Comparative Pharmacognostic and Preliminary Phytochemical Studies of *Tinospora cordifolia*

Arjun Patra[1,*], Richa Pathela[2] and Swaha Satpathy[1]

[1]*Institute of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur (C. G.), India*

[2]*School of Pharmaceutical Sciences, IFTM University, Moradabad, UP, India*

Abstract: In present study, pharmacognostical, physicochemical and preliminary phytochemical studies of *T. cordifolia* (Menispermaceae) stems, climbing on six different trees [*Anthocephalus cadamba* **(Tc1)**, *Ficus religious* **(Tc2)**, *Cassia fistula* **(Tc3)**, *Terminalia belerica* **(Tc4)**, *Ficus rumphi* **(Tc5)** and *Magnifera indica* **(Tc6)**] were evaluated and compared. Morphologically all the materials were similar, but they were having some differences in their microscopical features. Percentage total ash (Tc1: 6.7, Tc2: 5.6, Tc3: 5.9, Tc4: 5.0, Tc5: 4.3 and Tc6: 4.7% w/w), acid-insoluble ash (Tc1: 1.0, Tc2: 1.8, Tc3: 2.1, Tc4: 2.2, Tc5: 1.8 and Tc6: 2.4% w/w), water soluble extractive (Tc1: 22.1, Tc2: 11.6, Tc3: 12.8, Tc4: 10.6, Tc5: 18.4 and Tc6: 19.6% w/w), ethanol soluble extractive (Tc1: 4.3, Tc2: 8.1, Tc3: 13.6, Tc4: 3.3, Tc5: 7.6 and Tc6: 7.1% w/w), moisture content (Tc1: 8.0, Tc2: 9.2, Tc3: 8.2, Tc4: 5.7, Tc5: 7.1 and Tc6: 6.2% w/w) were determined. Preliminary phytochemical screening revealed the presence of inorganic constituents like calcium (Tc1 to Tc6), magnesium (Tc1), potassium (Tc2, Tc3 and Tc5), Iron (Tc1 to Tc6 except Tc4), sulphate (Tc1 to Tc6 except Tc3), phosphate (Tc1), carbonate (Tc4), and nitrates (Tc3); and organic constituents viz. carbohydrates, steroids, glycosides, flavonoids, alkaloids and tannins in the plant. Further, TLC of methanolic extract of the plant material showed 7, 5, 6, 7, 7 and 7 spots in Tc1, Tc2, Tc3, Tc4, Tc5 and Tc6 respectively. The different salient diagnostic features established in this study will help for proper identification and standardization of the drug in crude form, and then the plant material can be used for further studies. Further, the plant climbing on different trees could be explored for maximizing the significant phytoconstituents and the therapeutic potential of the plant.

Keywords: Morphology, microscopy, menispermaceae, preliminary phytochemical screening, *Tinospora cordofolia,* TLC fingerprinting, physicochemical parameters, fluorescence analysis.

1. INTRODUCTION

Medicinal herbs as potential source of therapeutics aids has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. It is estimated that nearly three fourth of the herbal drugs used worldwide were discovered following leads from local medicine. According to WHO about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated from plants. Almost, 70% modern medicines in India are derived from natural products. The basic uses of plants in medicine will continue in the future, as a source of therapeutic agents, and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes and food industries. In the dual role as a source of healthcare and income, medicinal plants make an important contribution to the larger development process. The major hindrance in the amalgamation of herbal medicine into modern medical practices is the lack of scientific and clinical data and better understanding of efficacy and safety of herbal products. To ensure the quality and safety of herbal products, standardization is of vital importance. Every herbal formulation must be standardized as per WHO guidelines. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of herbal medicines. In indigenous/ traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination etc. Therefore, to standardize the herbal material, a number of approaches have been undertaken at one time or another.

Tinospora cordifolia commonly named as “Guduchi” in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, sesquiterpenoids, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like

*Address correspondence to this author at the Institute of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur (C. G.), India; Tel: +917587471798;
E-mail: drarjunpatra22@gmail.com

E-ISSN: 2311-858X/16

anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, antiosteoporotic, radio-protective, hepatoprotective, diuretic, cardioprotective, gastrointestinal and anti-ulcer, antifertility, immunomodulatory and anti-neoplastic activities [1, 2]. The plant has been extensively studied for its phytochemistry and pharmacological properties, but maximum research papers related to *T. cordifolia* do not mention the plant on which this is growing. Pharmacognosy is closely related to both botany and plant chemistry and indeed both originated from the earlier scientific studies on medicinal plants. At the beginning of the 20th century pharmacognosy had developed mainly on the botanical side being particularly concerned with the description and identification of drugs, both in the whole state and in powder form, and with their history, commerce, collection, preparation and storage. Such branches are of fundamental importance which forms the basis for the rapid development in other areas particularly phytochemistry and pharmacology have enormously expanded the subject. As the crude drugs form the basis for the manufacture of wide range of medicinal preparations needed by people, the development of pharmacognostical research has become indispensable for getting therapeutically potent medicine prepared from genuine drug material. The pharmacognocists have a serious responsibility to take the initiative not only in correctly locating the plant mentioned in old treatises and pharmacopoeias but also making them available to scientist in other disciplines to put to test the use for which they are acclaimed [3, 4]. Hence, in the present study we aimed to explore the pharmacognostic and preliminary phytochemical studies of *T. cordifolia* growing on different trees.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Fresh plant/plant parts were collected randomly from Uttarakhand (Haridwar, Rishikesh and Tehri Garhwal) and Uttar Pradesh (Varanasi), India and authenticated through Regional Research Institute, Ranikhet, Uttarakhand, India (Specimen no. 23915) and voucher Specimen has been preserved for further reference. Plant material climbing on six different trees was collected as follow:

1. Tinospora cordifolia which climbs on Anthocephalus cadamba **(Tc1)**.
2. Tinospora cordifolia which climbs on Ficus religious (**Tc2**).
3. Tinospora cordifolia which climbs on Cassia fistula (**Tc3**).
4. Tinospora cordifolia which climbs on Terminalia belerica (**Tc4**).
5. Tinospora cordifolia which climbs on Ficus rumphi (**Tc5**).
6. Tinospora cordifolia which climbs on Magnifera indica (**Tc6**).

Tc1 and Tc4 were collected from Haridwar (Uttarakhand) in November, 2009; Tc3 was collected from Rishikesh, Uttarakhand in December, 2009; Tc6 was collected from Narendra Nagar, Uttarakhand in January, 2010; Tc2 was collected from Varanasi (Uttar Pradesh) in October, 2009 and Tc5 from Barielly (Uttar Pradesh) in December, 2009. The stems were washed under running tap water, air dried under shade and preserved for study. Some amount of plant material was coarsely powdered and kept in air-tight container for further use. Fresh stems were used for microscopic studies. All chemical reagents used were of analytical grade.

2.2. Morphology and Microscopy of Plant Material

The macromorphology of the stems were studied according to standard methods [5, 6]. Sections of stem was taken, stained and mounted following usual microtechniques [7] and representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan). The different powder characteristics were studied according to standard methods [8, 9]. Separate slides were prepared for observation of lignified tissues (phloroglucinol + HCl), starch (iodine solution) and non-lignified characters.

2.3. Determination of Physicochemical Parameters

Physico-chemical parameters i.e. percentage of moisture content, percentage of ash values and extractive values were performed according to the official methods [10] and the WHO guidelines on the quality control methods for medicinal plant materials [11]. Fluorescence analysis was carried out following reported methods [12, 13].

Figure 1: Macroscopic characters of *Tinospora cordifolia* stem.

