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Bindu Indira
Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

Suganya K
Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

Dr. Prakash NS
Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

Dr. Sundaram R
Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

Ilavarasu Arumugam
Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

Corresponding Author:
Bindu Indira
Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru, Karnataka, India

Evaluation of phytoconstituents in *Phyllanthus maderaspatensis* collected from the southern region of Tamil Nadu

Bindu Indira, Suganya K, Dr. Prakash NS, Dr. Sundaram R and Ilavarasu Arumugam

Abstract
Phyllanthus maderaspatensis belongs to Phyllanthaceae family is a highly valued medicinal plant and being used now a days in many polyherbal formulations. The plant being a vital component in the formulation, its quality and consistency is of prime importance. Thus it has become important to analyze certain value standard parameters to give a contribution to the valorisation of this natural source. The present study deals with the variation in the bioactive markers in *P. maderaspatensis* which are collected from different location of Tamil Nadu, South India. Simultaneous comparison of the fingerprints of the plant has been done using TLC technique. Ellagic acid and Rutin were used as reference standards for the identification and quantification of HPLC markers.

Keywords: *Phyllanthus maderaspatensis*, ellagic acid, rutin and analytical variations

1. Introduction
The geographical variations have an impact on active principles in medicinal plants hence, therapeutic efficacy also get influenced. Plant diversity has a considerable importance as a source of pharmaceutically active substance. Environmental conditions affect not only plant growth but also influences secondary metabolites. The medicinal plants show a marked variation in active ingredients during different seasons as these have been widely attributed to variations in environmental variables such as temperature and rainfall. It is difficult to establish quality control, yield and constituents in finished product because of the complex nature and variability in chemical constituent of plants. As the plant material varies in its percentage of active principles, its therapeutic efficacy also varies according to the different places of collection, different time of collection and environmental factors. The amount of phyto constituents varies at different seasons of the year and majority of plants are collected when the plants are at the peak maturity [1]. Many research have demonstrated that plants collected from different geographical regions are diverse in the chemical compositions [2, 3].

Plant 1: *P. maderaspatensis*

P. maderaspatensis is an annual herb, variable in habit, erect or decumbent below; stems low and herbaceous or erect, slender, woody with spreading branches. Leaves 6-25 mm, sub sessile, narrowly or broadly cuneately obovate, glaucous beneath. Flowers small in axillary fascicle with one larger female. Fruit very small, globose, dry. In India *P. maderaspatensis* is widely used for medicinal purposes to treat headache, bronchitis, earache and ophthalmia [4]. Powder from dried plant material mixed with milk is used to treat jaundice [5]. In Kenya, the smoke from the burning plants is used to kill caterpillars in maize. *P. maderaspatensis* is bitter in taste; possess several medicinal properties like astringent, deobstruent, stomachic, diuretic,

febrifugal and antiseptic properties [6]. The leaves are expectorant, diaphoretic useful in strangury and sweats [6]. The seeds have a bad taste used as carminative laxative, astringent to the bowels, tonic to the liver, diuretic, useful in bronchitis, earache, griping, ophthalmia and ascites [7].

The plant has shown anti-bacterial and anti-fungal activities [8]. The seeds have confirmed laxative, carminative and diuretic properties [9]. Many of the medicinal uses of the plant are related to the astringent action of the phytoconstituents. Clear deep yellow oil can be extracted from the seeds. It contains myristic, palmitic, stearic, oleic, linolenic acids and β-sitosterol [9]. The defatted seed cake contains fibrous mucilage which can be hydrolysed to galactose, arabinose, rhamnose and aldobionic acid. Extract of *Phyllanthus* were found to contain resins, steroids, triterpenoids, alkaloids, phenolic compounds, tannins and saponins but no glycosides [10, 11]. The plant also contains lignans namely Phyllanthin and Hypophyllanthin which are responsible for hepato-protective activity but in low concentration [12]. The plant is bitter in taste and possesses several medicinal properties like astringent, stomachic, diuretic, and antiseptic property [13].

The active principles and other constituents of medicinal plants are bound to fluctuate with seasons and geographic regions [14]. Since *P. maderaspatensis* is widely collected in all seasons, it was felt that a study on the geographic variations will identify those changes, if any, occurring in this plant [15]. Though the studies on this plant species are well recognised for medicinal uses, there is no suitable geographical condition to get effective properties [16]. The variation in the content of active markers with respect to geographical variation can be compared with analytical results [16]. Good agricultural and collection practice (GACP) was used for collecting the raw material in suitable geographic area for developing *P. maderaspatensis* as a reliable natural plant source.

2. Materials and Methods

2.1 Collection of plant material

The whole plant of *P. maderaspatensis* were collected from different locations of Tamil Nadu, South India and from different suppliers as shown in Table-1. The plant was identified and authenticated by Dr. Kannan, Botanist, R & D Centre, Himalaya Wellness Company, Bengaluru, Karnataka, India. The voucher specimen of this plant is deposited at the R&D archive centre of Himalaya Wellness Company.

Table 1: Geographical details for the collection of *Phyllanthus maderaspatensis*

