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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY OF LEAF OF PAVATE *(PAVETTA INDICA LINN)* A FOLK MEDICINAL PLANT

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ABSTRACT

Background & Objectives: *Pavetta indica Linn* is a stout bushy shrub of Rubiaceae family distributed throughout the India and used by the folklore practitioners in various conditions. The leaf of this plant possess many therapeutic uses such as analgesic and anti-inflammatory. It is also used in the form of paste *(kalka)* in *Agni lepa chikitsa* both internally and externally in folk practice. The present work has been designed to describe the Pharmacognostic and phytochemical characters of the leaves of *Pavetta indica Linn.* **Materials & Methods:** Macroscopic, microscopic evaluation and phytochemical study of the leaf of *Pavetta indica Linn* were carried out. **Results:** Pharmacognostic study showed various structures in leaf such as spongy parenchyma, calcium oxalate crystals etc. Phytochemical study showed presence of alkaloids, glycosides, carbohydrates, tannins, phenols and lipids. In qualitative analysis of HPTLC 7 peaks were detected at 366nm. Analytical study showed loss on drying (6.62%), total ash (2.46%), acid insoluble ash (1.14%), and water insoluble ash (2.86%) within API standards which shows standard quality of the drug. The aqueous and alcohol extractive values were 38.08%, 22.64% respectively.

KEYWORDS: Pharmacognostic, phytochemical, *Pavetta indica Linn*, folklore.

INTRODUCTION

Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name is given to two or more entirely different species. All these problems can be solved by pharmacognostic studies of medicinal plants. It is very important and in fact essential to lay down pharmacognostic specification of medicinal plants which are used in various drugs.

Modern medicine has evolved from folk and traditional medicines through chemical and pharmaceutical screening. The importance of medicinal plant in drug development is known to us and human have used them for different diseases from the beginning of human history. The biologically active compounds present in plants are called phytochemicals. These phytochemical are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as source of direct medicinal agents. Detailed phytochemical study of medicinal plant is needed prior its use, since therapeutic efficacy depends on the quality of active principles present in it. The highly advanced modern and evolved methods of experimentation if adopted will only widen the scope of Ayurvedic fundamentals and increase its applicability. Phytochemical and Analytical study helps to standardize the plant and differentiate the adulterants.

MATERIALS AND METHODS

I. Pharmacognostical Evaluation

The drug was collected from near the college premises of M. I. A. M. S Manipal and nearby places of Udupi and is authenticated by K. Gopalakrishna Bhat, (Retd.) Professor, Dept. of Botany, Poornaprajna College, Udupi. Pharmacognostical study has been carried out in S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi.

a) Macroscopic study

The external features of the test sample were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication. The macroscopic study reveals that leaves were opposite, petiolate, usually membranous with bacterial nodules: stipules intrapetiolar. Flowers white, odorous, in terminal sessile corymbose pubescent cymes; pedicels 4-6 mm long, densely pubescent; bracts broad membranous, buds oblong-clavate. Calyx densely pubescent. Corolla tube 13mm long. Style white, glabrous.

Stigma green, narrowly clavate, puberulous. Fruit 6-14mm diameter globose, black, smooth.[1]

b) Microscopic study

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and sections were stained saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse section was photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axiocam camera under bright field light. Magnification of the figures is indicated by the scale- bars.

II. Physico and Phytochemical Evaluation

Preparation of Powder Drug

The whole plant *Pavetta indica Linn* is cleaned properly, dried in shade and coarsely powdered and sieved using sieve No.180 as per WHO standards for medicinal plant materials. Drug is stored in clean air tight container.

a) Physico-chemical Evaluation

Tests such as Loss on drying, total ash, acid insoluble ash, water insoluble ash, aqueous and alcohol extractive values were conducted according pharmacopoeia standards of India[2-8] in R & D department of Muniyal Institute of Ayurveda Medical Sciences Manipal.

b) Preliminary Phytochemical Screening[9]

Tests for Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloids are traditionally defined as a basic (alkali-like), nitrogen-containing organic constituents that occur mainly in plants. Alkaloids often have pronounced bioactivities and are therefore thought to play an important role in the interaction of plants with their environment. Alkaloids and extracts of alkaloid-containing plants have been used throughout human history as remedies, poisons and psycho active weakly acidic drugs.

a. Dragendroff's test

To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent will be added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

b. Wagner's test

To a few mg of extract dissolved in acetic acid, few drops of Wagner's reagent will be added. A reddish brown precipitate formed indicates the presence of alkaloids.

c. Mayer's test

To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent will be added. A dull white precipitate formed indicates the presence of alkaloids.

d. Hager's test

To a few mg of extract dissolved in acetic acid, 3ml of Hager's reagent will be added, the formation of yellow precipitate indicate the presence of alkaloids.

