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Research Article

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES OF BARK OF *NYCTANTHES ARBOR – TRISTIS* LINN.

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ABSTRACT

Nyctanthes Arbor-tristis Linn (Nyctanthaceae) commonly called as night jasmine, found throughout in India, It is used for a wide range of diseases in folk medicine. The present study attempts pharmacognostic studies of bark, extraction, and identification of chemical constituents from pet. Ether, chloroform, ethanolic and aqueous extracts of bark of *Nyctanthes arbor-tristis* Linn. Macroscopic as well as microscopic studies of any crude drug are the primary steps to establish its botanical quality control before going to other studies. The Transverse section (T.S) features of the bark indicate the presence of Phellem, phelloids, tabular cells, Phloem rays. With some of the powder diagnostic features of the bark are the presence of sclerotic phelloid cells, thin walled phloem cells, Calcium oxalate crystals and physico-chemical characteristics. The presence of alkaloids, carbohydrate, glycosides, phytosterols, fixed oil, tannins, flavonoids, proteins and amino acids, gums and mucilage was confirmed during preliminary phytochemical screening. HPTLC profile of extracts will help in identification of the drug and also in isolating and identifying the biomarker compound responsible for the bioactivity. These observations would be of immense value in the botanical identification and standardization of the drug in crude form. This study would help distinguish the drug from its other species.

Keywords: *Nyctanthes Arbor-tristis* Linn, Pharmacognosy, Physiochemical constants, HPTLC.

INTRODUCTION

Nyctanthes arbor-tristis Linn (Nyctanthaceae) commonly called as night jasmine, a hardy large shrub or small tree widely distributed in outer Himalayan ranges from Chenab to Nepal, Assam, Burma, Bengal, Central India to Godavari, cultivated in many parts of India. The barks are intended for expectorant, anorexia, liver disorder, piles, worm infestation, blood disorder, oliguria, skin diseases fever and snake bite[1-4, 7, 10-13]. However pharmacognostical information about this plant has not been published, particularly there is necessary to define quality control procedures of the *Nyctanthes arbor-tristis* Linn as raw material. Hence the present investigation deals the pharmacognostical and preliminary phytochemical evaluation of the *N.arbor-tristis* Linn. The study includes morphological and anatomical, determination of physico-chemical constants and the preliminary phytochemical evaluation and establishment of HPTLC of the different extracts of *N.arbor-tristis* Linn[14].

MATERIALS AND METHODS

Collection of the plant

The plant was widely cultivated in gardens almost throughout India for the fragrant flowers. For the present work the plant was collected from sangameswarar temple near Bhavani (Erode District). The plant was identified by Dr.G.V.S.Murthy, Joint Director of Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore who authenticated the plant from available literature.

Chemicals and Instruments

Compound microscope, Camera lucida, stage and eyepiece micrometer, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Photomicrographs in different magnifications of all necessary cells and tissues were taken with Nikon Lab Phot- 2 microscopic Unit. Some crystals, starch grains and lignified wall photographs were taken under polarized light microscope. Solvents viz. Pet. ether, chloroform, ethanol and reagents such as Phloroglucinol, Glycerine, HCl, Chloralhydrate and Sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Pharmacognostical Evaluation

Exomorphology

The fresh (immediately after collection) leaves and peeled stem bark of *Nyctanthes arbor-tristis* Linn spreaded on a neat plastic sheet and the different organoleptic features was observed using modifying glass and ruler, the same procedure was repeated in the dried stem bark also.

Macro and Microscopic Studies

Macroscopic examination of the bark was carried out according to the standard procedures [15]. Fresh bark was selected for microscopical studies. Sections were cut on a microtone and by free hand sectioning; numerous temporary and permanent mounts of the microscopical sections of the bark specimen were made and examined microscopically. Photomicrographs of the microscopical sections were captured with the help of Motic photomicroscope (Canada) provided with Motic images plus 2.0 software.

Powder characteristics

The bark was dried in shade then finely powdered and passed through sieve No.125 and.180, separately, to obtain fine and very fine powder respectively and then subjected for microscopic examination. The powdered drug was separately treated with Phloroglucinol, Hydrochloric acid solution, glycerine and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains[16].

Fluorescence studies

Fluorescence study is an essential parameter for first line standardization of crude drug. The powder material was treated separately with different reagents and exposed to visible and ultraviolet light (short & long) to study their fluorescence behavior.

Physicochemical parameters

The total ash, acid insoluble ash and water soluble ash were calculated as per the ip, different extractive values were determined to find out the amount of soluble components in suitable solvents such as water, alcohol and ether.

Preliminary phyto-chemical screening

For the phytochemical studies, about 450g of the powder was extracted successively with petroleum ether, chloroform, ethanol and aqueous solvents using soxhlet apparatus for 24h. The percentage extractives were calculated with reference to air dried drug and tested for the presence or absence of the phyto constituents.

Chromatographic studies

HPTLC

HPTLC Profile for all the four different extracts (pet. Ether, chloroform, ethanol, aqueous) were carried out at the wavelength of 254nm.

RESULTS

The powdered bark of *Nyctanthes arbor-tristis* Linn has been investigated into a systematic way covering pharmacognostical and preliminary phytochemical aspects in an attempt to rationalize its uses drug of therapeutic importance.

Exomorphology

Nyctanthes arbor-tristis Linn vernacular name - *parijatham, Manjhapu* (Tamil), It is upto 10m height, young branches have angular stem. Leaves are simple, opposite, ovate, rough and thin. Margins serrate, apex acute. The flowers are delightfully fragrant, sessile, bisexual, hypogynous, corolla tube orange coloured, and lobes white, twisted. The seeds are orbicular, thin testa, brown coloured capsule 2cm long and compressed[2, 5-7].