Sl. No.	NPD Numbers	Parts collected	Geography	Source of the material	Colour	Soil type	Lat & Long.
1.	NPD/260/15	Whole plant	Enthan Patti	Agriculture land	Dull green	Black Soil	N 09°41.159' E 078°00.209'
2.	NPD/262/15	Whole plant	Melpatti	Agriculture land	Dull green	Black Soil	N 09°41.980' E 078°59.340'
3.	NPD/263/15	Whole plant	Odapatti	Agriculture land	Dull green	Black Soil	N 09°46.079' E 077°54.401'
4.	NPD/264/15	Whole plant	Sennampatti	Agriculture land	Dull green	Black Soil	N 09°40.814' E 078°00.455'
5.	NPD/265/15	Whole plant	Sennampatti	Agriculture land	Dull green	Black Soil	N 09°40.814' E 078°00.455'
6.	NPD/266/15	Whole plant	Vanjipattu	Agriculture land	Dull green	Black Soil	N 09°40.264' E 078°00.817'
7.	NPD/267/15	Whole plant	Painkulam	Agriculture land	Dull green	Black Soil	N 09°39.553' E 078°00.289'
8.	NPD/268/15	Whole plant	Vinivetti	Agriculture land	Dull green	Black Soil	N 09°38.681' E 077°59.203'
9.	NPD/269/15	Whole plant	Nallamanaickenpatti	Agriculture land	Dull green	Black Soil	N 09°39.544' E 077°57.935'
10.	NPD/270/15	Whole plant	Maittanpatti	Agriculture land	Dull green	Black Soil	N 09°40.230' E 077°57.734'
11.	NPD/271/15	Whole plant	Perumal Patti	Agriculture land	Dull green	Black Soil	N 09°42.052' E 078°00.401'
12.	NPD/272/15	Whole plant	S.P. Natham	Agriculture land	Dull green	Black Soil	N 09°43.223' E 078°00.056'
13.	NPD/274/15	Whole plant	Manthoppu	Agriculture land	Dull green	Black Soil	N 09°38.687' E 077°59.191'
14.	NPD/275/15	Whole plant	Karisalkalampatti	Agriculture land	Dull green	Black Soil	N 09°45.892' E 077°58.353'
15.	NPD/276/15	Whole plant	Sengipatti	Agriculture land	Dull green	Black Soil	N 09°45.027' E 077°58.161'
16.	NPD/278/15	Whole plant	T. Kallupatti	Agriculture land	Dull green	Black Soil	N 09°42.484' E 077°52.413'
17.	NPD/814/15	Whole plant	Narsingh herbals	unknown	Dull green	Unknown	Unknown
18.	NPD/410/16	Whole plant	Salaipatti	Agriculture land	Dull green	Not Known	NA
19.	NPD/222/15	Whole plant	SSK	unknown	Dull green	Unknown	Unknown
20.	NPD/813/15	Whole plant	Narsingh herbals	unknown	Dull green	Unknown	Unknown
21.	NPD/537/15	Whole plant	GMCL	unknown	Dull green	Unknown	Unknown
22.	NPD/152S/15	Stem	GMCL	unknown	Dull green	Unknown	Unknown
23.	NPD/154/16	Whole plant	Karisalkalampatti	Agriculture lands near village roads	Dull green	Black Soil	N 09°44.921' E 077°58.067'
24.	NPD/155/16	Whole plant	Sengapadai	Agriculture lands near village roads	Dull green	Black cotton Soil	N 09°45.468' E 077°56.685'
25.	NPD/156/16	Whole plant	Agathapatti	Agriculture lands near village roads	Dull green	Black cotton Soil	N 09°42.240' E 077°57.634'
26.	NPD/189/16	Whole plant	Odapatti	Agriculture lands near village roads	Dull green	Black Soil	N 09°41.244' E 077°59.971'
27.	NPD/160/16	Whole plant	Pullangal	Agriculture lands near village roads	Dull green	Black Soil	N 09°18.120' E 078°18.269'
28.	NPD/411/16	Whole plant	Vadakkampatti	Agriculture land	Dull green	Not Known	
29.	NPD/196/16	Whole plant	Poonam herbs	unknown	Dull green	Unknown	Unknown
30.	NPD/153/16	Whole plant	T. Kunnathur	Agriculture lands near village roads	Dull green	Black cotton Soil	N 09°45.408' E 077°53.705'
31.	NPD/412/16	Whole plant	Odaipatti	Agriculture land	Dull green	Not Known	NA
32.	NPD/178/16	Whole plant	Kallikudi	Agriculture lands near village roads	Dull green	Black Cotton Soil	N 09°42.221' E 077°58.317'
33.	NPD/159/16	Whole plant	Thuthinatham	Agriculture lands near village roads	Dull green	Black Soil	N 09°15.912' E 078°17.971'
34.	NPD/164/16	Whole plant	Karaikeni	Agriculture lands near village roads	Dull green	Black Clayey	N 09°42.728' E 077°51.823'
35.	NPD/166/16	Whole plant	Vannivelampatti	Agriculture lands near village roads	Dull green	Black Soil	N 09°44.391' E 077°60.983'
36.	NPD/168/16	Whole plant	Manthoppu	Agriculture lands near village roads	Dull green	Black Soil	N 09°38.606' E 078°01.839'
37.	NPD/169/16	Whole plant	Thandaiyarpettai	Agriculture lands near village roads	Dull green	Black Clayey	N 09°42.686' E 077°54.169'
38.	NPD/152/16	Whole plant	Ammapatti	Agriculture lands near village roads	Dull green	Black cotton Soil	N 09°42.732' E 077°50.305'
39.	NPD/152A/16	Aerial parts	Ammapatti	Agriculture lands near village roads	Dull green	Black cotton Soil	N 09°42.732' E 077°50.305'
40.	NPD/164A/16	Aerial parts	Karaikeni	Agriculture lands near village roads	Dull green	Black Clayey	N 09°42.728' E 077°51.823'
41.	NPD/152R/16	Root parts	Ammapatti	Agriculture lands near village roads	Brown	Black cotton Soil	N 09°42.732' E 077°50.305'
42.	NPD/164R/16	Root parts	Karaikeni	Agriculture lands near village roads	Dull green	Black Clayey	N 09°42.728' E 077°51.823'
43.	NPD/164S/16	Stem	Karaikeni	Agriculture lands near village roads	Dull green	Black Clayey	N 09°42.728' E 077°51.823'
44.	NPD/152S/16	Stem	GMCL	unknown	Dull green	Unknown	Unknown

2.2 Drying process
The aerial parts, stem and root are segregated from the whole plant of the freshly collected *Phyllanthus maderaspatensis.* The segregated plant parts and the whole plant were washed and dried under sunlight. In the present study forty-four samples were analysed in which thirty-seven whole plant, two aerial parts, two roots and three stems were studied.

2.3 Powdering of the material
The dried plant parts and whole plant of *P. maderaspatensis* were ground using a rotary grinder, sieved through 25 mesh sieves and stored in airtight HDPE container at room temperature. To minimize the analytical variations, the powdered samples were used throughout the study for the physico-chemical and phytochemical evaluation.

2.4 Chemicals and Reagents
All the chemicals used were Analytical reagent grade or HPLC grade. Trifluoroacetic acid was procured from Merck Life Science Pvt. Ltd., acetonitrile from Fischer Scientific, aluminium chloride from SD Fine Chemicals, ethyl acetate, acetic acid, and formic acid from Rankem, methanol from Standard Reagents Pvt. Ltd. and ethanol from Honey Intl, Inc,. Purified water which is used for the preparation of mobile phase is from Milli-Q water purification system (Millipore, Pure Lab, Classic, ELGA). Filtration membranes (0.45 µm, cellulose acetate/cellulose nitrate mixed esters) were purchased from Millipore. The standard of Rutin hydrate and Ellagic acid is procured from Sigma Aldrich.

