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PHARMACOGNOSTIC EVALUATION AND ICP-MS BASED METAL DETECTION IN *PERGULARIA DAEMIA* (AERIAL PART)

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ABSTRACT

The quality control standards of various medicinal plants used in indigenous systems of medicine are significant nowadays in view of the commercialization of herbal formulations. The major limitation of herbal medicines is the lack of standardization. The present study attempts to evaluate the identity, degree of purity and authenticity of the medicinal plant, *Pergularia daemia* (Forssk.) Chiov. It is a perennial twinning vine belonging to the family, Apocynaceae. The plant material was collected from three different geographical areas. Various pharmacognostic tests like anatomy, powder microscopy, physico-chemical and chromatography were executed by standard methods to evaluate the plant materials. High Performance Thin Layer Chromatographic (HPTLC) studies of the alcohol extracts were performed. Metal content present in these three accessions of the plant materials was also determined by employing ICP-MS. The evaluation of the physical constants of the drug is an essential parameter in detecting adulteration or improper handling of drugs. Pharmacogonstic parameters established the identity, purity and quality of the aerial part of *Pergularia daemia*. HPTLC fingerprint patterns unravelled the presence of various phytochemical constituents. These results help in the identification and standardization of the drug. The determined element concentrations were within the international safety limits. This makes the plant acceptable for human and animal consumption at nutritional as well as medicinal levels.

KEYWORDS: *Pergularia daemia*, microscopy, physico-chemical analysis, HPTLC, ICP-MS.

1. INTRODUCTION

Medicinal plants have been part and parcel of human society to combat diseases from the dawn of civilization. Many of these plants possess tremendous medicinal value and are used extensively for such purposes. The Siddha system is one of the important and oldest Indian traditional systems of medicine. Most of the Siddha formulations are prepared from herbs. The quality control standards of various medicinal plants, used in indigenous systems of medicine, are significant nowadays in view of the commercialization of formulations based on medicinal plants. The major limitation of herbal medicines is the lack of standardization techniques.

Standardisation of plants

Standardization of herbal drugs means confirmation of their identity, quality and purity. World Health Organization (WHO) has a set of specific Guidelines for evaluating the safety, efficacy and quality of herbal drugs. The standardization parameters of plant materials include macroscopy, microscopy, physico-chemical and chromatographic studies.[1] The availability of standardized herbs in the market would bring the trust of individuals towards herbal remedies and uniform therapeutic outcomes. In this study, the aerial part of *Pergularia daemia* (Forssk.) Chiov., (Fam: Asclepiadaceae), one of the important drugs being used in the Siddha system of medicine for various therapeutic

purposes, was taken for pharmacognostical standardisation.

P. daemia is a perennial twinning herb that grows widely along the roadsides of India and also in the tropical and subtropical regions. It is a slender, hispid, fetid smelling laticiferous twiner.[2] The Regional language names of the plant are Tamil-Vaeliparuthi; Malayalam – Veliparuthi; Bengali – Chhagalbete; Gujarathi-Nagaladudhi, Amaradudheli; Marathi -Mendhadhdhi, Utarana; Odia -Utrali; Panjabi-Karial,Siali; and Telugu – Dustuputige.[3] The plant possesses high medicinal value and is traditionally used in treating various ailments for human beings. Extract of this plant is taken orally for gastric ulcers, uterine and menstrual complaints. The stem bark of this plant is a good remedy for cold. It is also used to treat malaria and the twig is used as an antipyretic and appetizer. Dried roots are used for bronchitis, cough, asthma and constipation and also as an abortifacient, emetic while fresh roots are used to treat gonorrhoea. Leaf latex is used as pain killer and as a relief for toothache.[4] The leaves are useful in leprosy and haemorrhoids. The fresh, pulped leaves are applied as a poultice to relieve carbuncles. Leaf juice is used as amenorrhea and for catarrhal infections, dysmenorrheal, infantile diarrhoea and to reduce body pain. In addition the dried leaves are used for asthma, amenorrhea, dysmenorrheal, bronchitis, whooping cough, healing cuts and wounds and as an antirheumatic and also to facilitate parturition.[5] The medicinal properties of the plant depend on the presence of secondary metabolites. The leaves of the plant contain flavonoids, alkaloids, terpenoids, tannins, steroids, cardenolides, triterpenes, saponins, steroidal compounds and carbohydrates. The seeds contain uzarigenin, coroglaucigenin, calactin, calotropin and a bitter resin.[6,7] The objective of this study is to standardise the aerial part of *P. daemia*.An attempt has been made to study the plant material from the botanical, physico-chemical and chromatographical standardization points of view.

Fig.1: *Pergularia daemia* (Forssk.) Chiov.

2. MATERIALS AND METHODS

1. Collection of plant

P.daemia was collected from three different geographical areas. The first accession of the plant was collected from Siddha Medicinal Plants Garden, Mettur Dam (N11°47.859 E 77° 48.041). The second accession was taken up from Wayanadu in Kerala (N 11°80.659, E 76°021.912) and the third accession from Thirunelveli, Tamilnadu (N8°48.01 E77°44.34).

2. Pharmacognostic standardisation of plant material

a. Macroscopy

Macroscopic characteristics of plants were studied by observing under a Stereo zoom microscope.

b. Microscopy

1. Anatomical study

Microscopic studies of the three accessions of the aerial part of *P. daemia* were carried out by preparing thin sections of the samples. The thin sections were washed with water, stained with safranin and mounted in glycerine for observation under 10X and 40X objective of a Bright field microscope.[8]

2. Powder microscopy

The powdered form of plant materials was mounted in glycerin at room temperature for 24 hrs and observed under 10X and 40X objective of a Bright field microscope for powder characteristics.[8]

3. Determination of leaf constants

A number of leaf measurements are used to distinguish some closely related species not easily characterized by general microscopy. Stomatal number, stomatal index, palisade ratio, trichome number, vein islet and veinlet termination of leaves of the plant were observed under 4X, 20X objective of the microscope as per standard protocol.[9]

C. Physico-chemical analysis

Physico-chemical parameters such as loss on drying at 105ºC, total ash, acid insoluble ash, solubility in water and alcohol, pH of water extract, moisture content and volatile oil of the plant materials were determined by standard methods.[10,11]

D. High Performance Thin Layer Chromatographic(HPTLC) studies

HPTLC is an analytical separation and determination method with a wide application in herbal drug analysis.

Procedure: 1 gm of the coarsely powdered plant material of each accession was extracted with 10 ml of alcohol. These extracts were concentrated and used for HPTLC analysis. Each extract of 8 µl was applied as a band of a width of 10 mm on a TLC aluminium plate precoated with Silica gel 60 F_{254} (Merck) of 0.2 mm thickness. Each plate was developed up to a height of 8 cm in the solvent system, Toluene: Ethyl acetate: Formic acid (5: 2: 0.1). The developed plates were air dried and kept in CAMAG visualizer and the images were captured under UV light at 254 nm and 366 nm. Then the plates were scanned at 254 nm and 366 nm using TLC Scanner 4 and the fingerprint profiles were documented. The R_f values and fingerprint data were recorded with winCATS

software associated with the scanner. The plates were derivatised using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plates were visualized under white light and the chromatograms were documented. The plates were scanned at 575 nm and the fingerprint profiles were recorded.[12]

3. Estimation of Metals

The metal contents in the three accessions of the plant material were estimated using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (Make: Thermo Electron Corporation, Model :iCAP RQ) by outsourcing at 'Sophisticated Tests and Instrumentation Centre (STIC), Cochin University of Science and Technology, Cochin.

Procedure

ICP- MS working standard: Working standards, prepared from ICP single/ multi elements 1000 ppm certified standard solutions, are used for instrument calibration. Minimum of five working standards were prepared between 1 ppb to 250 ppb according to the concentration of the elements and matrix of the sample.

Sample analysis: Solid samples are typically digested in strong and hot acids (either open digestion or closed vessel digestion in a microwave digester). The acids range from simple nitric acid (for relatively simple matrices) to hydrofluoric acid (for samples containing high silicon dioxide content). Hydrogen peroxide may be added to the samples containing organic matter during digestion because H_2O_2 breaks down organic matter efficiently.

Modes of operation: Standard mode - the instrument performs like a standard ICP-MS. High sensitivity is achieved for all elements.

3. RESULTS

a. Macroscopy

The macroscopic characters of the three accessions of *P. daemia* are given in Table 1.

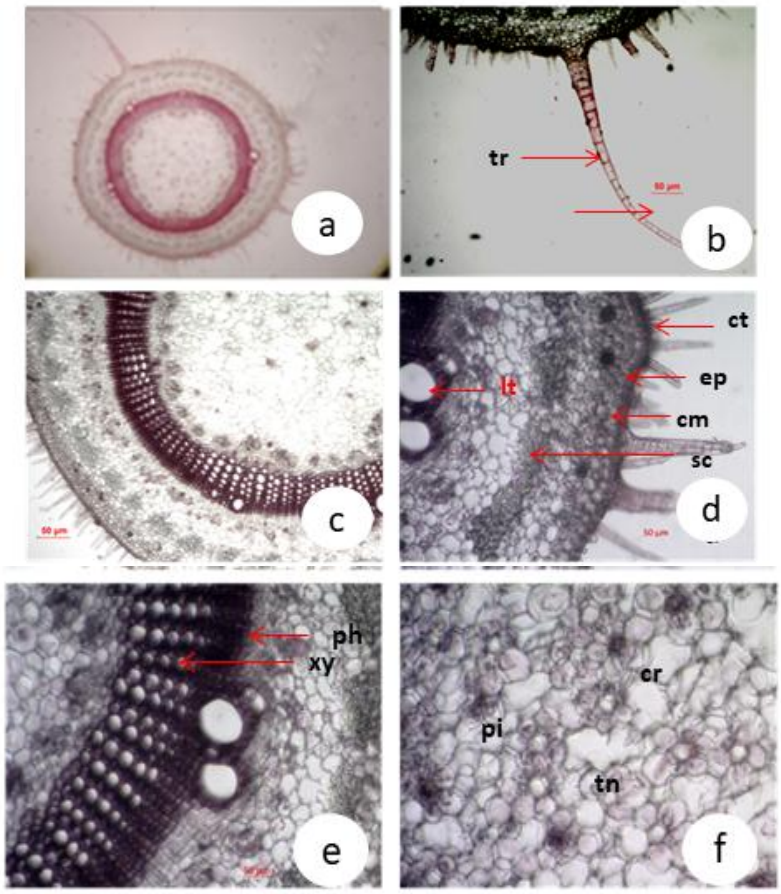
Table 1: Comparison of macroscopic characters of *Pergularia daemia* (Ariel Part).