The bark was externally grey or brownish white in colour, rough deep and irregularly fissured. [Fig.1a, 1b] Internally yellowish white or sandal colour, externally granular in texture and internally smooth, 8.2mm thick, bitter taste, odourless, curved or quill in shape, short fracture in outer bark and fibrous fracture in inner bark[8,9] and found in outer Himalayan ranges from Chenab to Nepal, Assam, Burma, Bengal, Central India to Godavari, cultivated in many parts of India.

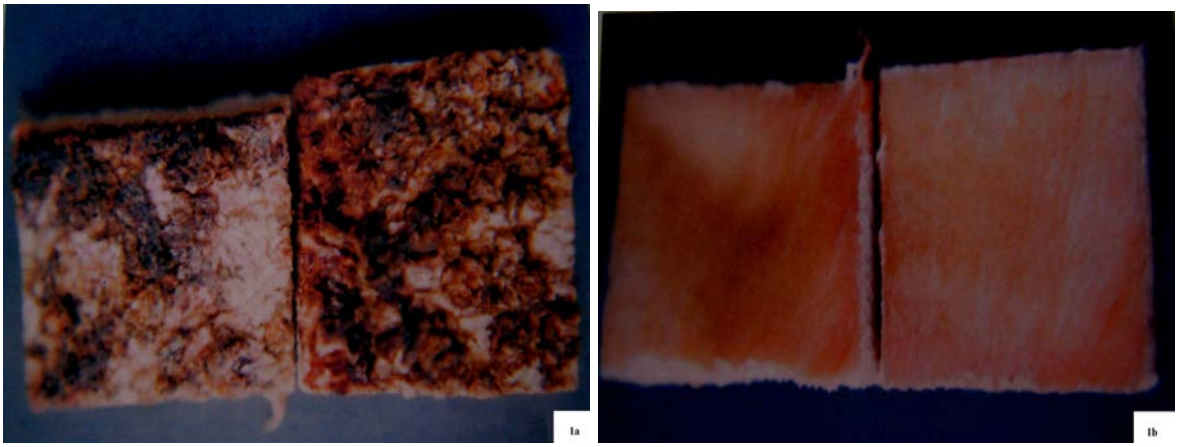

Fig. 1a,1b: Exomorphic features of young bark of *Nyctanthes arbour-tristis* Linn

Pharmacognostical Studies

Macro and Microscopic Studies

The surface of the bark is rough, deep, irregularly fissured externally grey or brownish in colour. Internally yellowish white or sandal coloured. Externally granular in texture and internally smooth, odourless and bitter in taste .The fractures are short in outer bark and fibrous in inner bark. The bark has total thickness of 8.2mm and differentiated into outer bark (periderm) and inner bark (secondary phloem). The periderm is superficial in position, it is wide, measuring 1-2mm thick, at certain places the periderm enters into the inner tissue forming wide bay (Fig.2). The periderm surface is irregularly fissured, the fissures are shallow. The periderm comprises of small, tabular phellem cells which are comprised of dark bands. In places where the phellem is heterogeneous, made up of thin, continuous tangential lines of phelloids (sclereids) and wider, thin walled, squarish or tabular cells (Fig.3a, 3b). Phelloderm is not evident. Periderm is immediately followed by secondary phloem. The secondary phloem consists of outer collapsed phloem and inner zone of non-collapsed phloem. The collapsed phloem is the widest part of the bark. The region beneath the periderm consists of circular, less compact parenchyma cells, narrow phloem rays and circular, prominent masses of sclereids (Fig 4a). Further inner to the phloem, here are obliquely radial dark thin lines which represent crushed and obliterated sieve elements or the collapsed phloem (Fig.4a). The non-collapsed phloem (Fig.4b) is 450 µm. It consists of intact sieve tube members, axial parenchyma and thin less prominent rays. The sieve tube members are small and are random or radial in orientation. The sieve tube members are 20-30 µm in diameter. The TLS view exhibits the collapsed and non-collapsed phloems. The collapsed phloem shows slightly wider rays which are three to many seriate short and wide, they are homocellular consisting of polygonal compact cells are intact. The sclereids are in thick vertical bands (Fig.5). The sieve tube members are crushed and appear in thick vertical dark lines. The rays are 200-250 µm in breadth. In the non-collapsed phloem region sclereids are absent; the phloem rays are smaller in size. The sieve tube members are short and narrow. They are 250µm in height. The sieve plate is simple and oblique (Fig.6). The phloem parenchyma cells of the phloem have wide simple pits (Fig.7). The RLS view exhibits horizontal ribbon like band of cells. These cells are horizontal oblong or squarish. The rays are homocellular. Some of the rays are heterocellular with horizontal procumbent cells and marginally upright cells (Fig.8).

Powder microscopy

The powdered bark shows phelloid cells, the periderm is broken into small bits in which sclerotic phelloid cells and thin walled phloem cells

are evident (Fig.9a). Calcium oxalate crystals of 2 types are seen in the powder (Fig9a). sphaerocrytals are spherical bodies formed by many pointed crystals(Fig.9b). Prismatic crystals include rectangular and spindle shaped crystals, they are equally abundant. (Fig.9b). The sclerenchyma components of the bark are the stone cell or branchy sclereids. They are squarish, irregular or rectangular in shape. They have thick lignified walls and wide lumen. Numerous simple circular pits are seen in the sclereids. (Fig.10) [10-19].

