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PHARMACOGNOSTICAL STUDIES OF *TINOSPORA CORDIFOLIA* (MIERS.) HK. F & TH. (STEM)

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Abstract

Tinospora cordifolia commonly known as Guduchi is used in traditional systems of medicine for general debility, dyspepsia, fevers and urinary diseases. The plant also possesses antiviral as well as antibacterial properties. The present study provides a detailed pharmacognostic study based on its physicochemical, macroscopic, microscopic and chromatographic features. The physicochemical parameters such as loss on drying, solubility in different solvents, ash content, acid insoluble ash, water soluble ash, volatile oil, fibre content etc. were determined by standard methods. Anatomical features of the stems of *Tinospora cordifolia* were determined. For this the sample was fixed in FAA, cast into paraffin blocks and sectioned with the help of Rotary Microtome. The stomata morphology, venation pattern and trichome distribution were studied. Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. Powder microscopy was carried out using standard methods. HPTLC profile of the methanolic plant extract was carried out in short UV, long UV and using anisaldehyde - sulphuric acid as detection reagent. The R_f values of the spots developed were noted which is an important parameter for identification of plant materials. The pharmacognostical parameters

along with the HPTLC profile may be utilized to identify the drug material and for laying down the pharmacopoeial standards.

Keywords:

Tinospora cordifolia, pharmacognostic study, microscopic features, physicochemical parameters, HPTLC profile.

Introduction

Tinospora cordifolia (Miers.) Hk. F & Th. is a large, glabrous, deciduous climbing shrub found throughout tropical India, belonging to the family Menispermaceae[1,2,3], ascending to an altitude of 300 m. Stems are succulent with long filiform fleshy aerial roots from the branches. Bark is greyish brown or creamy white and warty. Leaves are membraneous, cordate with a broad sinus. Flowers are small, yellow or greenish yellow, appearing when the plant is leafless, in auxillary and terminal raceme or racemose panicles. Male flowers are clustered and female flowers are usually solitary. Drupes are ovoid, glossy, succulent, red and pea-sized. Seeds are curved[4].

Tinospora cordifolia is mentioned as a constituent of several compound preparations, used in general debility, dyspepsia, fevers and urinary diseases. The plant also possesses antiviral as well as antibacterial properties. Different constituents reported to be present in the stem are alkaloids including berberine, a bitter glucoside giolin, giloinin, gilo-sterol, columbin, chasamanthin, palmarin, tinisporon, tinosporic acid and tinosporol. The vernacular names of the plant are:- Sanskrit – Amrita, guduchi, jwarari; Hindi – Amrita, giloe, gulancha, gulbel, guloh, gurcha, jiwantika; Bengali – Golacha; Marathi – Gulvel; Gujarathi – Gulvel, Telugu – Tippateege; Tamil – Amudom, chindil; Kannarese – Amrutaballi, madhuparne, uganiballi; Malayalam – Amrytu, chittamriyham; Oriya – Gulochi. Dry twigs with bark intact, constitute the drug. Stem is a constituent of several medicines used in general debility, dyspepsia, fevers and urinary diseases. Bitter principles present in the drug show antispasmodic, antipyretic, and anti-inflammatory properties. Root is a powerful emetic and used for visceral obstruction. Its watery extract is used in leprosy[5]. The drug is used in scorpion-sting. An infusion prepared from the stem and root is a valuable tonic in deliberating diseases, intermittent fever and dyspepsia[6]. The stem powdered and made into infusion used as alterative and aphrodisiac[7]. Pharmacognostical studies of the medicinal plants are very important to assure the purity of the drug and to avoid the adulterations of the medicinal plants. Structure of the plant organs has the significance bearing on its functions. The utilization of medicinal plants and its management with infectious diseases are the old practices in the world. Although, so many medicinal

plants are used medicinally and commercially, the reports regarding the biological activity of many medicinal plants and their mode of actions are evidently lacking. But, recently the medicinal plant extracts are receiving the great importance, due to the efficacy, safety and low cost.

Materials and Methods

The stem of *Tinospora cordifolia* (Fig 1) was collected from the out skirts of Chennai. The plant material was washed in flowing water, dried in shade, cut, crushed and kept in airtight bottle for experimental purpose.

Fig 1: *Tinospora cordifolia*: Stem with fruits.

Reagents and Chemicals

All the chemicals and solvents were purchased from SRL Chemicals, India. All the reagents used were of GPR grade.