Characters	1st Accession	2nd Accession	3rd Accession
Stem	Pubescent, pale green to green, 1- 3 mm diameter and the internodal length 5 – 15 cm.	Pubescent, pale green to green, 1- 2 mm diameter and the internodal length 6 – 10 cm.	Pubescent, pale green to green, 1- 3 mm diameter and the internodal length 5 – 15 cm.
Leaf	Simple, opposite, pubescent, lamina 5- 10 cm long & 4 -9 cm broad, cordate, green colour Long petiole, 3 – 6 cm length, hairy	Simple, opposite, pubescent, lamina 4- 8 cm long & 3 -7 cm broad, cordate, dark green colour. Long petiole, 3 – 5 cm length, hairy	Simple, opposite, pubescent, lamina 5- 12 cm long & 5 -10 cm broad, cordate, pale green colour. Long petiole, 3 – 6 cm length, hairy
Flowers	Greenish- yellow colour	Greenish- yellow colour	Greenish- yellow colour
Fruit	Follicles slightly curved, usually in pairs, green, having thick soft, short, and spines throughout with soft spines.	Follicles slightly curved, usually in pairs, green, having thick soft, short, and spines throughout with soft spines. Seeds pale to brown in colour 4-6 mm. in length, pubescent.	Follicles slightly curved, usually in pairs, green, having thick soft, short, and spines throughout with soft spines. Seeds pale to brown in colour 4-6 mm in length, pubescent.

b. Microscopy

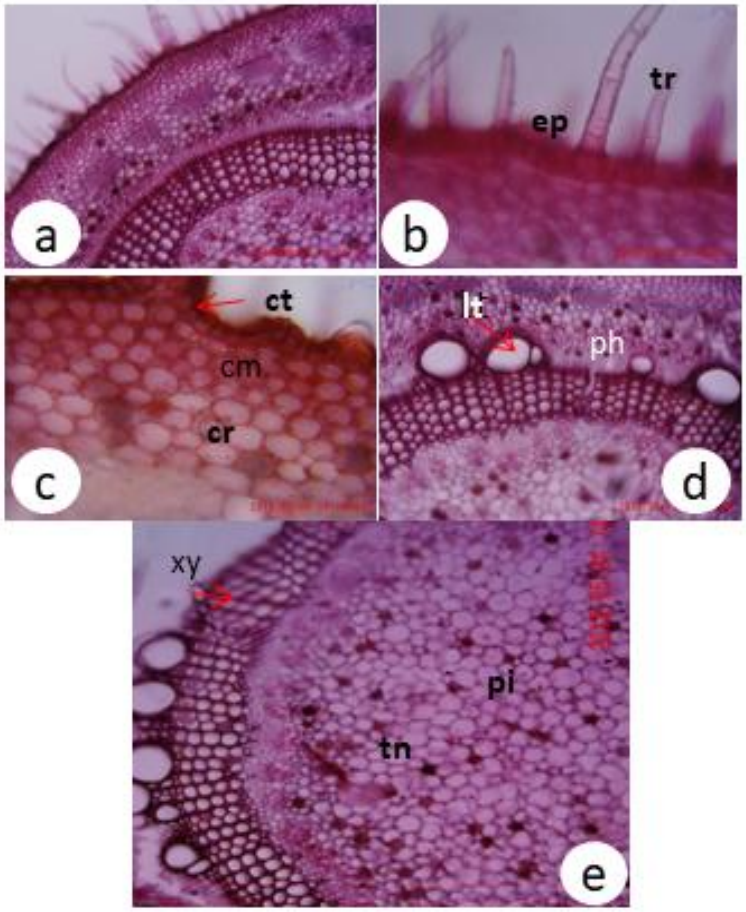
Stem

Anatomical features of the stem of the three accessions of *P. daemia* are given in Fig. 2 & Table 2**.**



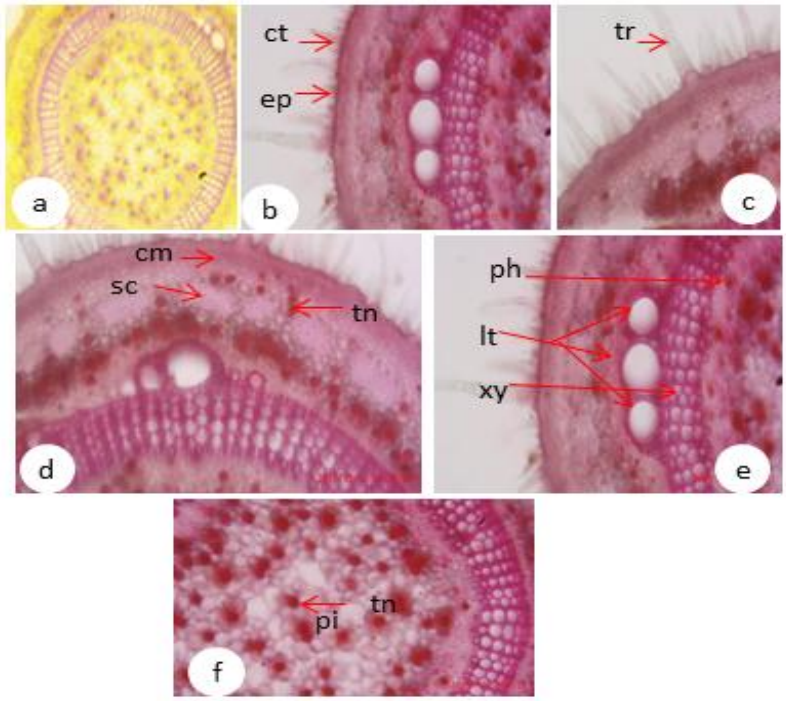
1st Accession

a: full view, b: uniseriate multicellular trichome (tr), c: T.S of stem (portion enlarged), d: cuticle (ct), epidermis (ep), collenchyma cells (cm), sclerenchyma patches (sc), leaf trace (lt) e: xylem (xy), phloem (ph), f: pith (pi), tannin (tn), crystal (cr)



2nd Accession

a: full view, b: epidermis (ep) with multicellular trichome (tr), c: cuticle (ct), collenchyma cells(cm), calcium oxalate crystal (cr), d: leaf trace (lt), phloem, e: xylem (xy), pith (pi), tannin (tn)



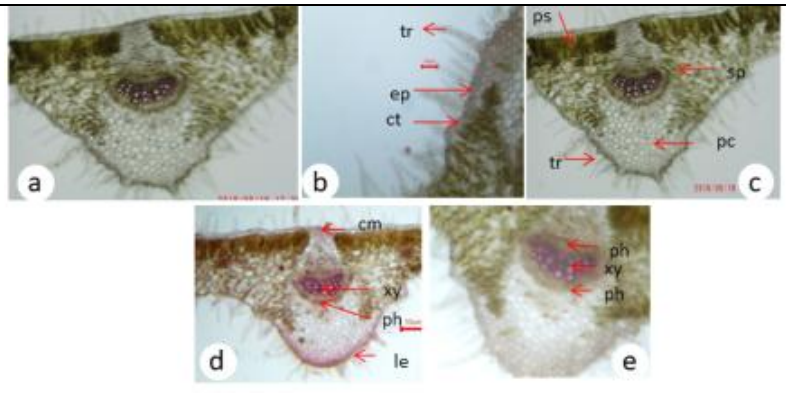
3rd Accession
a: full view, b: cuticle (ct), epidermis (ep), c: uniseriate multicellular trichome (tr), d: collenchyma (cm), sclerenchyma (sc), tannin content (tn), e: xylem (xy), phloem (ph), leaf traces (lt), f: pith (pi), tannin content (tn)
Fig. 2: T.S of stem of three accessions of *Pergularia daemia*

Table 2: Comparison of the anatomy of stem of three accessions of *Pergularia daemia.*

