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PHARMACOGNOSTICAL STUDY AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF *DECHASCHISTIA CROTONIFOLIA WIGHT & ARN.*

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ABSTRACT

Dechaschistia crotonifolia Wight & Arn. (Ebaenaceae) is commonly grown in the deciduous forests of penincular India. The name *Dechaschistia* is derived from Greek word "deka" meaning ten and "schistos" meaning cleft as it consists of 10 celled locucidal capsule Genus. The current study is to evaluate pharmacognostical aspects and preliminary analysis of chemical constituents along with physical parameters findings of stem and root of *Dechaschistia Crotonifolia Wight & Arn.* to establish the standardization of this particular plant. Pharmacognostical examination was carried out in terms of macroscopical and microscopical aspects. Preliminary phytochemical investigation was carried over for the presence of primary and secondary metabolites and physical parameters were also evaluated viz., Ash values, extractive values, foreign organic matter, crude fibre content etc., The structural features of stem and root of *Dechaschistia crotonifolia Wight & Arn.* were figured out. The preliminary phytochemical examination revealed the presence of flavonoids, steroids and other inorganic compounds. The physical parameters were examined like ash values (6.4%), Acid insoluble ash (1.5%), water soluble ash (3.2%), moisture content (8.1%) and crude fibre content (3.4%). The pharmacognostical findings of *Dechaschistia Crotonifolia Wight & Arn.* helps to pursue the research in the way of ethanobotanical aspects and phytochemical study helps to ensure the quality and minimizes the adulteration the crude drug.

INTRODUCTION

Plant materials remain an important component in combating serious diseases in the world; for the therapeutic approach to several pathologies. Interest in medicinal plants has been overwhelming in the recent times especially as an important source of medication/health care. By 2000, World Health Organization had assessed that 80% of inhabitants of the world were estimated who were only relies on traditional medicines for the needs of primary health care and it also presumed that the major portion of traditional Healing involves by utilizing the extracts or active constituents of plants [1]. The Trinorcadalenes, parviflorals A and B (1 and 2), and four bis-trinorcadalenes, parviflorals C–F (3–6), together with the known trinorcadalenes, syriacusins, scopoletin and stigmasterol were isolated from roots of Decaschistia parviflora . Their structures were established by spectroscopic techniques and further their structures were confirmed by a single-crystal X-ray crystallographic analysis [2].

The extensive literature review regarding the genus *Dechaschistia* shown a very limited research and the findings shown, lack in knowledge of pharmacognostical , phytochemical aspects along with physical parameters. Hence this attempt was made inorder to develop the standardization of *Dechaschistia crotonifolia Wight & Arn.*

MATERIAL AND METHODS:

Plant material:

The stem and roots of plant *Dechaschistia crotonifolia Wight & Arn.* belonging to the family to Ebaenaceae were collected from surroundings of Tirumala, Andhra Pradesh, India in the month of June and it was authenticated by Dr. K. Madhava Chetty, Head of Department, Department of Botany, SV University, Tirupati. The stem and roots of *Dechaschistia crotonifolia Wight & Arn.* were shade dried for 7 days and stored in air tight containers.

Description of the plant:

It is a shrub with stems and branches densely whitish woolly. Ovate lance shaped leaves, 3-6 cm long, 2-4 cm wide, have heart shaped or rounded bases, pointed tip and coarsely toothed margins. Leaves are velvety on both sides and are carried on 1.5cm long stalks. Yellow flowers occur singly in leaf axils. Sepal cup is bell shaped with sepals 1-1.5cm long. Flowers have a dark maroon center and are 5-7cm across wit obovate petals, 3-4 x 2-2.5cm. Capsules are enclosed in the sepal cup and seeds are kidney-shaped. It is common in the deciduous forests of peninsular India. Flowering takes place in the month of March to June (Figure 1).

Pharmacognostic evaluation:

Organoleptic Evaluation:

Organoleptic characteristics like size, shape, color, odor and taste were studied. The macroscopical features of root and stem was observed with the help of magnifying lens (10X).