2.4. Preliminary Phytochemical Screening

The shade dried and coarsely powdered stem were extracted with methanol using soxhlet apparatus and the marc was extracted with water by decoction. Different extracts were screened for the presence of various groups of phytoconstituents using different chemical tests [14-18]. The ash of the powder was prepared by incinerating the powder in muffle furnace. 50% v/v HNO_3 was added to the ash and kept for 2hr then filtered and the filtrate was used for conforming the presence or absence of different inorganic elements [17].

2.5. TLC Fingerprint Profile

Thin layer chromatography of the petroleum ether, chloroform, alcoholic and aqueous extracts was studied [19] and the R_f values were determined.

3. RESULTS

3.1. Morphological Characteristics

The pieces of stems are cylindrical and of varying thickness ranging from 0.5-2.5cm in diameter. Young stems are green with smooth surfaces and swelling at nodes, while older ones have light brown surface marked with warty protuberances due to circular lenticels. The stem wood is dull and light in colour as well as porous and soft. Bark is thin, easily separable and papery. Lenticels are oblong having short vertical slit; adventitious root originate from it. The fracture is fibrous; odor faint and taste is intensely bitter (Figure **1**).

3.2. Microscopic Characteristics

3.2.1. Transverse Section

Structurally the woody climber's stem consists of cork, cork cambium, collenchymatous cells, parenchymatous cells, pericycle, phloem, cambium, xylem, medullary rays and pith. Transverse section of stem shows outer-most layer of cork with thick walled brownish and compressed cells. Cork contains thin walled colourless, tangentially arranged cells. Cork is broken at some places due to opening of lenticels. Cork is followed by cortex region; outer zone of cortex consists of chlorophyllous collenchymatous cells and inner zone has rounded parenchymatous cell in upper part and elongated parenchymatous cells in lower part. Starch grains and calcium oxalate crystals are present in cortex. Pericyclic fibres consist of lignified

Table 1: Comparative Microscopic Features of Transverse Section of *T. cordifolia* stem

Number of Layers of Cells in						No. of Pericyclic
Cork	**Cork Cambium**	**Collenchyma**	**Rounded Parenchyma**	**Elongated Parenchyma**	**Pericycle**	**Fibres in Pericycle**
2-3	3-4	4-5	5-6	7-8	9	18
1-2	4-5	5-6	6-7	6-7	9	13
1-2	3-4	3-4	6-7	7-8	9	13
2-3	4-5	5-6	7-8	7-8	9	16
2-3	3-4	4-5	5-6	6-7	9	13
2-3	4-5	4-5	4-5	7-8	9	18

Table 2: Microscopical Measurements of Different Cells and Tissues (in Micron)

Cell/tissue	Parameter		Tc-1	Tc-2	Tc-3	Tc-4	Tc-5	Tc-6
Cork cambium	Length	Range	13.6 - 27.2	13.6 - 34	13.6 - 27.2	13.6 - 27.2	13.6 - 40.8	13.6 - 27.2
		Avg.	15.98	21.42	15.98	16.66	26.86	20.4
	Width	Range	27.2 - 54.2	13.6 - 54.4	20.4 - 54.4	20.4 - 54.4	34.0 - 61.2	34.0 - 68.0
		Avg.	35.7	35.04	39.1	39.44	45.22	43.18
Collenchyma	Length	Range	13.6 - 27.2	13.6 - 27.2	13.6 - 34	13.6 - 40.8	13.6 - 27.2	13.6 - 27.2
		Avg.	15.98	18.7	18.02	25.16	17.68	18.36
	Width	Range	13.6 - 40.8	27.2 - 54.4	20.4 - 47.6	27.2 - 68	20.4 - 47.6	20.4 - 47.6
		Avg.	27.2	40.8	34.68	47.94	32.98	32.3
Rounded parenchyma	Diameter	Range	27.2 - 54.4	27.2 - 68	13.6 - 40.8	40.8 - 81.6	27.2 - 54.4	27.2 - 54.4
		Avg.	36.72	47.6	29.24	57.8	41.14	41.14
Elongated parenchyma	Length	Range	40.8 - 68.0	40.8 – 68	40.8 - 81.6	34.0 - 68.0	27.2 - 68	40.8 - 81.6
		Avg.	56.44	51.68	55.42	46.92	51.34	63.58
	Width	Range	13.6 - 47.6	27.2 - 54.4	13.6 - 40.8	27.2 - 95.2	27.2 - 54.4	27.2 - 54.4
		Avg.	31.28	38.76	29.58	72.76	34.68	38.76
Xylem vessels	Diameter	Range	40.8 - 163.2	68.0 - 421.6	40.8 - 244.8	40.8 - 326.4	27.2 - 285.6	54.4 - 217.6
		Avg.	86.36	207.4	126.13	193.8	149.6	120.7
Pith	Diameter	Range	47.6 - 122.4	47.6 - 95.2	34.0 - 95.2	54.4 - 95.2	27.2 - 74.8	40.8 - 136.0
		Avg.	80.58	66.3	58.14	77.52	52.36	82.62

sclerenchymatous cells, forming a continuous circle of arches. These fibres are having pointed ends. Vascular bundles are open and collateral. Vascular zone is composed of wedge-shaped strips of xylem, externally surrounded by semi-circular strips of phloem. A strip of thin tangentially elongated cambium cells is present between xylem and phloem in each vascular bundle. Xylem consists of vessels, tracheids, parenchyma and fibres. Medullary rays are multiseriate thin walled parenchymatous cells, separating the xylem patches. Pith is composed of round to oval thin walled parenchymatous cells, loaded with starch grains.

Various differences were observed among Tc1, Tc2, Tc3, Tc4, Tc5 and Tc6 during microscopical study (Table **1**). The microscopical measurements viz. length, width and diameter of different cells were also measured (Table **2**). Microscopical features of Tc1, Tc2, Tc3, Tc4, Tc5 and Tc6 stem have been shown in Figure **2, 3, 4, 5, 6** and **7** respectively.

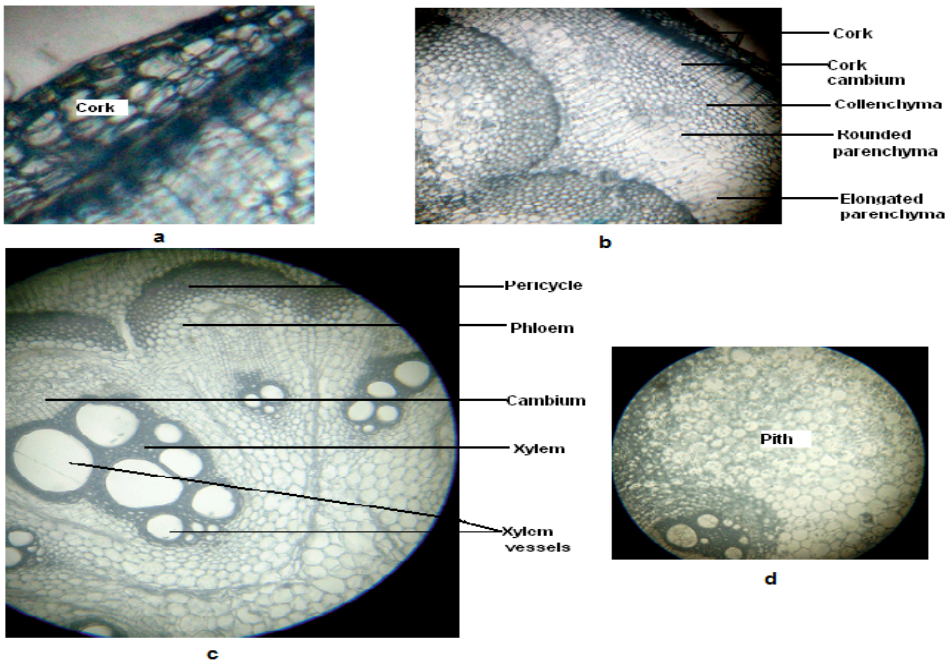

Figure 2: Transverse sections of *Tinospora cordifolia* (Tc1) stem (100X); **a:** Transverse section showing cork; **b:** Transverse section showing cork, cork cambium, collenchyma, rounded parenchyma and elongated parenchyma; **c:** Transverse section showing pericycle, phloem, cambium, xylem and xylem vessels; **d:** Transverse section showing pith.

