



Research Article

PHYTO-PHARMACOGNOSTICAL STANDARDIZATION WITH HPTLC FINGERPRINTING OF
ROOT OF CITRAKA (*PLUMBAGO ZEYLANICA* L.)

Akhilraj A.R.^{1*}, Rukmini S.², Amalraj A.R.³

¹Research Fellow (Ayurveda), Ethnomedicine & Ethnopharmacology Division, KSCSTE-Jawaharlal Nehru Tropical Botanic Garden and Research Institute (KSCSTE - JNTBGRI), Palode, Thiruvananthapuram, Kerala, India.

²Assistant Surgeon, Primary Health Centre (PHC), Ittiva, Kollam, Kerala, India.

³Medical Officer, Community Health Centre (CHC), Kallara, Thiruvananthapuram, Kerala, India.

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ABSTRACT

Citraka or *Sveta Citraka* (*Plumbago zeylanica* L.) is an important medicinal plant mentioned in Ayurvedic classics, belongs to the family Plumbaginaceae. The root of the plant exhibits medicinal properties. It has a broad range of pharmacological activities, including anti-inflammatory, antibacterial, antifungal, anticancer, antidiabetic, antioxidant, hepatoprotective, wound healing and cytotoxic.

Objective: The aim of the study was to evaluate phyto-pharmacognostical standardization with HPTLC fingerprinting of root of *P. zeylanica*, one of the most accepted and beneficial medicinal plant in Ayurveda.

Materials and Methods: *P. zeylanica* was collected from Konni, Kerala, India during the month of May 2018 and its root was studied for macroscopic, microscopic, physico-chemical, preliminary phytochemical screening and HPTLC fingerprinting following the standard protocol recommended by The Ayurvedic Pharmacopoeia of India and Quality Standards of Indian Medicinal Plants.

Results: The root is long, stout, cylindrical and reddish to dark brown in colour. The transverse section of root showed 5-7 rows of small cubical or rectangular celled cork with light yellow walls, polygonal or slightly tangentially elongated cortex cells containing starch grains and coloured contents, phloem containing small groups of fibres, radially arranged xylem vessels and narrow, 1-4 seriate medullary rays. Physico-chemical analysis showed water soluble extractive value as 26.11±0.01 w/w and alcohol soluble extractive value as 15.85±0.00 w/w. Preliminary phytochemical analysis of root decoction showed the presence of alkaloids, carbohydrates, tannins, saponins, resin and quinones.

Conclusion: The information obtained from this research work may be useful to establish the botanical as well as analytical standards for the root of *P. zeylanica*.

INTRODUCTION

Medicinal plants play a significant role both in developed and developing countries in providing health benefits to human beings. India's medical heritage, as evident in the four codified systems of Indian medicine namely Ayurveda, Siddha, Unani and Sowa-Rigpa, is well acknowledged as one of the oldest living traditions of health care.

A large segment of our population continues to depend upon and benefit from these practices even today. This unique medicinal heritage is largely dependent upon the use and availability of a very wide variety of botanical materials obtained from a diversity of plant species. In recent years, the worldwide interests in plant based drugs have grown significantly particularly for delivering improved health care. This led to large scale commercial production of both classical and proprietary herbal formulations in the country. As a result, the demand for herbal raw material also increased. The raw material is being procured from wild and cultivated sources. Several factors are known to influence the quality of the herbal raw material. In addition, there is a possibility of

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deliberate adulteration or substitution in the genuine raw material. Hence, to maintain quality and to ensure efficacy standardization parameters are to be defined.

Citraka or *Sveta Citraka* (*Plumbago zeylanica* L.) commonly known as Ceylon leadwort, is a well-known medicinal plant mentioned in Ayurvedic classics, belongs to the family Plumbaginaceae. *Citraka* is used in treating dyspepsia, helminthiasis, cough, colic, inflammations, bronchitis, elephantiasis, haemorrhoids, leprosy, chronic and intermittent fever, leukoderma, scabies, ring-worm, hepatosplenomegaly, anaemia and amenorrhoea.^[1] In Ayurveda, the root of the plant is used for medicinal purpose.^[2] It is a large perennial under shrub, found in Sri Lanka (Ceylon), widely in India and occasionally grown in the gardens.^[3] *P. zeylanica* has long, tuberous root, stem 0.6 to 1.5m long, somewhat woody, spreading, striate and glabrous. Leaves are simple, alternate, ovate, exstipulate, short-petiolate and amplexicaul at the base. The plant bears pure white flowers in terminal spikes, rachis glandular, striate, ovate bracteoles, acuminate, calyx- 1 to 1.3cm long, narrowly tubular, persistent, densely covered with stalked glands; corolla- white, slender, tube 2 to 2.5cm long, lobes 8mm long, obovate-oblong, acute, apiculate; filaments as long as the corolla tube and anthers exerted just beyond the throat. Fruit of *P. zeylanica* is one seeded membranous capsule, oblong, pointed, pericarp thin below, thick and hardened above. *P. zeylanica* contain Plumbagin as the chief chemical constituent belonging to the class naphthoquinone.^[4]

Standardization of herbal drug refers to confirmation of its identity and determination of its quality, purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations.^[5] Detailed pharmacognostical and phytochemical studies of root of this plant have not reported till now, hence the present study have been undertaken to analyze the macroscopical, microscopical, physico-chemical, phytochemical and HPTLC fingerprinting of root of *P. zeylanica*. The findings of the present study may be a tool for the authentication and quality control assessment of root collected for pharmaceutical industry.

MATERIALS AND METHODS

Collection of plant material

The plant *Citraka* (*Plumbago zeylanica* L.) was collected from the plains of Konni (Latitude 9°15' 53.1288" N, Longitude 76°47' 13.3476" E and Altitude 400-600m ASL) in Pathanamthitta, Kerala, India during the summer season. The plant was authenticated by taxonomists at Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram, India, where the voucher

specimen was deposited (TBGT 78452 dated 09/05/2018). The root was segmented and shade dried. After the completion of dehydration (within 10-14 days), the root was powdered to suitable size with the help of a multipurpose grinder and passed through mesh no. 60 and made into coarse power.

Chemicals

All reagents, chemicals and solvents were of analytical grade and purchased from Hi Media, Mumbai, India. Marker Plumbagin (P7262 - 100mg) was obtained from Sigma Aldrich, USA.

Macroscopical Analysis

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora (Flora of Pathanamthitta)^[6] for authentication.

Microscopical Analysis

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 sections using a sharp blade and stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures were indicated by the scale-bars.

Powder Microscopy Analysis

A pinch of root powder previously sieved was put on the slide and mounted in glycerine and powder characters were observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physico-chemical Analysis

The Physico-chemical parameters such as pH with Eutech Instruments pH Tutor, Loss on drying (LOD), total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value and water soluble extractive value were carried out following standard procedures recommended by The Ayurvedic Pharmacopoeia of India and Quality Standards of Indian Medicinal Plants.

Preliminary Phytochemical Screening

The coarse power of the root of *P. zeylanica* was made in to decoction as per The Ayurvedic Pharmacopoeia of India (Part I, Vol I). The decoction was used for preliminary phyto-chemical screening with a set of various chemical tests viz., Dragendroff's, Wagners's, Mayer's, Hager's tests for alkaloids; Libermann-Burchard, Salkowski tests for steroids; Molisch's, Fehling's, Benedict's tests for carbohydrates; Ferric chloride test for tannins; Shinoda's test for flavonoids; Magnesium test for saponins; Thionyl

chloride test for triterpenoids; 2 N Sodium hydroxide test for coumarins; Alcoholic ferric chloride test for phenol; Sodium bicarbonate test for carboxylic acid; Ninhydrine test for amino acid; Acetone test for resin; 0.5% of Sodium hydroxide test for quinone. These parameters were performed following the standard procedure.

HPTLC

Standard Preparation: The standard Plumabagin marker compound was prepared in different concentration range (10-100ng) in ethanol (absolute 99.9%).

Sample preparation

1g each of root powder of *Citraka* (*P. zeylanica*) was extracted with 10ml of alcohol. 2µl (sample) of each of the above extract were applied on a pre-coated silica gel F₂₅₄ on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.9: 0.1) solution. The developed plates were visualized under short UV, long UV, then derivatised with vanillin sulphuric acid (observed under white light), scanned under UV

272nm. R_f, colour of the spots and densitometric scan were recorded. Marker Plumbagin (P7262 - 100mg) was obtained from Sigma Aldrich, USA.