Analytical studies

Physico-chemical parameters such as loss on drying at 105^0C, total ash, acid insoluble ash, solubility in water and alcohol, pH of water extract, volatile oil, and fibre content were determined as per standard methods[8]. Estimation of tannin in the drug was carried out by Folin Dennis method[9]. The quantitative analysis of sugar present in the plant was carried out by Fehling's solution method[10].

Preliminary phytochemical study

In order to examine the presence of different natural products in the plant, characteristic phytochemical tests for different classes of compounds were performed using different extractives of the plant[11,12].

Chromatographic studies

(HPTLC) provides the most convenient and rapid analytical technique in the case of solid products and is used for determining the purity of materials and also for the preliminary identification purpose. It is a powerful separation tool

for quantitative analysis with high sample throughput; HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and is the simplest separation technique today available to the analyst[13].

Sample preparation for TLC and HPTLC

Extracts of the plant was prepared by boiling 1g the plant material in 10 ml methanol. The filtrates were concentrated on a water bath to 1 ml. These extracts were used for chromatographic studies[14].

Development of HPTLC profile

HPTLC is a micro analytical separation and determination method which has a wide application in herbal drug analysis. Methanolic extract of the plant material was spotted in the form of bands with Camag microlitre syringe on a precoated silica gel G F_{254} plate with Camag Linomat V applicator. Mobile phase used was Toluene: Ethyl acetate (5:1.5). Linear ascending development was done in twin trough glass chamber saturated with mobile phase. Densito metric scanning was performed on Camag TLC scanner III in the absorption mode at 254nm[14].

Anatomical studies of *Tinospora cordifolia*

The samples were fixed in FAA (Formalin-5 mL + Acetic acid-5 mL + 70% Ethyl alcohol-90 mL). After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per standard procedure[15]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens was sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections by customary procedure[15]. The sections were stained with toluidine blue as per the method[16] published by O'Brien *et al*. (1964). 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 suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary the sections were also stained with safranin and Fast green and IKI (for starch).

To study the stomata morphology, venation pattern and trichome distribution, para dermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial

maceration employing Jeffrey's maceration fluid were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 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 appeared bright against dark background. Magnifications of the figures were indicated by the Scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books[17].

Powder Microscopy

Powder microscopy was carried out by the methods of Wallis, 1985[17]. To study the epidermal tissues, fragments of leaves measuring 1-2 mm^2 were treated with Jeffrey's maceration fluid (5% chromic acid + 5% nitric acid in equal volumes) and partial maceration resulted in the separation of upper and lower epidermis. The peelings were stained with safranin and mounted on drop of glycerine.

To visualize the venation system under the microscope, small bits of the lamina were boiled in alcohol to remove chlorophyll. Then, the material was soaked in warm 10% sodium hydroxide for several hours till the materials became transparent. After total clearing was achieved, the material was washed to remove the alkali from the cells. The cleared materials were stained and mounted in glycerine. Maceration of xylem elements was carried out with the maceration fluid mentioned earlier.

Results and Discussion

The physico-chemical parameters of the stem of *Tinospora cordifolia* were determined and the values obtained are given in Table I.

Table I: physico-chemical parameters.

Sl.No.	Test	Result
1	Loss on drying at 105˚C (%)	11.33
2	Ash value (%)	6.18

3	Acid insoluble ash (%)	0.28
4	Extractable matter in water (%)	16.06
5	Extractable matter in alcohol (%)	13.78
6	pH of water extract	6.10
7	Volatile oil (%)	2.20
8	Fibre content	18.32
9	Tannin content	0.724
10	Sugar content	0.324

Preliminary phytochemical study

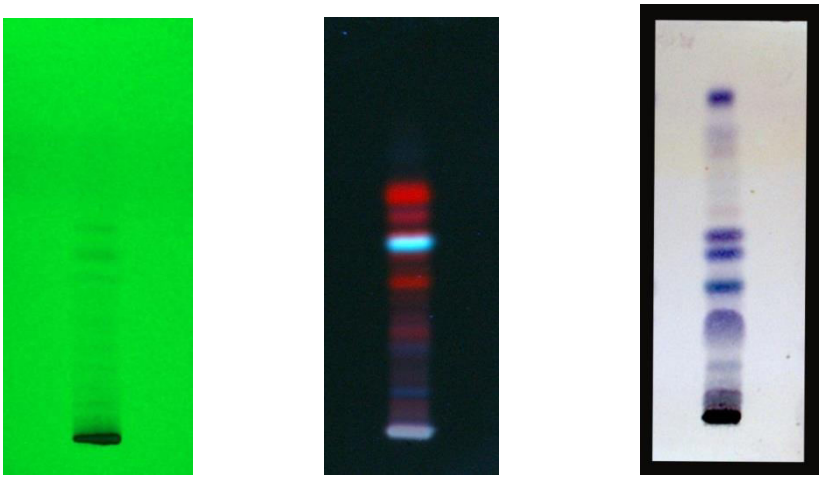
Phytochemical tests revealed the presence of sugar, poly phenol, mucilage, steroid and flavonoid and are recorded in the Table II.