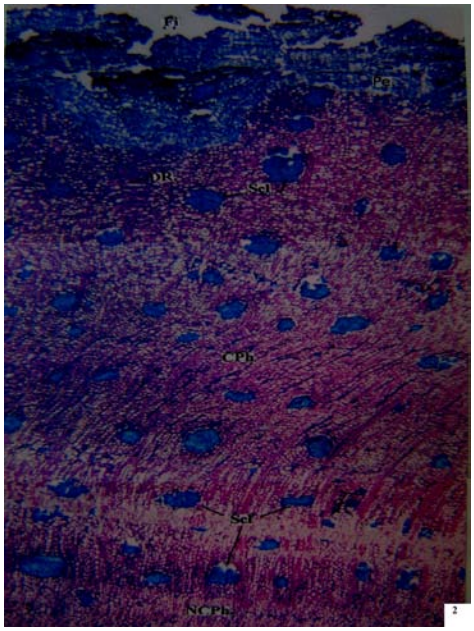

Fig. 2: T.S. of bark of *N. arbor-tristis* Linn entire view

Fi: Fissures; Pe: Periderm, DR: Dilated ray, Scl: Scelerids, Cph: Collapsed phloem, Neph: Non collapsed phloem.

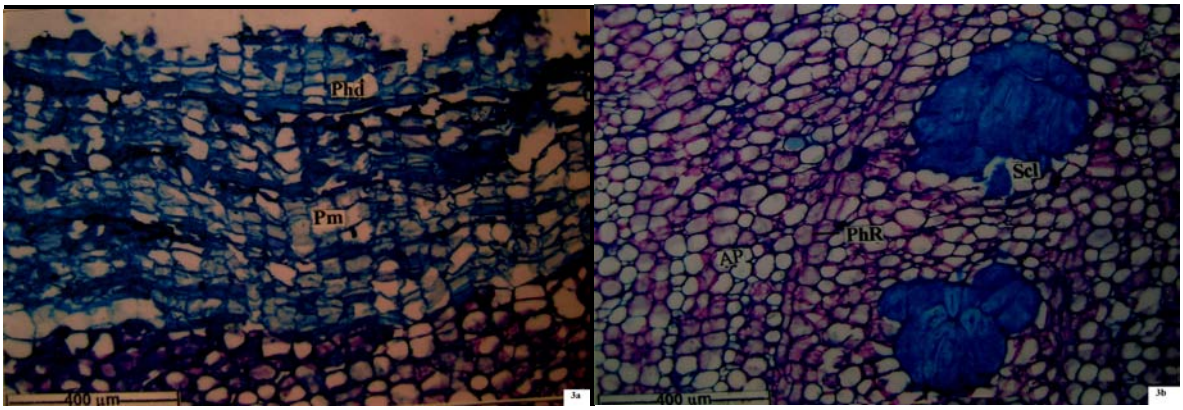

Fig. 3a, 3b: Microscopy of periderm and outer collapsed phloem

Phd: Phelloid; Pm: Phellem; Scl: Sclereid; PhR: Phloem rays; AP: Axial parenchyma