Microscopic Evaluation:

Transverse Section Preparation:

The stem and roots of *Dechaschistia crotonifolia Wight & Arn.* were made into pieces at the various portions and they were fixed with formalin, acetic acid and 70% alcohol (5:5:90). The solution was subjected for dehydration with the mixture of tertiary butanol and ethanol. The prepared specimens treated with paraffin wax and transferred to the blocks of paraffin. Further they were made sectioned with the help of 10-12µm using rotary microtome. These sections were stained with Toluidine blue followed by safranin. The clear sections mounted and focused under light microscope. The snapshots were taken with the help of Nikon Lab Photo microscopic unit in diversed magnification [3].

Powder microscopy:

To the powder of stems and roots of *Dechaschistia crotonifolia Wight & Arn.* in a watch glass, 1-2 drops of 0.1% phloroglucinol solution and concentrated HCL(1:1) was added. The stained powder was mounted with glycerol and observed under microscope with 10x10 magnifications.

Physical evaluation:

Estimation of crude fiber:

2gm of powdered drug was taken and heated by adding 50ml of 10 % v/v nitric acid with continous stirring and strained. To the residue 50 ml of 2.5% v/v sodium hydroxide solution was added, heated and maintained at boiling point for 30 seconds.[5] After it was strained and the residue is weighed. The percentage of crude fibers was determined.

Moisture content:

10gm of accurately weighed roots and stems of *Dechaschistia crotonifolia Wight & Arn.* was placed in a tared porcelain dish and dried at 105°c for 5 hrs and weighed. Drying and weighing is continued at an interval of one hour until two successive weighing is constant.

Total ash:

Determination of total ash:

2gm of root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* was taken in tared silica crucible and incinerated at a temperature not more than 450°C until free from carbon. The obtained ash was cooled and weighed. The percentage of ash was calculated with reference to the air dried drug.[6,7]

Acid-insoluble ash:

The total ash obtained from 2gm of root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* boiled with 25 ml of dilute hydrochloric acid for 5 minutes and the insoluble mater was collected on an ashless filter paper. It was washed, ignited and weighed. The percentage of acid insoluble ash

was calculated with reference to the air dried drug.

Water soluble ash:

The total ash obtained from 2g of root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* was boiled with 25ml of water for 5 minutes and the insoluble matter was collected on an ashless filter paper. It was washed, ignited and weighed. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of alcohol soluble extractive:

5g of accurately weighed root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* was taken and macerated with 100 ml of 95% alcohol for 24 hr. The contents were frequently shaken during the first 6hr and allowed to stand for 18hr. After 24 hr, 25ml of extract was filtered and evaporated. The extract was dried at 105℃ to a constant weight.

Determination of water soluble extractive:

5g of accurately weighed root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* was taken and macerated with 100 ml of chloroform water for 24 hr. The contents were frequently shaken during the first 6hr and allowed to stand for 18hr. After 24 hr, 25ml of extract was filtered and evaporated. The extract was dried at 105℃ to a constant weight.

Preparation of extract:

500g of dried coarsely powdered root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* was packed in soxhlet apparatus and was defatted with petroleum ether (50-60ºC). The marc left subsequently extracted with ethanol. The root and stem extracts obtained were concentrated using rotary evaporator and dried with the help of freeze drier.

Preliminary Phytochemical Screening:

The extracts were subjected to preliminary Phytochemical screening for the detection of various plant constituents viz. Primary metabolites like carbohydrates, gums & mucilages, proteins, fats & oils, organic acids & inorganic compounds, enzymes and vitamins and Secondary metabolites like glycosides, flavonoids, steroids, alkaoloids, tannins & phenolic compounds[6-12].

Fluorescence analysis of the powdered drug:

The fluorescence analysis of the powdered root and stem powder of *Dechaschistia crotonifolia Wight & Arn.* was done by placing dry powdered leaves on a slide and observed by treating with several drops of different chemical reagents to detect the color changes under UV and Visible light[13].