Table II: Preliminary phytochemical tests.

Sl. No.	Natural products	Test performed	Inference
1	Sugar	Molisch' test	+ve
2	Starch	Iodine test	+ve
3	Poly phenol	Neutral $FeCl_3$ test	+ve
4	Saponin	Foaming in water	+ve
5	Mucilage	Swelling in water	+ve
6	Steroid	Liebermann's test	+ve
7	Alkaloid	Mayer's reagent test	+ve
8	Flavonoid	Shinoda test	+ve

Chromatographic studies: The HPTLC of the methanol extract of the plant material was carried out. The plates were viewed under UV short, UV long and developed in anisaldehyde - sulphuric acid reagent. HPTLC profile is a valuable parameter for identification of plant materials. HPTLC profile of *Tinospora cordifolia* is given in Figure 2. The scanned

Peak table at 254nm is given in Table III, 366nm is given in Table IV, at 580nm after derivatisation using anisaldehyde - sulphuric acid and heating at105ºC for 5 minutes is given in Table V.



(a) **(b)** **(c)**

Figure 2: HPTLC profile of methanol extract *Tinospora cordifolia*

at (a) 254 nm, (b) 366 nm and (c) Day light after derivatisation and heating at 105°C for 5 minutes (spray reagent - Anisaldehyde sulphuric acid) and scanned it at 580 nm

Table III: Scanned Peak table-After development the plate was scanned at 254nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	399.2 AU	0.02 Rf	590.1 AU	58.90 %	0.07 Rf	0.1 AU	18378.1 AU	62.42 %
2	0.09 Rf	0.5 AU	0.12 Rf	42.0 AU	4.20 %	0.15 Rf	0.1 AU	963.4 AU	3.27 %
3	0.18 Rf	0.0 AU	0.20 Rf	11.1 AU	1.11 %	0.21 Rf	9.8 AU	178.0 AU	0.60 %
4	0.21 Rf	10.3 AU	0.23 Rf	22.2 AU	2.21 %	0.25 Rf	9.7 AU	495.9 AU	1.68 %
5	0.25 Rf	9.7 AU	0.28 Rf	19.3 AU	1.93 %	0.30 Rf	11.4 AU	651.3 AU	2.21 %
6	0.30 Rf	11.7 AU	0.33 Rf	32.9 AU	3.29 %	0.36 Rf	0.1 AU	793.1 AU	2.69 %
7	0.37 Rf	0.1 AU	0.40 Rf	11.8 AU	1.18 %	0.41 Rf	10.3 AU	212.1 AU	0.72 %
8	0.41 Rf	10.5 AU	0.42 Rf	13.6 AU	1.36 %	0.45 Rf	0.0 AU	209.5 AU	0.71 %
9	0.46 Rf	4.1 AU	0.49 Rf	38.7 AU	3.87 %	0.51 Rf	22.3 AU	1085.8 AU	3.69 %
10	0.51 Rf	22.4 AU	0.55 Rf	82.8 AU	8.26 %	0.57 Rf	55.0 AU	2652.0 AU	9.01 %
11	0.57 Rf	55.1 AU	0.57 Rf	56.2 AU	5.61 %	0.61 Rf	32.3 AU	1690.5 AU	5.74 %
12	0.61 Rf	32.4 AU	0.64 Rf	37.7 AU	3.77 %	0.64 Rf	36.0 AU	753.6 AU	2.56 %
13	0.64 Rf	36.0 AU	0.67 Rf	43.2 AU	4.31 %	0.68 Rf	40.0 AU	1381.0 AU	4.69 %