Tests for Carbohydrates

a. Molisch's test

To the extract, 1 ml of α-naphthol solution and conc. Sulphuric acid will be added along the sides of test tube. Violet colour formed at the junction of two liquids indicates the presence of carbohydrates.

b. Fehling's test

A few mg of extract will be mixed with equal quantities of Fehling's solution A and B. The mixture should be warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

b. Benedict's test

To 5 ml of Benedict's reagent, a few mg of extract will be added, and boil for two minutes and should be cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Test for Steroids

Steroids comprise a natural product widely distributed throughout the plant and animal world. They are defined as compound having cyclopentanoperhenanthrene nucleus. Steroids are included in such compound as acid, cardiac glycoside, steroid hormones and vitamins.

a. Libermann-Burchard test

To the extract dissolved in chloroform, 1ml of acetic acid and 1ml of acetic anhydride will be added, then heat it on water bath and cool. Few drops of conc.Sulphuric acid should be added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

b. Salkowski test

The extract should be dissolved in chloroform and equal volume of conc. Sulphuric acid will be added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for Tannins

Tannins represent a wide variety of compounds that can be found in fruits, vegetables, tea etc. Tannins also known as pro-anthocyanidins possessing useful properties such as antioxidant, antiapoptosis, anti-ageing, anti-carcinogenic, anti-inflammatory, as well as anti-atherosclerosis and cardiovascular protection

Procedure

To the extract, a few drops of dilute solution of ferric chloride will be added, formation of dark blue colour shows the presence of tannins.

Test for Flavonoids

The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and iso-flavonoids. Together with carotenes, flavonoids are also responsible for the colouring of fruits, vegetables and herbs.

Shinoda's test

To the extract in alcohol, a few magnesium turnings and few drops of con. Hydrochloric acid will be added and heat on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for Phenol

To the extract in alcohol, a few drops of alcoholic ferric chloride. Formation of blue to bluish black indicates the presence of phenol.

Test for Glycosides

Keller-Kiliani test

1ml of glacial acetic acid containing traces of ferric chloride and 1ml of conc. sulphuric acid was added to the extract and was observed for the formation of reddish brown colour at the junction of two layers. Appearance of bluish green colour in the upper layer indicates the presence of glycosides.

Test for Amino acids

Ninhydrin test

The Ninhydrin reagent is 0.1% w/v solution of Ninhydrin in n-butanol. A little of this reagent was added to the test extract. A violet or purple colour indicates the presence of amino acids.

Test for proteins

Biuret test

A few mg of the residue was taken in water and 1ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

Xanthoproteic test

A little residue was taken with 2ml of water and 0.5ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

III. Methods of High Performance Thin Layer Chromatography[10]

High performance thin layer chromatography (HPTLC) is a form of thin-layer chromatography (TLC) that provides separation power using optimized coating material, novel procedures for mobile-phase feeding, layer conditioning and improved sample application. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase and efficient data acquisition and processing. This study has been carried out in the SDM Centre for Research in Ayurveda and Allied Sciences Udupi.

1.0g of powdered leaf sample of *Pavetta indica Linn* was suspended in 20.0ml of *ethanol* kept for 24 hours and cold maceration was followed. The filtrate was reduced to 10.0ml. 3,6 and 9µl of each of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 6m using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (1.0: 1.0). The developed plates were visualized under short UV, long

UV and then derivatised with vanillin Sulphuric acid and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were detected.

RESULTS AND DISCUSSION

For validation of any scientific principle or fact it is vital for it to be based on methodical discussion and conclusion. Hence discussion forms a crucial part of any scientific research work.