2.5 Physico-chemical analysis
2.5.1 Loss on drying
10g of powdered sample was transferred in a tared evaporating dish and dried at 105 ºC for 5 hours and weighed. The process was continued until difference between two successive weights corresponded to not more than 0.25%.

2.5.2 Total ash
2g of powdered material was weighed in a previously weighed silica crucible and incinerated, gently at first, and gradually increased the temperature to 675 ºC until free from carbon.

2.5.3 Acid insoluble ash
The ash obtained was treated with 25 ml of 2M hydrochloric acid and boiled for 5 minutes. The insoluble matter was collected, washed with hot water and ignited at 600 ºC in a silica crucible.

2.5.4 Water soluble extractive value
5g of powdered material was macerated with 100 ml of chloroform-water mixture in a closed flask for 24 hours, continuously shaken for 6 hours and allowed to stand for 18 hours. The solution was filtered, and 25 ml of the filtrate was dried completely in a tared flat-bottomed shallow dish at 105 ˚C and weighed which gives the percentage of water-soluble extractive value.

2.5.5 Alcohol soluble extractive value
5g of powdered material was macerated with 100 ml of absolute alcohol in a closed flask for 24 hours. It was continuously shaken for 6 hours and kept still for 18 hours. The solution was filtered, 25 ml of the filtrate was transferred into a flat-bottomed flask and dried at 105 ºC and weighed which gives the alcohol soluble extractive value.

2.6 Total Flavonoids by UV
Total flavonoids were estimated by colorimetry (Shimadzu UV 1700 series) using rutin as a standard. Flavonoids give a yellow colour complex when treated with aluminium chloride reagent. The samples were extracted with methanol and subjected to reaction with aluminium chloride reagent. The intensity of the colour complex was measured at 410 nm against the prepared reagent blank.

2.7 HPLC analysis
A method coupling high performance liquid chromatography (HPLC) with photo diode array detector (PDA) was used for the separation and identification of ellagic acid and rutin in *P. maderaspatensis*. Shimadzu HPLC prominence I equipped with a photo diode array detector, SIL-20ACHT auto sampler, DGU-20A5 degasser, LC-20AD pump, CBM-20 A system controller, CTO-10 ASVP oven and LC solutions software was used. The results indicated that the best chromatographic condition was achieved using 0.1% v/v trifluoro acetic acid in water (mobile phase A) and 0.1% v/v trifluoro acetic acid in acetonitrile and water in the ratio 70:30 (mobile phase B). The linear gradient elution was performed with the following ratio of mobile phase B (0%, 15 minutes, 10-30%, 15-18 minutes, 30-40%, 18-22 minutes, 40-80%, 22-26 minutes, 80%, 26-32 minutes) at a flow rate of 1 ml/minute with a column oven temperature of 35 °C. Ellagic acid and Rutin were identified and quantified by comparing the chromatographic peaks with the retention time of the standard at the wavelength of 254 nm and 354 nm respectively (Figures 1 to 10).

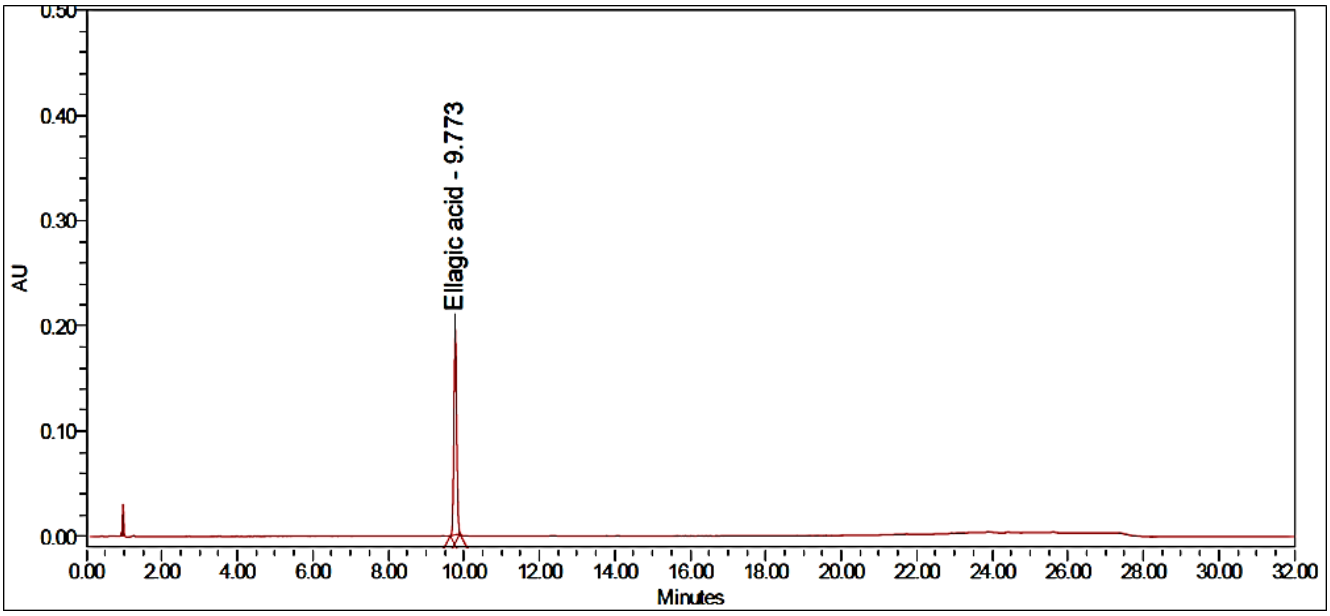


Fig 1: HPLC chromatogram of Standard Ellagic acid at 254 nm.