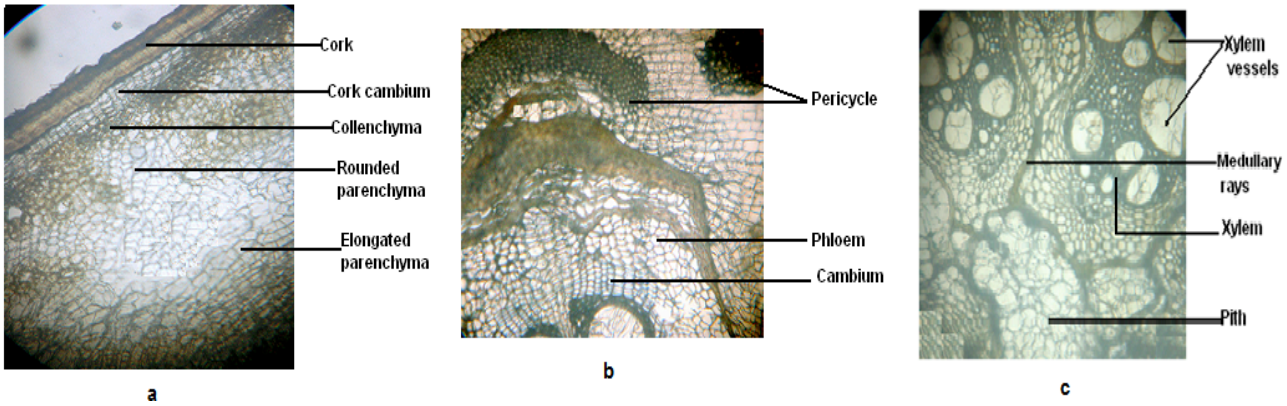

Figure 3: Transverse sections of *Tinospora cordifolia* (Tc2) stem (100X); **a:** Transverse section showing cork, cork cambium, collenchyma, rounded parenchyma and elongated parenchyma; **b:** Transverse section showing pericycle, phloem and cambium; **c:** Transverse section showing xylem vessels medullary rays, xylem and pith.

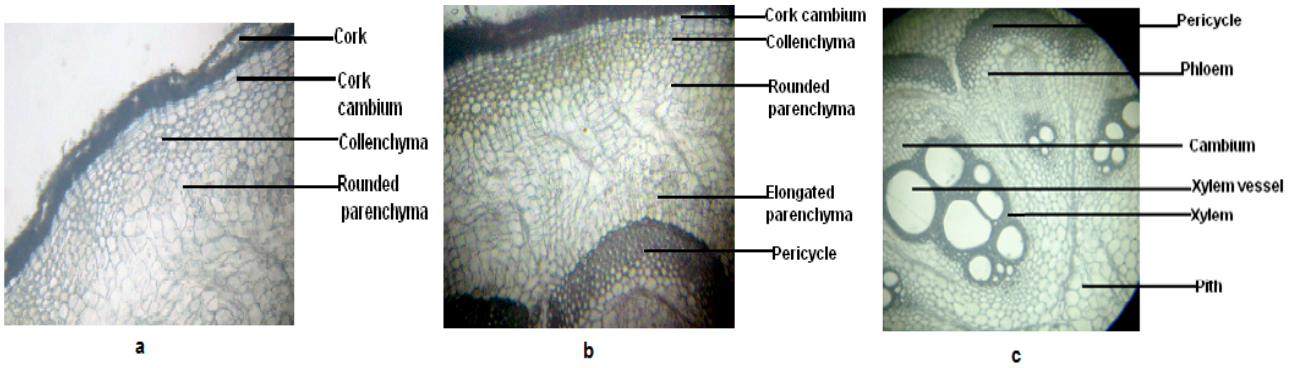

Figure 4: Transverse sections of *Tinospora cordifolia* (Tc3) stem (100X); **a:** Transverse section showing cork, cork cambium, collenchyma and rounded parenchyma; **b:** Transverse section showing cork cambium, collenchyma, rounded parenchyma, elongated parenchyma and pericycle; **c:** Transverse section showing pericycle, phloem cambium, xylem vessels, xylem and pith.

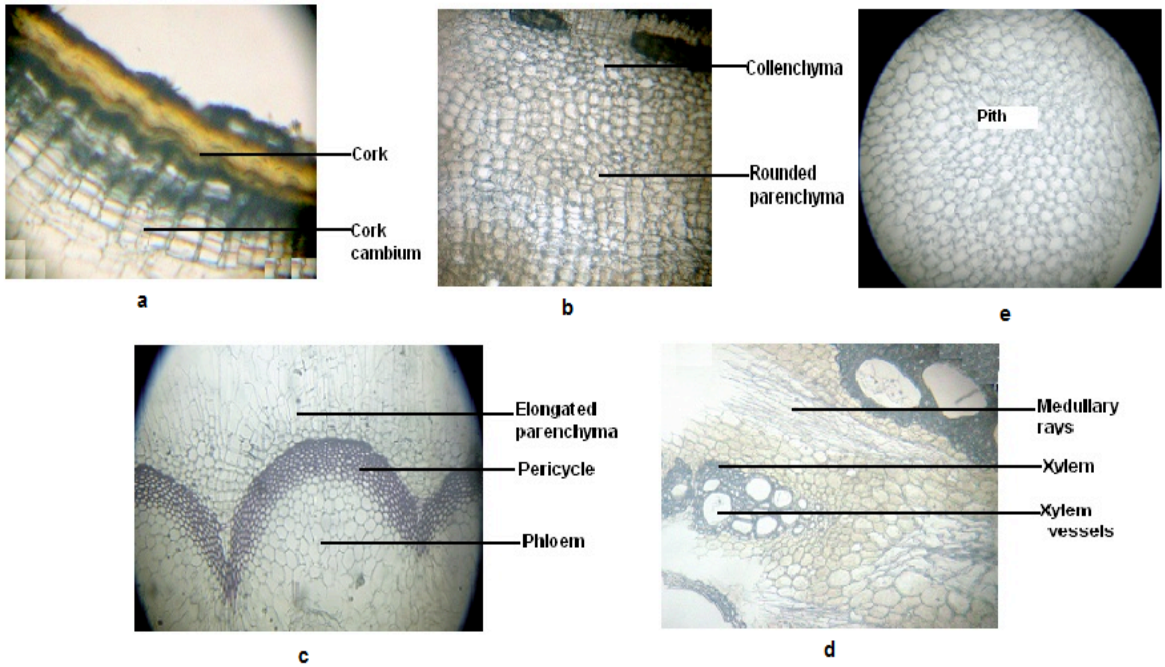

Figure 5: Transverse sections of *Tinospora cordifolia* (Tc4) stem (100X); **a:** Transverse section showing cork and cork cambium; **b:** Transverse section showing collenchyma and rounded parenchyma; **c:** Transverse section showing elongated parenchyma, pericycle and phloem; **d:** Transverse section showing medullary rays, xylem and xylem vessels; **e:** Transverse section showing pith.