- **Pharmacognosy**
  - **Microscopic study**

T.S. of Leaf: It shows Lamina and Midrib

Leaf Lamina-Upper epidermis is continuous single layer of epidermal cells followed by elongated palisade cells, inner to which there is spongy parenchyma containing loosely arranged parenchyma cells with intercellular space containing chlorophyll, underneath there is single layer of lower epidermal cell.

Midrib region- Midrib region consist of upper epidermis and lower epidermis continuous with lamina. Below the upper epidermis there is 3-4 layer of closely arranged collenchymatous cells, which is also present above the lower epidermis. The ground tissue is mainly parenchymatous it contains rosette crystals of calcium oxalate. Centrally there is vascular bundle located. Pericyclic fibres are present on outer side. Vascular bundle arrangement consist phloem on outer side and xylem on inner side. Xylem consist of xylem vessels and xylem fibres.

- **Phytochemical analysis**
  - Physico-chemical analysis of Pavetta indica Linn leaf powder revealed following results.

The loss on drying test was designed to measure the amount of water and volatile matter in sample and it showed 6.62%. The Ash value indicates the presence of inorganic material in the sample. Here we can observe that Ash value is 2.46% and acid insoluble ash, water insoluble ash are 1.14% and 2.86% respectively. Extractive value plays an important role in evaluation of crude drugs. Water soluble extractive value is 38.08% w/w and acid soluble extractive value is 22.64% w/w which was in normal limits as per API guidelines.

- Qualitative analysis of Pavetta indica Linn showed following active constituents, Aqueous, Alcohol, Ether and Chloroform extract of Pavetta indica Linn was prepared and preliminary phytochemical tests were conducted.

In the above test Alkaloids, Glycosides, Carbohydrates, Tannins, Phenols, Lipids were found. While Flavonoids, Protein and Amino acids were absent.

Table No: 1: Results of Physico-Chemical Parameters.

Parameter	**Results n = 3 %w/w**
	Pavetta indica Linn
Loss on drying	6.62
Total Ash	2.46
Acid Insoluble Ash	1.14
Water Soluble Ash	2.86
Alcohol Soluble Extract	22.64
Water Soluble Extract	38.08

Table no: 2: Observations of Preliminary Phytochemical Tests.

Sl.No	**Tests**	**Colour if positive**	***Pavetta indica Linn***
1	**Alkaloids**		
	Dragendroff's test	Orange precipitate	Orange precipitate
	Wagner's test	Red precipitate	Reddish brown colour
	Mayer's test	Dull white precipitate	Dull white precipitate
	Hager's test	Yellow precipitate	Yellow precipitate
2	**Carbohydrates**		
	Molisch's test	Violet ring	Violet ring
	Fehling's test	Brick red precipitate	Brick red precipitate
	Benedict's test	Red precipitate	Reddish brown precipitate
3	**Steroids**		
	Liebermann-Buchard test	Bluish green	Slight bluish green
	Salkowski test	Bluish red to cherry red	Dark red at junction
4	**Tannin**		
	With FeCl3	Dark blue or green or brown	Dark blue colour solution
5	**Flavonoids**		
	Shinoda's test	Red to pink	No red or pink colour
6	**Glycosides**		
	Keller-Kiliani test	Bluish green colour	Bluish green colour
7	**Phenols**		
	With alcoholic ferric chloride	Green or blue colour	Green colour
8	**Amino acid**		
	Ninhydrin test	Purple colour	Green colour
9	**Proteins**		
	Biuret test	Violet colour	No violet colour
	Xanthoproteic test	Pink colour	No pink colour

Table no: 3: Results of Preliminary Phytochemical Tests.

Sl.No	Test	Extract of *Pavetta indica Linn*			
		Methanol	water	Pt.Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	-	+
3	Steroids	+	+	+	+
4	Flavonoids	_	_	_	_
5	Tannin	+	+	+	_
6	Phenol	+	_	_	_
7	Glycosides	+	_	+	+
8	Amino acids	_	_	_	_
9	Proteins	_	_	_	_