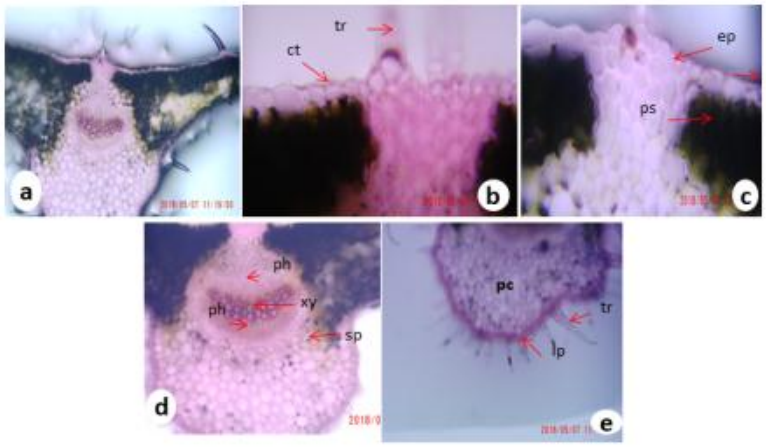
Characters	1st Accession	2nd Accession	3rd Accession
Stem			
Cuticle	Thin walled	Thin walled	Thin walled
Epidermis	Single layered	Single layered	Single layered
Trichomes	Unicellular and multicellular-uniseriatetrichomes. 9 – 12 long trichomes and numerous short trichomes	Unicellular and multicellular-uniseriatetrichomes. 3 – 5 longtrichomes and numerous short trichomes	Unicellular and multicellular-uniseriatetrichomes. 3 - 5 long trichomes and numerous short trichomes
Cortex	2 – 3 layers of collenchyma cells, 5-12 layers of parenchyma cells. 6 – 8 leaf traces	3 – 6 layers of collenchyma cells, 5-12 layers of parenchyma cells. 8 – 16 leaf traces	2 – 3 layers of collenchyma cells, 5-12 layers of parenchyma cells. 6 – 8 leaf traces
Stelar region	Well-developed vascular bundle, phloem patches present internal to the xylem and in the periphery of the pith	Well-developed vascular bundle, phloem patches present internal to the xylem and in the periphery of the pith	Well-developed vascular bundle, phloem patches present internal to the xylem and in the periphery of the pith
Pith	Parenchymatous, circular to polygonal with intercellular spaces	Parenchymatous, circular to polygonal with intercellular spaces	Parenchymatous, circular to polygonal with intercellular spaces
Tannin and crystals	scattered in the pith and cortex	Crystals were scattered in the cortex and tannin was scattered in the pith	Tannin cells were scattered in the pith and cortex.

Leaf

Anatomical features of the leaf of the three accessions of *P. daemia* are given in Fig. 3 & Table 3.

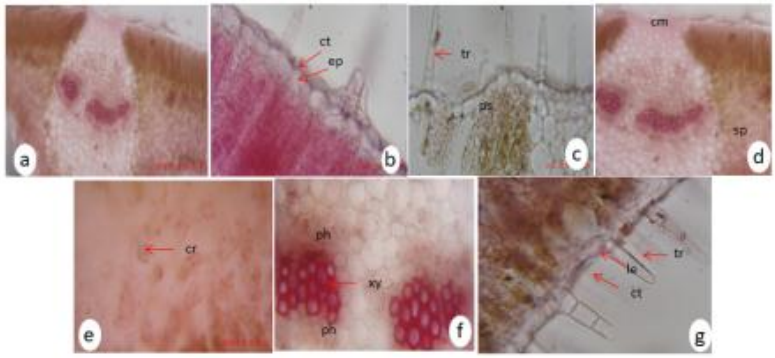


1st Accession
a: full view, b: cuticle (ct), epidermis (ep) with trichome (tr), c: palisade cell (ps), trichome (tr), spongy parenchyma (sp), parenchyma (pc), d: collenchyma (cm), xylem (xy), phloem (ph), lower epidermis (le), e: enlarged xylem (xy) and phloem (ph)



2nd Accession

a: full view, b: cuticle (ct), trichome (tr), c: epidermis (ep), palisade cell (ps), d: xylem (xy), phloem (ph), spongy parenchyma (sp), e: parenchyma (pc), lower epidermis (lp), trichome (tr)



3rd Accession

a: full view, b: epidermis (epi) with cuticle (ct) , c: palisade cell (ps), trichomes (tr), d: collenchyma cell (cm), spongy parenchyma (sp), e: calcium oxalate crystal (cr), f: xylem (xy), phloem (ph), g: lower epidermis (le), cuticle (ct), trichome (tr)

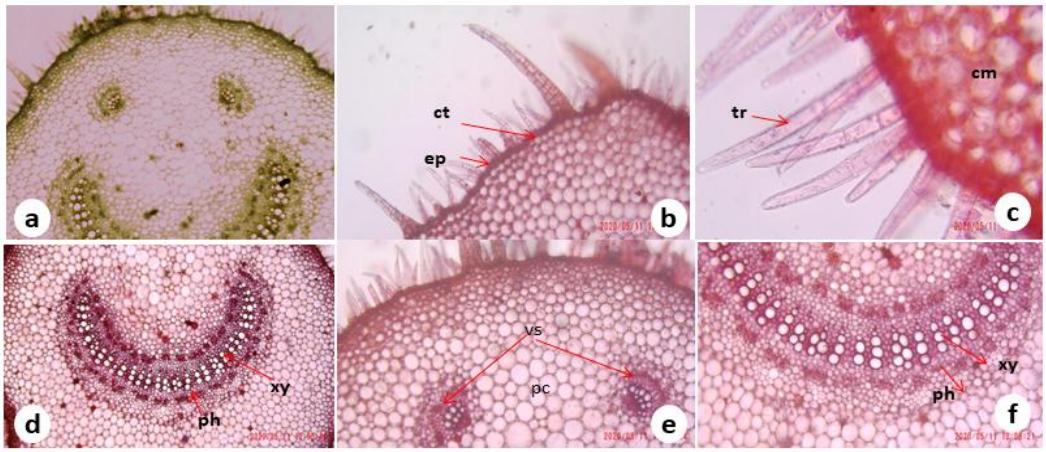
Fig. 3: T.S of leaf of three accessions of *Pergularia daemia.*

Table 3: Comparison of anatomy of leaf of three accessions of *Pergularia daemia.*

Characters	1st Accession	2nd Accession	3rd Accession
Leaf			
Cuticle	Thick-walled cells	Thick-walled cells	Thick-walled cells
Epidermis (upper)	Single layered basal shaped thick-walled cells	Single layered basal shaped thick-walled cells	Single layered basal shaped thick-walled cells
Epidermis (lower)	Single layered small thin-walled cells	Single layered small thin-walled cells	Single layered small thin-walled cells
Trichome	Unicellular and multicellular-uniseriate trichomes present on the upper and lower epidermis	Unicellular and multicellular-uniseriate trichomes present on the upper and lower epidermis But lower epidermis has numerous trichomes.	Unicellular and multicellular-uniseriate trichomes present on the upper and lower epidermis
Mesophyll	Double-layered elongated palisade cells and polygonal irregularly arranged spongy parenchyma cells	Double-layered elongated palisade cells and polygonal irregularly arranged spongy parenchyma cells	Double-layered elongated palisade cells and polygonal irregularly arranged spongy parenchyma cells
Vascular bundle	Single, crescent-shaped bicollateral vascular bundle	Single, crescent-shaped bicollateral vascular bundle	Two bicollateral vascular bundle

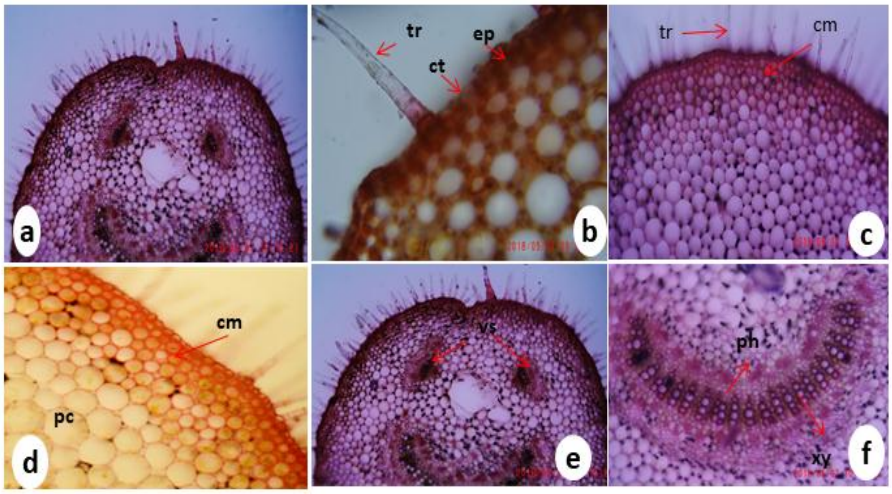
Petiole

Anatomical features of the petiole of the three accessions of *P. daemia* are given in Fig. 4 & Table 4.



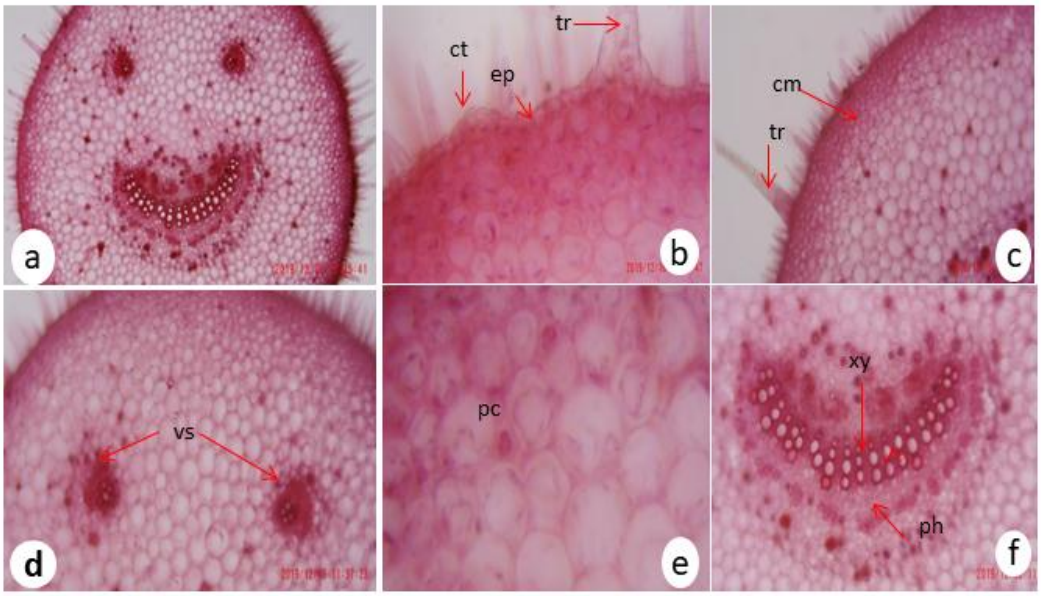
1st Accession

a: full view, b: cuticle (ct), epidermis (ep), c: collenchyma (cm), trichome (tr), d: xylem (xy), phloem (ph), e: vascular strand (vs), parenchyma cells (pc), f: enlarged xylem (xy), phloem (ph)