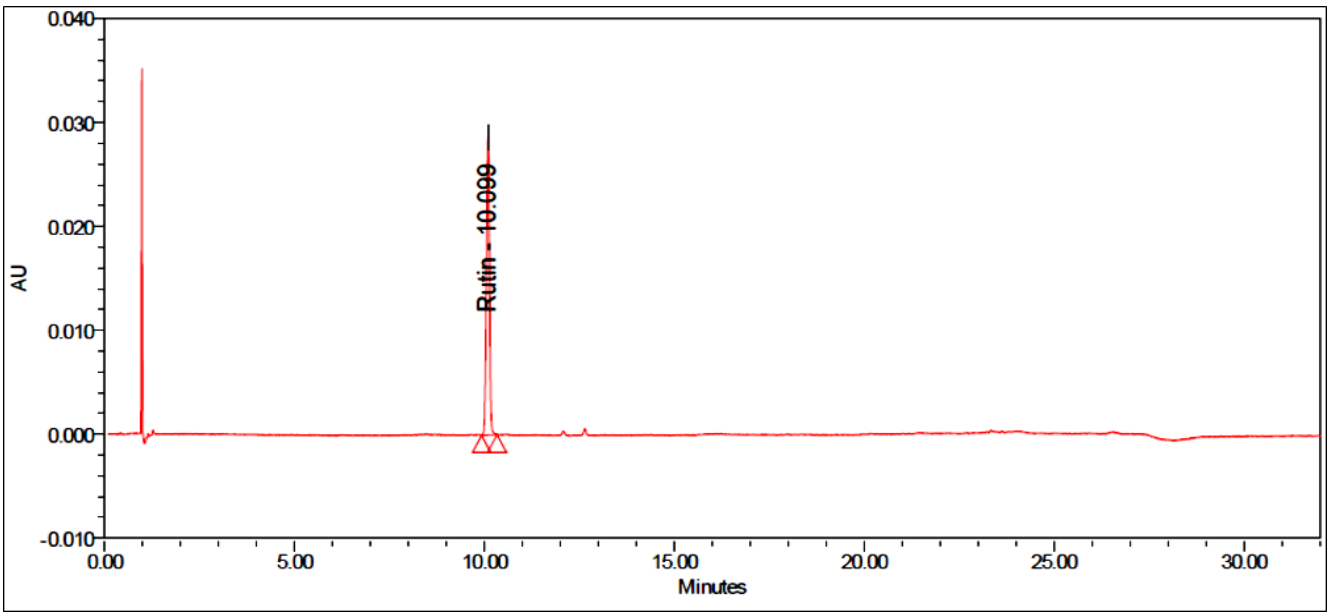


Fig 2: HPLC chromatogram of Standard Rutin at 354 nm

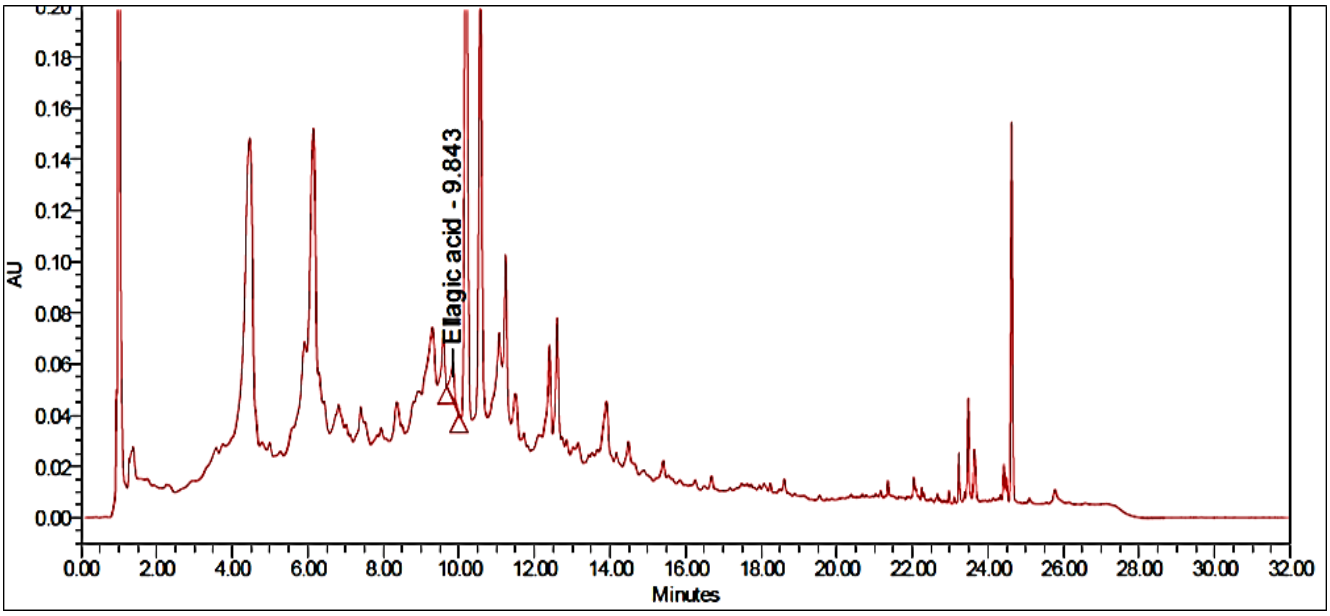


Fig 3: HPLC chromatogram of Ellagic acid in *P. maderaspatensis* whole plant (NPD/278/15)

