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Research Article

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION ON STEMS OF *TEPHROSIA VILLOSA* PERS

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ABSTRACT

The objective of present studies deals with the macroscopically and microscopically studies of stems of *Tephrosia villosa* Pers. Some distinct and different characters were observed with section of young thin stems. Physiochemical parameter and Preliminary phytochemical studies of the stems powder were also carried out. The present study on Pharmacognostical investigation of *Tephrosia villosa* Pers. stems 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. stems, Pharmacognostical, Physiochemical studies

INTRODUCTION

Humankind first utilized materials found in environment on an empirical basis to cure various ailments. Natural products from plants and animals traditionally have provided the pharmaceutical industry with one of its important sources of lead compounds in search of new drugs and medicines. The search for new pharmacologically active agents from natural resources such as plants, animals and microbes led to discovery of many clinically useful drugs [1]. Over the past two decades, researchers have also turned to many of the traditional folk medicines – invariably a "cocktail" of natural products to uncover the scientific basis of their remedial effects, which improves the efficacy as to enhance modern medical practices [2]. The growing awareness of the harmful side-effects of chemotherapy, has made people to explore the time tested remedies from traditional alternative medicine. India being a tropical country is blessed with vast natural resources and ancient knowledge for its judicious utilization. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and understand the mechanism of action [3]. There is a need for documentation of research work carried out on traditional medicines [4]. 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 [5].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 [6].These standards are of utmost importance not only in finding out gentry, but also in detection of adulterants in marketed drug [7]. *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 [8, 9].The plant contains rotenoids, prenylated flavonone [10].

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

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 [11]. 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 thickness of the sections were 10-12 μm. De waxing of the sections were done by customary procedure [12].The sections were stained with Toluidine blue as per the method [13]. 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 subring, 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 [14].

Qualitative and Quantitative Investigations

Quantitative stems of *Tephrosia villosa* Pers. were determined such as Total ash, Acid insoluble ash, Water soluble ash, Sulphated ash, moisture content etc, alcohol soluble extractive and water soluble extractive were determined [15,16]. Physicochemical parameter of Fluorescence characteristic of the powder is an essential parameter for first line standardization of crude drug. The powdered drug of stems of *Tephrosia villosa* Pers. was treated separately with different reagents and exposed to visible, UV light (short and long) to study their fluorescence behavior [17].

Preliminary Phytochemical Investigations

Conventional standard protocols for detecting the presence of different chemical constituents in the plant extract were employed. The tests for the secondary metabolites viz. alkaloids, tannins, sterols, saponins, amino acids, glycosides, proteins, sterols /

terpenes, resins, flavonoids and phenols were carried out with the different extracts of stems of *Tephrosia villosa* Pers. using preliminary phytochemical screening [18,19].

RESULTS AND DISCUSSION

Macroscopy

Tephrosia villosa is an annual or perennial bushy herb, 0.3-1.3 m tall. Stem white tomentose and hairy surface. Stem is generally green when young and later often become woody and dark brown. Pod strongly curved, up to 4 cm x 6 mm, densely silvery or brown-tomentose, hairs to 2 mm long, 4-10-seeded. Seed 12-16, rectangular, black and smooth with short hard excrescences, up to 4.5 mm x 2.5-2.75 mm. The specific name '*villosa*' means covered in white soft hair in Greek [Figure 1].

Fig. 1: *Tephrosia villosa* Pers. Plant

Microscopy

T.S. of Stem [Figure 2.1, 2]

The stem is more or less angular in cross section; small, short and blunt ridges are present at the angles of the stem. Stem showed initial phase of secondary growth. Periderm is not evident and the epidermal cells are intact, the surface of stem is covered with dense mat of trichomes, the epidermis is thick and unistratose, the epidermal cells are small, cubical and thick walled. The epidermis is followed by a narrow cortex composed of 4 to 5 layers of compact, angular collenchymas cells. Cortex is followed by thin discontinuous bands or strips of perivascular sclerenchyma cells, which surround the vascular cylinder [Figure 2.1, 3.1].