RESULTS

Pharmacognostic evaluation:

Macroscopical examination:

Stems are woody, round shaped, pale green to grey, whitish-stellate-tomentose, more so on younger parts measures about 14- 30cm long, 2-4mm in diameter and the root system is tap root system they appear in brown in color showing fissures measures about 2-3mm in diameter. (Figure 1)

Microscopical examination:

Stem of Decaschistia crotonifolia:

The stem is circular in cross sectional outline measuring 1.6mm in thickness. The surface of the stem is densely hairy covered with stellate. The epidermal layer consists of vertically oblong rectangular thick walled cells. Inner to the cortex are four layers of horizontally oblong rectangular cells arranged in compact layers. Inner to the cortical zone is a thick discontinuous layer of sclerenchyma cells. The vascular cylinder is hollow enclosing a wide pith. The vascular cylinder has thick outer cylinder of secondary phloem. The secondary phloem includes alternating layers of phloem elements and their rectangular blocks of phloem fibres. There are thin radial lines of phloem rays. Secondary xylem is compact hollow cylinder. It includes numerous thin radial lines of vessels, thick xylem rays and xylem fibres. Most of the vessels are in radial multiples (arranged in continuous radial rows). The vessels are circular, angular, elliptical or ovate in outline. The vessels have these lignified walls. The xylem fibres are small angular cells with thick lignified walls. The xylem rays have radially elongated rectangular thick walled cells. Pith is wide with angular thin walled parenchyma cells. Some of the pith cells are wide and circular and possess dense mucilage. Some of the cells also possess calcium oxalate druses (Figure 2).

Root of Decaschistia crotonifolia:

Both thin and thick roots were studied. The thin root is 2.6mm in diameter. It consists of a very thick continuous layer of homocellular periderm which is 300µm thick.

Table 1: Preliminary phytochemical Screeing of ethanaolic stem and root extract of *Decaschistia crotonifolia* for primary metabolites:

S. No.	Phytoconstituents	Observations in Stem	Observations in Root
1	Carbohydrates	+ve	+ve
	Gums	-ve	-ve
	Mucilage	+ve	+ve
2	Proteins	-ve	-ve
	Fats & Oils	+ve	-ve
	Inorganic Compounds: a) Ca, Mg, P, carbonate & nitrates.	+ve	+ve
	b) Na, K, Fe, Cl and suphates.	-ve	-ve
5	Organic Acids	-ve	-ve
6	Enzymes	-ve	-ve
7	Vitamins	-ve	-ve

+ ≡ Presence, – ≡Absence.

Table 2: Preliminary phytochemical Screeing of ethanaolic leaf extract of *Decaschistia crotonifolia* for secondary metabolites

S. No.	Phytoconstituents	Observations in Stem and Root
1.	Glycosides	-ve
2.	Flavonoids	+ve
3.	Alkaloids	-ve
4.	Tannins & Phenolic Compounds	+ve
5.	Steroids	+ve

+ ≡ Presence, – ≡Absence.

Table 3: Physical Parameters of stem and roots of *Decaschistia crotonifolia*

S. No.	Physico Chemical Parameters	Stem %w/w	Root %w/w
1.	Ash Values		
	Total Ash	8.0	10
	Acid Insoluble Ash	1.1	1.3
	Water soluble Ash	5.0	6.0
2.	Solvent Extractive Values		
	Ethanol Soluble Extractive	15.14	14.0
	Water Soluble Extractive	6.5	7.4
3.	Loss on Drying	6.5	5.5
4.	Crude Fibre Content	13	12

Table 4: Fluorescence analysis of stem and root powder of *Decaschistia crotonifolia*