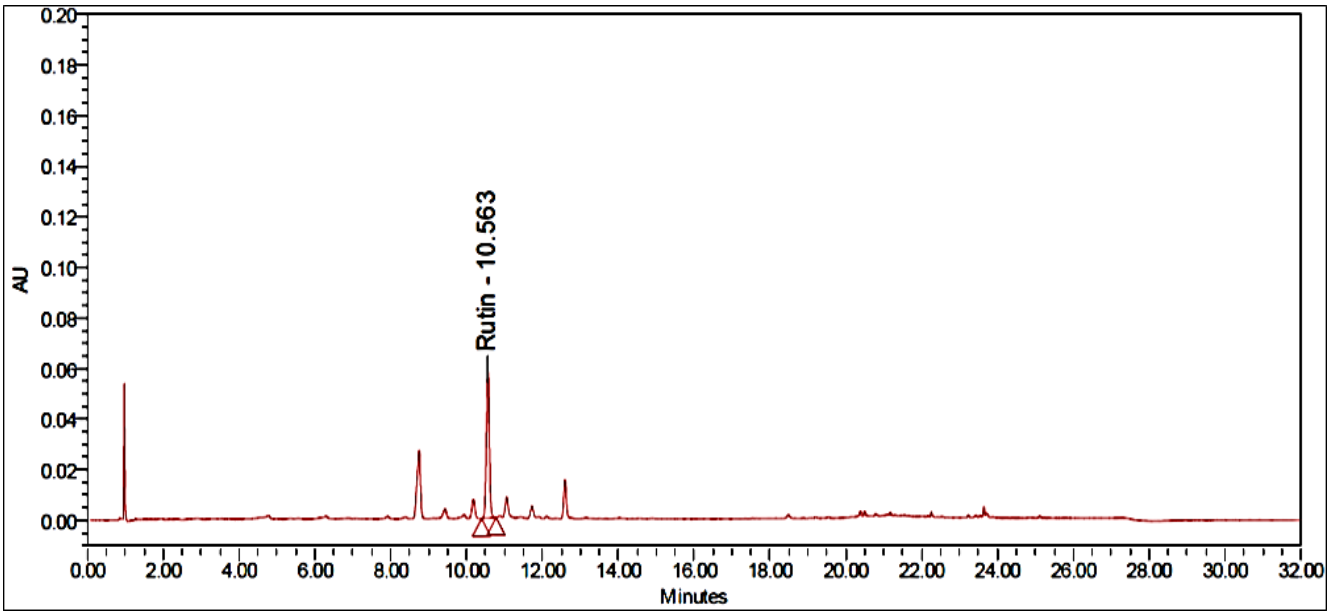


Fig 4: HPLC chromatogram of Rutin in *P. maderaspatensis* whole plant (NPD/196/16)

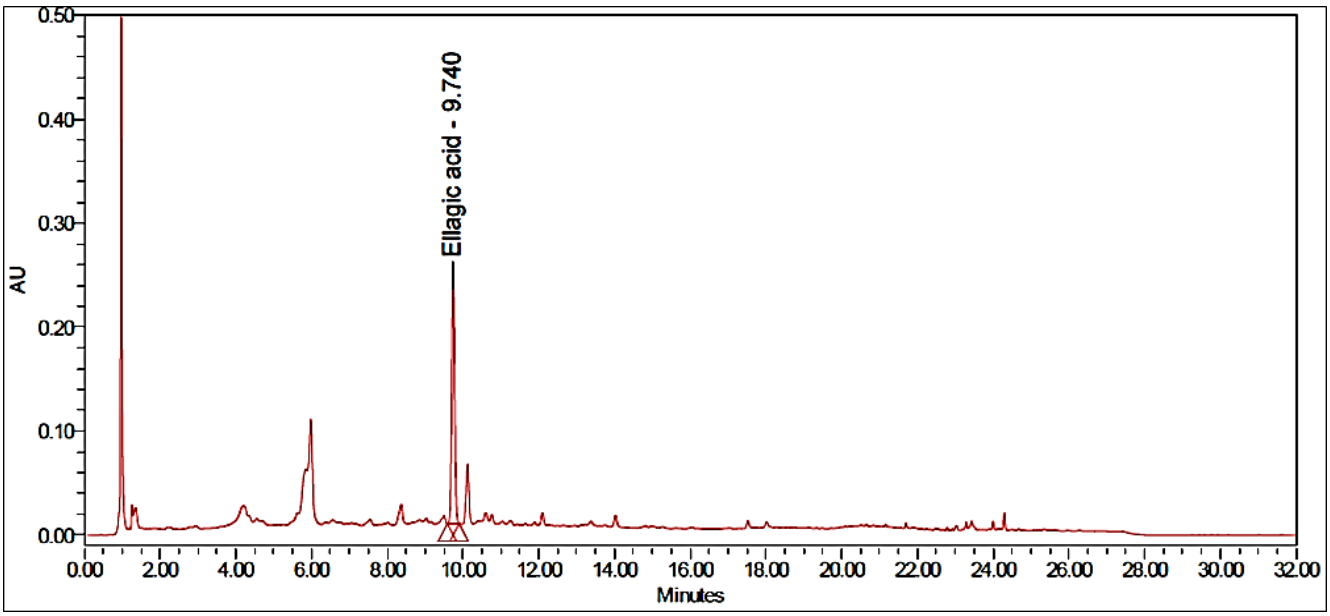


Fig 5: HPLC chromatogram of Ellagic acid in *P. maderaspatensis* aerial parts (NPD/164A/16)

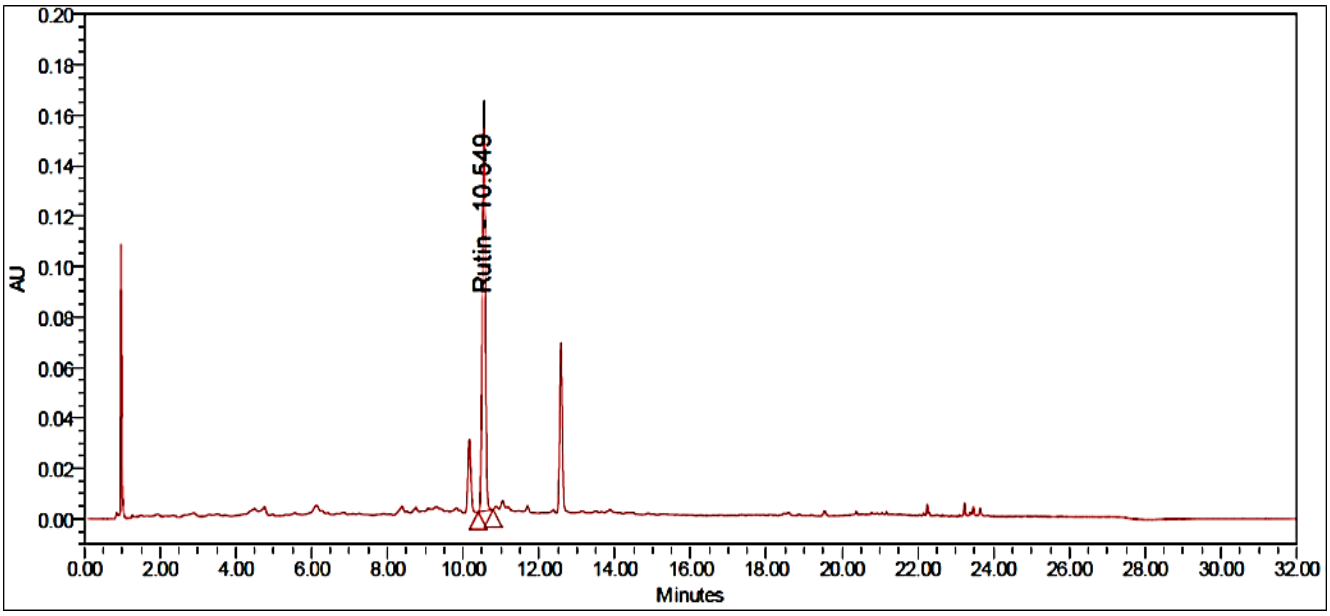


Fig 6: HPLC chromatogram of Rutin in *P. maderaspatensis* aerial parts (NPD/164A/16)