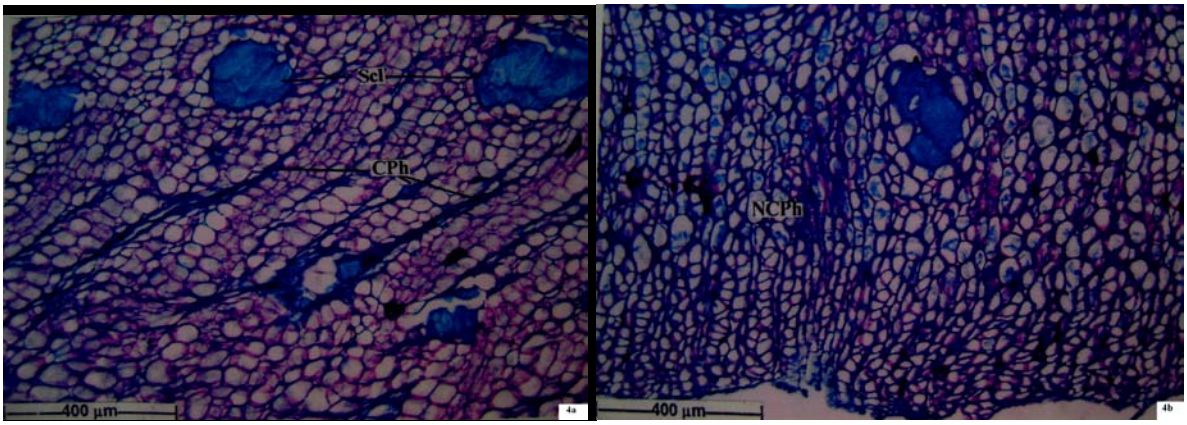

Fig. 4a, 4b: Anatomy of collapsed and non-collapsed phloem

Scl: Scelerids; Cph: Collapsed phloem; Ncph: Non- collapsed phloem

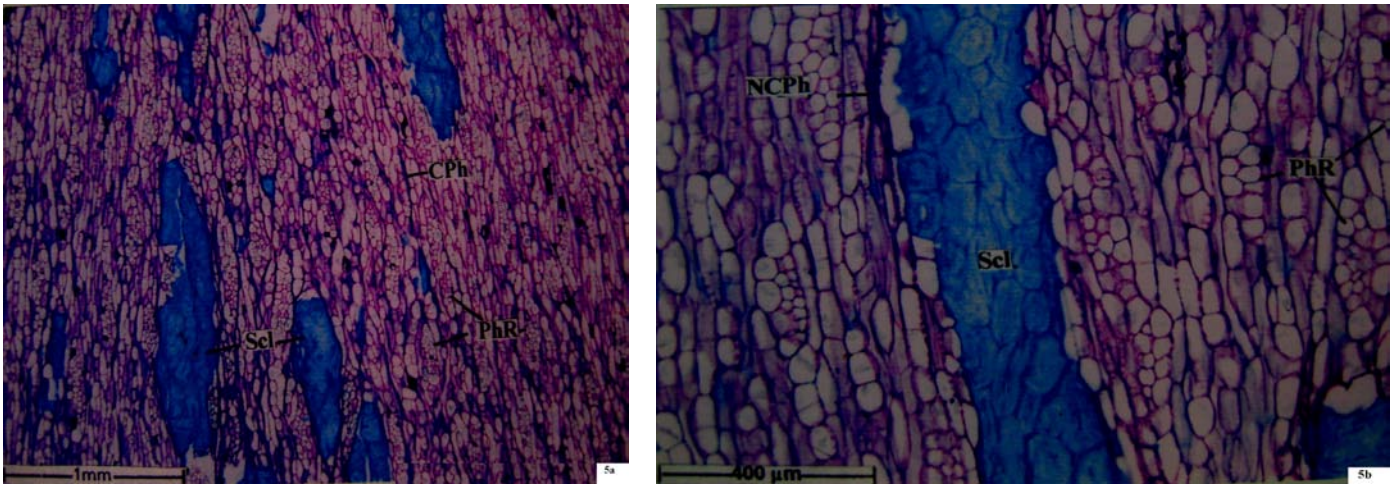

Fig. 5a, 5b: TLS view of collapsed phloem

Cph: Collapsed phloem: PhR: Phloem rays; Scl: Scelerids

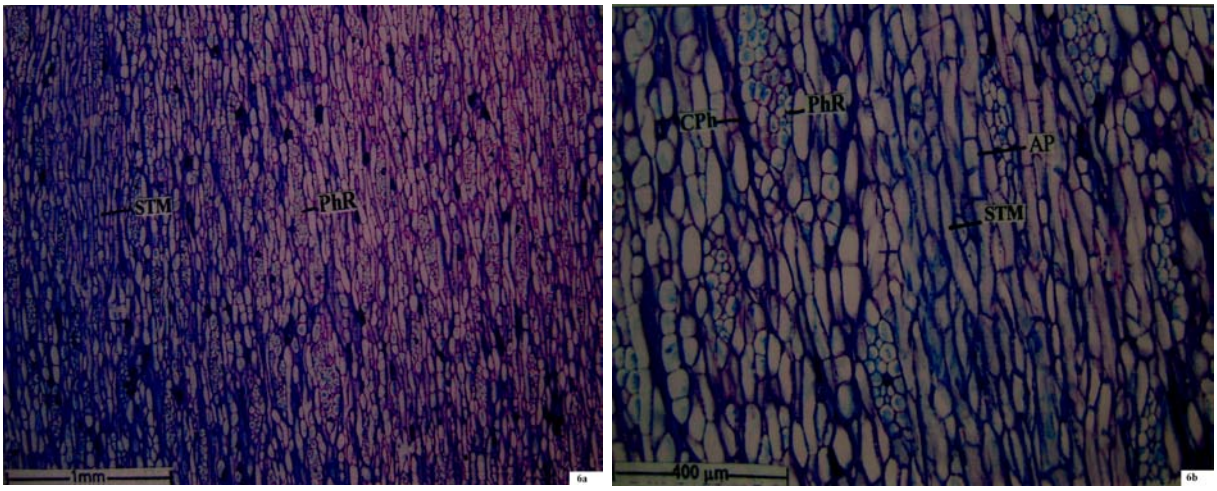

Fig.6a, 6b: TLS view of non-collapsed phloem

STM: Sieve tube members; PhR: Phloem rays; Cph: Collapsed phloem: AP: Axial parenchyma