Method

Standard Plumbagin and *P. zeylanica* was applied in different concentrations. Marker concentration was ranging from 1 -10µl (10-100ng). *P. zeylanica* was applied in 2µl concentration. Densitometric scanning was carried out at 272nm, where dark green colour band appeared. The calibration curve was plotted for the above mentioned concentration of Plumbagin marker.

RESULTS AND DISCUSSION

Macroscopical analysis

The Macroscopical analysis of root of *P. zeylanica* exhibits,

- Size : 5 to 6 mm in diameter, 3 to 7cm in length
- Shape : Stout, cylindrical, sometimes irregularly bent
- Colour : Reddish to dark brown externally
- Odour : Disagreeable
- Taste : Acrid



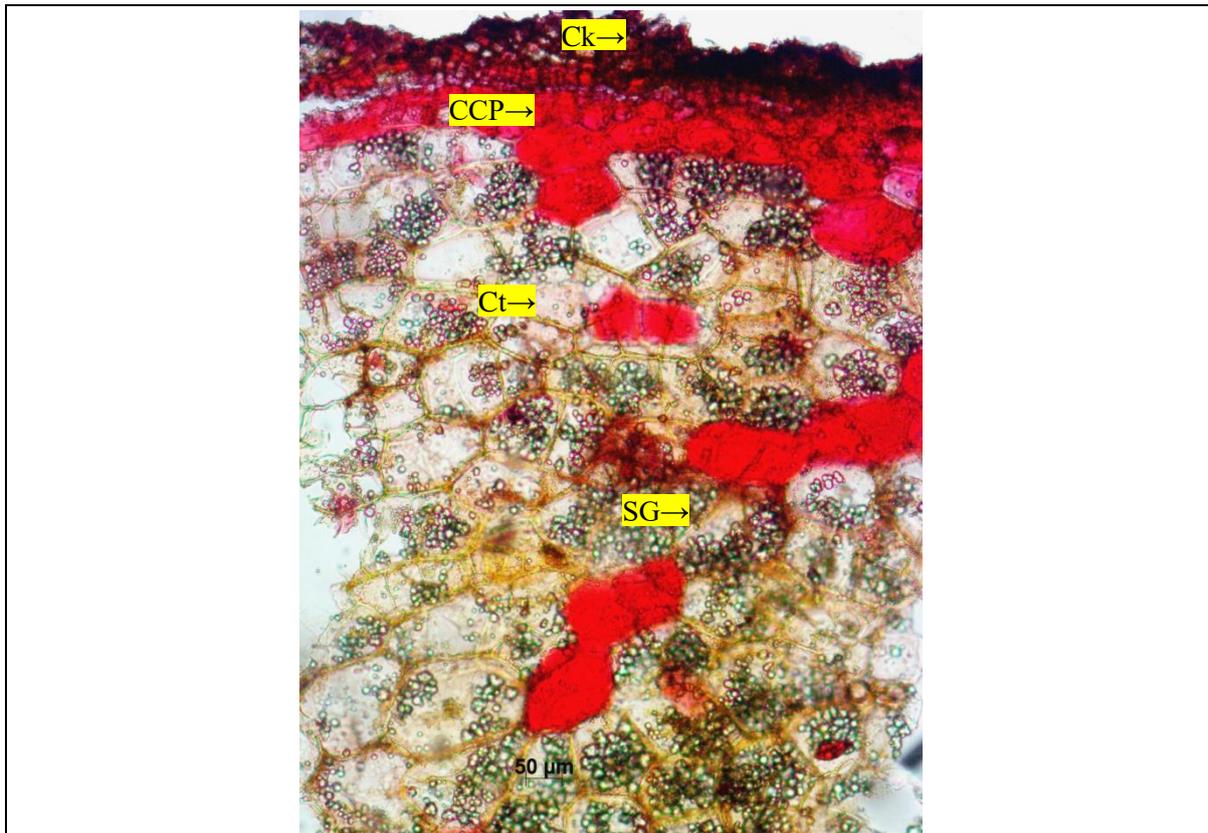
Fig 1: *Plumbago zeylanica* L.

Microscopic Analysis

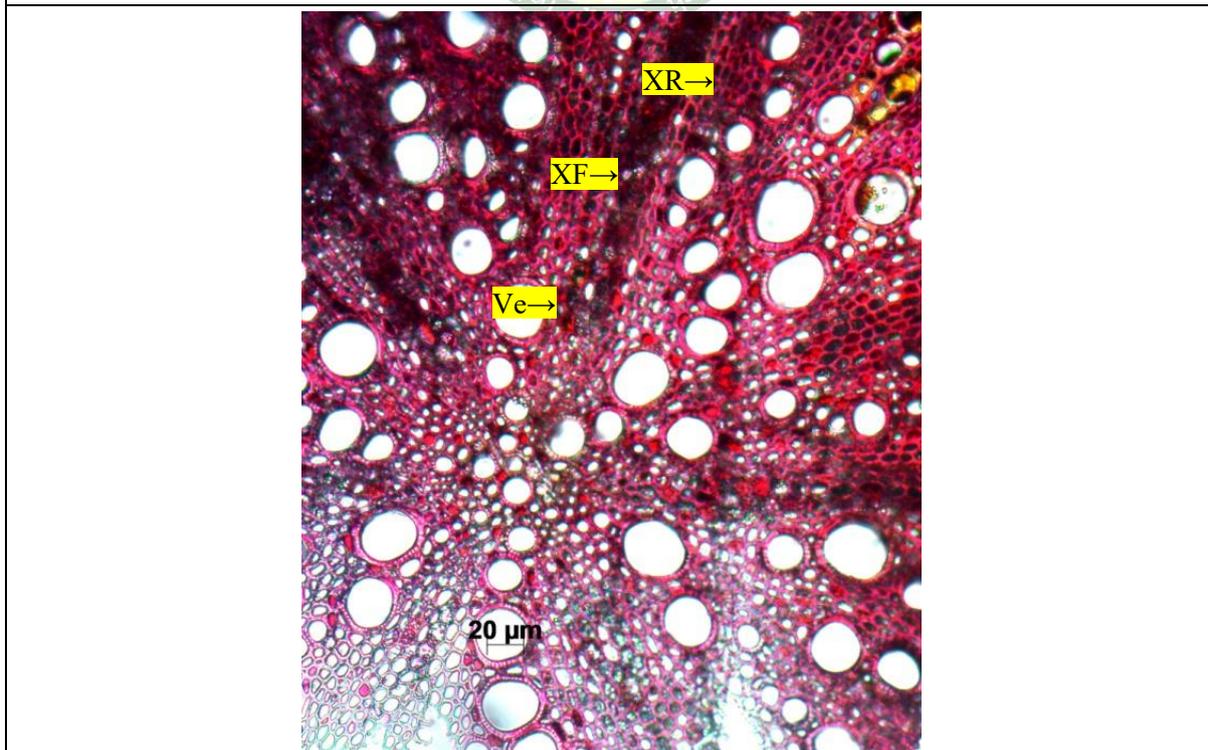
The transverse section of the root of *P. zeylanica* which is circular in outline, showed a very thin cork consisting of 5 to 7 rows of small cubical to rectangular cells some of which contain dark brown contents. Their cell walls were light yellow in colour, those of the outermost row of cells being darker. The phellogen which could be distinguished in some specimens was composed of a single row of almost cubical cells. The cortex was wide and about 1.4mm in thickness. The cortical cells were large, polygonal or slightly tangentially elongated, but the cells were not uniform in size. Most of the cells were densely packed with starch grains while a few cells contained the yellow cell contents. The phloem situated just inner to the fibre groups was very narrow. Its cells were small

polygonal and thin walled. Cells containing the yellowish contents were not found in this region. The wood in the centre was comparatively narrow. It had a light yellow colour. The xylem vessels were not wide and arranged mostly radially in nature. Wood fibres were numerous and thick walled. Medullary rays were few in number; almost straight, 1 to 4 seriate and reached up to the cortex. The ray cells were radially elongate and filled with starch grains. The primary xylem was tetrarch.