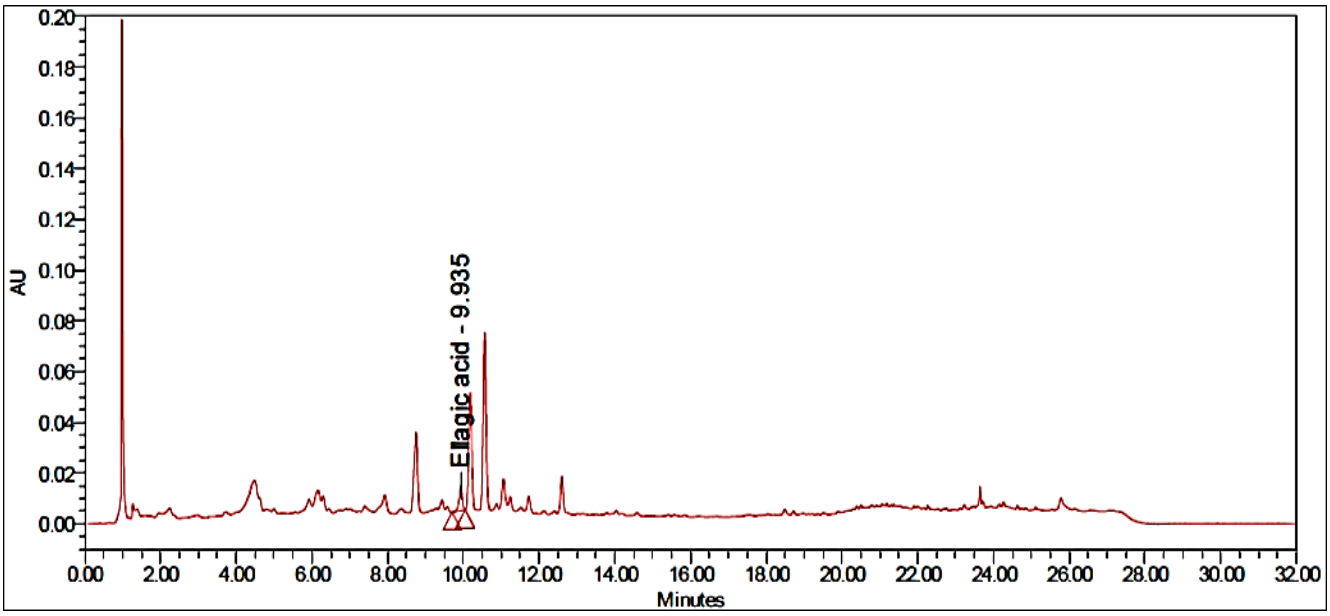


Fig 7: HPLC chromatogram of Ellagic acid in *P. maderaspatensis* stem (NPD/152S/115)

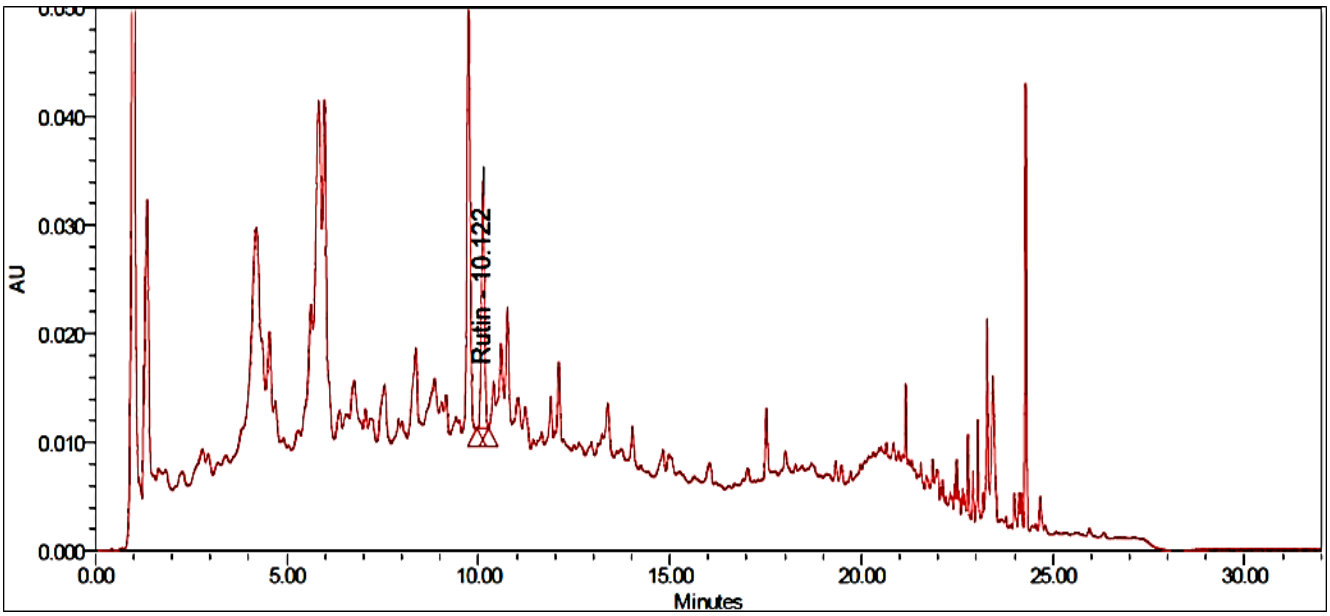


Fig 8: HPLC chromatogram of Rutin in *P. maderaspatensis* stem (NPD/152S/16)