S. No.	Reagents	Day Light	At Short Wavelength 254nm		Long Wavelength 366 nm	
			Stem	Root	Stem	Root
1.	Distilled water	Pale Green	Pale Green	Brown	Pale Green	Brown
2.	1 N NaOH	Brown	Dark greenish yellow	Pale yellow	Blackish Green	Brown
3.	1N HCl	Green	Pale green	Dark Black	Dark Green	Dark Black
4.	Ethanol	Brownish green	Green	Brown	Dark Green	Brown
5.	50% HNO_3	Green	Pale Yellow	Brownish grey	Dark Green	Brownish grey

6.	$FeCl_3$	Yellowish Green	Green	Brownish Yellow	Green	Brownish Yellow
7.	$CHCl_3$	Green	Pale Green	Pale Brown	Blackish green	Pale Brown
8	Picric acid	Green	Green	Brown	Blackish green	Brown

Table 5: Fluorescence Analysis of Stem and root extract of Decaschistia crotonifolia

Name of the plant species	Plant part	Solvent Used	Day light	Short UV	Long UV
Decaschistia crotonifolia	Stem	Ethanol	Light Green	Green	Greyish Green
	Root	Ethanol	Brown	Brown	Dark Brown

Figure 1: A, B. Twig of *D. Crotonifolia*

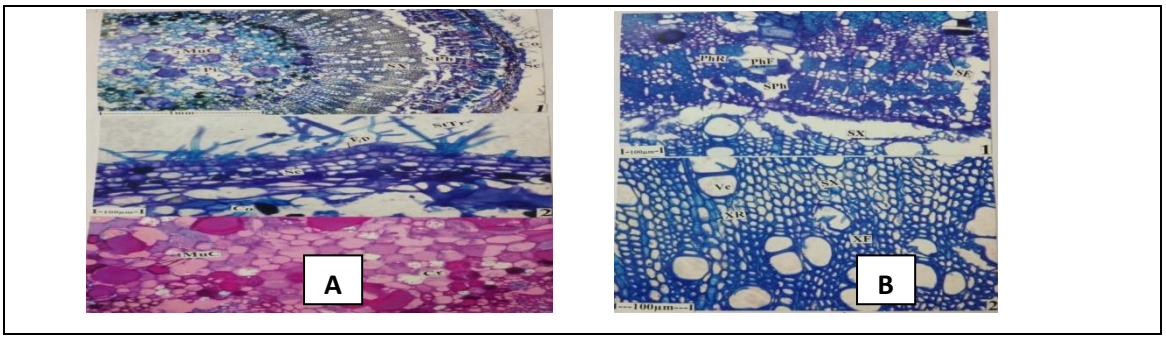

Figure 2: Stem of *Dechaschistia crotonifolia.* **A.** Pith region Showing cortex, Crystal, Mucilage cavity, Sclereids, Secondary phloem, Secondary Xylem and Stellate Trichome. **B.** Secondary xylem and Phloem showing Phloem fibre, Phloem Ray, Sieve Element, Secondary Xylem, Secondary Phloem, Vessel, Xylem Ray and Xylem Fibre.

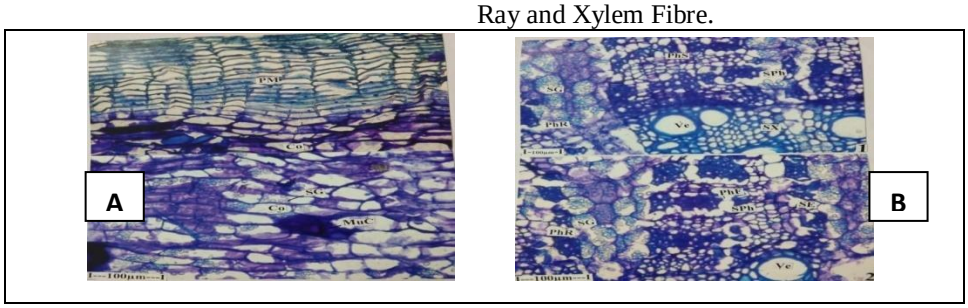

Figure 3: Thin Root of Dechaschistia crotonifolia **A.** Thin root showing Palisade Mesophyll, Cortex, Starch grains and Mucilage Cavity. **B.** Root showing Periderm, Xylem Fibre, Fissure, Cortex zone, Secondary Xylem and Secondary Phloem and Vessels.