2nd Accession

a: full view, b: epidermis (ep), cuticle (ct), trichome (tr), c: trichome (tr), collenchyma (cm), d: collenchyma cells (cm), parenchyma (pc) e: small vascular strant (vs), f: xylem (xy), phloem (ph)



3rd Accession

a: full view, b: cuticle (ct), epidermis (ep), trichome (tr), c: collenchyma (cm), trichome (tr), d: vascular strant (vs) e: parenchyma (pc), f: xylem (xy), phloem (ph)

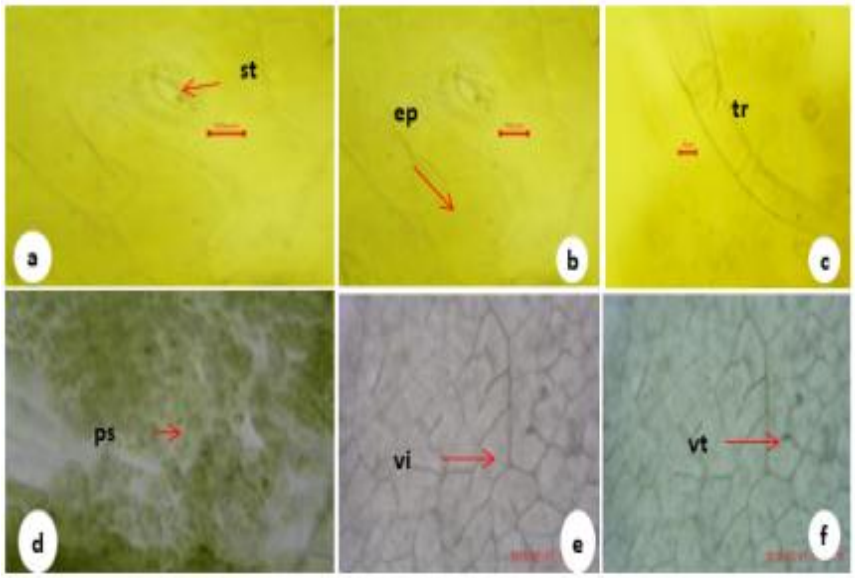
Fig. 4: T.S of petiole of three accessions of *Pergularia daemia*

Table 4: Comparison of petiole of three accessions of *Pergularia daemia.*

Characters	1st Accession	2nd Accession	3rd Accession
Petiole			
Cuticle	Thick-walled cuticle	Thick-walled cuticle	Thick-walled cuticle
Epidermis	Single layered	Single layered	Single layered
Ground tissue	Parenchyma cells	Parenchyma cells	Parenchyma cells
Vascular bundles	25 vertical rows of xylem and phloem patches	22 vertical rows of xylem and phloem patches	17 vertical rows of xylem and phloem patches

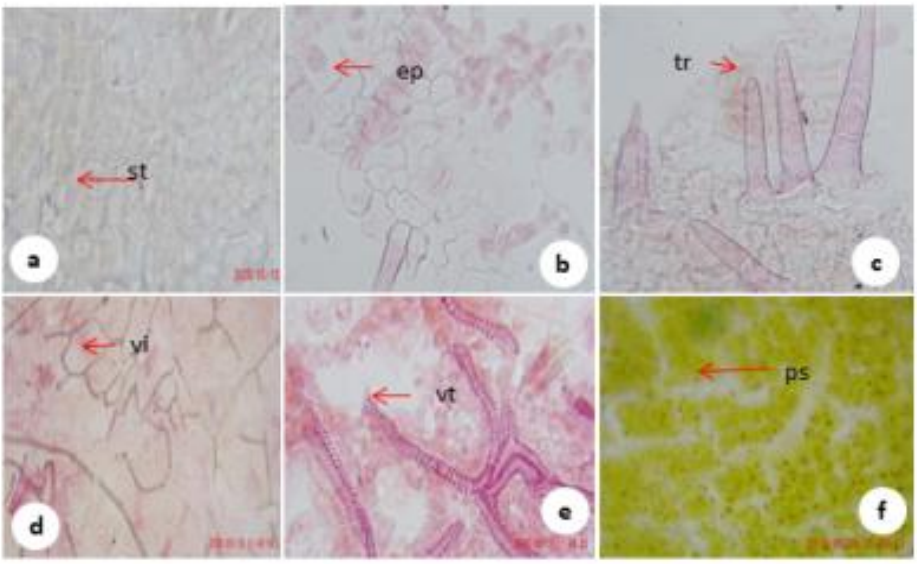
c.Determination of leaf constants

Leaf constants of the three accessions of *P. daemia* are given in Fig. 5 & Table 5.



1st Accession

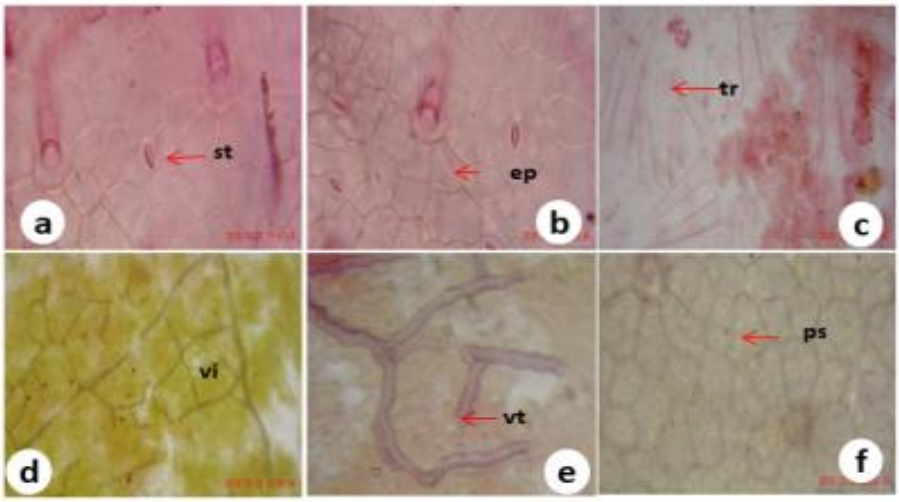
a: anomocytic stomata (st), b: epidermis (ep), c: trichome (tr), d: palisade cell (ps), e: vein islet (vi), f: vein let termination (vt)



2nd Accession,

a: anomocytic stomata, b: epidermis (ep), c: trichome (tr), d: vein islet (vi), e: vein

let termination (vt), f: palisade cell (ps)



3rd Accession

a: anomocytic stomata (st), b: epidermis (ep), c: trichome (tr), d: vein islet (vi), e: vein let termination (vt), f: palisade cell (ps)

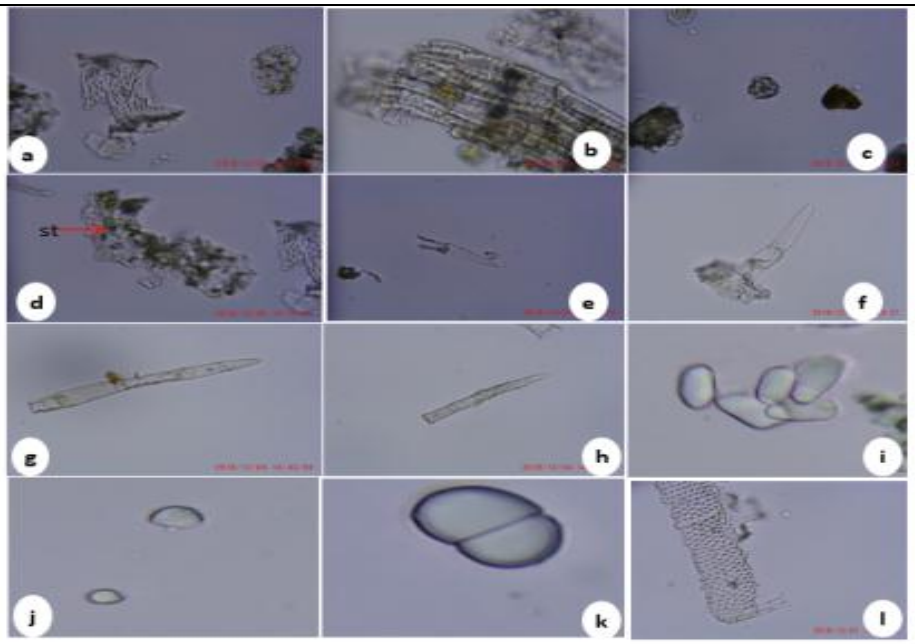
Fig. 5: Leaf constants of three accessions of *Pergularia daemia*

Table 5: Parameters of leaf constants of three accessions of *Pergularia daemia.*

Sl. No.	Parameters	Result		
		1st Accession	2nd Accession	3rd Accession
1	Stomatal number	10.6	13	12
2	Stomatal index	25	25	28.8
3	Palisade ratio	3	2.9	29.3
4	Vein islet number	28	14.6	5.3
5	Vein let termination	32	18.6	30.6
6	Epidermal number	100	45.3	3.81
7	Trichome number	21.3	9.3	36