Fig 2: Microscopy of *P. zeylanica* root

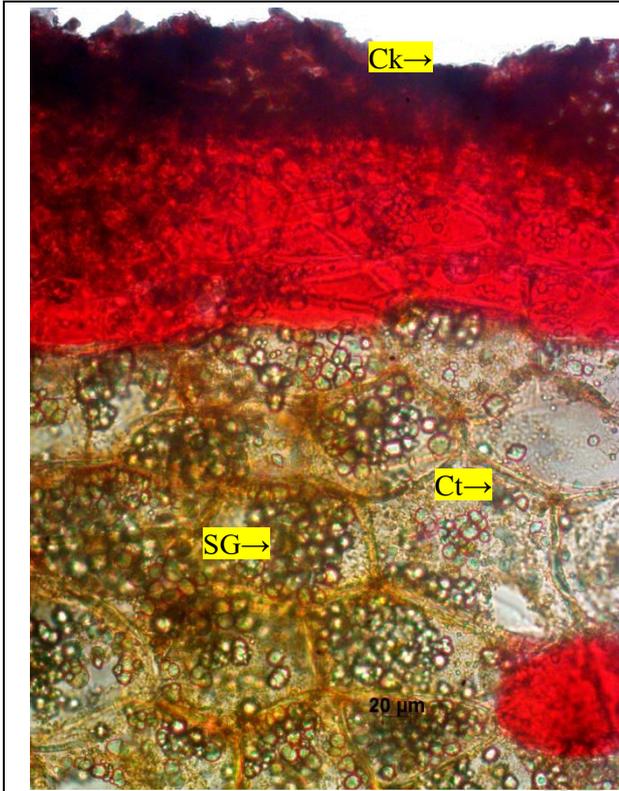


2a - Outermost cork and cortex

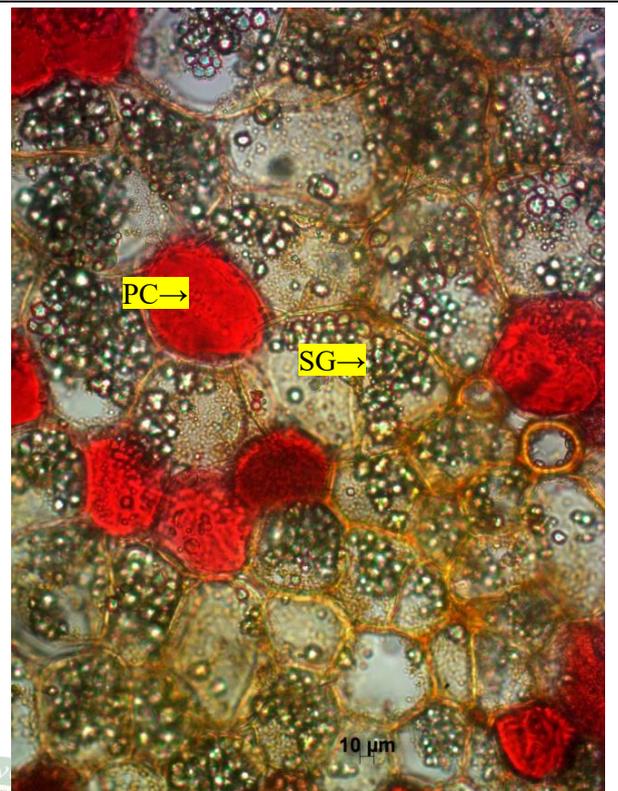


2b - Xylem

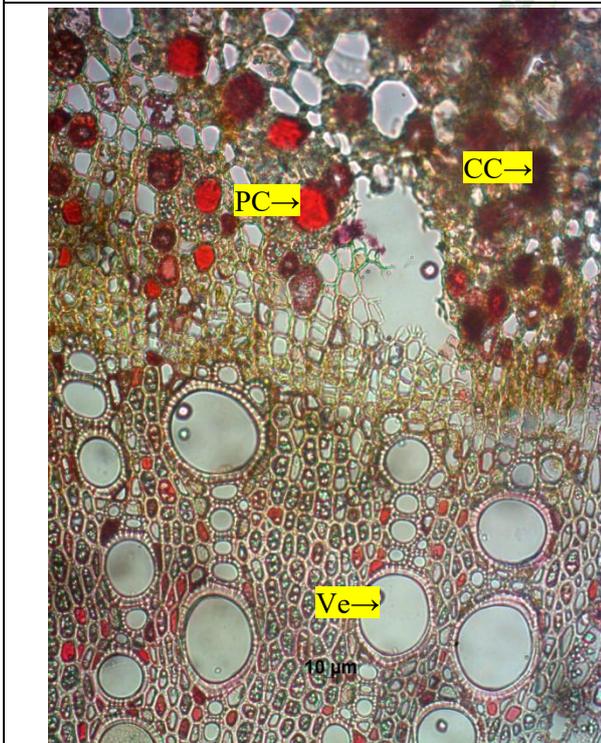
CCP – cells containing pigment; Ck – cork; Ct – cortex;; Ph – phloem; SG – starch grains; Ve – vessels; XF – fibres; XR – xylem rays.