+ POSITIVE **- NEGATIVE**

HPTLC

Under UV 254nm 5 peaks detected. The major peaks were at 1, 2, and 3 corresponding to Rf 0.03(23.57%), 0.05 (42.91%), 0.17 (28.15%). At 366nm under fluorescent light 7 peaks detected. Major peaks were 1, 2, and 3 0.03Rf (15.03%), 0.05 Rf (30.44%), 0.17Rf (43.55%) respectively. After derivatisation 7 peaks were detected. Major peaks were 1 and 3, corresponding to Rf 0.04 (24.07%), 0.17Rf (58.06%) these peaks may be corresponding to either one of the phytoconstituents

Table No: 7.5 Rf value of HPTLC sample of Leaf of *Pavetta indica Linn.*

Short UV	Long UV	Post derivatisation
0.12 (L. green)	0.12 (F. red)	-
-	-	0.14 (Green)
-	0.17 (F. red)	-
-	0.33 (F. red)	-
-	0.36 (F. purple)	0.36 (L. purple)
-	-	0.41 (L. purple)
-	0.80 (F. red)	0.80 (Purple)
-	-	0.86 (Purple)
-	-	0.91 (Purple)

***L** – light; **D** – dark; **F** – fluorescent

Pavetta indica Linn

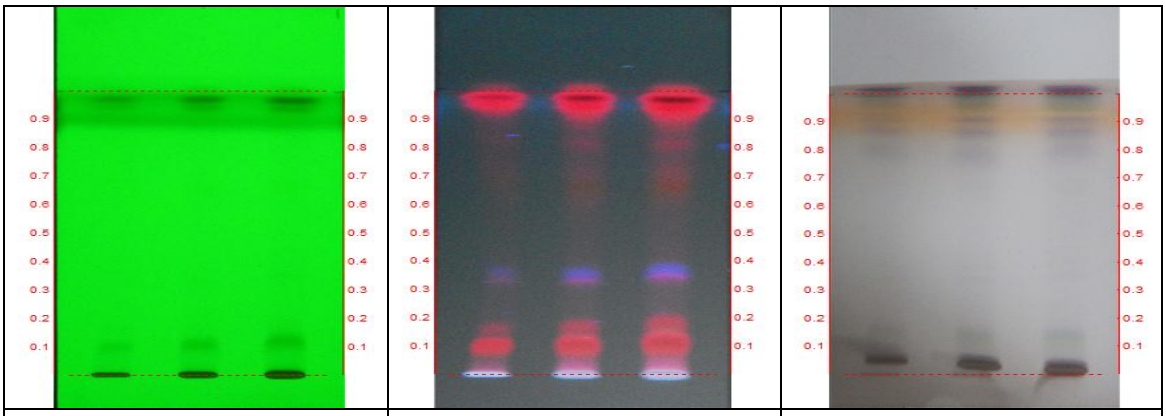
Figure No 1

Figure No 2

Figure No 3

Figure No 4

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY



Short UV	Long UV	Post derivatisation

Figure No 5a.

Track 1: Leaf of *Pavetta indica* - 3µl

Track 2: Leaf of *Pavetta indica* - 6µl

Track 3: Leaf of *Pavetta indica* - 9µl

Solvent system- Petroleum ether: Ethyl acetate (1.0: 1.0)