The vascular cylinder of the stem consists of narrow cylinder of secondary xylem and secondary phloem [Figure 2.2]. Secondary xylem is composed of vessels, xylem fibers and xylem parenchyma cells, xylem vessels are narrow lumen, thick walled, circular or angular and occur in radial multiples of 2to3 vessel elements, xylem vessels are fairly well developed in the fascicular region than in the interfascicular region [Figure 2.2, 3.2]. Xylem fibers are narrow, thick walled and lignified, the secondary phloem occur as narrow band around the xylem cylinder and consist of sieve tube members, phloem fibers and phloem parenchyma, the sclerenchymatous bundle cap of the vascular bundles are still evident around the phloem [Figure 3.1]. The Centre of stem is occupied by wide, thin walled, angular or polygonal shaped parenchyma cells.

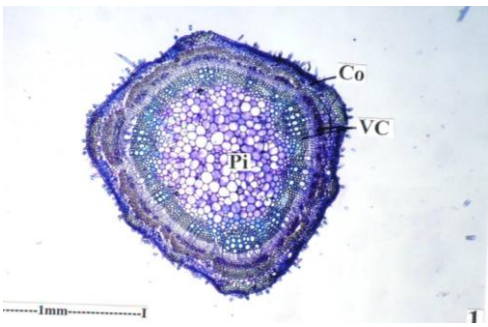

Fig. 2.1: T.S. of Stem

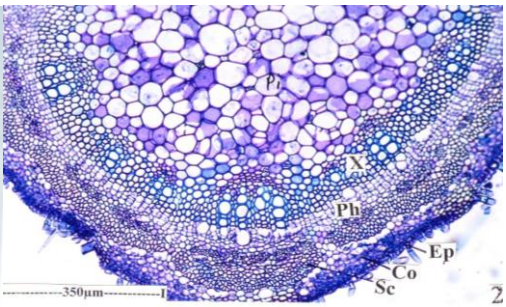

Fig. 2.2: T.S. of stem (enlarged sector)

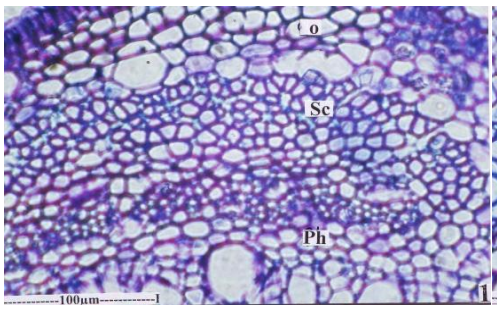

Fig. 3.1: T.S. of stem (cortex phloem enlarged)

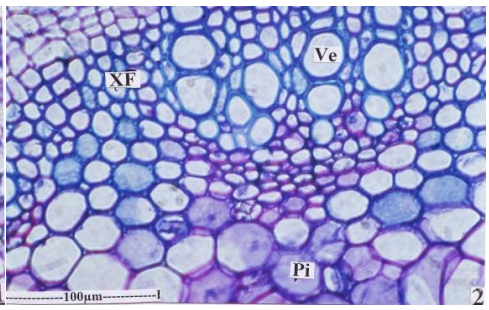

Fig 3.2: T.S. of stem (xylem portion)

(Co-Cortex, Ph- Phloem, Sc- Sclerenchyma, Ve - Vessel, XF-Xylem Fiber, Pi - Pith, X- Xylem, Ep - Epidermis, VC - Vascular Cylinder,)

Powder Microscopy

Maceration

Macerated elements show the types vessels are narrow, thick walled with simple perforation plate, lateral wall pitting of the vessels are distinct [Figure 4.1, 2] vessel length ranges from 150µm to 170 µm and width from 30 µm to 40 µm, fibers are long, thick walled, narrow lumen and lignified and the length of the fibers ranges from 300µm to 500 µm [Figure 5.2].

Calcium oxalate crystals

Calcium oxalate crystals are sparsely seen in the parenchyma cells of the stem [Figure 5.1]. The crystals are prismatic types. They are located in the cells in the sclerenchyma xylem tissues.