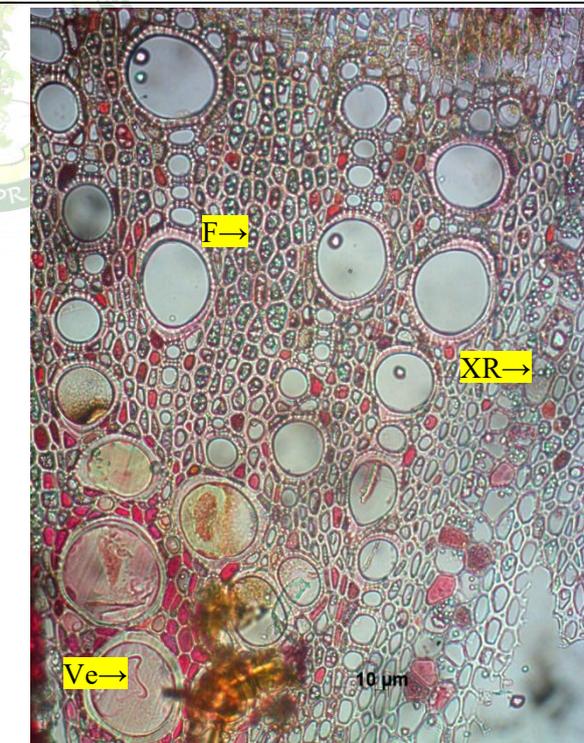
2c - Cork and cortex



2d - Cortex

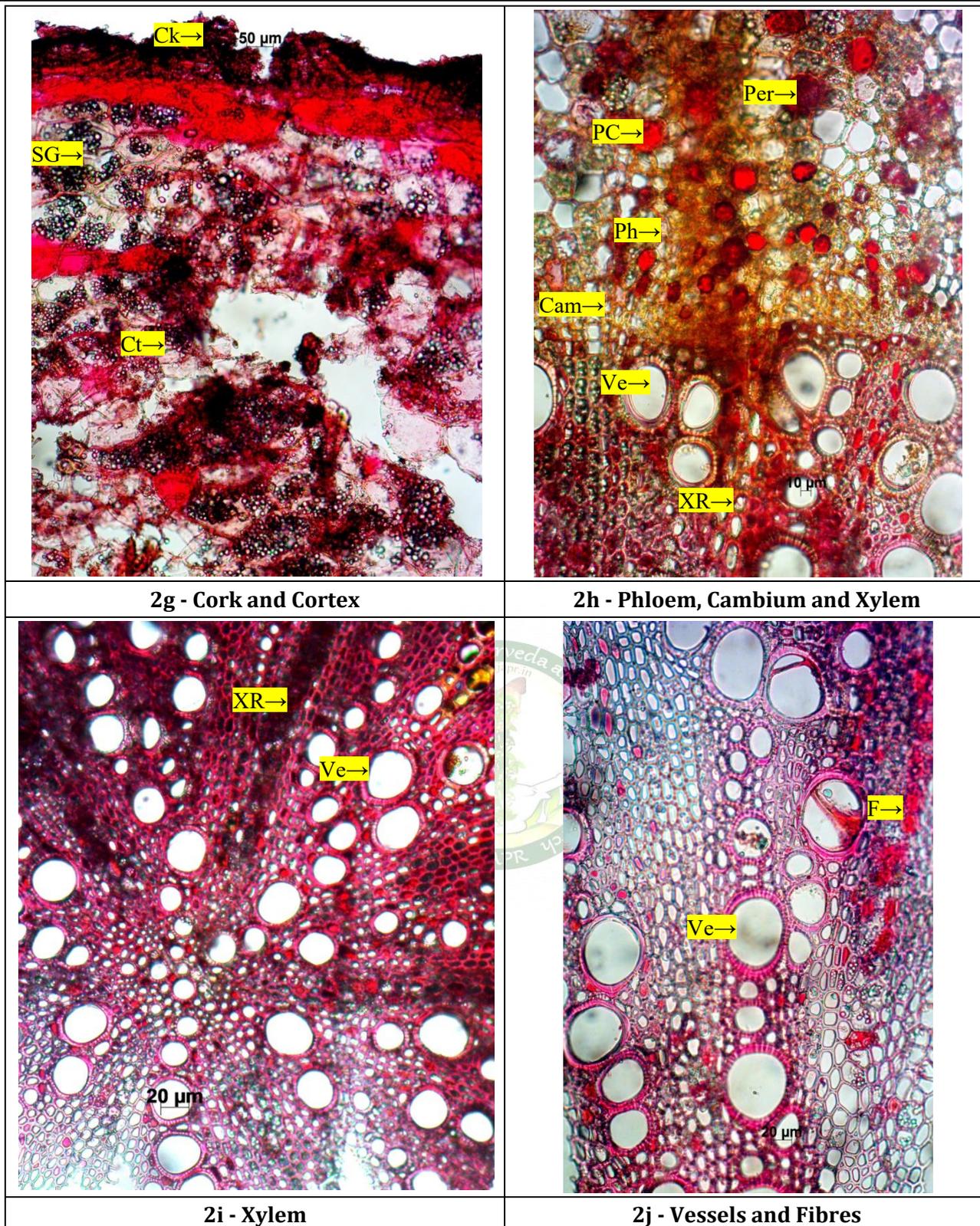


2e - Phloem and xylem



2f - Xylem

Ck – cork; Ct – cortex; F – fibres; PC – pigment cells; Ph – phloem; SG – starch grains; Ve – vessels; XR – xylem rays.



Cam – cambium; **Ck** – cork; **Ct** – cortex; **F** – fibres; **PC** – pigment cells; **Per** – pericyclic fibres; **Ph** – phloem; **SG** – starch grains; **Ve** – vessels; **XR** – xylem rays.

Powder microscopy analysis

The root powder of *P. zeylanica* showed fragments of cork in surface and sectional view; pitted thin-walled, lignified fibres; fragments of pitted vessel; tangentially cut fragment of pitted, lignified, medullary rays; parenchyma filled with dark brown contents; simple and compound starch grains with distinct hilum embedded in parenchymatous cells scattered as such throughout the powder and embedded in the parenchymatous cells.

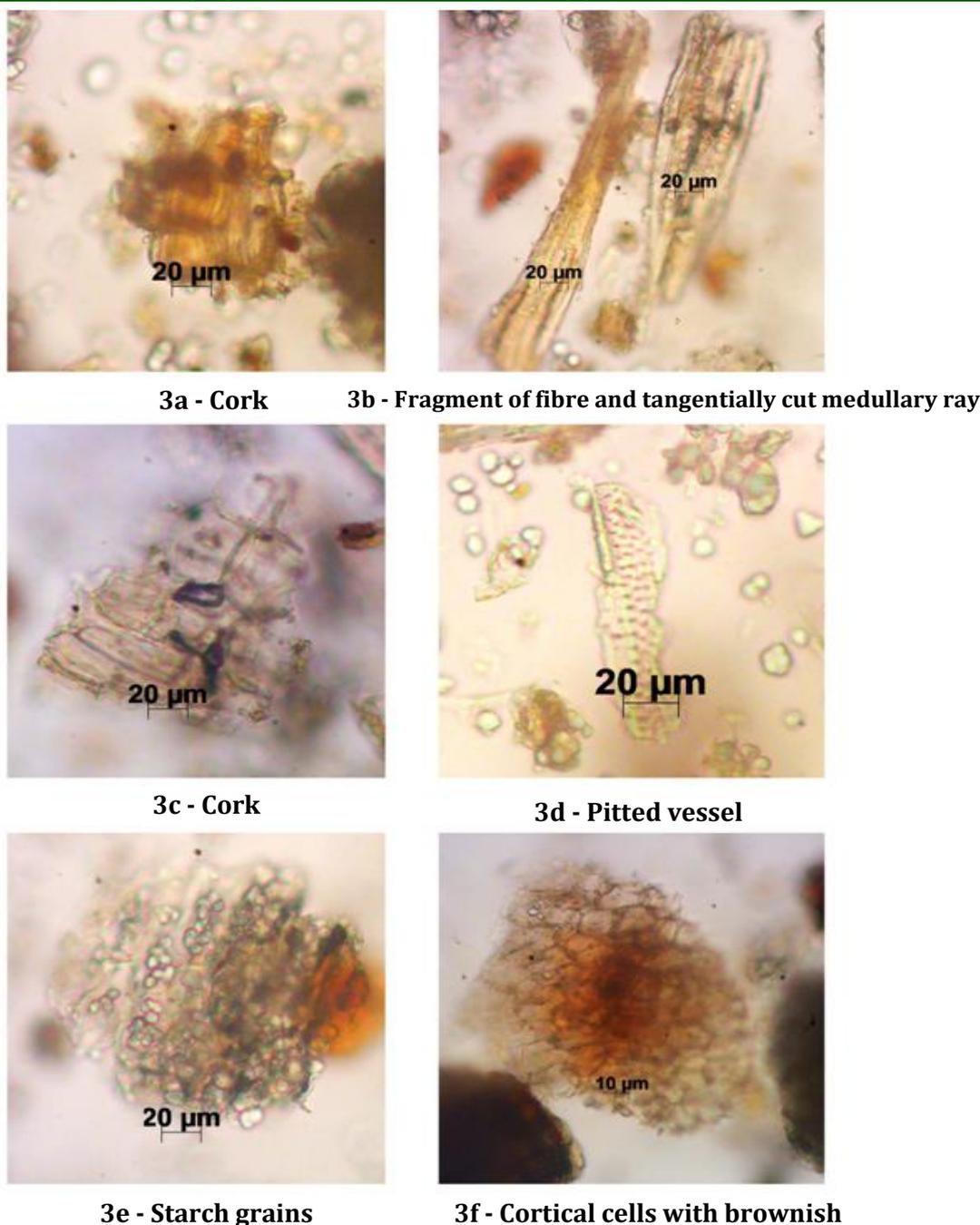


Fig 3: Powder microscopy of *P. zeylanica* root

Physico-chemical analysis

The root powder had a dark brown colour, disagreeable odour and acrid taste. The powder was coarse in texture and not much free flowing. The physicochemical standards like pH, loss on drying, total ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value were determined (Table 1).

Table 1: Physico-chemical parameters of powdered root of *P. zeylanica* (Avg±SEM, n=3 % w/w)

| Sl. No. | Parameters | Value (% w/w) |
|---------|----------------------------------|---------------|
| 1 | pH | 5.55 |
| 2 | Loss on drying | 10.80±0.01 |
| 3 | Total ash | 2.20±0.00 |
| 4 | Acid insoluble ash | 0.19±0.00 |
| 5 | Water soluble ash | 0.69±0.01 |
| 6 | Alcohol soluble extractive value | 15.85±0.00 |
| 7 | Water soluble extractive value | 26.11±0.01 |

Preliminary phytochemical screening

Phytochemical screening of root of *P. zeylanica* in decoction form confirmed the presence of primary metabolites such as carbohydrates and secondary metabolites such as alkaloids, tannins, flavanoids, saponins, terpenoids, resins and quinones (Table 2).