Table IV: Scanned Peak table-After development the plate was scanned at 366nm nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	7.8 AU	0.02 Rf	73.9 AU	6.05 %	0.04 Rf	1.1 AU	1199.0 AU	3.33 %
2	0.04 Rf	0.5 AU	0.07 Rf	34.6 AU	2.83 %	0.10 Rf	23.2 AU	1199.2 AU	3.34 %
3	0.10 Rf	23.2 AU	0.11 Rf	36.5 AU	2.99 %	0.14 Rf	20.1 AU	896.8 AU	2.49 %
4	0.16 Rf	19.1 AU	0.17 Rf	20.6 AU	1.68 %	0.19 Rf	14.3 AU	489.0 AU	1.36 %
5	0.20 Rf	14.4 AU	0.24 Rf	21.9 AU	1.79 %	0.25 Rf	20.0 AU	856.5 AU	2.38 %
6	0.25 Rf	20.1 AU	0.27 Rf	38.1 AU	3.12 %	0.30 Rf	25.1 AU	1208.3 AU	3.36 %
7	0.30 Rf	25.2 AU	0.31 Rf	27.3 AU	2.24 %	0.33 Rf	15.1 AU	566.4 AU	1.58 %
8	0.34 Rf	15.2 AU	0.36 Rf	20.3 AU	1.66 %	0.37 Rf	20.0 AU	502.1 AU	1.40 %
9	0.37 Rf	20.1 AU	0.40 Rf	102.1 AU	8.35 %	0.45 Rf	23.7 AU	3447.8 AU	9.59 %
10	0.45 Rf	23.7 AU	0.51 Rf	637.7 AU	52.16 %	0.55 Rf	23.7 AU	18363.3 AU	51.07 %
11	0.55 Rf	24.0 AU	0.57 Rf	47.4 AU	3.88 %	0.59 Rf	28.0 AU	1258.5 AU	3.50 %
12	0.59 Rf	28.5 AU	0.63 Rf	161.9 AU	13.25 %	0.71 Rf	0.7 AU	5967.2 AU	16.60 %

Table V: Scanned Peak table-After development the plate was derivatised using anisaldehyde sulphuric acid as spray reagent and heated at105ºC for 5m) and scanned it at 580nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	81.2 AU	0.01 Rf	112.1 AU	6.44 %	0.03 Rf	0.8 AU	1226.4 AU	2.00 %
2	0.04 Rf	1.4 AU	0.05 Rf	38.0 AU	2.18 %	0.06 Rf	12.7 AU	477.5 AU	0.78 %
3	0.06 Rf	13.3 AU	0.08 Rf	90.6 AU	5.20 %	0.10 Rf	1.1 AU	1195.3 AU	1.95 %
4	0.10 Rf	0.1 AU	0.11 Rf	5.8 AU	0.33 %	0.12 Rf	0.1 AU	35.1 AU	0.06 %
5	0.12 Rf	0.1 AU	0.15 Rf	89.5 AU	5.14 %	0.18 Rf	0.4 AU	1872.8 AU	3.06 %
6	0.19 Rf	0.7 AU	0.28 Rf	267.7 AU	15.37 %	0.31 Rf	55.6 AU	15678.6 AU	25.62 %
7	0.31 Rf	56.7 AU	0.36 Rf	315.0 AU	18.09 %	0.40 Rf	31.0 AU	11795.7 AU	19.28 %
8	0.40 Rf	61.0 AU	0.45 Rf	345.6 AU	19.84 %	0.47 Rf	10.2 AU	12951.7 AU	21.17 %
9	0.47 Rf	211.2 AU	0.50 Rf	303.4 AU	17.43 %	0.54 Rf	6.7 AU	9418.1 AU	15.39 %
10	0.54 Rf	6.7 AU	0.56 Rf	27.7 AU	1.59 %	0.60 Rf	0.6 AU	659.5 AU	1.08 %
11	0.60 Rf	0.5 AU	0.63 Rf	26.7 AU	1.53 %	0.64 Rf	26.3 AU	512.7 AU	0.84 %
12	0.64 Rf	26.4 AU	0.65 Rf	28.0 AU	1.61 %	0.67 Rf	21.1 AU	524.0 AU	0.86 %
13	0.67 Rf	20.1 AU	0.73 Rf	91.4 AU	5.25 %	0.78 Rf	0.1 AU	4845.2 AU	7.92 %

Anatomical studies of *Tinospora cordifolia*

Stem

The stem measuring 6 mm in diameter with well-developed secondary growth was studied. The surface of the stem is smooth and even accepting the places where wide lenticels are present (Fig. 3). The lenticels are 1.65 mm broad and 600 μm deep. The stem shows the following tissue zones:

1. Epidermis

It is very thin and broken at several places due to periderm formation.