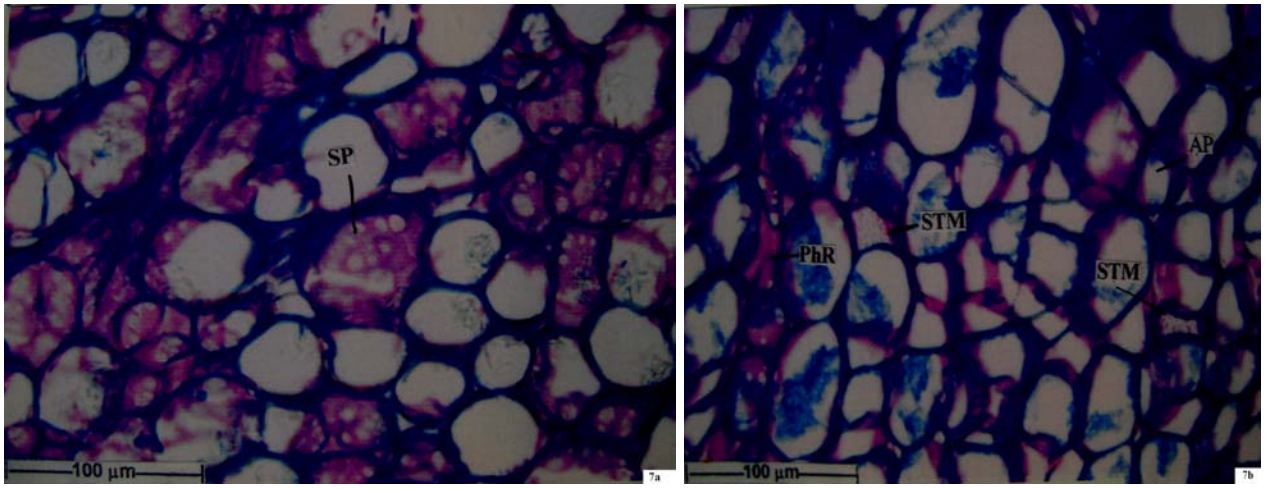

Fig .7a, 7b: TLS view of non-collapsed phloem

SP: Simple pits; AP: Axial parenchyma PhR: Phloem rays; STM: Sieve tube members

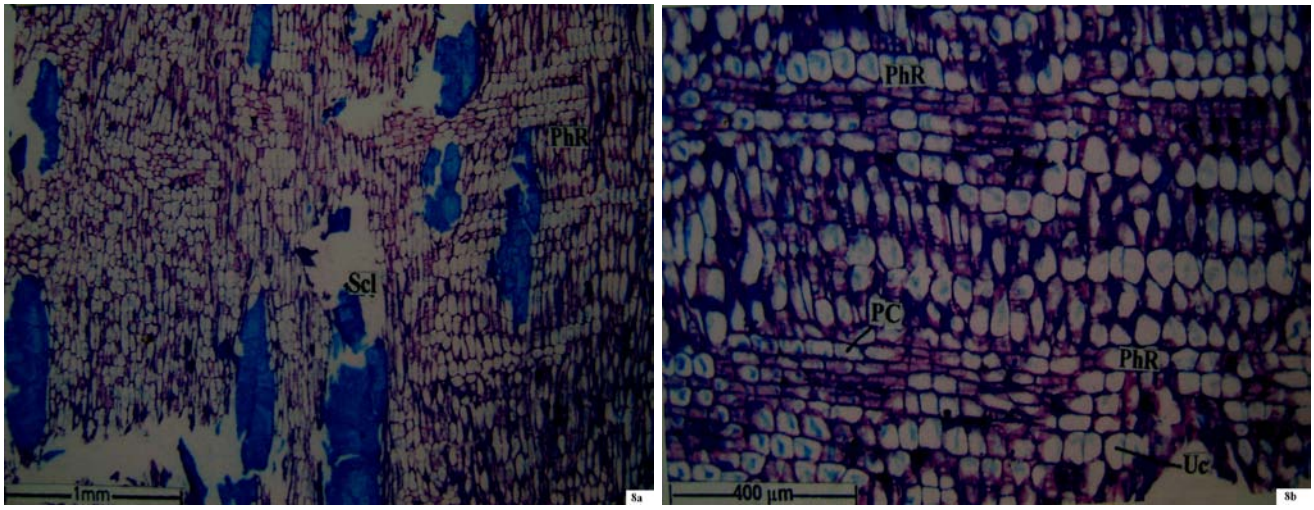

Fig. 8a, 8b: RLS view of phloem

PhR: Phloem rays Scl: Scelerids PC: Procumbent cells UC: Upright cells

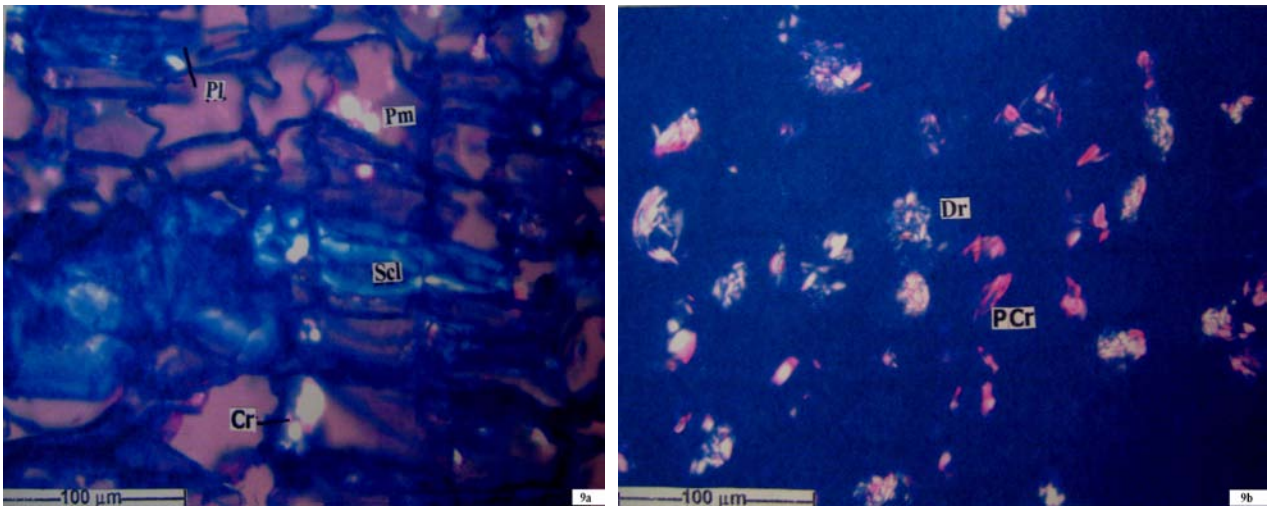

Fig. 9a, 9b: Powder microscopy of bark of *N.arbor-tristis* Linn

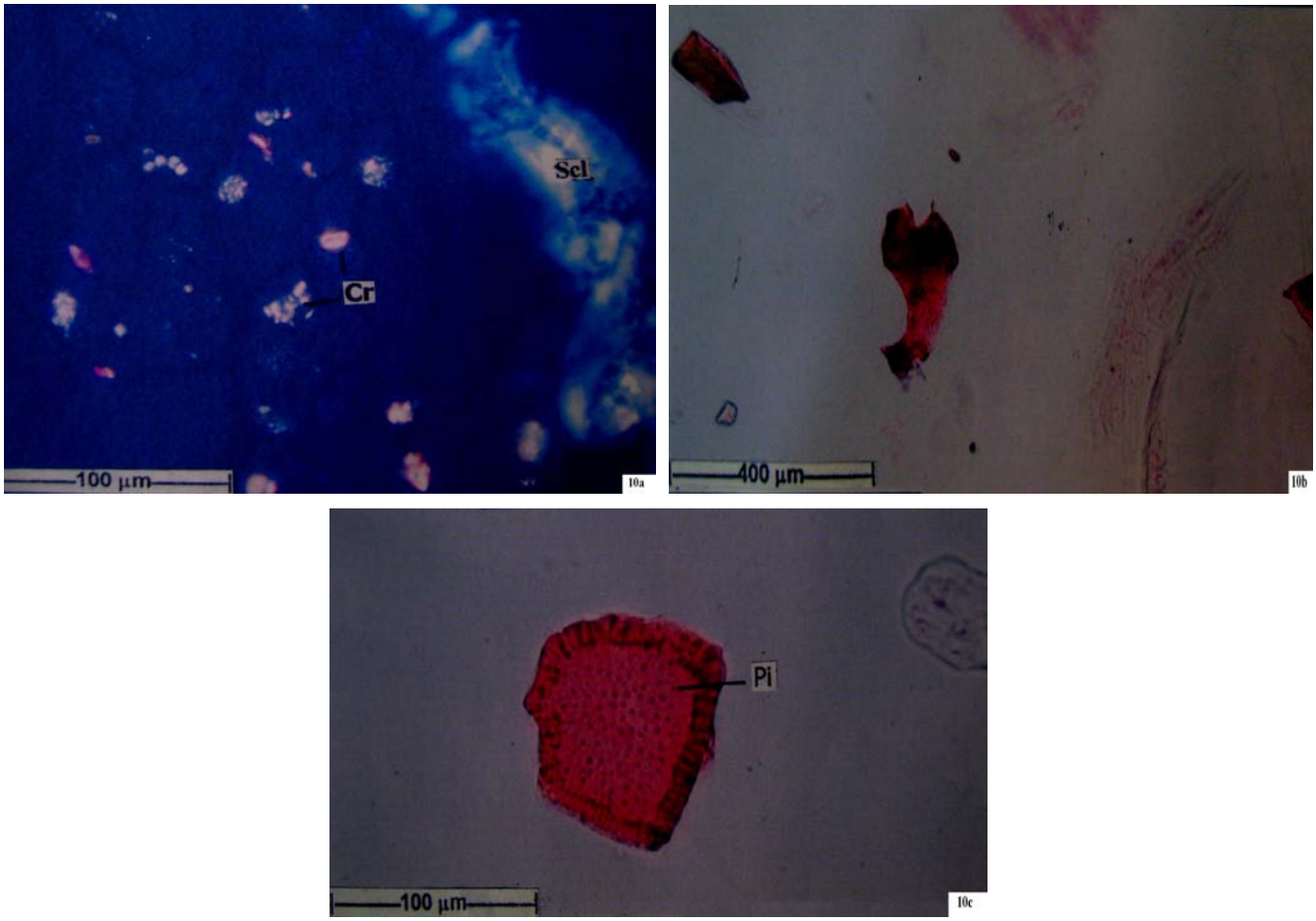

Fig .10a, 10b, 10c: Powder microscopy of *N.arbor-tristis* Linn

Scl: Scelerids; Cr: Crystal; Pi: Pits

Fluorescence Analysis

The fluorescence studies for the bark powder by treating it with different chemical reagents and the results were reported (Table 1). The fluorescence studies for the bark powder by treating it with different solvent extracts of bark were also performed under day light (Table 2). Data for extractive values are given in (Table 3). The above studies enable the identification of the plant material for future investigation and form an important species of drug studies[20-24].

Table 1: Fluorescence analysis

Treatment	Day light	UV light (254nm)
Powder as such	Light brown	Light green
Powder + 1N HCL	Yellowish brown	Light green
Powder + Aq. 1N NaOH	Yellow	Dark green
Powder +Alcoholic 1N NaOH	Pale yellow	Yellowish green
Powder + 5% I_2 solution	Bluish black	Dark brown