Table 2: Qualitative phytochemical analysis of decoction of *P. zeylanica* root

| Sl. No. | Phytoconstituents | Tests | Nature of colour change | Degree of colour change |
|---------|-------------------|-----------------------------------|---|-------------------------|
| 1 | Alkaloids | Dragendroff's test | Orange red precipitate | +++ |
| | | Wagner's test | Reddish brown precipitate | ++ |
| | | Mayer's test | Dull white precipitate | ++ |
| | | Hager's test | Yellow precipitate | ++ |
| 2 | Steroids | Liebermann- buchard test | No Bluish green colour | - |
| | | Salkowski test | No Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer | - |
| 3 | Carbohydrates | Molisch's test | Violet ring | ++ |
| | | Fehling's test | Brick red precipitate | + |
| | | Benedict's test | Red precipitate | ++ |
| 4 | Tannins | With FeCl ₃ | Brown colour | + |
| 5 | Flavanoids | Shinoda's test | Golden yellow colour | - |
| 6 | Saponins | With water | Stable froth | ++ |
| 7 | Triterpenoids | Tin and thionyl chloride test | Buff colour | - |
| 8 | Coumarins | With 2 N NaOH | Red colour | - |
| 9 | Phenol | With alcoholic ferric chloride | Brown color | - |
| 10 | Carboxylic acid | With water and NaHCO ₃ | No effervescence | - |
| 11 | Amino acid | With ninhydrine reagent | Brown color | - |
| 12 | Resin | With aqueous acetone | Turbidity | ++ |
| 13 | Quinones | 0.5% NaOH | Red color | +++ |

+ = slightly present, ++ = moderately present, +++ = highly present, - = absent; All the tests were carried out three times. Observations were based on the colour intensity and precipitation with appropriate reagents.

HPTLC

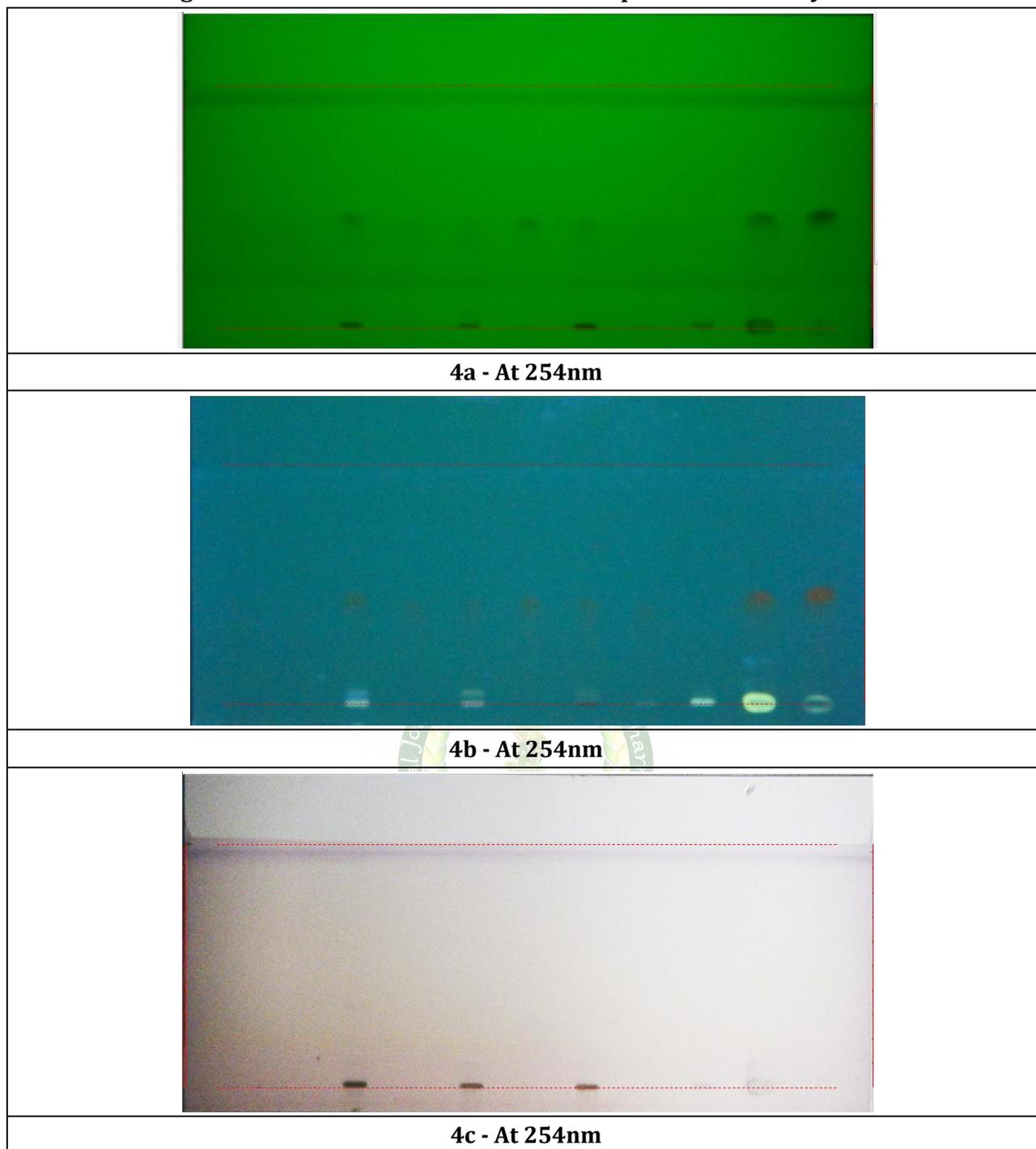
In the HPTLC chromatogram for quantification of Plumbagin (marker) which was identified at Rf of 0.50±0.02, for different standard concentrations range of 10-100ng/spot with R²±SD = 0.97±23.75 (regression via height) and R²±SD = 0.99±11.50 (regression via area). The 2µl (200µg) concentration of *P. zeylanica* was 30.67ng via height and 28.19ng via area.

The HPTLC method development was precise, specific, accurate and robust for determination of Plumbagin in *P. zeylanica*. The comparison of bio marker compound showed that, it was present in concentration of 0.015% in *P. zeylanica*.

Table 3: HPTLC quantification of Plumbagin in root of *P. zeylanica*

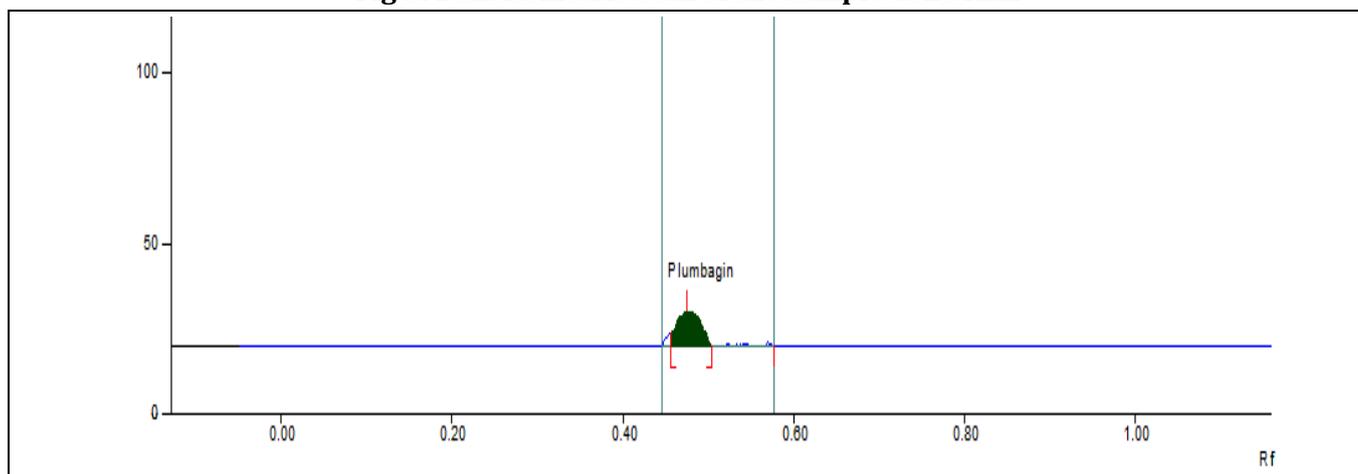
| Sample | X (calc) via Height (µg) | X (calc) via Area (µg) | Average (µg) | Percentage (%) |
|---------------------|--------------------------|------------------------|--------------|----------------|
| <i>P. zeylanica</i> | 0.0153 | 0.0140 | 0.0146 | 0.015% |