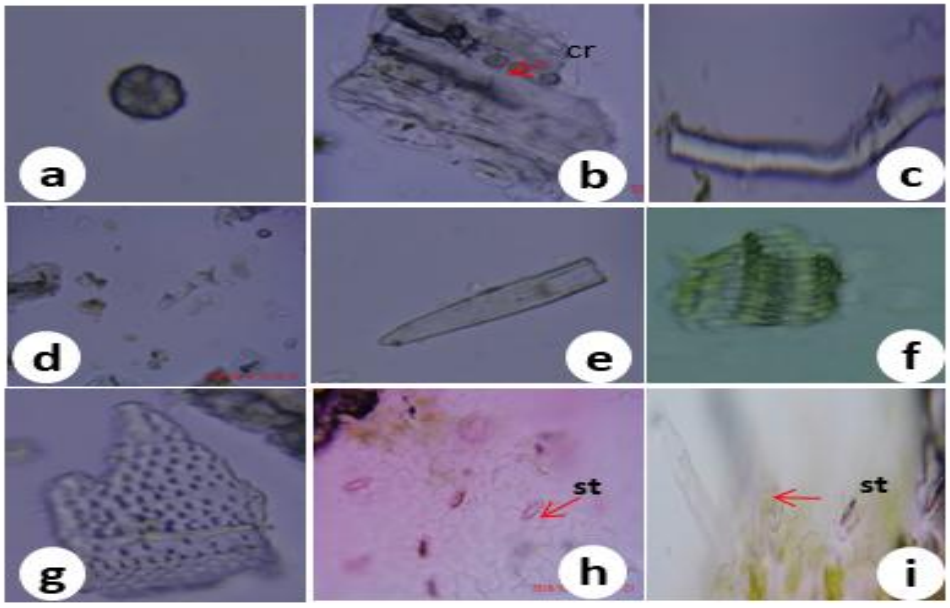
d. Powder microscopy

Powder microscopies of three accessions of P. daemia are given in Fig. 6 & Table 6.



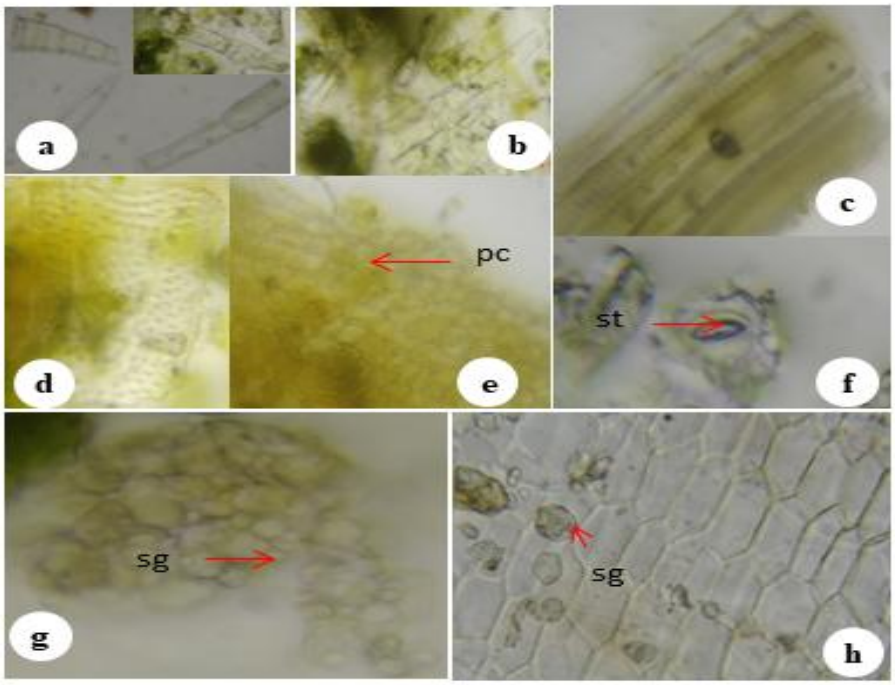
1st Accession

a: simple pitted vessel, b: xylem vessel with annular thickening, c: rosette shaped calcium oxalate crystals, d: anomocytic stomata, e-h: unicellular multicellular uniseriate trichomes, I – k: starch grains, l: xylem vessel with simple pits.



2nd Accession

a & b: rosette shaped calcium oxalate crystals, c-e: trichomes, f: xylem vessel with spiral thickening, g: xylem vessel with simple pits, h & i: anomocytic stomata.



3rd Accession

a & b: trichomes, c: xylem vessels with spiral thickening, d: simple pitted vessels, e: parenchyma cell (pc), f: anomocytic stomata, g: group of oval-shaped starch grains in the parenchyma cells, h: starch grains in the elongated parenchyma cells.

Fig. 6: Powder microscopy of Aerial part of three accessions of *Pergularia daemia*.

Table 6: Comparison of powder characteristics of three accessions of *Pergularia daemia* (Aerial part).

Characters	**1st Accession**	**2nd Accession**	**3rd Accession**
Colour	Light brown	Light brown	Brown
Odour	No odour	No odour	No odour
Characters			
	Anomocytic stomata	Anomocytic stomata	Anomocytic stomata
	1 or 2 rosette shaped calcium oxalate crystals	1 or 2 rosette shaped calcium oxalate crystals	Numerous rosette shaped calcium oxalate crystals
	Numerous unicellular and multicellular uniseriate trichomes	1 or 2 unicellular and multicellular uniseriate trichomes	Numerous Unicellular and multicellular uniseriate trichomes
	Xylem vessels with Simple pits	Xylem vessels with Simple pits	Xylem vessels with Simple pits
	Xylem vessels with spiral thickening	Xylem vessels with spiral thickening	Xylem vessels with spiral thickening
	Simple to compound starch grains	Not observed	Starch grains

e. Physico-chemical Analysis

The physico – chemical parameters of three accessions of *Pergularia daemia* (Aerial part) are recorded in Table 7.

Table 7: Physico – chemical parameters of three accessions of *Pergularia daemia* (Aerial part)

Parameters	**1st Accession**	**2nd Accession**	**3rd Accession**
Foreign matter	< 2	< 2	< 2
Loss on drying at 105ºC %	13.29	8.68	8.75
Total ash %	8.54	6.86	10.75
Acid insoluble ash %	1.34	1.80	4.76
Water soluble extractive %	14.67	12.00	15.78
Alcohol soluble extractive %	8.86	4.96	8.45
P^H of water extract	9.0	8.8	6.2
Moisture content %	12.88	8.48	8.56
Volatile oil %	Nil	Nil	Nil

d. High Performance Thin Layer Chromatographic (HPTLC) Profile

HPTLC studies of the alcohol extracts of the aerial part of the three accessions of *Pergularia daemia* were carried out and the results are given in Fig. 7 & Fig. 8.

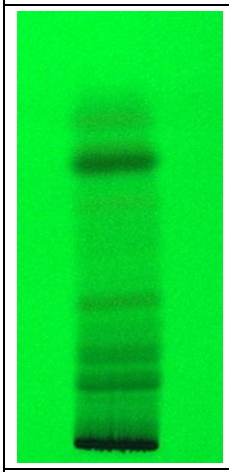
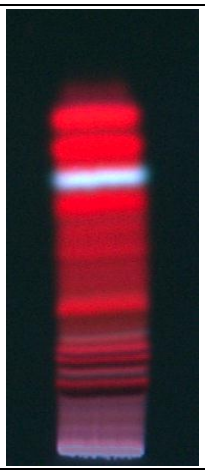
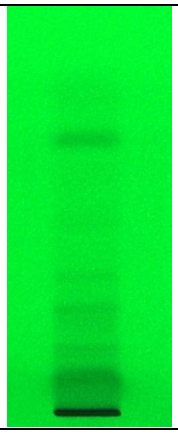
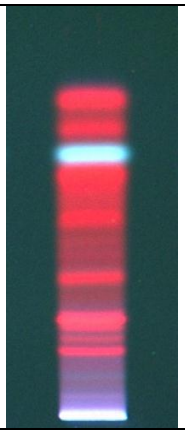
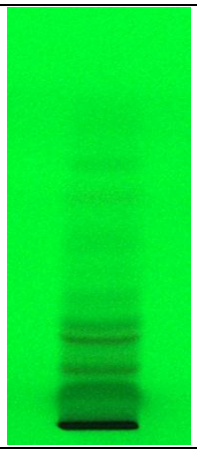
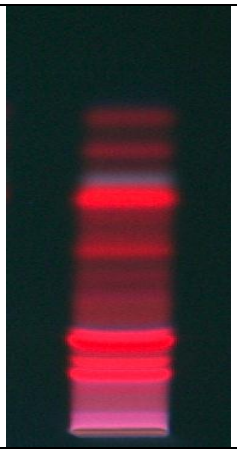
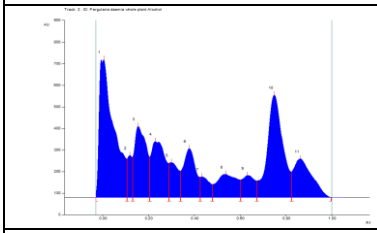
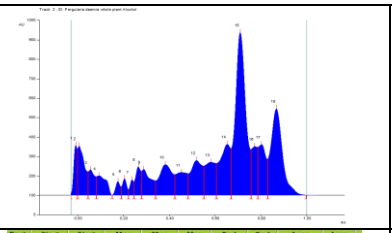
Accession 1		
		
Viewed in UV short	**Viewed in UV long**	**Viewed in visible light after derivatisation**
Accession 2		
		
Viewed in UV short	**Viewed in UV long**	**Viewed in visible light after derivatisation**
Accession 3		
		
Viewed in UV short	**Viewed in UV long**	**Viewed in visible light after derivatisation**