Powder + 50% HNO_3	Yellowish orange	Light green
Powder + 50% H2SO4	Yellowish orange	Light green
Powder + Methanol	Pale yellow	Emerald green
Powder + 5% FeCl3 solution	Violet colour	Fluorescent green

Table 2: Fluorescence study

Extracts	Day light	UV light (254nm)
Petroleum-ether extract	Brown	Light green
Chloroform extract	Yellowish brown	Dark green
Ethanolic extract	Yellowish orange	Light green
Aqueous extract	Brownish black	Dark green

Physicochemical parameters

The physiochemical parameters such as extractive values in various solvents and ash values were evaluated (Table 3 &Table 4).

Table 3: Extractive values

Extractive value	Percentage (w/w)
Alcohol soluble extractive	6.5 %
Water soluble extractive	4.76 %
Ether Soluble extractive	0.11 %

Table 4: Ash values

Ash Values	%w/w
Total Ash	7.00
Acid Soluble Ash	0.50
Water Insoluble Ash	0.45
Sulphated Ash	3.00

Preliminary Phytochemical Studies

The shade dried bark of *Nyctanthes arbor-tristis* Linn was powdered and successfully extracted with different solvents and the yields are calculated (Table 5). The extracts are subjected to preliminary phytochemical test to find out the active constituents and the data are given in Table 6. It revealed that presence of alkaloids, carbohydrate, glycosides, phytosterols, fixed oil, tannins, flavonoids, proteins and amino acids, gums and mucilage [14, 20, 24].