Fig 4: HPTLC Photo-documentation of sample of root of *P. zeylanica*



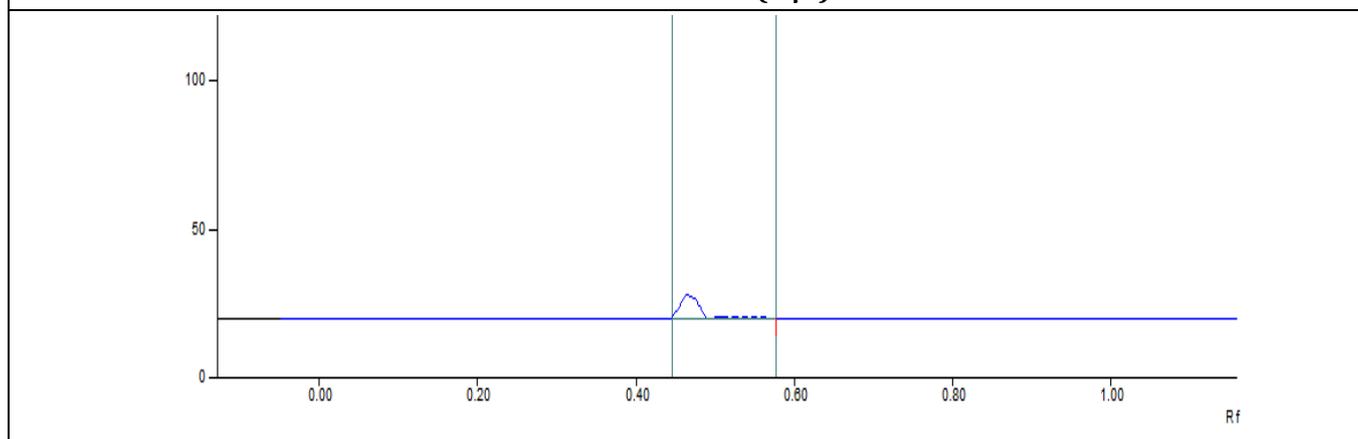
- Track 1: *Marker plumbagin* (Standard 1) - 1 μ l
 - Track 2: *Marker plumbagin* (Standard 1) - 1 μ l
 - Track 3: *P. zeylanica* - 2 μ l
 - Track 4: *Marker plumbagin* (Standard 2) - 2 μ l
 - Track 5: *P. zeylanica* - 2 μ l
 - Track 6: *Marker plumbagin* (Standard 3) - 4 μ l
 - Track 7: *P. zeylanica* - 2 μ l
 - Track 8: *Marker plumbagin* (Standard 4) - 8 μ l
 - Track 9: *P. zeylanica* - 2 μ l
 - Track 10: *Marker plumbagin* (Standard 5) - 10 μ l
 - Track 11: *Marker plumbagin* (Standard 5) - 10 μ l
- Solvent system- Toluene: Formic acid (9.9:0.1)

Fig 5: Densitometric scan of the sample at 254nm



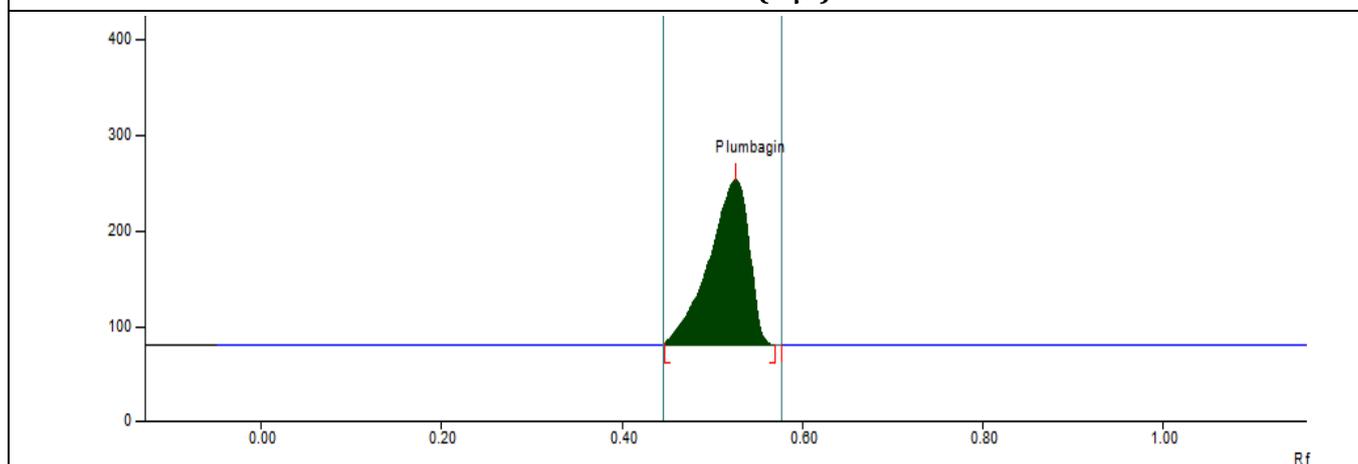
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|----------|----------|
| 1 | 0.46 Rf | 3.9 AU | 0.48 Rf | 10.3 AU | 100.00 % | 0.50 Rf | 0.1 AU | 210.7 AU | 100.00 % |

5a - Standard 1 (1 µl)



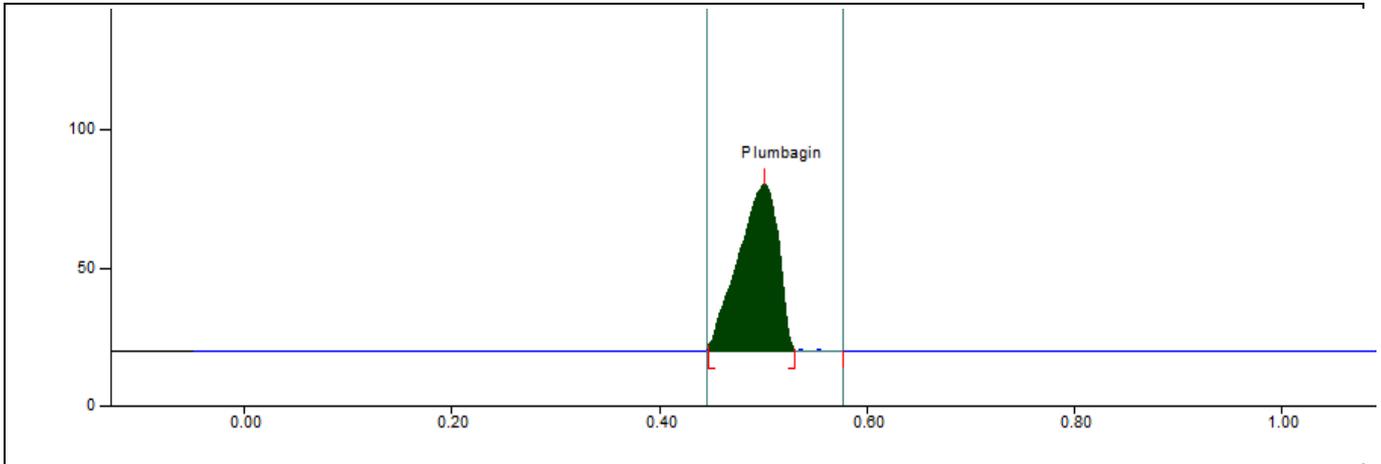
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|----------|----------|
| 1 | 0.46 Rf | 3.9 AU | 0.48 Rf | 10.3 AU | 100.00 % | 0.50 Rf | 0.1 AU | 210.7 AU | 100.00 % |

5b - Standard 1 (1 µl)