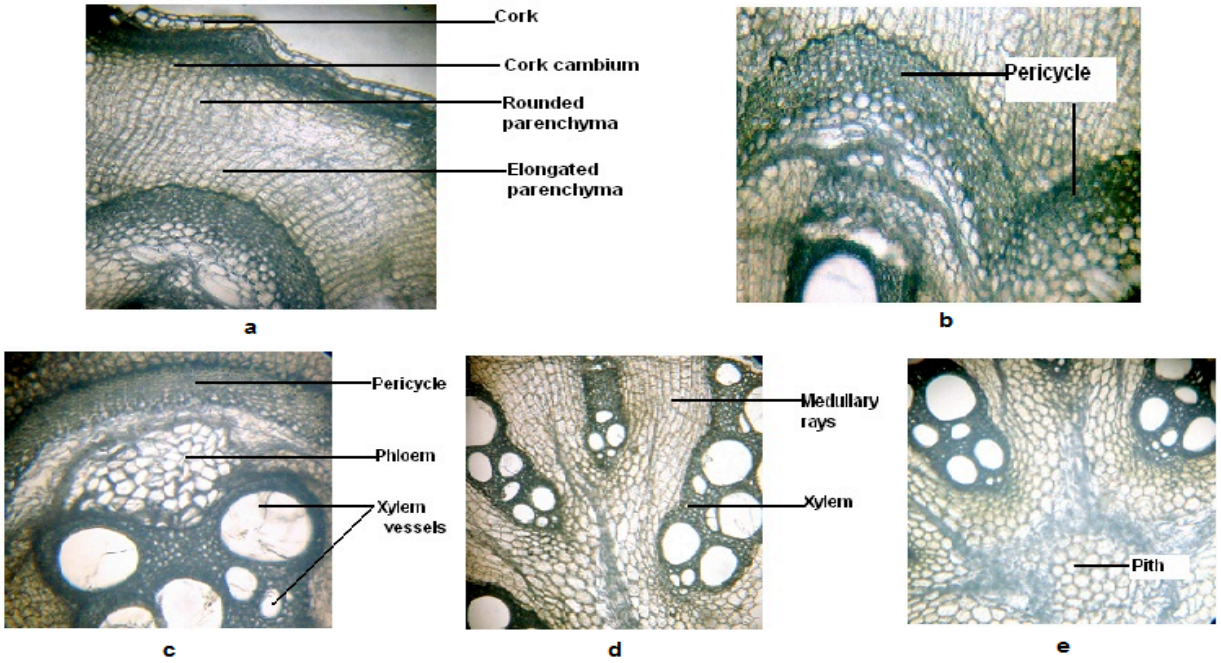

Figure 6: Transverse sections of *Tinospora cordifolia* (Tc5) stem (100X); **a:** Transverse section showing cork, cork cambium, rounded parenchyma and elongated parenchyma; **b:** Transverse section showing pericycle; **c:** Transverse section showing pericycle, phloem and xylem vessels; **d:** Transverse section showing medullary rays and xylem; **e:** Transverse section showing pith.

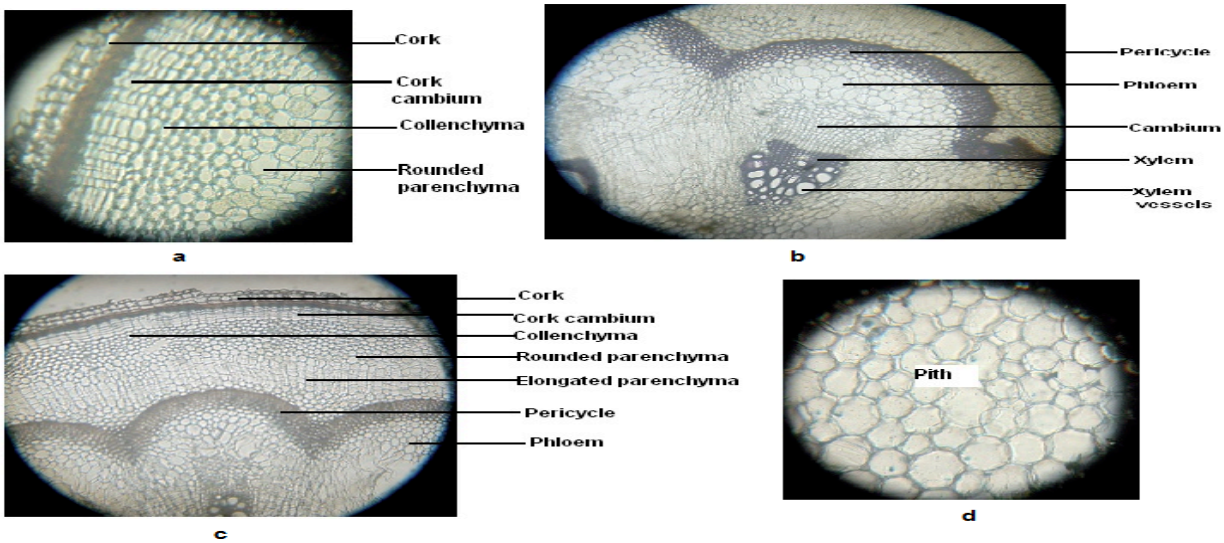

Figure 7: Transverse sections of *Tinospora cordifolia* (Tc6) stem (100X): **a:** Transverse section showing cork, cork cambium, collenchyma and rounded parenchyma; **b:** Transverse section showing pericycle, phloem, cambium, xylem and xylem vessels; **c:** Transverse section showing cork, cork cambium, collenchyma, rounded parenchyma, elongated parenchyma, pericycle and phloem; **d:** Transverse section showing pith.

3.2.2. Powder Microscopy

The well-dried homogenous drug samples of stems were subjected to grinding. The powder so obtained was sieved through No. 120 sieve. The powders were kept in air tight glass containers for further studies. The powder was yellowish brown in colour. Small quantity of the powder was taken on the slide. Different slides were prepared by using different reagents like phloroglucinol + conc. HCl (1:1) (for lignified cork, xylem region, fibre and sclerenchymatous cells of pericycle), iodine solution (for starch), acetic acid (calcium oxalate insoluble) and hydrochloric acid (calcium oxalate soluble). These slides were observed under the microscope. Prismatic crystals of calcium oxalate, starch grain (simple and compound), cork cells, lignified reticulate xylem vessels, fibres and

lignified sclerenchymatous cells were observed. In *Tc*-6, both reticulate and annular types of lignified xylem vessels were observed. The powder characteristics of Tc1, Tc2, Tc3, Tc4, Tc5 and Tc6 have been depicted in Figure **8, 9, 10, 11, 12** and **13** respectively.

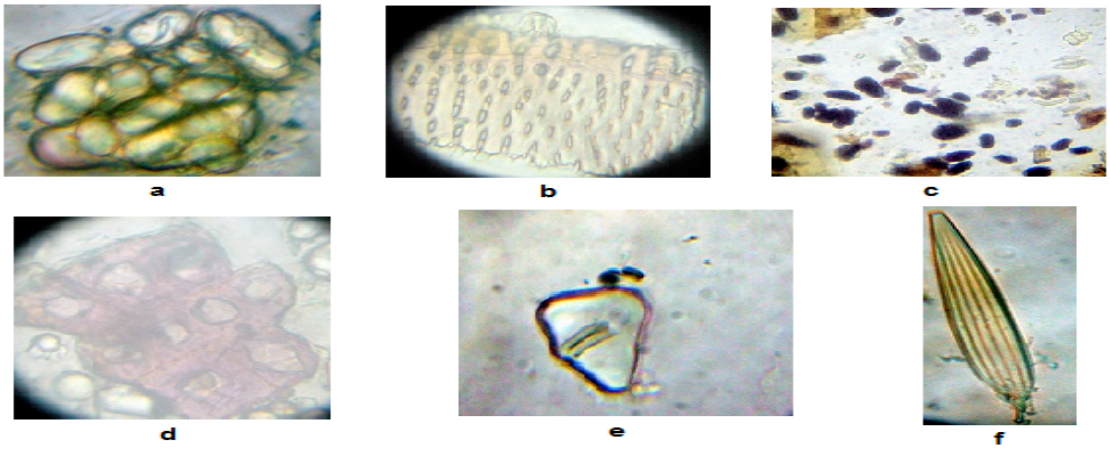

Figure 8: Powder microscopy of *Tinospora cordifolia* (Tc1) stems (100X); **a:** Cork cells; **b:** Reticulate xylem vessel; **c:** Starch grains; **d:** Sclerenchymatous cells; **e:** Prismatic calcium oxalate crystal; **f:** Fibre having blunt end.