Fig. 7: HPTLC profile of alcohol extracts of three accessions of *Pergularia daemia* (Aerial part); Solvent system – Toluene: Ethyl acetate: Formic acid (5: 2: 0.1); Volume applied – 8 µl

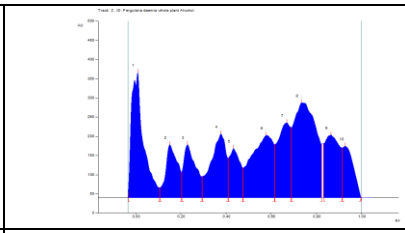
Accession 1



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	12.0 AU	0.01 Rf	636.8 AU	23.09 %	0.11 Rf	77.9 AU	28611.2 AU	25.46 %
2	0.11 Rf	178.7 AU	0.12 Rf	195.1 AU	7.05 %	0.13 Rf	86.2 AU	2832.8 AU	2.52 %
3	0.13 Rf	188.3 AU	0.16 Rf	329.0 AU	11.89 %	0.20 Rf	86.5 AU	11712.0 AU	10.42 %
4	0.21 Rf	186.5 AU	0.23 Rf	258.6 AU	9.35 %	0.29 Rf	58.2 AU	11211.8 AU	9.98 %
5	0.29 Rf	158.7 AU	0.30 Rf	161.3 AU	5.83 %	0.34 Rf	22.2 AU	4411.0 AU	3.92 %
6	0.34 Rf	122.7 AU	0.38 Rf	226.6 AU	8.19 %	0.43 Rf	93.9 AU	8448.5 AU	7.52 %
7	0.43 Rf	94.0 AU	0.44 Rf	95.5 AU	3.45 %	0.48 Rf	60.4 AU	2609.5 AU	2.32 %
8	0.48 Rf	60.5 AU	0.54 Rf	107.0 AU	3.87 %	0.60 Rf	81.4 AU	6610.0 AU	5.88 %
9	0.61 Rf	81.6 AU	0.63 Rf	101.3 AU	3.66 %	0.67 Rf	76.5 AU	3799.7 AU	3.38 %
10	0.68 Rf	76.5 AU	0.75 Rf	474.5 AU	17.15 %	0.82 Rf	19.0 AU	22165.4 AU	19.72 %
11	0.83 Rf	119.6 AU	0.87 Rf	178.8 AU	6.46 %	1.00 Rf	0.3 AU	9985.8 AU	8.88 %



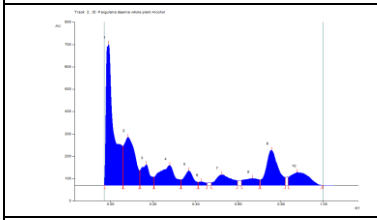
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	3.5 AU	-0.01 Rf	256.5 AU	6.53 %	0.00 Rf	37.2 AU	2705.2 AU	2.59 %
2	0.00 Rf	238.0 AU	0.01 Rf	253.3 AU	6.45 %	0.05 Rf	20.8 AU	5183.5 AU	4.96 %
3	0.05 Rf	120.9 AU	0.06 Rf	130.1 AU	3.31 %	0.08 Rf	95.8 AU	2510.7 AU	2.40 %
4	0.08 Rf	96.2 AU	0.09 Rf	99.9 AU	2.54 %	0.15 Rf	0.3 AU	2955.8 AU	2.83 %
5	0.15 Rf	0.3 AU	0.18 Rf	70.1 AU	1.79 %	0.19 Rf	43.6 AU	921.9 AU	0.88 %
6	0.19 Rf	44.1 AU	0.20 Rf	84.7 AU	2.16 %	0.22 Rf	28.6 AU	1127.4 AU	1.08 %
7	0.22 Rf	28.8 AU	0.24 Rf	79.0 AU	2.01 %	0.25 Rf	68.7 AU	910.5 AU	0.87 %
8	0.25 Rf	69.6 AU	0.27 Rf	145.8 AU	3.71 %	0.28 Rf	22.9 AU	2380.3 AU	2.28 %
9	0.28 Rf	124.2 AU	0.29 Rf	133.2 AU	3.39 %	0.34 Rf	74.4 AU	3571.0 AU	3.41 %
10	0.34 Rf	74.5 AU	0.38 Rf	157.2 AU	4.00 %	0.43 Rf	05.9 AU	6235.4 AU	5.96 %
11	0.43 Rf	106.0 AU	0.46 Rf	116.2 AU	2.96 %	0.48 Rf	11.3 AU	3966.3 AU	3.79 %
12	0.49 Rf	111.4 AU	0.52 Rf	178.1 AU	4.54 %	0.55 Rf	50.5 AU	6246.5 AU	5.97 %
13	0.55 Rf	150.8 AU	0.58 Rf	169.2 AU	4.31 %	0.61 Rf	63.7 AU	5387.8 AU	5.15 %
14	0.61 Rf	163.9 AU	0.65 Rf	261.7 AU	6.66 %	0.67 Rf	40.1 AU	8270.3 AU	7.91 %
15	0.67 Rf	240.4 AU	0.71 Rf	836.4 AU	21.30 %	0.76 Rf	35.2 AU	26391.4 AU	25.23 %
16	0.76 Rf	235.3 AU	0.77 Rf	250.4 AU	6.38 %	0.79 Rf	46.6 AU	4174.8 AU	3.99 %
17	0.79 Rf	247.0 AU	0.80 Rf	260.2 AU	6.63 %	0.83 Rf	84.1 AU	6076.7 AU	5.81 %
18	0.83 Rf	185.8 AU	0.87 Rf	444.6 AU	11.32 %	1.00 Rf	0.2 AU	15592.8 AU	14.91 %



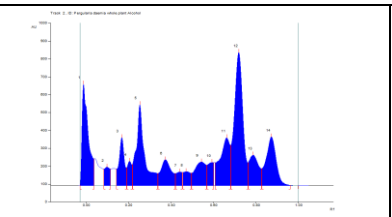
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	12.6 AU	0.01 Rf	326.7 AU	18.15 %	0.11 Rf	25.9 AU	12124.0 AU	14.84 %
2	0.11 Rf	26.0 AU	0.15 Rf	136.1 AU	7.67 %	0.20 Rf	66.6 AU	5027.3 AU	6.15 %
3	0.20 Rf	67.4 AU	0.23 Rf	136.1 AU	7.68 %	0.29 Rf	55.2 AU	5222.3 AU	6.39 %
4	0.30 Rf	55.3 AU	0.38 Rf	166.5 AU	9.25 %	0.41 Rf	03.3 AU	7707.7 AU	9.43 %
5	0.41 Rf	103.4 AU	0.44 Rf	128.0 AU	7.11 %	0.48 Rf	77.6 AU	4285.6 AU	5.25 %
6	0.48 Rf	77.7 AU	0.58 Rf	162.5 AU	9.03 %	0.62 Rf	38.6 AU	10866.4 AU	13.30 %
7	0.62 Rf	138.7 AU	0.67 Rf	195.3 AU	10.85 %	0.69 Rf	82.9 AU	7639.2 AU	9.35 %
8	0.69 Rf	183.0 AU	0.74 Rf	247.3 AU	13.74 %	0.82 Rf	39.6 AU	16915.3 AU	20.71 %
9	0.83 Rf	140.7 AU	0.87 Rf	163.3 AU	9.07 %	0.92 Rf	30.1 AU	7610.7 AU	9.32 %
10	0.92 Rf	130.3 AU	0.93 Rf	133.9 AU	7.44 %	1.00 Rf	3.1 AU	4296.4 AU	5.26 %

At 254 nm | **At 366 nm** | **At 575 nm after derivatisation**

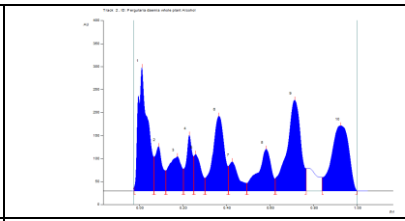
Accession 2



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	5.1 AU	-0.00 Rf	632.0 AU	45.06 %	0.06 Rf	75.9 AU	15851.3 AU	34.47 %
2	0.06 Rf	176.4 AU	0.09 Rf	215.0 AU	15.33 %	0.14 Rf	65.6 AU	7754.8 AU	16.87 %
3	0.14 Rf	66.2 AU	0.17 Rf	92.7 AU	6.61 %	0.20 Rf	36.6 AU	2615.5 AU	5.69 %
4	0.21 Rf	36.8 AU	0.28 Rf	89.1 AU	6.35 %	0.33 Rf	25.1 AU	4372.6 AU	9.51 %
5	0.34 Rf	25.2 AU	0.37 Rf	65.0 AU	4.63 %	0.41 Rf	13.7 AU	1874.6 AU	4.08 %
6	0.41 Rf	13.9 AU	0.43 Rf	15.8 AU	1.12 %	0.46 Rf	10.4 AU	354.5 AU	0.77 %
7	0.48 Rf	11.9 AU	0.52 Rf	47.2 AU	3.36 %	0.60 Rf	20.7 AU	2348.8 AU	5.11 %
8	0.62 Rf	22.4 AU	0.67 Rf	30.6 AU	2.18 %	0.70 Rf	25.3 AU	1422.0 AU	3.09 %
9	0.71 Rf	25.0 AU	0.76 Rf	157.3 AU	11.22 %	0.82 Rf	35.4 AU	5927.9 AU	12.89 %
10	0.84 Rf	36.5 AU	0.88 Rf	57.9 AU	4.13 %	1.00 Rf	0.5 AU	3457.1 AU	7.52 %