Table 5: Successive extraction values

Extracts	Yield (gm)	% Yield (w/w)
Petroleum- ether extract	0.57	0.19%
Chloroform extract	0.46	0.15%
Ethanolic extract	30.0	10%
Aqueous extract	19.0	6.3%

Table 6: Qualititative phytochemical analysis

Phytoconstituents	Pet. Etherextract	Chloroformextract	Ethanolextract	Aqueousextract
Alkaloids	-	+	+	-
Saponins	-	-	-	-
Glycosides	-	-	+	+
Carbohydrates	-	-	+	+
Tannins and phenolic compounds	-	-	+	+
Flavonoids	-	-	+	+
Phytosterols	+	-	-	-
Proteins and amino acids	-	-	-	+
Triterpenoids	-	-	-	-
Fixed oils and fats	+	-	-	-
Gums and mucilage	-	-	+	+

(+): Present , (-): Absent

Chromatographic Studies

HPTLC profile for all different extracts of the bark was carried out. At 254 nm petroleum ether extract showed 10 phytoconstituents at R_f 0.03, 0.09, 0.16, 0.29, 0.33, 0.38, 0.46, 0.52, 0.63, 0.69 (Fig. 11). At 254 nm chloroform extract revealed 7 phytoconstituents at R_f 0.03, 0.05, 0.23, 0.29, 0.45, 0.59, 0.88 (Fig. 12). At 254 nm Ehtanolic extract revealed 10 phytoconstituents at R_f 0.07, 0.20, 0.25, 0.28, 0.40, 0.47, 0.62, 0.70, 0.76, 0.85 (Fig. 13). At 254 nm aqueous extract revealed 8 phytoconstituents at R_f 0.05, 0.21, 0.33, 0.46, 0.59, 0.70, 0.73, 0.86 (Fig. 14). Results obtained from the HPTLC studies showed that the different extracts of *N.arbor tristis* Linn possess the various phtyoconstitoents.