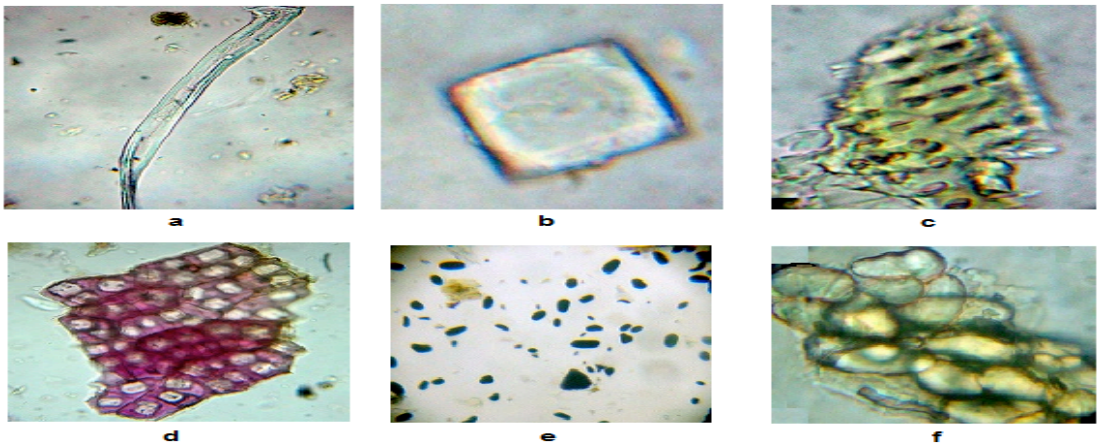

Figure 9: Powder microscopy of *Tinospora cordifolia* (Tc2) stems (100X); **a:** Fibre; **b:** Calcium oxalate crystal; **c:** Reticulate xylem vessel; **d:** Sclerenchymatous cells; **e:** Starch grains; **9**, Cork cells.

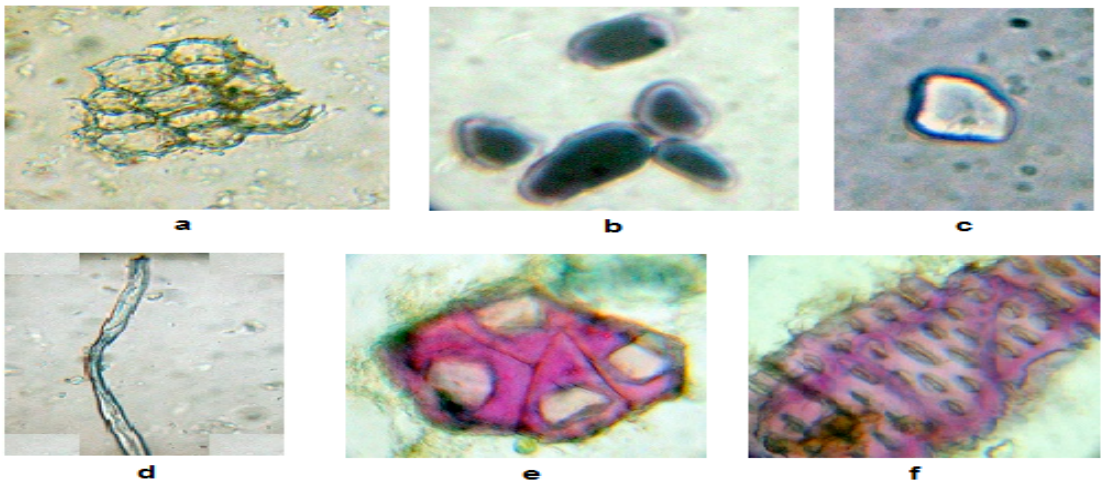

Figure 10: Powder microscopy of *Tinospora cordifolia* (Tc3) stems (100X); **a:** Cork cells; **b:** Starch grains; **c:** Calcium oxalate crystals; **d:** Fibre; **e:** Sclerenchymatous cells; **f:** Reticulate xylem vessel.

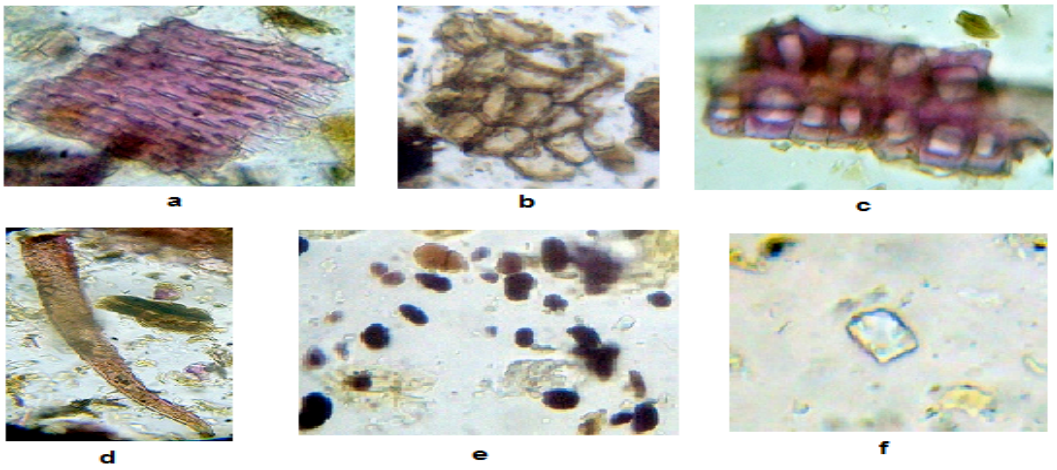

Figure 11: Powder microscopy of *Tinospora cordifolia* (Tc4) stems (100X); **a:** Reticulate xylem vessel; **b:** Cork cells; **c:** Sclerenchymatous cell; **d:** Fibre; **e:** Starch grains; **f:** Calcium oxalate crystal.

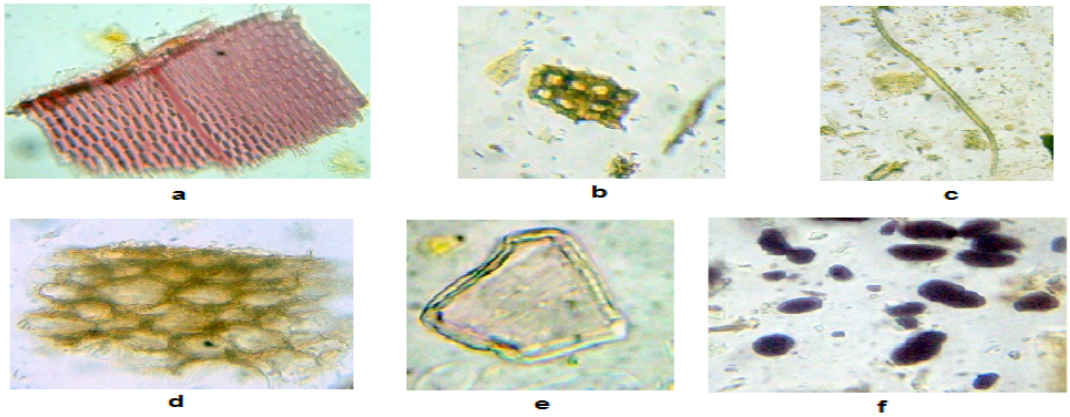

Figure 12: Powder microscopy of *Tinospora cordifolia* (Tc5) stems (100X); **a:** Reticulate xylem vessel; **b:** Sclerenchymatous cell; **c:** Fibre; **d:** Cork cells; **e:** Calcium oxalate crystal; **f:** Starch grains.