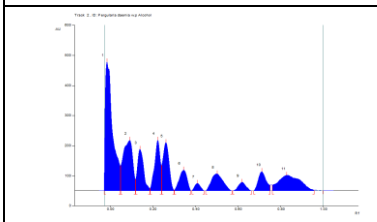
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	3.9 AU	-0.01 Rf	568.7 AU	16.02 %	0.04 Rf	49.9 AU	11886.2 AU	12.85 %
2	0.09 Rf	92.6 AU	0.10 Rf	104.1 AU	2.93 %	0.12 Rf	93.0 AU	1961.1 AU	2.12 %
3	0.14 Rf	93.3 AU	0.17 Rf	268.9 AU	7.57 %	0.19 Rf	98.2 AU	4738.1 AU	5.12 %
4	0.19 Rf	100.6 AU	0.21 Rf	136.9 AU	3.86 %	0.22 Rf	15.9 AU	1963.3 AU	2.12 %
5	0.22 Rf	118.5 AU	0.25 Rf	452.7 AU	12.75 %	0.34 Rf	68.7 AU	12290.2 AU	13.28 %
6	0.34 Rf	68.9 AU	0.38 Rf	144.7 AU	4.08 %	0.42 Rf	67.2 AU	5056.7 AU	5.47 %
7	0.42 Rf	67.2 AU	0.44 Rf	75.8 AU	2.14 %	0.46 Rf	74.8 AU	1463.1 AU	1.58 %
8	0.46 Rf	74.4 AU	0.47 Rf	78.6 AU	2.21 %	0.50 Rf	69.3 AU	1800.5 AU	1.95 %
9	0.50 Rf	69.4 AU	0.55 Rf	133.1 AU	3.75 %	0.57 Rf	15.2 AU	4868.7 AU	5.26 %
10	0.57 Rf	115.5 AU	0.60 Rf	129.6 AU	3.65 %	0.60 Rf	28.0 AU	2495.1 AU	2.70 %
11	0.61 Rf	127.6 AU	0.66 Rf	267.9 AU	7.55 %	0.68 Rf	25.0 AU	8611.7 AU	9.31 %
12	0.68 Rf	225.5 AU	0.72 Rf	745.1 AU	20.99 %	0.76 Rf	27.6 AU	21044.6 AU	22.75 %
13	0.77 Rf	129.1 AU	0.79 Rf	170.8 AU	4.81 %	0.83 Rf	95.1 AU	5242.6 AU	5.67 %
14	0.83 Rf	95.5 AU	0.88 Rf	273.1 AU	7.69 %	0.96 Rf	1.7 AU	9098.9 AU	9.83 %



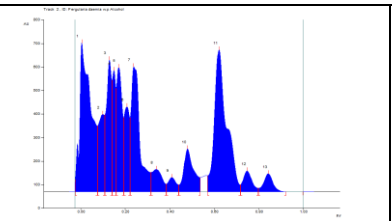
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	7.9 AU	0.01 Rf	268.4 AU	20.68 %	0.07 Rf	74.1 AU	8012.8 AU	17.51 %
2	0.07 Rf	74.3 AU	0.09 Rf	96.8 AU	7.46 %	0.12 Rf	44.1 AU	2330.3 AU	5.09 %
3	0.12 Rf	44.1 AU	0.18 Rf	74.9 AU	5.77 %	0.20 Rf	47.8 AU	2948.0 AU	6.44 %
4	0.20 Rf	47.9 AU	0.23 Rf	121.6 AU	9.38 %	0.25 Rf	75.5 AU	2401.7 AU	5.25 %
5	0.25 Rf	75.3 AU	0.26 Rf	79.9 AU	6.16 %	0.30 Rf	28.0 AU	1782.6 AU	3.90 %
6	0.30 Rf	28.1 AU	0.37 Rf	162.4 AU	12.51 %	0.41 Rf	53.4 AU	5912.4 AU	12.92 %
7	0.41 Rf	53.6 AU	0.43 Rf	63.4 AU	4.89 %	0.49 Rf	16.9 AU	1962.2 AU	4.29 %
8	0.50 Rf	17.0 AU	0.58 Rf	90.8 AU	7.00 %	0.63 Rf	26.6 AU	3852.1 AU	8.42 %
9	0.63 Rf	27.1 AU	0.71 Rf	197.3 AU	15.21 %	0.77 Rf	48.8 AU	8752.8 AU	19.13 %
10	0.84 Rf	29.5 AU	0.93 Rf	142.0 AU	10.94 %	1.00 Rf	0.8 AU	7799.5 AU	17.05 %

At 254 nm | **At 366 nm** | **At 575 nm after derivatisation**

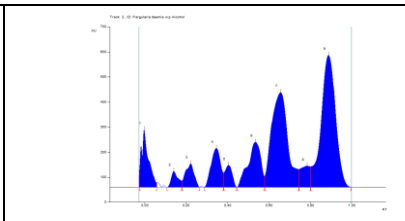
Accession 3



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	0.0 AU	-0.01 Rf	427.9 AU	31.59 %	0.05 Rf	83.3 AU	10463.2 AU	29.72 %
2	0.05 Rf	83.9 AU	0.09 Rf	167.8 AU	12.39 %	0.12 Rf	36.8 AU	5321.0 AU	15.11 %
3	0.12 Rf	38.1 AU	0.14 Rf	137.9 AU	10.19 %	0.19 Rf	7.6 AU	2798.2 AU	7.95 %
4	0.19 Rf	8.3 AU	0.22 Rf	168.0 AU	12.40 %	0.24 Rf	84.2 AU	3071.8 AU	8.72 %
5	0.24 Rf	85.3 AU	0.26 Rf	160.1 AU	11.82 %	0.30 Rf	2.6 AU	3240.6 AU	9.20 %
6	0.30 Rf	2.6 AU	0.34 Rf	68.8 AU	5.08 %	0.38 Rf	0.4 AU	1722.7 AU	4.89 %
7	0.38 Rf	0.9 AU	0.41 Rf	24.7 AU	1.82 %	0.44 Rf	0.0 AU	460.3 AU	1.31 %
8	0.45 Rf	0.1 AU	0.50 Rf	55.7 AU	4.11 %	0.57 Rf	0.1 AU	2127.3 AU	6.04 %
9	0.58 Rf	0.0 AU	0.62 Rf	28.1 AU	2.08 %	0.67 Rf	0.0 AU	665.4 AU	1.89 %
10	0.68 Rf	0.0 AU	0.71 Rf	63.9 AU	4.72 %	0.75 Rf	18.8 AU	1651.5 AU	4.69 %
11	0.76 Rf	19.0 AU	0.83 Rf	51.3 AU	3.79 %	0.96 Rf	1.7 AU	3689.3 AU	10.48 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	52.9 AU	0.01 Rf	631.9 AU	13.85 %	0.08 Rf	79.3 AU	22047.4 AU	20.08 %
2	0.08 Rf	279.6 AU	0.10 Rf	329.0 AU	7.21 %	0.11 Rf	24.9 AU	6232.2 AU	5.68 %
3	0.11 Rf	326.2 AU	0.13 Rf	560.8 AU	12.29 %	0.14 Rf	72.4 AU	9129.9 AU	8.32 %
4	0.14 Rf	474.6 AU	0.15 Rf	514.7 AU	11.28 %	0.16 Rf	39.2 AU	5338.0 AU	4.86 %
5	0.16 Rf	439.7 AU	0.17 Rf	527.4 AU	11.56 %	0.19 Rf	13.5 AU	8776.8 AU	8.00 %
6	0.19 Rf	317.4 AU	0.21 Rf	360.5 AU	7.90 %	0.22 Rf	13.0 AU	5802.9 AU	5.29 %
7	0.22 Rf	315.8 AU	0.24 Rf	530.8 AU	11.64 %	0.32 Rf	82.0 AU	14693.6 AU	13.39 %
8	0.32 Rf	82.2 AU	0.34 Rf	95.0 AU	2.08 %	0.38 Rf	32.8 AU	3007.8 AU	2.74 %
9	0.39 Rf	33.2 AU	0.41 Rf	61.3 AU	1.34 %	0.44 Rf	29.6 AU	1555.2 AU	1.42 %
10	0.44 Rf	29.7 AU	0.48 Rf	181.8 AU	3.98 %	0.54 Rf	60.5 AU	5694.7 AU	5.19 %
11	0.57 Rf	68.9 AU	0.62 Rf	602.8 AU	13.21 %	0.72 Rf	29.1 AU	22848.1 AU	20.81 %
12	0.72 Rf	29.6 AU	0.75 Rf	88.5 AU	1.94 %	0.80 Rf	14.9 AU	2409.2 AU	2.19 %
13	0.80 Rf	15.0 AU	0.85 Rf	77.0 AU	1.69 %	0.92 Rf	0.0 AU	2239.6 AU	2.04 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	59.8 AU	0.00 Rf	230.8 AU	12.82 %	0.06 Rf	18.1 AU	5424.5 AU	7.40 %
2	0.11 Rf	0.3 AU	0.14 Rf	63.7 AU	3.54 %	0.18 Rf	25.1 AU	1438.3 AU	1.96 %
3	0.18 Rf	25.2 AU	0.22 Rf	94.1 AU	5.23 %	0.27 Rf	0.2 AU	2738.1 AU	3.73 %
4	0.29 Rf	0.2 AU	0.35 Rf	155.5 AU	8.63 %	0.38 Rf	57.2 AU	4959.9 AU	6.77 %
5	0.38 Rf	57.9 AU	0.41 Rf	87.5 AU	4.86 %	0.45 Rf	0.7 AU	2125.0 AU	2.90 %
6	0.45 Rf	0.0 AU	0.54 Rf	179.4 AU	9.96 %	0.58 Rf	43.2 AU	7822.7 AU	10.67 %
7	0.58 Rf	44.2 AU	0.66 Rf	378.5 AU	21.02 %	0.75 Rf	71.3 AU	20653.1 AU	28.17 %
8	0.75 Rf	71.5 AU	0.79 Rf	84.3 AU	4.68 %	0.80 Rf	82.2 AU	2722.9 AU	3.71 %
9	0.81 Rf	82.2 AU	0.89 Rf	527.1 AU	29.27 %	1.00 Rf	0.0 AU	25427.6 AU	34.68 %

At 254 nm | **At 366 nm** | **At 575 nm after derivatisation**

Fig. 8: HPTLC fingerprint profile of 8 µl of alcohol extract of three accessions of *Pergularia daemia* (Aerial part)

The HPTLC profile of alcohol extracts of three accessions are compared and the R_f values and colour observed are given in Table 8.

