and mostly unbranched and rarely branched the terminations are slightly.

Powder Microscopic Results

Maceration

Macerated elements show the types of fibers, their width and length and vessel types, lateral wall thickenings of the vessels, length and width of vessel elements, perforation plate, epidermal trichome leaf vein etc. in tephrosia the epidermal trichomes are long, narrow, unbranched with pointed tip [Figure 6.1, 2, 3] the trichomes are 10 μm in width and 200-400 μm in length [Figure 6].

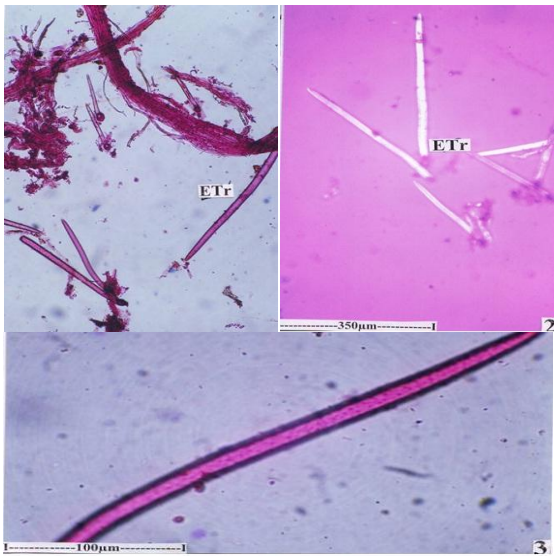

Fig. 6: Trichomes; 6.1 Epidermal trichomes; 6.2 Epidermal as seen under the polarised light to show the lignified walls; 6.3 A portion of the trichomes (enlarged)

(ETr- Epidermal Trichomes)

Fragments of lamina with venation

Leaflets and fragmentary lamina are after seen in the powder. The lamina has thick straight midrib with the lateral veins originating from the midvein and converging at the apical part of the lamina [Figure 5.1]. Vein islets and vein terminations are also seen in the broken lamina [Figure 5.2].

Physicochemical Parameters

Leaves of *Tephrosia villosa* Pers. powder showed the presence of total ash 9.34% w/w, acid insoluble ash 4.28% w/w, water soluble ash be 0.72% w/w, sulphated ash 5.20% w/w , alcohol soluble extractive 8% w/w water soluble extractive 20% w/w and moisture content 6.10% w/w.

Preliminary Phytochemical Studies

Ethanolic extract of leaves of *Tephrosia villosa* Pers. Showed the presence of various Phytoconstituents such as carbohydrates, saponins, coumarin glycosides, flavanoids and phytosterol.

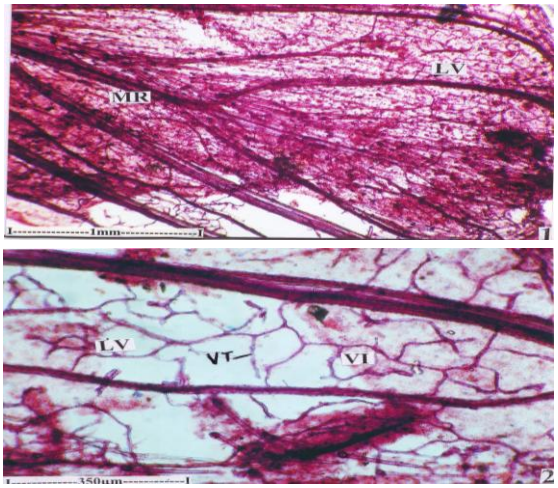

Fig. 5: Powder microscopy; 5.1 Leaflet showing Veins 5.2 Lamina showing vein islet and vein terminations

(LV- Lateral Vein, VT- vein Termination, VI- Vein Islet, MR-Midrib, LV- Lateral Vein)

CONCLUSION

The present Pharmacognostical studies of roots of *Tephrosia villosa* Pers. might be useful to supplement assumed significantly in the way of acceptability of herbal drugs in present scenario that lacks regulatory laws to control quality of herbal drugs.

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