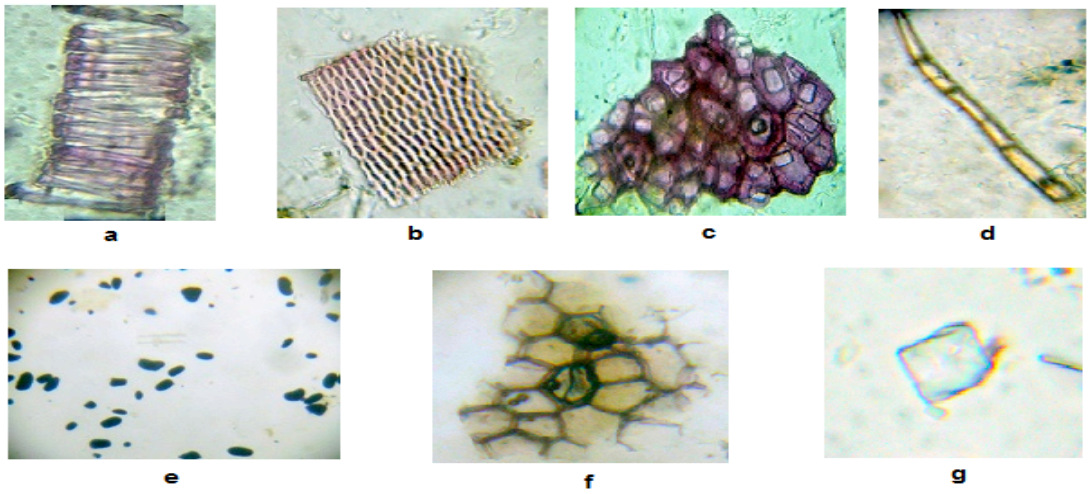

Figure 13: Powder microscopy of *Tinospora cordifolia* (Tc6) stems (100X); **a:** Annular xylem vessel; **b:** Reticulate xylem vessel; **c:** Sclerenchymatous cell; **d:** Fibre; **e:** Starch grains; **f:** Cork cells; **g:** Calcium oxalate crystal.

3.3. Physicochemical Parameters

Physico-chemical constants like percentage of moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractive and ethanol soluble extractive were determined and depicted in Table **3-5**.

Table 3: Ash Values of Different *Tinospora cordifolia* stems

Parameters	(% w/w)*					
	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6
Total ash	6.75	5.6	5.92	5.07	4.3	4.75
Acid insoluble ash	1.05	1.85	2.15	2.25	1.8	2.4
Water soluble ash	1.15	1.45	1.57	1.75	2.1	3.2

*Values are average of three readings.

Table 4: Extractive Values of Different *Tinospora cordifolia* stems

Extractive values	(% w/w)*					
	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6
Water soluble extractive	22.16	11.6	12.8	10.64	18.4	19.6
Ethanol soluble extractive	4.36	8.16	13.68	3.32	7.68	7.12

*Values are average of three readings.

Table 5: Moisture Content of Different *Tinospora cordifolia* stems

Plant	Moisture Content (% w/w)*
Tc1	8.08
Tc2	9.28
Tc3	8.22
Tc4	5.72
Tc5	7.13
Tc6	6.22

*Values are average of three readings.

3.4. Fluorescence Analysis

Powder of *T. cordifolia* stems were observed under visible and ultraviolet (254 and 365nm) light. The powder were also treated with different chemicals and observed under visible and ultraviolet (254 and 365nm) light. The co lour of powders was different with different reagents. Observations are recorded in Table **6**.

3.5. Extraction and Preliminary Phytochemical Screening

About 100-150gm of *T. cordifolia* stems was coarsely powdered and this coarse powder was extracted with methanol by using soxhlet apparatus and then subsequent decoction was also prepared. The percentage yield of each extract was calculated and is shown in Table **7**. The colour and consistency of each extract is shown in Table **8**. Preliminary phytochemical screening revealed the presence of carbohydrates, steroids, alkaloids, glycosides, flavonoids, tannins and phenolics in the plant (Table **9**). Again, the inorganic constituents present in the plant were found to be calcium, magnesium, potassium, iron, sulphate, phosphate, carbonate and nitrate (Table **10**).

Table 7: Percentage Yield of Different Extracts

Plant	Yield (%w/w)	
	Methanolic Extract	Aqueous Extract
Tc1	5.8	1.66
Tc2	5.9	4.36
Tc3	8.19	3.57
Tc4	4.91	4.91
Tc5	6.11	5.8
Tc6	3.35	9.39

Table 6: Fluorescence Analysis of Different *Tinospora cordifolia* stem

Treatment	Visible Light						UV Light											
							254 nm						365 nm					
	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6
Powder	Yellowish brown	Blackish brown	Creamish green	Dark brown	Dark brown	Creamish green	Light green	Blackish green	Light green	Dark green	Dark green	Dull brown	Black	Reddish brown	Brown	Reddish brown	Reddish brown	Dark brown
Powder + 5% KOH	Light brown	Greenish brown	Brownish green	Greenish brown	Light brown	Light green	Green	Emerald green	Light green	Dark green	Light green	Blackish green	Black	Reddish brown	Black	Black	Dark brown	Dark grey
Powder + 5% NaOH	Brown	Greenish black	Greenish brown	Dark brown	Yellowish brown	Light green	Emerald green	Light green	Dark green	Blackish green	Blackish green	Dark green	Black	Black	Dark brown	Maroon	Black	Black
Powder + Conc. HCl	Yellowish brown	Brownish black	Light green	Brownish green	Dark brown	Dark green	Blackish green	Blackish green	Emerald green	Blackish green	Dark green	Blackish green	Black	Reddish brown	Black	Black	Chocolate brown	Blackish brown
Powder + Conc. H_2SO_4	Reddish brown	Brownish black	Blackish brown	Chocolate brown	Chocolate brown	Dark brown	Green	Dark green	Blackish green	Dark green	Greenish black	Dark green	Black	Dark brown	Black	Crimson red	Black	Black
Powder + Conc. HNO_3	Brownish red	Reddish brown	Brownish yellow	Yellowish orange	Yellowish orange	Yellowish brown	Dark green	Light green	Emerald green	Emerald green	Light green	Dark green	Black	Dark brown	Dark brown	Black	Reddish brown	Black
Powder + Conc. NH_4	Dark brown	Greenish black	Light green	Brownish green	Yellowish brown	Light green	Dark green	Light green	Dark green	Dark green	Dark green	Creamish green	Brownish Black	Brownish black	Brown	Reddish brown	Chocolate brown	Light grey
Powder + Dil. HCl	Light brown	Blackish brown	Light green	Light brown	Light brown	Greenish brown	Dark green	Dark green	Dark green	Dark green	Light green	Light green	Black	Black	Brown	Black	Black	Dark brown
Powder + Dil. H_2SO_4	Brownish black	Dark brown	Dark brown	Chocolate brown	Chocolate brown	Chocolate brown	Dark green	Dark green	Dark green	Blackish green	Dark green	Brownish green	Black	Brownish black	Black	Dark brown	Reddish brown	Black
Powder + Dil. HNO_3	Light brown	Light brown	Creamish green	Yellowish brown	Orange brown	Reddish brown	Light green	Blackish green	Light green	Dark green	Blackish green	Dark green	Brownish Black	Brownish black	Brown	Chocolate brown	Chocolate brown	Black
Powder + $FeCl_3$ Solution	Yellowish brown	Brownish green	Greenish yellow	Dark brown	Dark brown	Yellowish brown	Light green	Brown	Dark green	Black	Dark green	Dark green	Black	Black	Black	Reddish brown	Black	Black
Powder + Iodine Solution	Greenish black	Yellowish brown	Dark green	Chocolate brown	Black	Dark green	Dark green	Blackish green	Green	Dark green	Blackish green	Chocolate brown	Black	Dark brown	Black	Reddish brown	Dark brown	Black