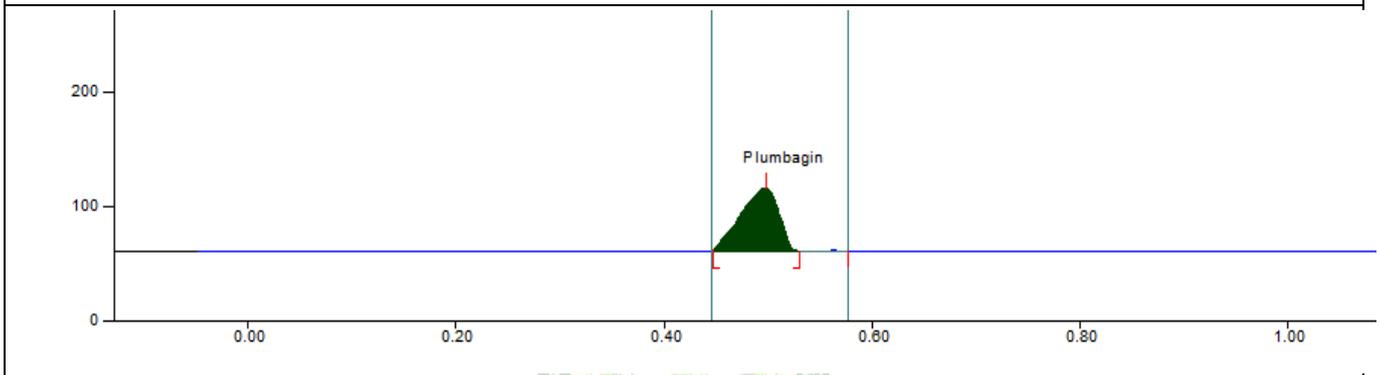
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|-----------|----------|
| 1 | 0.45 Rf | 1.5 AU | 0.53 Rf | 172.3 AU | 100.00 % | 0.57 Rf | 0.4 AU | 5522.3 AU | 100.00 % |

5c - *P. zeylanica* (2 µl)



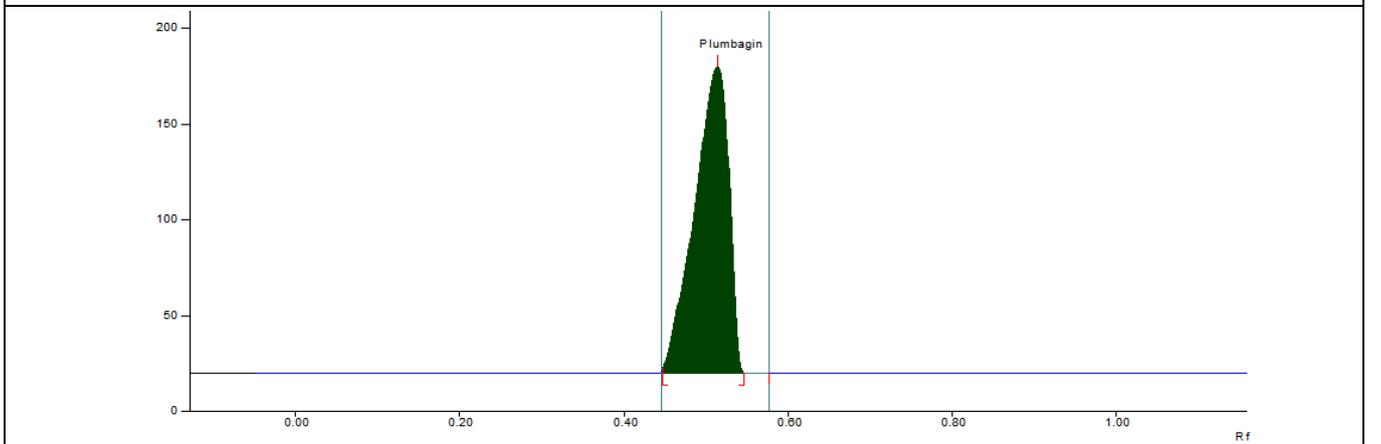
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|-----------|----------|
| 1 | 0.45 Rf | 1.6 AU | 0.50 Rf | 60.2 AU | 100.00 % | 0.53 Rf | 0.4 AU | 1672.9 AU | 100.00 % |

5d - Standard 2 (2 µl)



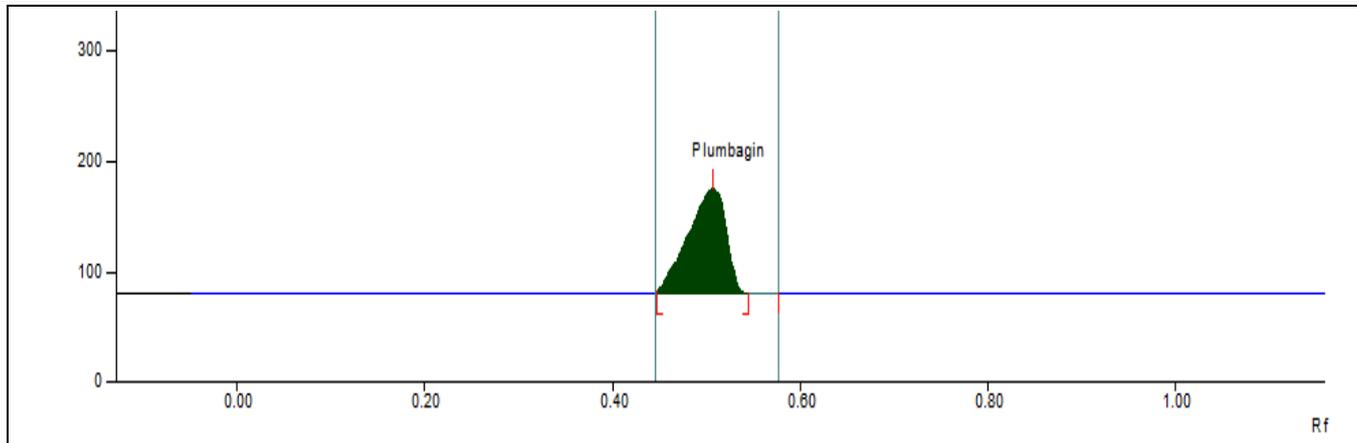
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|-----------|----------|
| 1 | 0.45 Rf | 1.7 AU | 0.50 Rf | 55.5 AU | 100.00 % | 0.53 Rf | 0.4 AU | 1477.5 AU | 100.00 % |

5e - *P. zeylanica* (2 µl)



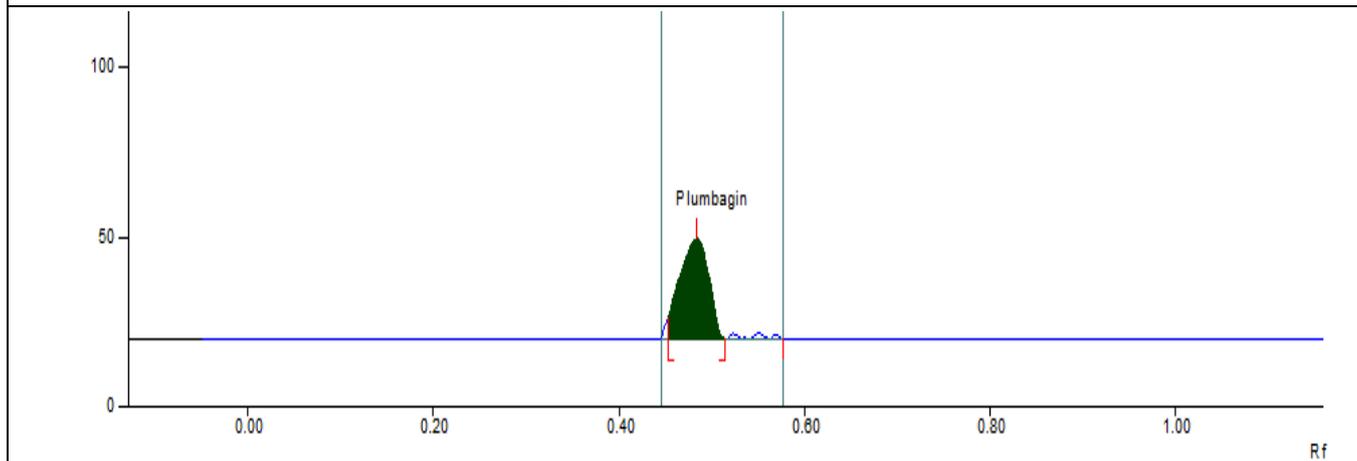
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|-----------|----------|
| 1 | 0.45 Rf | 2.3 AU | 0.51 Rf | 159.8 AU | 100.00 % | 0.55 Rf | 0.3 AU | 4828.0 AU | 100.00 % |