Table 8: R_f values and colour of significant bands of alcohol extract of three accessions of *Pergularia daemia* (Aerial part)

Wavelengths	Accession 1		Accession 2		Accession 3	
	R_f	colour	R_f	colour	R_f	Colour
254 nm	-	-	0.09	Dark green	0.09	Dark green
	0.12	Light green	-	-	0.14	Dark green
	0.16	Dark green	0.17	Light green	-	-
	0.23	Dark green	-	-	0.22	Dark green
	0.30	Light green	0.28	Light green	0.26	Dark green
	0.38	Dark green	0.37	Light green	0.34	Light green
	-	-	0.43	Light green	0.41	Light green
	0.54	Light green	0.52	Light green	0.50	Light green
	0.63	Light green	0.67	Light green	0.62	Light green
	0.75	Dark green	0.76	Dark green	0.71	Light green
	0.87	Light green	0.88	Light green	0.83	Light green
366 nm	0.09	Pink	0.10	Light blue	0.10	Pink
	0.18	Dark pink	0.17	Dark pink	0.17	Dark pink
	0.20	Violet	0.21	Dark pink	0.21	Dark pink
	0.24	Brown	0.25	Dark pink	0.24	Dark pink
	0.27	Dark pink	-	-	-	-
	0.29	Dark pink			-	-
	0.38	Red	0.38	Red	0.34	Brown
	-	-	0.44	Brown	0.41	Brown
	0.46	Red	0.47	Brown	0.48	Brown
	0.52	Red	0.55	Red	-	-
	0.58	Red	0.60	Red	0.62	Bright red
	0.65	Red	0.66	Red	0.69	Light blue
	0.71	Fluorescent blue	0.72	Fluorescent blue	-	-
	0.77	Red	0.79	Red	0.75	Brownish red
	0.87	Red	0.88	Red	0.85	Brownish red
575 nm after derivatisation	-	-	0.09	Light purple	-	-
	0.15	Green	0.18	Light purple	0.14	Green
	0.23	Green	0.23	Blue	0.22	Green
	-	-	0.26	Brown	-	-
	0.38	Light yellow	0.37	Light purple	0.35	Light purple
	0.44	Light purple	0.43	Light purple	0.41	Light purple
	0.58	Light purple	0.58	Light purple	0.54	Light purple
	0.67	Light purple	-	-	0.66	Purple
	0.74	Light purple	0.71	Purple	-	-
	0.77	Light green	-	-	0.79	Light purple
	0.87	Light yellow	-	-	0.89	Purple
	0.93	Light purple	0.93	Light purple		

3. Estimation of metals in the aerial part of *Pergularia daemia*

Estimation of metals in the three accessions of the plant material was carried out using ICP-MS and the results are documented in Table 9.

Table 9: Metal content in *Pergularia daemia* (Aerial part) as estimated by ICP-MS.

Name of plant	Accessions	Metal content (ppm)				
		Pb	Fe	Cu	As	Cd
Pergularia daemia (Aerial part)	**1**	1.56	560.68	132.57	0.09	0.09
	2	2.29	3126.13	39.31	0.14	0.17
	3	0.85	245.20	13.55	0.28	0.08

Three accessions of the plant material studied contain Lead, Iron, Copper, Arsenic & Cadmium in the detectable range. The metal concentrations in every accession of the plant material vary from one another. The Lead content varied from 0.85 ppm to 2.29 ppm, Arsenic from 0.09 to 0.28ppm and Cadmium from 0.08 to 0.17ppm in the different accessions. In the case of Iron content, the second accession showed the highest value and the third accession showed the lowest. The copper in the first accession is too high compared to other accessions. There is no regularity or order in the quantity of metals present among the accessions of the plant.

DISCUSSION

Powder microscopy is the simplest method for establishing the correct identity of the source materials. Unique powder characteristics ensure the accurate botanical authentication of the plant materials. In this plant material, powder characteristics such as rosette calcium oxalate crystals, starch grains, pitted vessels, trichomes etc. were observed. Calcium oxalate crystals on rosette shape were observed in three accessions of *P. daemia*. Crystal formation is usually associated with membranes, chambers, or inclusions within cell vacuoles. Calcium oxalate crystals protect plants against herbivores by their association with irritating chemicals or with proteolytic toxins.[13] The presence and absence of crystals, and dimensions are helpful in the correct identification of crude drugs as well as in the detection of adulterants. Another unique cellular character found in *P. daemia* is the trichome. In the present study, unicellular and multicellular type of trichomes was seen. Trichomes are the specialized epidermal structure which protects the plant from biotic and abiotic stresses.[14] Many types of trichomes are generally seen in plants based on shape, number of cells etc. Likewise, pitted xylem vessel is a diagnostic character observed that acts as the channel for the transport of water and minerals between adjacent cells.[15] Hence these unique powder characteristics ensure the correct botanical authentication of the plant material.

The evaluation of the physical constants of the drug is an essential parameter in detecting adulteration or improper handling of drugs. Ash values are helpful in determining the purity of drugs. Total ash estimates the presence of various inorganic components. Total ash usually consists of carbonates, phosphates, silicates and silica, which includes both physiological ash derived from the plant tissue itself and non-physiological ash, which is the residue of sand and soil adhering to the plant surface.[16] In this study, total ash (10.75%) and acid insoluble ash (4.76%) of accession 3 are high compared to other accessions.The acid insoluble ash measures the amount of silica present; especially sand, indicating contamination with earthy material. These two parameters indicate that the inorganic matter and silica were present in the selected drug. Alcohol and water soluble extractive value for accession 2 is low and that of other accessions are almost the same. Most of the highly polar secondary metabolites are extracted with water and alcohol. The water-soluble extractive value indicates the presence of sugar, acids and inorganic compounds in the plant. The alcohol-soluble extractive values indicate the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc. The extractive values are helpful to evaluate the chemical constituents present in the plants.[17] The test for loss on drying determines both water and volatile matter present in the plants and the moisture content determines only the water content. Loss on drying is high for accession 1 and volatile oil content was found absent in all accessions. The herbal drug should be free from visible signs of contamination by moulds, insects, and other animal contamination, including animal excreta. The presence of contamination may also be due to faulty collection of crude drugs or due to deliberate mixing. Hence, the foreign matter, if any, should be separated from the single drugs during the analysis ensures accurate results.[18] The foreign matter was found to be less than 2% which complies with the guidelines of WHO. P^H of the water extract of accession 3 is 6.2 and that of the other two accessions have values higher than 7 indicating the alkaline nature of the plant extract.

HPTLC is a convenient tool for finding out the distribution pattern of phytoconstituents which is unique to each plant extract. HPTLC profiling of the extract confirms the presence of various phytochemicals. It is an accepted fact that the qualitative analysis of crude herbal extracts constitutes an essential and reliable part of quality control protocol, as any change in the quality of extract directly affects the constituents. From the results obtained by the analysis of this study, it is found that the patterns obtained for all three accessions of the plant extract were somewhat similar and also showed slight variations in the chromatograms. Even though variations occurred, many bands with identical or almost similar R_f values were obtained for all the accessions of the plant. The presence of a band at the same R_f position in the alcohol extract of three accessions of the plant may substantiate the existence of a specific compound.[19] Along with the prominent bands observed, all other colour bands also indicate the presence of various compounds that could attribute the bio efficacy of the plant.[20,21]

The results of the metal content of three accessions of the plant material were compared. The metal content in all three accessions of the plant was observed to vary without any specific pattern. The high Lead level was observed in the second accession (2.29 ppm) and the low Lead level was observed in the third accession (0.85 ppm).The Lead content in all three accessions of the plant was found to be within the permissible limit (<10 ppm), which is in agreement with WHO and FDA guidelines. Similarly, Iron and Cadmium content is high in the second accession (Fe-3126.13ppm and Cd-0.17ppm and low in the third accession (Fe-245.20ppm and Cd-0.08ppm). In the case of Copper content, the first

accession showed a high level of 132.57ppm and the third accession showed a low level of 13.55ppm. Out of three accessions of plant material studied, the third accession has more Arsenic of 0.28 ppm and the first accession has the least value (0.09ppm). The Arsenic and Cadmium content in all three accessions of the plant was found to be within the permissible limit (<3ppm for As and <0.3 ppm for Cd) which is in agreement with WHO and FDA guidelines. The implication of the present findings can be taken into consideration whilst dealing with the medicinal herbs for human or animal consumption. Mineral and trace elements present in considerable amounts are directly or indirectly useful in the management of different diseases.[22]

CONCLUSION

Pharmacognostic evaluation of the aerial part of the three accessions of *Pergularia daemia*, based on anatomical, powder microscopical, physico-chemical and chromatographical studies, established the identity, purity and quality of the studied drug. HPTLC fingerprint patterns unravelled the presence of various phytochemical constituents in the plant material. These results help in the identification and standardization of the drug. Moreover scientifically validated the concentration of the metals present in the plant material using ICP-MS and was found within the international safety limits. This proves the level of safety of the plant - *Pergularia daemia* and makes it acceptable for human and animal consumption at nutritional as well as medicinal levels.

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