Table 8: Characteristics of Different Extracts

Plant	Methanolic Extract				Aqueous Extract			
	Colour				Colour			
	Visible Light	UV Light		Consitency	Visible Light	UV Light		Consitency
		254nm	365nm			254nm	365nm	
Tc1	Chocolate Brown	Dark Brown	Reddish Brown	Sticky Semi-solid	Dark Brown	Dark Green	Reddish Brown	Sticky Powder
Tc2	Chocolate Brown	Dark Brown	Black	Sticky Solid	Blackish Brown	Black	Black	Sticky Powder
Tc3	Greenish Brown	Black	Reddish Brown	Stick Semi-solid	Chocolate Brown	Light Green	Reddish Brown	Sticky Powder
Tc4	Chocolate Brown	Reddish Brown	Black	Sticky Solid	Blackish Brown	Reddish Brown	Black	Sticky Powder
Tc5	Blackish Brown	Black	Black	Stick Semi-solid	Blackish Brown	Reddish Brown	Dark Brown	Sticky Powder
Tc6	Blackish Green	Black	Black	Stick Semi-solid	Dark Brown	Reddish Brown	Blackish Brown	Sticky Powder

Table 9: Preliminary Phytochemical Screening of *T. cordifolia* stem Extracts (Organic Elements)

Phytoconstituents	Tc1		Tc2		Tc3		Tc4		Tc5		Tc6	
	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.	Meth.	Aq.
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	-	-	-	-	-	-	-	-	-	-	-	-
Amino acids	-	+	-	-	+	+	+	-	-	+	+	+
Steroids												
Salkowaski test	+	-	+	-	+	-	+	-	+	-	+	-
Libermann - Burchard test	-	-	-	-	+	-	+	-	+	-	+	-
Glycosides												
Cardiac glycosides	+	+	+	+	+	+	+	-	+	+	+	+
Anthraquinone glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Flavanoids												
Shinoda test	-	-	-	-	-	-	-	-	-	-	-	-
Lead acetate test	+	+	+	+	+	-	+	+	+	+	+	-
Alkaloids												
Dragendorff's test	+	-	+	-	+	-	+	+	+	-	+	+
Mayer's test	+	-	+	+	+	-	+	-	+	+	+	-
Hager's test	-	+	+	-	+	+	-	+	-	-	+	-
Wagner' test	+	-	+	+	+	-	-	+	+	-	+	+
Tannins and Phenolics												
Ferric chloride test	+	-	-	-	-	-	-	-	-	-	-	-
Lead acetate test	-	-	+	-	-	+	+	-	-	-	-	+
Dilute iodine solution test	-	-	+	-	+	+	+	-	+	-	+	+

Meth. –Methanolic extract, Aq. – Aqueous extract; (+) Sign indicates presence, (-) Sign indicates absence.

Table 10: Inorganic Elements of *T. cordifolia* stem

Elements	Tc1	Tc2	Tc3	Tc4	Tc5	Tc6
Calcium	+	+	+	+	+	+
Magnesium	+	-	-	-	-	-
Sodium	-	-	-	-	-	-
Potassium	-	+	+	-	+	-
Iron	+	+	+	-	+	+
Sulphate	+	+	-	+	+	+
Phosphate	+	-	-	-	-	-
Chloride	-	-	-	-	-	-
Carbonate	-	-	-	+	-	-
Nitrates	-	-	+	-	-	-

(+) Present, (-) Absent.

3.6. TLC Finger Print Profile

Thin layer chromatography of the methanolic extract was carried out using Heptane: Benzene: Ethyl acetate: chloroform (16.0: 14.0: 4.0: 1.0) and the Rf values were recorded. The maximum number of constituents separated from the methanolic extract of Tc1, Tc2, Tc3, Tc4, Tc5 and Tc6 were 7, 5, 6, 7, 7 and 7 respectively (Table **11** and Figure **14**).

4. DISCUSSION

Morphological and microscopical characteristics play a vital role in plant systemic study and are used as a tool for the classification of a taxon [20]. The morphological and microscopical features obtained in this study are more or less similar in all the plant samples with slight variation in number of layers of cell in different regions, number of pericyclic fibres, and microscopical measurements of various cells and tissues. Different physicochemical parameters like ash values, extractive values, moisture content etc could help for botanical identification, quality control as well as the therapeutic potential of plant materials [21-23]. Organic molecules absorb light usually over a specific range of wavelength; many of them re-emit such radiations. So if the powder is treated with different chemical reagents and seen in the UV- chamber under shorter and longer wavelength different colour will be

Table 11: TLC Fingerprinting Profile of Different of *Tinospora cordifolia*

Extract	Solvent System	No. of Spots and their Rf Values		
		Naked Eye	Iodine Chamber	UV (365 nm)
Methanolic Extract (Tc-1)	Heptane: Benzene: Ethyl acetate: chloroform (1.6: 1.4: 0.4: 0.1)	Two (0.39, 0.68)	Seven (0.09, 0.19, 0.29, 0.34, 0.39, 0.55, 0.68)	One (0.39)
Methanolic Extract (Tc-2)	Heptane: Benzene: Ethyl acetate: chloroform (1.6: 1.4: 0.4: 0.1)	One (0.62)	Five (0.12, 0.30, 0.48, 0.62, 0.88)	No spot
Methanolic Extract (Tc-3)	Heptane: Benzene: Ethyl acetate: chloroform (1.6: 1.4: 0.4: 0.1)	Three (0.14, 0.64, 0.88)	Six (0.14, 0.44, 0.56, 0.64, 0.76, 0.88)	One (0.14)
Methanolic Extract (Tc-4)	Heptane: Benzene: Ethyl acetate: chloroform (1.6: 1.4: 0.4: 0.1)	Two (0.56, 0.78)	Seven (0.15, 0.22, 0.35, 0.48, 0.56, 0.70, 0.78)	One (0.56)
Methanolic Extract (Tc-5)	Heptane: Benzene: Ethyl acetate: chloroform (1.6: 1.4: 0.4: 0.1)	Three (0.40, 0.60, 0.80)	Seven (0.20, 0.29, 0.40, 0.45, 0.60, 0.73, 0.80)	No spot
Methanolic Extract (Tc-6)	Heptane: Benzene: Ethyl acetate: chloroform (1.6: 1.4: 0.4: 0.1)	Two (0.16, 0.42)	Seven (0.11, 0.16, 0.32, 0.42, 0.49, 0.65, 0.79)	No spot