Parenchyma cells

Parenchyma cells are also frequently met with in the powder [Figure 5.1]. The cells elongated and scale like. They often contain dense accumulation of starch grain.

Fig. 4.1 and 4.2: Powder microscopy (Vessel element)

(PP –Perforation Plate, Fi - Fiber, LWP –Lateral Wall Pits, VE –Vessel Element)

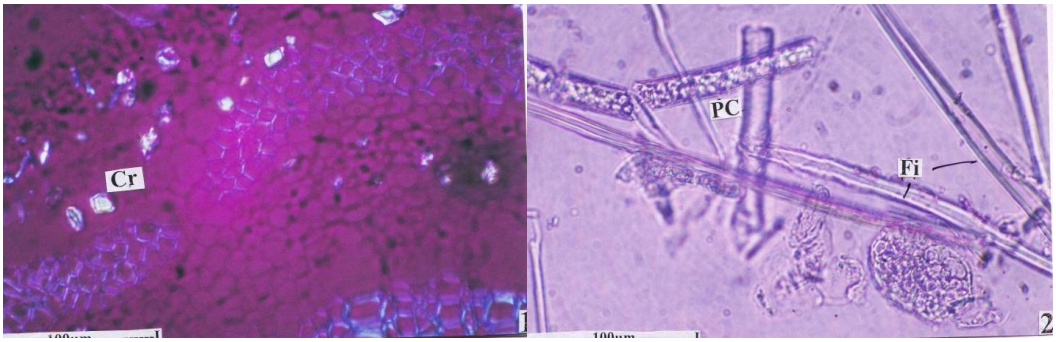

Fig 5.1: Crystal in stem (Polarized light)

Fig. 5 .2: Fibers and Parenchyma cells

(Cr- Crystals, PC- Parenchyma Cell, Fi- Fibers)

Table 1: Results of quantitative microscopy and Physical parameters of the stems of Tephrosia *villosa* Pers.

S. No.	Parameter	Result (%)
1.	Total ash value	3.5
2.	Acid insoluble ash	0.5
3.	Water soluble ash	2.5
4.	Sulphated ash	4.5
5.	Moisture content	6.8
6.	Extractive value (water soluble)	8
	(Alcohol soluble)	1.6

Table 2: Fluorescence characters of the powdered stems *Tephrosia villosa* Pers. under UV light.

Treatments	Colour developed under UV light	
	Short (254 nm)	Long (366 nm)
Powder as such	Pale yellow	Green
1N HNO3	Pale green	Pale yellow
5N NaOH in water	Green	Pale yellow
1N HCl	Yellow	Green
50% HNO3	Yellow	Dark green
Acetic acid	Pale Yellow	Pale green green
Fecl3 (5%w/v aqueous solution)	Yellow	Dark green
50% H2SO4	White yellow	Dark green
1N NaOH in ethanol	Pale yellow	Green

Table 3: Preliminary Phytochemical screening of various extracts of stems of *Tephrosia villosa* Pers.

S. No.	Plant constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Carbohydrates	+	-	-	+	-
2.	Protein	-	-	-	+	-
3.	Amino acids	+	-	-	-	-
4.	Glycosides	-	+	+	+	-
5.	Flavonoids	-	+	+	+	-
6.	Tannins and Phenolic compound	+	+	+	+	+
7.	Alkaloids	-	+	+	+	-
8.	Saponins	-	-	-	+	+
9.	Coumarin	-	-	+	+	+
10.	Lipid	+	-	-	-	-
11.	Phytosterol	-	-	-	-	-

Qualitative and Quantitative Evaluation Parameters

The calculated quantitative values and physiochemical parameters of the stems of *Tephrosia villosa* Pers. are presented in Table 1. The fluorescence characters of the powdered stem with different chemical reagents are shown in Table 2.

Preliminary Phytochemical Investigation

The preliminary phytochemical analysis of the stems of *Tephrosia villosa* Pers. showed the presence of alkaloids, amino acids, flavonoids, phenol, proteins, sterols/terpenes and tannins [Table 3]. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for the various actions of Tephrosia *villosa* Pers.

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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