5f - Standard 3 (4 µl)



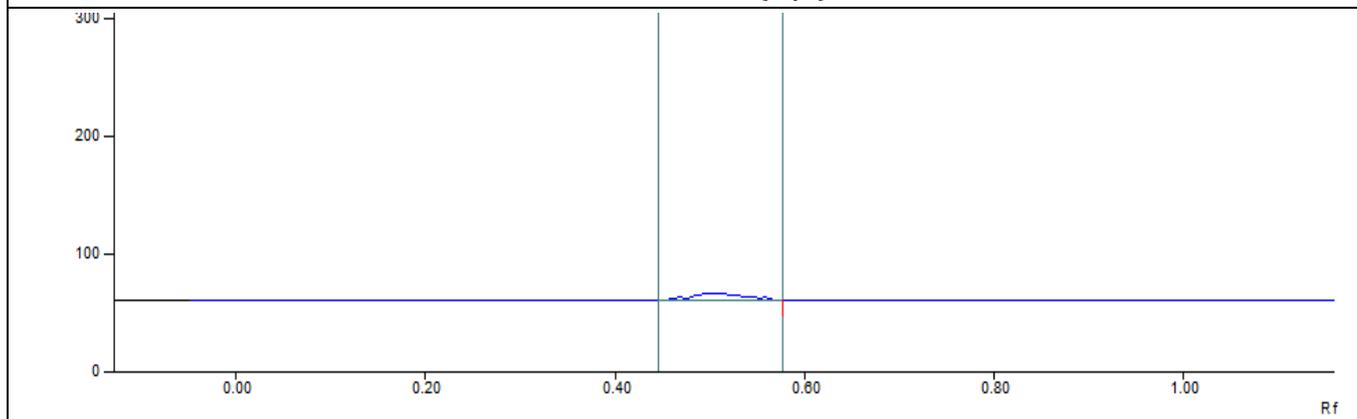
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|-----------|----------|
| 1 | 0.45 Rf | 1.4 AU | 0.51 Rf | 95.2 AU | 100.00 % | 0.55 Rf | 0.2 AU | 2765.2 AU | 100.00 % |

5g - *P. zeylanica* (2 µl)



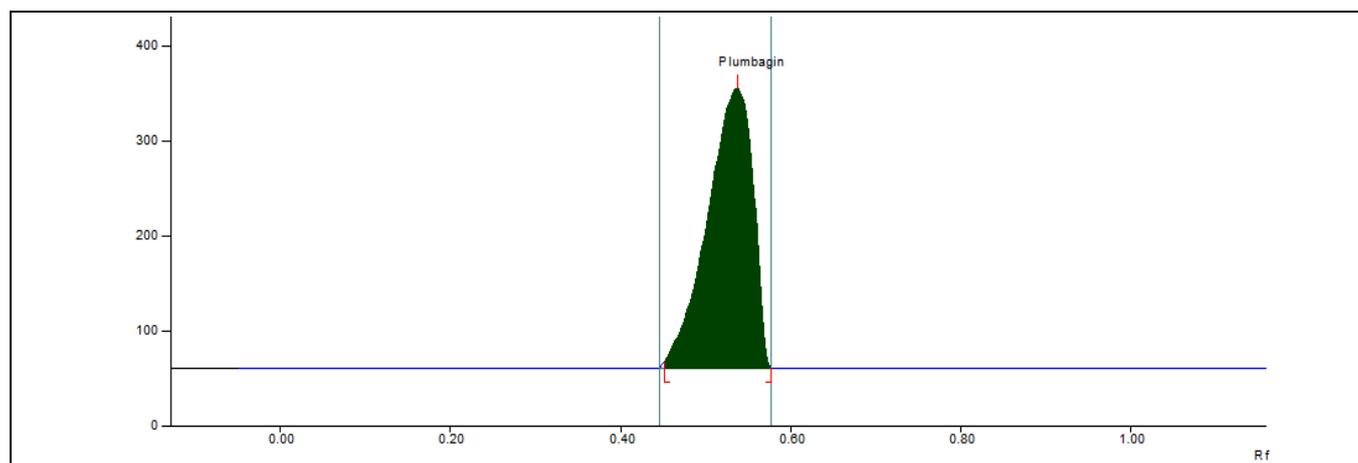
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|----------|----------|
| 1 | 0.45 Rf | 6.4 AU | 0.48 Rf | 29.7 AU | 100.00 % | 0.51 Rf | 0.0 AU | 676.7 AU | 100.00 % |

5h - Standard 4 (8 µl)



| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|-------|--------------|------------|------|--------|
|------|----------------|--------------|--------------|------------|-------|--------------|------------|------|--------|

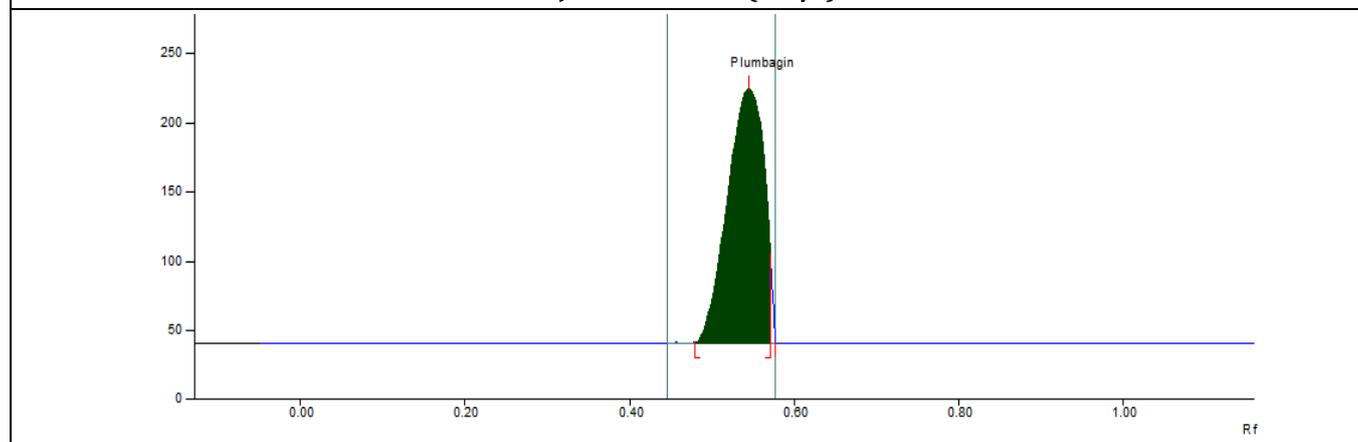
5i - *P. zeylanica* (2 µl)



Track 10, ID: Standard5

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|------------|----------|
| 1 | 0.45 Rf | 6.3 AU | 0.54 Rf | 294.4 AU | 100.00 % | 0.58 Rf | 0.0 AU | 11347.4 AU | 100.00 % |

5j - Standard 5 (10 µl)



| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|------|----------------|--------------|--------------|------------|----------|--------------|------------|-----------|----------|
| 1 | 0.48 Rf | 1.2 AU | 0.55 Rf | 183.6 AU | 100.00 % | 0.57 Rf | 64.1 AU | 5911.7 AU | 100.00 % |

5k - Standard 5 (10 µl)

CONCLUSION

The present study on Pharmacognostical, Preliminary phytochemical screening and HPTLC fingerprinting of root of *Citraka* (*P. zeylanica*) of family Plumbaginaceae can act as an important key for its identification. Generated data can be used for determining correct identity, purity of raw drug and detection of adulteration as well as may be useful to establish certain botanical standards. Thus, pharmacognostic studies can increase the quality and reliability of phytodrugs and would extend the research scope nationally and internationally leading to the discovery more bioactive compounds.

ACKNOWLEDGEMENT

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***Address for correspondence**

Dr. Akhilraj A.R.

Research Fellow (Ayurveda),
Ethnomedicine & Ethnopharmacology
Division, KSCSTE-Jawaharlal Nehru
Tropical Botanic Garden and Research
Institute (KSCSTE - JNTBGRI), Palode,
Thiruvananthapuram, Kerala, India.
Email: akhilrajar999@gmail.com

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