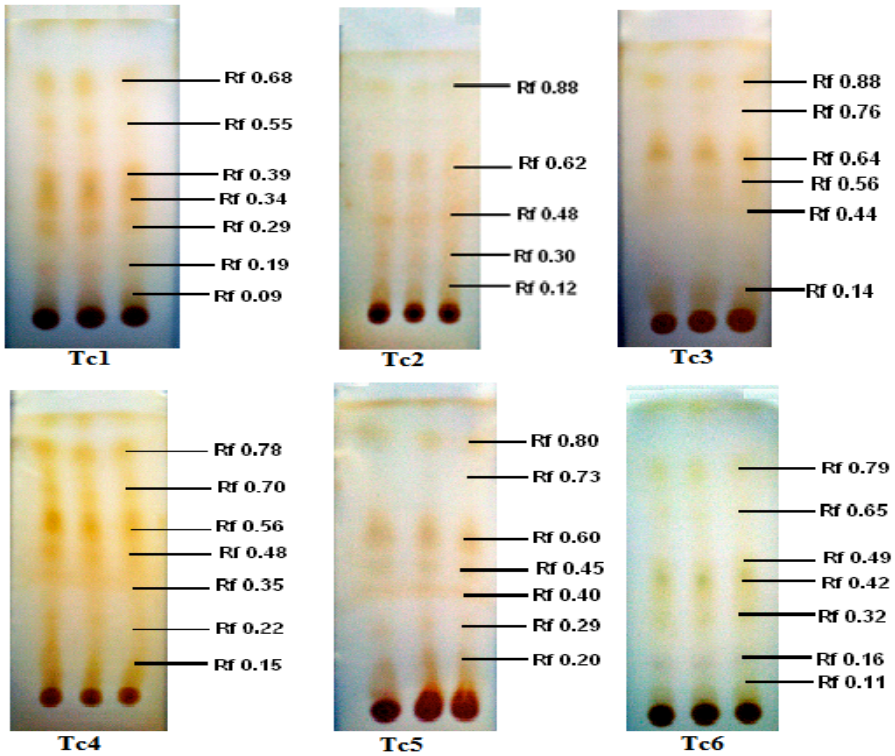

Figure 14: TLC fingerprinting profile of methanolic extract of different of *Tinospora cordifolia*; Solvent system- heptane: benzene: ethyl acetate: chloroform (16.0:14.0:4.0:1.0).

produced. Therefore, fluorescence analysis can be used for the identification of the drug and adulteration can also be determined [12, 13, 24]. Preliminary phytochemical screening confirms the different group of phytoconstituents in a sample whereas TLC is used for qualitative and quantitative determination of phytoconstituents. The variation in the inorganic constituents in different plant samples may be because they are growing on various plants. The maximum number of constituents separated from the methanolic extract of Tc1, Tc2, Tc3, Tc4, Tc5 and Tc6 were 7, 5, 6, 7, 7 and 7 respectively by thin layer chromatography using Heptane: Benzene: Ethyl acetate: chloroform (16.0:14.0:4.0:1.0). The therapeutic potential of a medicinal plant is mainly due to the active ingredient present in it, but unfortunately the concentration of active ingredients are very less in the herbal medicines. In the present study TLC revealed that the plant samples analysed could have different phytoconstituents and further studies are required to quantify the bioactives in different samples. These standardized parameters would certainly help for selection of the right sample of plant material for its phytopharmacological studies.

CONCLUSION

Herbal medicines are promising choice over modern synthetic drugs because they show minimum/no side effects and are considered to be safe. Generally herbal formulations involve 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. The major hindrance in the amalgamation of herbal medicine into modern medical practices is the lack of scientific and clinical data and better understanding of efficacy and safety of herbal products. To ensure the quality and safety of herbal products, standardization is of vital importance. In the present study we observed some microscopical, physicochemical and phytochemical variations of *T. cordifolia* stem growing on different trees. Hence, therapeutic potential and the content of important active constituents could be enhanced by further pharmacological and phytochemical analyses of the plant materials growing on different plants. The plant could be grown on some other plants and standardized for their phytopharmacological potential. The plant is

used in various Ayurvedic formulations viz. Guduchyadi churna, Guduchi taila, Dashmoolarishtha, Sanjivani vati, Kantakari avaleha, Chyavanaprasha, Guduchi sattva, Brihat guduchi taila, Stanyashodhana kashaya churana, Punchnimba churana, Guduchi ghrita, Amritaguggulu, Amritashtaka churna, etc. which are useful for treatment of different ailments [2]. Again, Selection of the right raw material (with high phytochemical and pharmacological property) for the formulations will be of paramount importance.

REFERENCES

[1] Saha S and Ghosh S. *Tinospora cordifolia*: One plant, many roles. Ancient Sci Life 2012; 31: 151-159. http://dx.doi.org/10.4103/0257-7941.107344

[2] Upadhyay AK, Kumar K, Kumar A and Mishra HS. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi) - Validation of the Ayurvedic pharmacology through experimental and clinical studies. Int J Ayurveda Res 2010; 1: 112-121. http://dx.doi.org/10.4103/0974-7788.64405

[3] Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacogn Phytochem 2014; 2(5): 69-73.

[4] Dineshkumar C. Pharmacognosy can help minimize accidental misuse of herbal medicine. Curr Sci 2007; 3: 1356-1358.

[5] Brain KR and Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright-Scientechnica, Bristol 1975.

[6] Mukherjee PK. Quality Control of Herbal Drugs. Business Horizon's Pharmaceutical Publishers, New Delhi 2002.

[7] Johansen DA. Plant Microtechniques. 1 ed. McGraw-Hill Book Company, New York and London 1940.

[8] Kokate CK. Practical Pharmacognosy. 1 ed. Vallabh Prakashan, New Delhi 1986.

[9] Agarwal SS and Paridhavi M. Herbal Drug Technology. University Press (India) Private Limited, Himayatnagar, Hyderabad 2007.

[10] Indian Pharmacopoeia. 4 ed. Vol II, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi 1996.

[11] WHO/PHARM/92.559/rev.1., Quality Control Methods for Medicinal Plant Materials. Organisation Mondiale De La Sante, Geneva 1992.

[12] Chase CR and Pratt R. Fluorescence of powdered vegetable drugs with particular references to development of system of identification. J Am Pharm Assoc 1949; 38(6): 324-331. http://dx.doi.org/10.1002/jps.3030380612

[13] Kokaski CJ, Kokaski RJ and Slama FT. Fluorescence of powdered vegetable drugs under ultraviolet radiation. J Am Pharm Assoc 1958; 47(10): 715-717. http://dx.doi.org/10.1002/jps.3030471010

[14] Harborne JB. Methods of extraction and isolation. In: Phytochemical Methods. Chapman and Hall, London 1998.

[15] Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy. 20 ed. Nirali Prakashan, Pune 2002.

[16] Ali M. Text Book of Pharmacognosy. 2 ed. CBS Publishers and Distributers, New Delhi 2003.

[17] Khandelwal KR. Practical Pharmacognosy. 14 ed. Nirali Prakashan, Pune 2005.

[18] Ahmed F, Shahid IZ, Baksi B and Sadhu SK. Antinociceptive and antidiarrhoeal activities of *Sonneratia caseolaris*. Orient Pharm Exp Med 2007; 7: 274-279. http://dx.doi.org/10.3742/OPEM.2007.7.3.274

[19] Stahl E. Thin Layer Chromatography, a Laboratory Hand-book. Springer, Verlag Berlin Heidelberg New York 1969.

[20] Jayalakshmi S, Satpathy S and Patra A. Pharmacognostical standardization and high performance thin layer chromatography (HPTLC) of root of *Saccharum officinarum* Linn. (Poaceae). Glob J Bot Sci 2015, 3: 25-30.

[21] Sahu R, Gahlot K, Patra A and Joshi R. Pharmacognostocal Standardization of Leaves of *Xanthium strumarium* Linn. Phcog J 2010; 2(12): 492-497. http://dx.doi.org/10.1016/S0975-3575(10)80037-9

[22] Patra A, Jha S and Murthy PN. Pharmacognostical standardization of leaves of *Hygrophila spinosa* T. Anders. Phcog J 2009; 1(2): 82-87.

[23] Gahlot K, Patra A and Sharma A. Pharmacognostical studies on *Gelsemium sempervirens* Ait. Root. Pharmacogn Mag 2008; 4(16)(suppl.): S212-S215.

[24] Denston TC. A Text book of Pharmacognosy. 5th edition, Pitman Medical Publishing Co. Ltd., London 1951.

Received on 11-02-2016 Accepted on 04-03-2016 Published on 31-07-2016

DOI: http://dx.doi.org/10.12974/2311-858X.2016.04.01.2