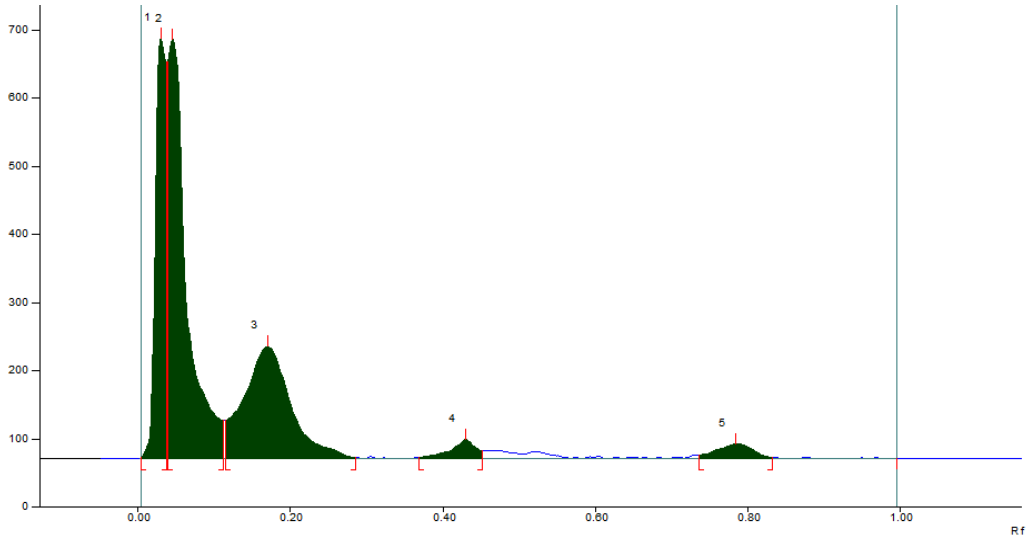
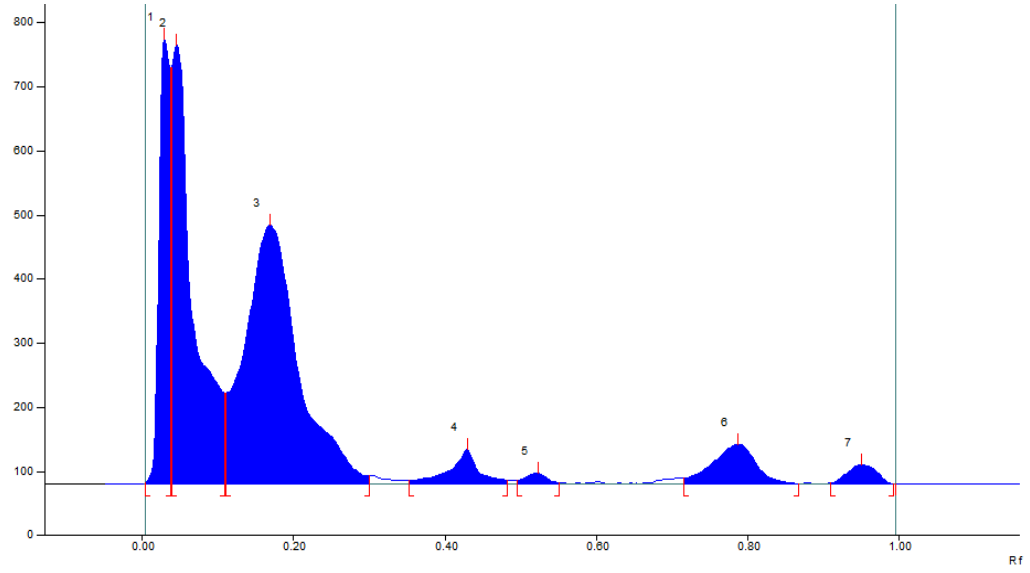


Figure no

Track 3, ID: Pavetta indica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.1 AU	0.03 Rf	616.3 AU	42.63 %	0.04 Rf	81.8 AU	6135.9 AU	23.57 %
2	0.04 Rf	582.3 AU	0.05 Rf	614.7 AU	42.52 %	0.11 Rf	55.9 AU	11169.2 AU	42.91 %
3	0.11 Rf	56.0 AU	0.17 Rf	164.5 AU	11.38 %	0.29 Rf	1.3 AU	7329.2 AU	28.15 %
4	0.37 Rf	1.2 AU	0.43 Rf	28.5 AU	1.97 %	0.45 Rf	12.0 AU	661.7 AU	2.54 %
5	0.74 Rf	4.9 AU	0.79 Rf	21.6 AU	1.50 %	0.83 Rf	1.4 AU	736.0 AU	2.83 %