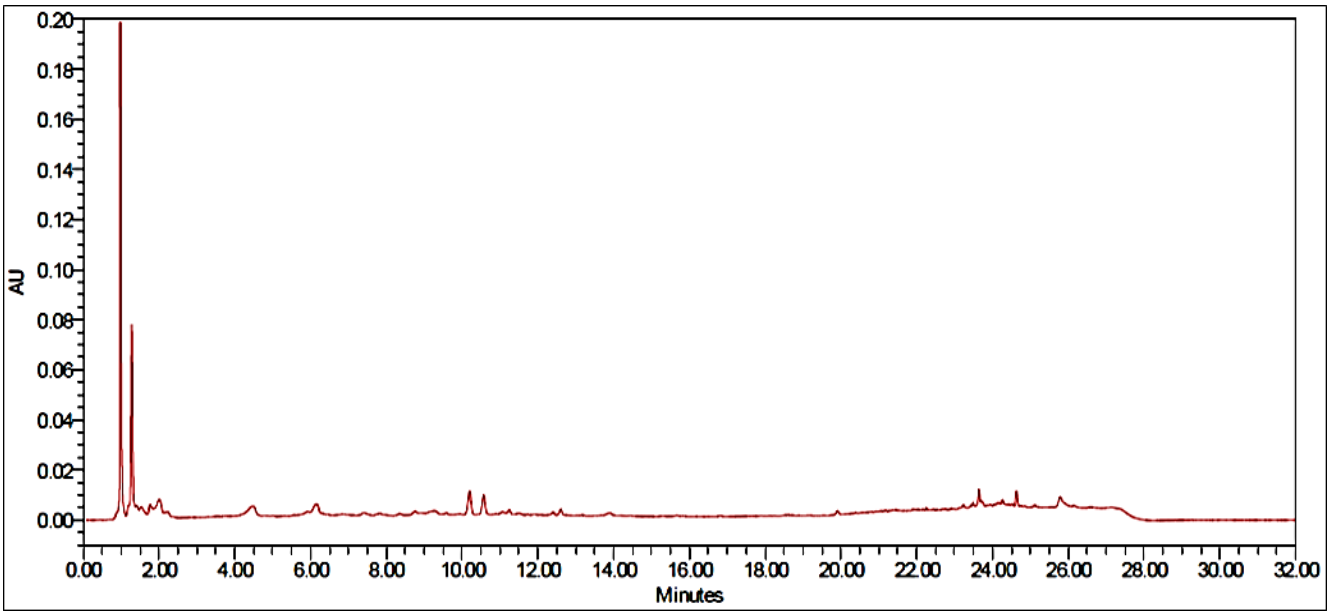


Fig 9: HPLC chromatogram of Ellagic acid in *P. maderaspatensis* root (NPD/152R/16)

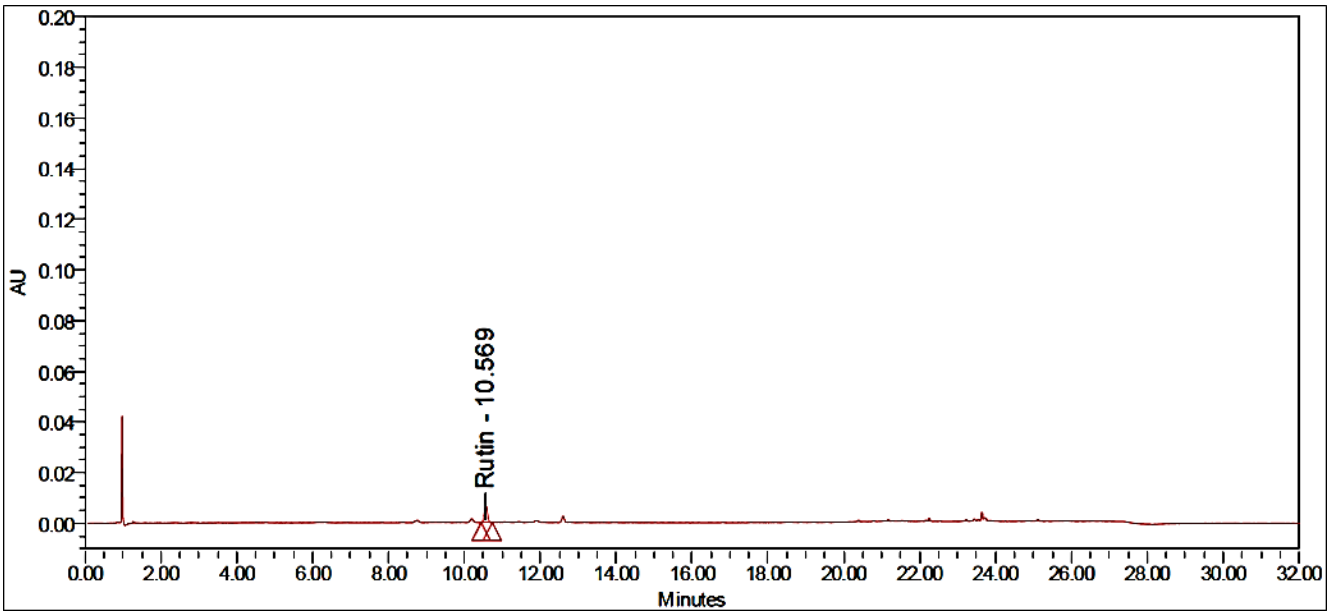


Fig 10: HPLC chromatogram of Rutin in *P. maderaspatensis* root (NPD/152R/16)

2.8 TLC analysis

A CAMAG TLC system with winCATS software including photo documentation unit, densitometric scanner and TLC spotter was used for the study. TLC parameters for plate layout, sample application, conditioning of the plate, development and visualization were in line with USP general chapter < 203 >.

The test solutions and standard rutin and ellagic acid were prepared by dissolving in methanol. The samples were spotted on precoated silica gel plate (Merck) using CAMAG Linomat V sample applicator. The mobile phase employed was Ethyl acetate: Acetic acid: Formic acid: Water in the ratio of 100:11:11:26. The plates were developed and visualized under UV 254 nm, 366 nm and at 366 nm after derivatization with aluminium chloride reagent.

3. Results and Discussion

P. maderaspatensis were collected from different geographical conditions and different variance such as associated species, source of material, main crop, colour, soil type, latitude and longitude has been considered for the collection of the plant as shown in Table 1. The study has been conducted in whole plant, aerial parts, stem and root.

3.1 Physico-chemical analysis

The physico-chemical analysis was performed as per the method mentioned in section 2.5. The physico-chemical parameters include loss on drying, total ash, acid insoluble ash, and extractive values. These parameters are screened with the reference to tests on herbal products of Indian Pharmacopoeia (2.6). The findings are depicted in Table 2. The minimum and maximum water extractive value for the whole plant was observed as 8.54% (NPD/270/15) and 22.11% (NPD/196/16) respectively. In aerial parts, the percentage of water extractive value was found to be 7.01% and 7.20% whereas in stem, it was 6.98%, 6.92% and 6.82% respectively. In the root samples, the percentage of water extractive value found was 6.98% and 7.05%.