2. Periderm

It is 130 μm wide and consists of outer heavily thick walled lignified cells and inner five or six layers of thin walled tabular cells. Beneath these lenticels thin periderm is wider and the cells are sclerotic (Fig. 4 a)

3. Cortex

It is 450 μm wide; it consists of outer zone of circular or angular compact parenchyma cells and circular secretory canals. The canals are schizogenous type and have 5 or more epithelial cells. Inner to this zone, the cortical cells are the regular radial compact files of parenchyma cells (Fig. 4 b).

Vascular system

The vascular system exhibits wide secondary xylem and secondary phloem, which are organized in an unusual or anomalous pattern (Fig. 5 a). There are 12 discrete vascular bundles arranged closely around narrow central pith. These bundles are of unequal size; they are radially oblong with wide outer part and narrow conical inner part (Fig.5 b). The radial bands of vascular tissues are separated laterally from each other by narrow radial passages of parenchyma cells (Fig. 5 c).

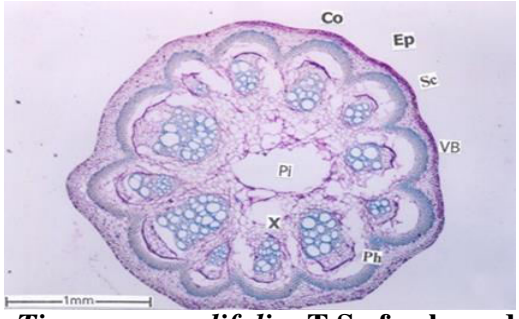


Fig. 3: *Tinospora cordifolia*: T.S of enlarged stem.

Co- cortex; Pe – periderm; Sc- sclerenchyma; Le- Lenticel; SX- Secondary xylem; SPh- Secondary phloem

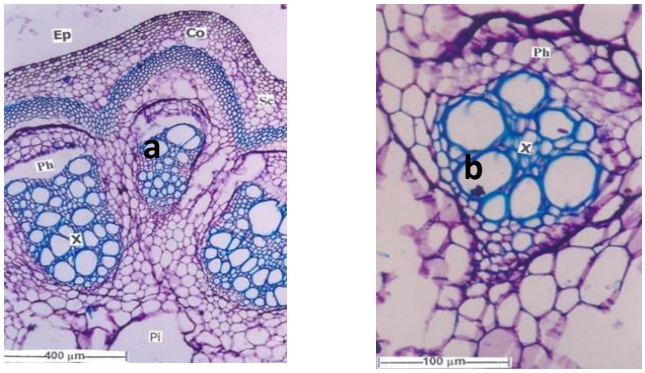


Fig 4: *Tinospora cordifolia:* Anatomy of the old stem

a. T.S. of stem - cortex enlarged; b. T.S. of stem - lenticell enlarged

Co – Cortex; Le – Lenticells; Pe – Periderm; Sc- Scelerenchyma; Sph – Secondary phloem; Sx- Secondary xylem; Ph- Phloem; X- Xylem; Ep- Epidermis; Pi- Pith; VB- Vascular bundle.

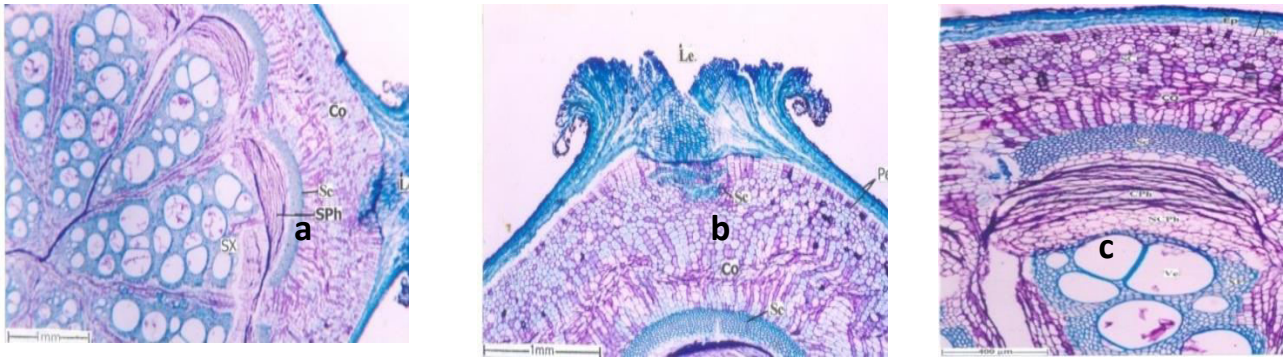


Fig 5: *Tinospora cordifolia*: Vascular band and crystal distribution

a. One vascular band enlarged; b. Crystal's distribution in the old stem; c. Crystals in the xylem fibres

Cr- Crystal; Sc- sclerenchyma; CPh- Collapsed phloem; NCPh- Non collapsed phloem;Ve- Vessel; XF – Xylem fibres; Ep- Epidermis; Pe- Periderm.