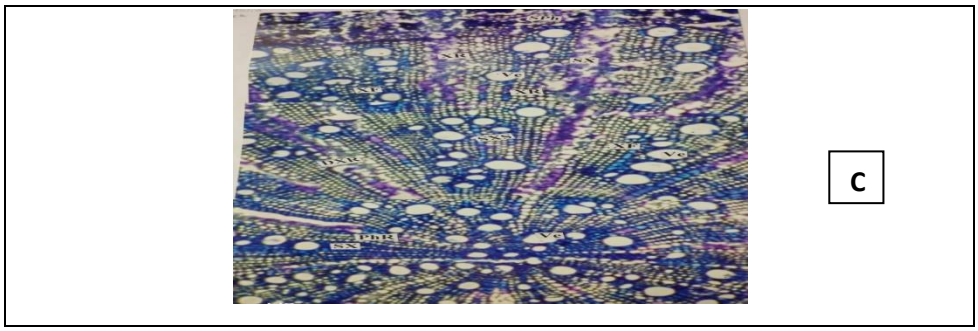

Figure 4: Thick Root of Dechaschistia crotonifolia.
XR: Xylem Ray, XF: Xylem Fibre, Ve: Vessel, SXS: Secondary Xylem Series, SPh: Secondary Phloem, SX: Secondary Xylem, PhR: Phloem Ray, DXR: Dilated Xylem Ray.