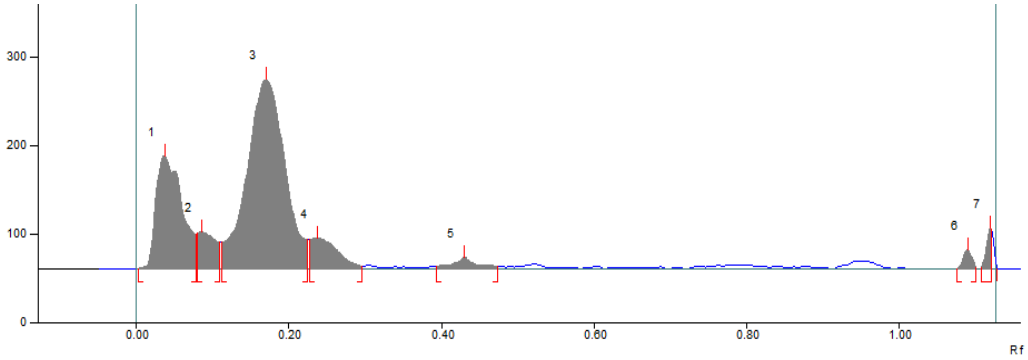
Figure No 5b



Track 3, ID: Pavetta indica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.0 AU	0.03 Rf	694.1 AU	35.66 %	0.04 Rf	48.2 AU	7115.0 AU	15.03 %
2	0.04 Rf	648.5 AU	0.05 Rf	684.9 AU	35.19 %	0.11 Rf	41.6 AU	14408.1 AU	30.44 %
3	0.11 Rf	141.6 AU	0.17 Rf	404.4 AU	20.78 %	0.30 Rf	12.1 AU	20612.9 AU	43.55 %
4	0.35 Rf	5.1 AU	0.43 Rf	54.4 AU	2.80 %	0.48 Rf	6.3 AU	1474.7 AU	3.12 %
5	0.50 Rf	6.0 AU	0.52 Rf	16.4 AU	0.84 %	0.55 Rf	1.8 AU	340.7 AU	0.72 %
6	0.72 Rf	9.2 AU	0.79 Rf	62.2 AU	3.20 %	0.87 Rf	0.2 AU	2539.8 AU	5.37 %
7	0.91 Rf	0.9 AU	0.95 Rf	29.8 AU	1.53 %	0.99 Rf	0.1 AU	836.0 AU	1.77 %

Figure No 5c



Track 3, ID: Pavetta indica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.6 AU	0.04 Rf	127.7 AU	25.58 %	0.08 Rf	39.7 AU	3166.0 AU	24.07 %
2	0.08 Rf	40.2 AU	0.09 Rf	41.6 AU	8.33 %	0.11 Rf	30.3 AU	702.7 AU	5.34 %
3	0.11 Rf	30.8 AU	0.17 Rf	213.4 AU	42.74 %	0.22 Rf	33.3 AU	7637.7 AU	58.06 %
4	0.23 Rf	33.7 AU	0.24 Rf	35.2 AU	7.04 %	0.30 Rf	3.2 AU	922.7 AU	7.01 %
5	0.39 Rf	3.2 AU	0.43 Rf	13.5 AU	2.71 %	0.47 Rf	3.2 AU	300.3 AU	2.28 %
6	1.08 Rf	0.4 AU	1.09 Rf	22.0 AU	4.40 %	1.10 Rf	1.6 AU	187.0 AU	1.42 %
7	1.11 Rf	1.3 AU	1.12 Rf	45.9 AU	9.20 %	1.12 Rf	45.5 AU	239.1 AU	1.82 %

Figure No 5d.