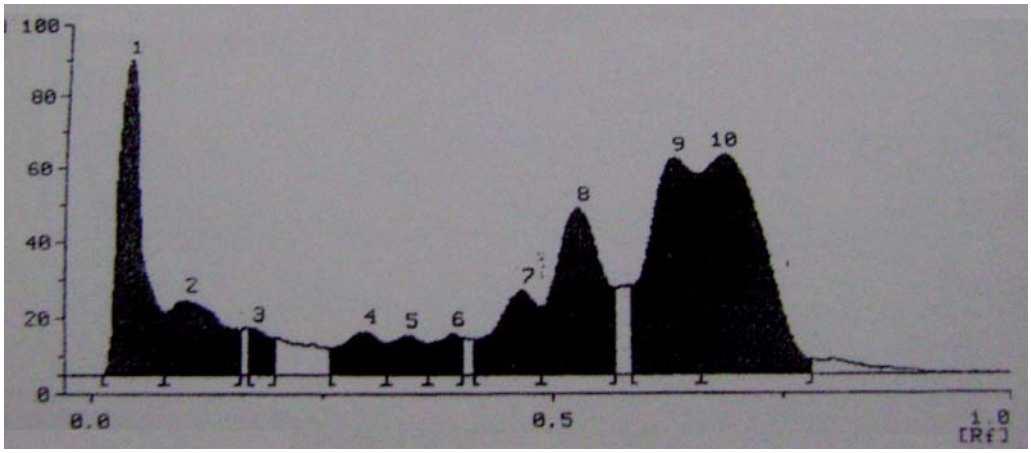

Fig.11: HPTLC profile for Pet ether extract

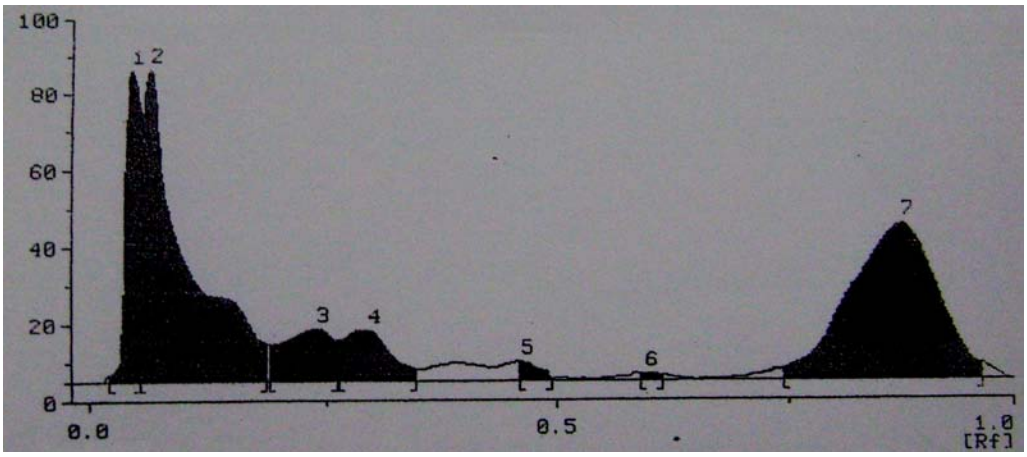

Fig. 12: HPTLC profile for chloroform extract

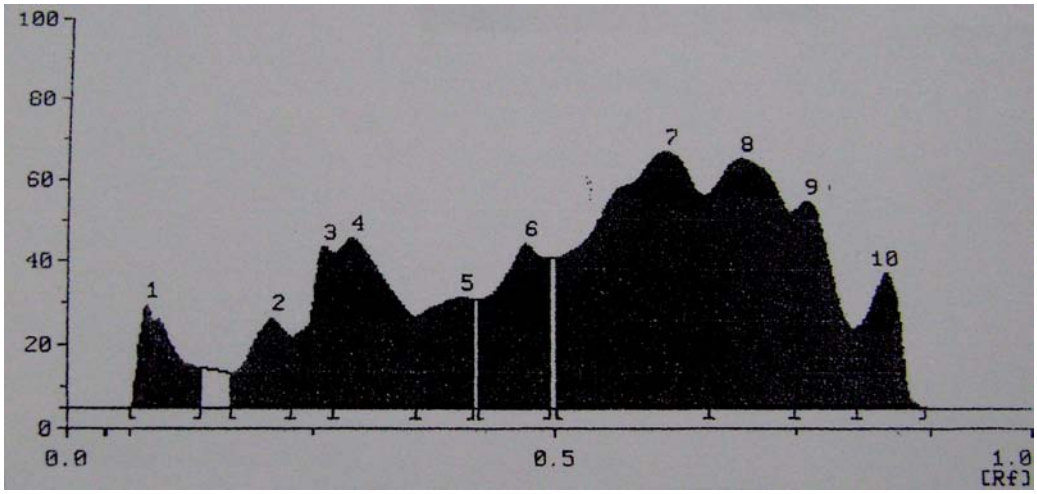

Fig. 13: HPTLC profile for ethanolic extract

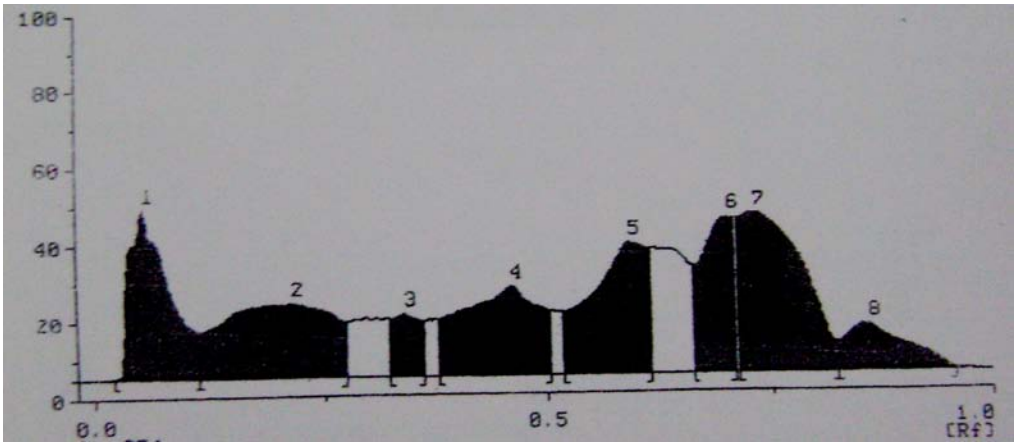

Fig. 14: HPTLC profile for aqueous extract

REFERENCES

1. Gosh GK. Herbs of Manipur. Vol-I. New Delhi: APH Publishing Corporation; 2000: 415-18.
2. Anonymous. "The Wealth of India", Raw materials, National Institute of Science Communication and Information Resources.6th ed. CSIR; New Delhi; 2003. p. 265-285.
3. Bhattacharjee. Handbook of Aromatic plants. 3rdrevised ed. Jaipur, India: Pointer publishers; 2001: 315.
4. Central Council for Research in Homeopathy (CCRH), http://www.Cerhindia.org/common-Indian-Plants-1. Eva Wallander. Systematic Botany and Plant Ecology. Oct 2000.
5. Wallander E, Albert VA. Phylogeny and classification of Oleaceae based on rps 16 and trnL-F sequence data. American J.Botany. 2000; 87: 1827-41.
6. Yoganarasimhan SN. Medicinal Plants of India. Tamilnadu, Regional research institute (Ay) Bangalore, India 2000;2: 379- 80.
7. Kirthikar KR and Basu BD. Indian Medicinal Plants. Dehradun India. 2003; 2(3) 1526 – 1528.
8. Orient Longman. Indian Medicinal Plants. Madras: Orient Longman Ltd. 1995;1: 149.
9. Esu K. Plant Anatomy. John Wiley and Sons, New York. 1965: 787.
10. Esu K. Plant anatomy of seed plants. NewYork: John wiley and sons. 1979: 550.
11. Foster A.S. The use of tannic acid and iron chloride for staining walls in meristematic tissue. Stain Tech. 1934;9: 91-92.
12. Johansen DA. Plant Microtechnique. New York: Mc.Graw Hill Book Co. 1940: 523.
13. Metcalfe CR and Chalk, L. Anatomy of the Dicotyledone, Clarendon Press, Oxford: 1950.
14. WHO (world health organization). Quality control methods for medicinal plants. Delhi: AITBS publishers and distributors. 2002: 10-11.
15. Yoganarasimhan SN. Madhavan V. Hema Basnett and Gurudeva. M|R. Pharmacognostical evaluation of *Drosera burmannii* Vahl. Ind J of Trad Know. 2009;8(3): 326-333.
16. Metcalfe CR. and Chalk L. Anatomy of the Dicotyledone. Vol-I: Clarendon Press, Oxford 1979: 276.
17. O' brien TP, Feder N, Mc and Cull ME. Polychromatic staining of plant cell walls by toluidine blue-O.Protoplasm 1964;9: 364-73.
18. Sass JE. Elements of Botanical Microtechnique. New York: MC Graw Hill. 1940: 222.
19. Solereder H. Systemic anatomy of the Dicotyledons. Oxford Clarendon press; 1899.
20. Willis JC. A Dictionary of the flowering plants and ferns. London: Cambridge University Press. 1973;8: 1214.

21. Evans WC. Trease and Evan's Pharmacognosy, London: ELBS printing; 2002; 15(414): 98-99,103.
22. Dr.Kokate CK. Practical Pharmacognosy. Vallabh Prakashan: Reprint; 2001. p. 107 -11,130.
23. Roy SK, Ali M, Sharma MP, Ramachandran R. Phytochemical investigations of *Desmotrichum fimbrianum* Blume. Indian J. Nat Prod. 2002; 18(1): 13-17.
24. Chase CR and Pratt RF. Fluorescence of powdered vegetable drugs with particular reference to the development of identification. J. American Pharm. Assoc.1949; 38: 324-333.
25. Kokashi CJ, Kokoshi RJ and Sharma FJ. Fluorescence of powdered vegetable drugs under UV Radiation. J. American Pharm. Assoc. 1958; 48(10): 715-717.