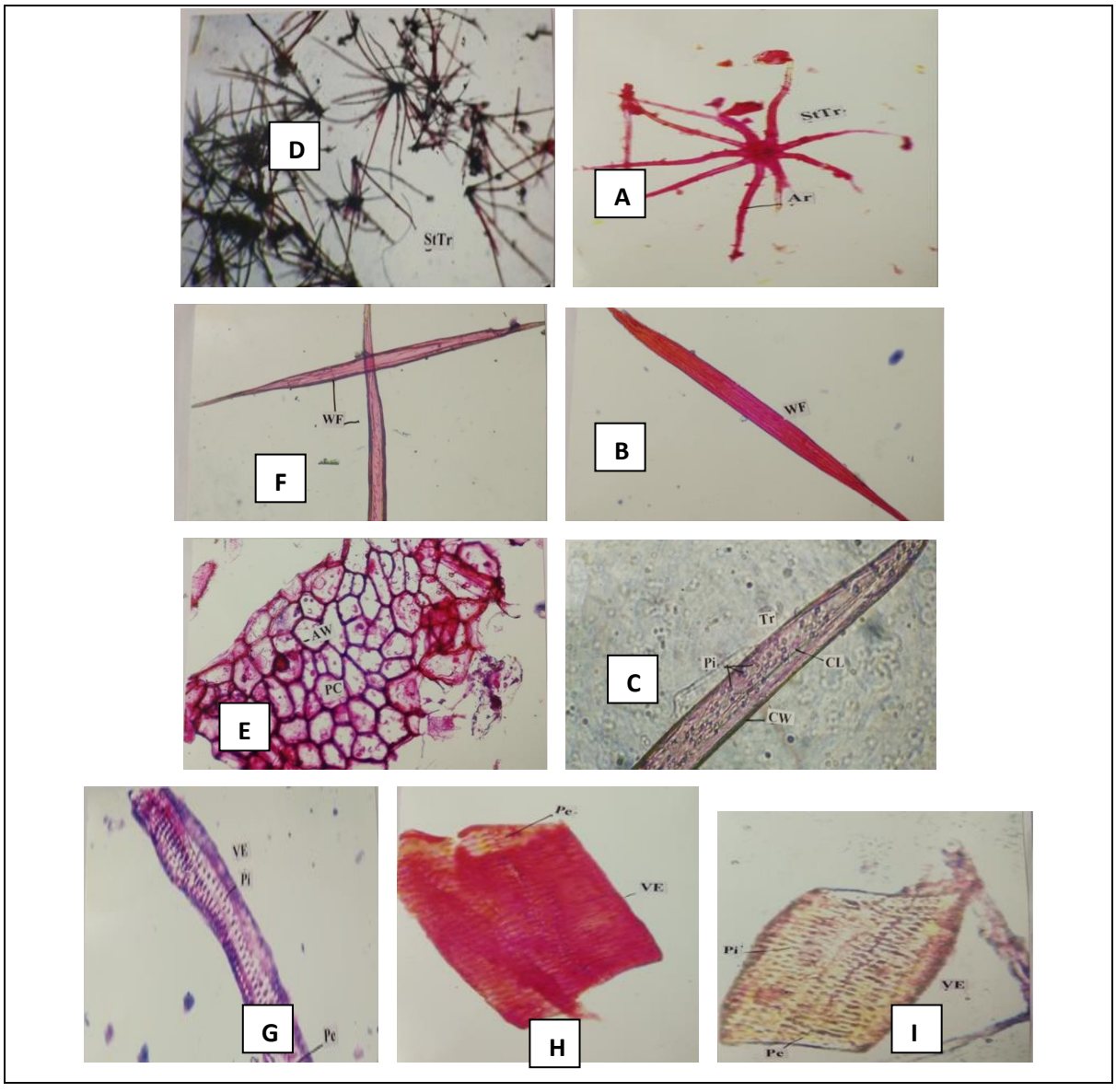

Figure 5: Powder Microscopy of Root of *Dechaschistia crotonifolia*.
A &B. Stellate Trichomes with arms, **C & D.** Libriform Fibres and Wide Fibre, **E.** Parenchymatous cell showing anticlinal wall, **F.** Tracheids showing Cell Lumen, Cell Wall and Piths and **G, H & I.** Xylem vessel showing perforation.

The periderm consists of only phloem cells which are in radial rows of rectangular narrow thin walled cells. No phelloderm is found. The cortical zone is narrow comprising a few layer of parenchyma cells. The cortical cells have starch grains. The vascular cylinder is wide and the phloem and xylem cylinders are cleaved radially into about eight fan shaped segments by dilation of the rays. Xylem and phloem tissues are in continuous radial segment. Phloem includes alternating blocks of fibres and phloem. The fibres are in compact units, the ccells being squarish, thick walled and lignified. Phloem elements are small angular and thick walled with darkly stained walls. The cells are located at the corners of the sieve elements (Figure 3). The thick root is 6.6mm in diameter. It consists of a wide, uniform type of phellem cells in several compact lines. The phellam tissue has narrow rectangular, horizontally elongated cells. The phellem zone is 400µm thick. Phelloderm derivatives are narrow with three or more cell layers. Cortical zone is not Widen‌t. As in the thin root, the thick root has several long, vascular segments which are very narrow in the center and become progressively wider towards the periphery. The vascular segments are separated from each other by dilated rays which are narrow in the center and become dilated towards periphery. Each vascular segment has xylem unit and secondary phloem unit at extereme outer end of the xylem . Phloem consists of horizontal blocks of phloem fibres and alternating with the fibre block occurs phloem segments. The entire phloem segments is narrowly conical with outer region due to dilated phloem rays. Phloem includes sieve elements with companion cells. The secondary xylem has numerous radical multiples or solitary vessels which are mostly circular in outline. The vessels include both narrow and wide vessels. The vessels ensheated by thick layer of these are also which are seen in long straight radicals lines located within the xylem radical blockers. The narrow vessels 10µm in diameter and these wide vessels are 80µm in diameter (Figure 4).

Powder Microscopy of Decaschistia Crotonifolia: The powder preparation of the plant was examined under the microscope; the following inclusion was observed.

Stellate Trichomes:

Cluster of many unbranced,unicellular needle shaped trichomes are abundant in the powder. The trichomes arise from epiderm of cells, which are modified into circular, thick wall group of cells. The trichomes spread horizontally and appear star shaped when viewed from above .hence, they are called stellate or star shaped trichomes.