Microscopy of leaf of *Pavetta indica*

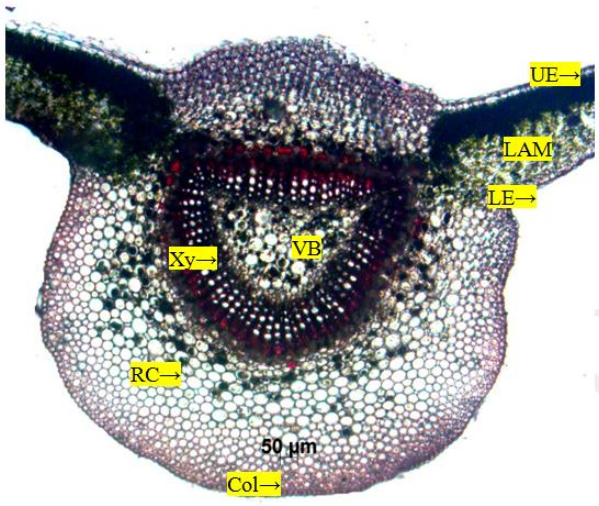


Fig 6a: T.S of leaf

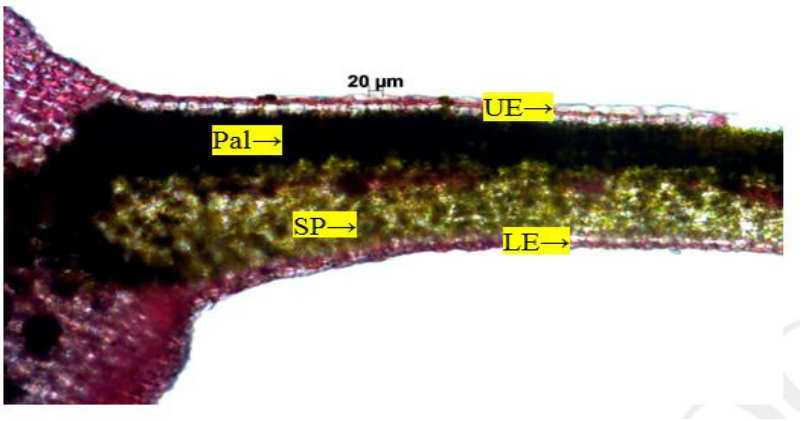


Fig 6b. Lamina.

Col – collenchma; UE – uppr epidermis; LAM – lamina; LE – lower epidermis; Pal – palisade; RC –rosette crystals; SP – spongy parenchyma; VB–vascular bundle; Xy – xylem.

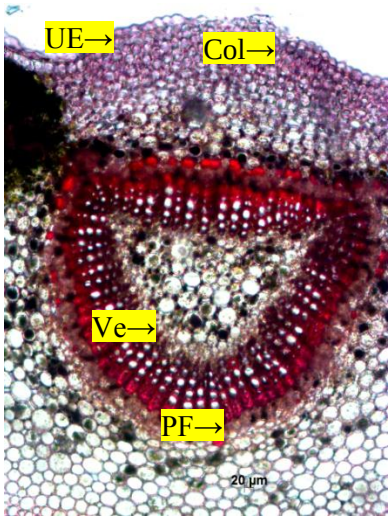


Fig 6c. Upper portion of midrib enlarged.

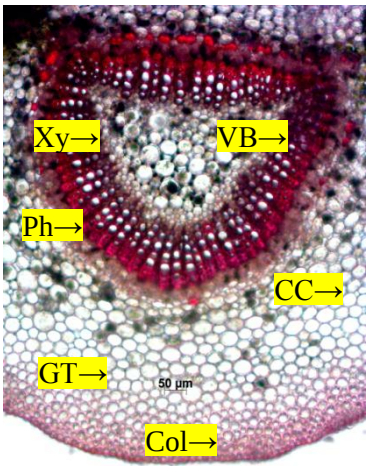


Fig 6d. Lower portion of midrib enlarged.

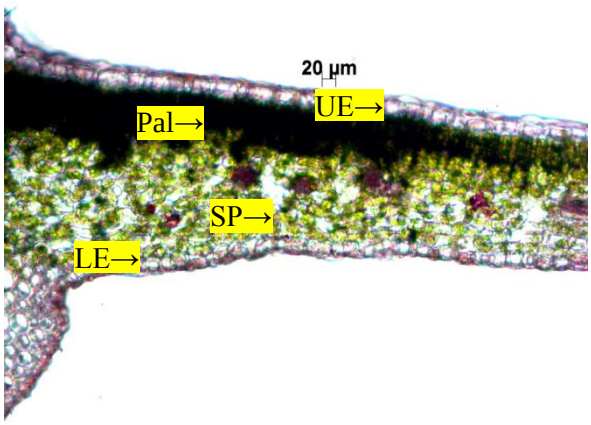


Fig 6e. Lamina enlarged.

Col – collenchymas; CC – cluster crystals; GT – ground tissue; LE – lower epidermis; Pal – palisade; PF – 947ericyclic fibres; Ph – phloem; UE – upper epidermis; VB–vascular bundle; Ve – vessels; Xy – xylem.

CONCLUSION

The drug *Pavetta indica Linn* is known by Pavate. Though it is a folk medicine references regarding this shrub are available in books of modern era. Phytochemical study revealed presence of phytoconstituents like alkaloids, steroids, glycosides and tannin. The morphological and microscopical study provided scientific data with respect to the identification of *Pavetta indica Linn.*

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