Powder microscopy of *Tinospora cordifolia*

The powder of the stem part is characterized by the following microscopic elements (Fig. 6).

1. Vessel elements: The vessel elements are common component; they are wide and shortly cylindrical. They have simple perforation plate, which is horizontal or slightly oblique. The lateral walls have several vertical rows of pits, which are elliptical in shape. The vessel elements age 200-250 µm long.

2. Tracheids: These are long, narrow, cylindrical cells. They differ from the vessels in the absence of endwall perforations; but the lateral walls of the tracheids have well developed pits as those on the vessels. The tracheids may be straight or much wavy; the wavy tracheids are seen associated with the vessels. The tracheids are 500-800 µm long.

3. Parenchyma cells: These are squarish or oblong cells with thick walls. Most of the parenchyma cells have simple pits. The cells occur in vertical files. Thin walled squarish parenchyma cells without pits are also seen in the powder.

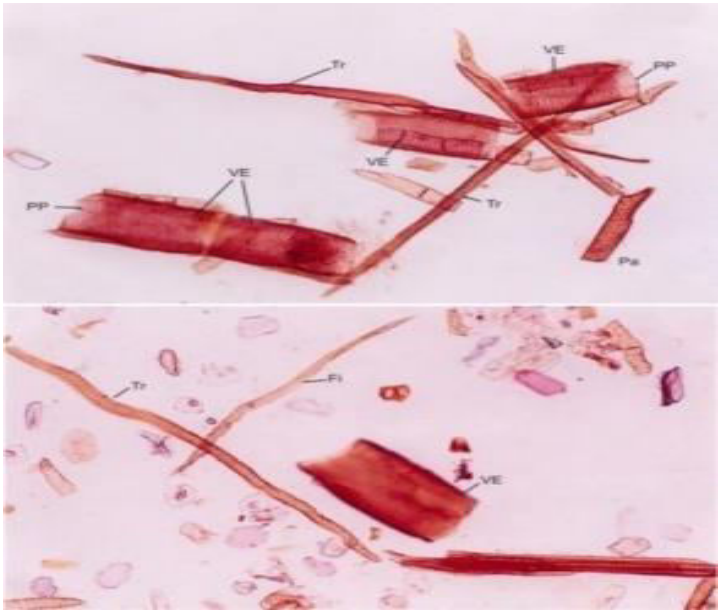


Fig 6: *Tinospora cordifolia:* Elements in the powder of the stem.

Fi- Fibre; pa- parenchyma cell; pp- perforation plate; Tr- Tracheid; VE- Vessel element.

The salient diagnostic features of the stem of *Tinospora cordifolia are as follows:-*

The stem part has about 12 discrete wedges shaped vascular bundles arranged in a ring. The surface of the stem part is smooth and even accepting the places where wide lenticels are present. The canals are schizogenous type and have 5 or more epithelial cells. There are 12 discrete vascular bundles arranged closely around narrow central pith. The xylem strands have wide circular, cluster of metaxylem vessels and conical cylinder of protoxylem vessels. The lateral walls of vessel element have several vertical rows of pits, which are elliptical in shape. The tracheids may be straight or much wavy; the wavy tracheids are seen associated with the vessels. Most of the parenchyma cells have simple pits.

Conclusion

The need of standardisation of crude drugs for identification and authentication of the drug is the need of the hour. The lack of standardisation technique fails to identify the drug from its originality which there by exploits the usage of drug from its Traditional System of Medicine. *Tinospora cordifolia* is popular in Siddha and Ayurveda for their stimulant, tonic and strengthening properties. Besides these properties, the plants are used to cure many other diseases. Hence these plants were taken up for detailed study. The HPTLC profile and R_f values obtained are important parameters for

standardisation. HPTLC studies of the plant gave characteristic patterns which can be used to establish the identity of the drug. HPTLC profiles developed along with the results of macroscopic and microscopic studies, powder analysis and physico- chemical analysis can be conveniently used as a tool for the proper identification and standardization of *Tinospora cordifolia* (stem).

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