Libriform Fibres:

Long, needle shaped, unicellular, unbranchedfibres are abundant with powder. The fibres are wide and thin walled and fibres are wide fibres. Tthe wide fibres have wide cell lumen and someof them have narrow slit like simple pits. The wide fibres are 620to 750 µm long nd 40 µm thick in the mid portion . A part from wide fibers, these are narrow fibres are very thin, these and like with ends. Some narrow fibres are uniformly thin and fibrous are thick in middle and narrow along terminal part. Thefibres have thick lignifed walls and narrow cell lumen. No pits are evident in the narrow fibres. These fibres are 780 to 1020µm long 10 µm thick.

Ground Parenchyma Cells:

These are small fragments of arenchyma cells which are angular, thin walled and compared. Their anticlinal walls are thin and straight some of the cells have small, less prominent nulei.

Tracheid:

These are rarely seen the trachid , which is imperforate tracheary element. It is thick and wide and conical ends .the walls are thick and lignified .the cell lumen in wide and have wide circular bordered pits .the trachid is about 450 µm long and 30 µm thick.

Xylem Ray:

Thick bundle of xylem ray with horizontal system and vertical system of cells in rarely seen in the powder .the vertical system of ray cells are vertically elongated and compactly arranged. the horizontal system of cells are wide , thin walled cells with dense simple on the radical walls. The systems of cells are firmly attached with each other forming a complex system.

Vessel Element:

Single isolated vessel elements as well as bundle of vessel elements and fibres are frequently seen in the powder .isolated vessel

elements are either long , narrow or cyclindrical or short, wide, barrel shaped vessel element are seen in the powder. Vessel elements have simple , wide, either obliquet or horizontal end wall perforation. Lateral wall pits are multiseriate and alternate .the pits are wide and circular or horizontally elongated and spindle shaped. The narrow cylindrical vessel element are 400µm long , some vessel elements are 850 µm long they are 40 µm wide the short wide barrel shaped elements are 170µm long and 100µm wide (Figure 5).

Preliminary Phytochemical Screening:

The preliminary phytochemical screening of ethanolic extract for the presence of primary & secondary metabolites was carried out and the results obtained. (Table 1 & 2)

Physical Constants Evaluation:

Inorder to establish standards to the crude drug of *Dechaschistia crotonifolia Wight & Arn.* the physical constants like Ash values viz., Total ash, Acid insoluble ash and water soluble ash, Solvent Extractive values viz., Ethanol soluble extractive, water soluble extractive, Loss on drying and crude fibre content were evaluated and their values (Table 3).

Fluorescence Analysis:

The fluoresecence analysis of both the stem and root powder and stem and root extracts of *Dechaschistia crotonifolia Wight & Arn.* were studied and it was observed the behavior of stem and root powder to different organic solvents or reagents in Day light and Ultraviolet at shorter wavelength of around 254nm and as well as longer wavelength around 366nm (Table 4 & 5).

DISCUSSION

In the present study, pharmacognostical parameters of stem and root of *Dechaschistia crotonifolia Wight & Arn.* were evaluated which serves as an establishment for the monograph of the crude drug. The fluorescence analysis of the powdered leaf was examined and the various behavioral changes had observed with different reagents at both UV and Visible light. The phytochemical analysis of ethanolic extract of root and stem of *Dechaschistia crotonifolia Wight & Arn.* were examined and it revealed the presence of steroids and flavonoids predominantly which gains an importance in development of lead molecules.

CONCLUSION

The Phytochemical studies reported in the present study need further scientific investigation to ascertain its identity up to compound level. Pharmacognostical characters and preliminary phytochemical investigations which are studied will be helpful in quantitative and qualitative standardization of *Dechaschistia crotonifolia Wight & Arn.* However detailed differential studies using chromatographic techniques, molecular and chemical markers are required for their authentication especially in their drug form.

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