The alcohol extractive value analysed in the whole plant was found maximum (21.31%) in the NPD/265/15 and minimum was observed in NPD/178/16 (4.38%). In aerial parts, the percentage of alcoholic extractive value was found to be 5.60% and 6.10% whereas in stem, it was 7.55%, 4.59% and 4.42% respectively. In the root samples, the percentage of alcohol extractive value observed was 5.80% and 5.60%.

In the whole plant, the highest percentage of ash value observed was 10.22% (NPD/264/15) and lowest was 3.20% (NPD/168/16). The maximum value of acid insoluble ash was 4.76% (NPD/268/15) and minimum 0.13% (NPD/537/15). The higher and lower value for loss on drying observed was 9.68% (NPD/410/15) and 6.46% (NPD/268/15 & NPD/271/15) respectively.

In the aerial parts, the percentage of ash value estimated was 3.20% and 4.01% whereas in stem it was 1.78%, 2.99% and 2.23%. In roots, the percentage of ash calculated was 4.25% and 5.2% and in stem it was 1.78%, 2.99% and 2.23%.

In aerial parts, the amount of acid insoluble ash found was 0.81% and 0.98%. The observation of percentage of acid insoluble ash in root was 0.89% and 0.75% whereas in stem it was 0.34%, 0.29% and 0.26%.

In the aerial parts, the percentage of moisture estimated was 6.85% and 6.25% whereas in stem it was 7.22%, 7.90% and 6.12%. In roots, the percentage of moisture calculated was 5.98% and 6.02%.

The physico-chemical analysis data were compared with the various standard limits of Ayurvedic Pharmacopoeia of India and quality standards of Indian Medicinal Plants. The data reveals that the samples comply with the standards.

Table 2: Physico-chemical analysis data of *Phyllanthus maderaspatensis*

S. No.	Batch no	Description	Water extractive value (% w/w)	Alcohol extractive value (% w/w)	Ash value (% w/w)	Acid insoluble Ash (% w/w)	Loss on drying (% w/w)
1.	NPD/260/15	Whole plant	14.26	12.85	7.61	0.40	7.29
2.	NPD/262/15	Whole plant	13.48	16.39	6.87	1.73	8.38
3.	NPD/263/15	Whole plant	11.88	13.48	5.84	1.77	7.64
4.	NPD/264/15	Whole plant	13.64	13.60	10.22	4.57	7.82
5.	NPD/265/15	Whole plant	16.18	21.31	9.18	4.08	8.36
6.	NPD/266/15	Whole plant	14.36	15.06	4.69	1.33	7.29
7.	NPD267/15	Whole plant	14.31	14.66	6.50	2.75	7.92
8.	NPD/268/15	Whole plant	16.97	10.89	9.73	4.76	6.46
9.	NPD/269/15	Whole plant	17.81	15.46	5.80	1.56	7.97
10.	NPD/270/15	Whole plant	8.54	10.75	6.58	3.16	7.55
11.	NPD/271/15	Whole plant	12.57	10.89	4.55	0.94	6.46
12.	NPD/272/15	Whole plant	11.04	8.53	5.56	1.18	7.98
13.	NPD/274/15	Whole plant	11.80	13.62	5.73	2.26	7.68
14.	NPD/275/15	Whole plant	11.69	9.13	4.79	0.86	8.22
15.	NPD/276/15	Whole plant	14.68	8.15	6.02	2.10	8.51
16.	NPD/278/15	Whole plant	14.68	7.86	5.89	0.74	8.13
17.	NPD/154/16	Whole plant	10.46	5.63	3.31	0.43	7.80
18.	NPD/155/16	Whole plant	15.84	8.21	4.06	0.33	7.53
19.	NPD/814/15	Whole plant	16.26	6.62	3.35	0.16	7.55
20.	NPD/410/15	Whole plant	8.73	6.46	4.43	1.00	9.68
21.	NPD/156/16	Whole plant	9.17	4.98	3.49	0.96	7.29
22.	NPD/222/15	Whole plant	12.68	8.47	3.52	0.81	7.51
23.	NPD/189/16	Whole plant	21.35	9.90	4.68	0.78	7.99
24.	NPD/160/16	Whole plant	15.36	9.79	3.49	0.3	7.82
25.	NPD/411/16	Whole plant	17.38	9.42	3.82	0.51	9.12
26.	NPD/196/16	Whole plant	22.11	10.16	4.47	0.26	8.00
27.	NPD/153/16	Whole plant	18.65	7.07	4.17	0.41	7.19
28.	NPD/813/15	Whole plant	19.39	5.63	6.13	2.67	8.19
29.	NPD/412/16	Whole plant	13.56	5.74	4.11	0.57	9.12
30.	NPD/178/16	Whole plant	17.12	4.38	6.65	2.59	9.16
31.	NPD/159/16	Whole plant	16.45	6.86	4.18	0.69	7.93
32.	NPD/164/16	Whole plant	14.91	7.82	5.09	1.23	7.65
33.	NPD/166/16	Whole plant	11.89	5.27	4.12	0.36	7.39
34.	NPD/168/16	Whole plant	14.54	7.06	3.20	0.32	7.80
35.	NPD/169/16	Whole plant	11.52	6.68	3.36	0.65	7.82
36.	NPD/537/15	Whole plant	12.01	6.46	4.41	0.13	8.2
37.	NPD/152/16	Whole plant	11.83	9.41	3.46	0.92	7.45
38.	NPD/152A/16	Aerial parts	7.01	5.60	3.2	0.81	6.85
39.	NPD/164A/16	Aerial parts	7.20	6.10	4.01	0.98	6.25
40.	NPD/152R/16	Root	6.98	5.80	4.25	0.89	5.98
41.	NPD/164R/16	Root	7.05	5.60	5.2	0.75	6.02
42.	NPD/152S/15	Stem	6.98	7.55	1.78	0.34	7.22
43.	NPD/152S/16	Stem	6.92	4.59	2.99	0.29	7.90
44.	NPD/164S/16	Stem	6.82	4.42	2.23	0.26	6.12

3.2 Total flavonoids

Flavonoids are phytoconstituents with a wide spectrum of health promoting effect and are widely used for different biological applications. The content of flavonoids was estimated by calorimetry using spectrophotometric method. The addition of aluminium chloride to the plant extract gives a yellow colour complex with flavonoids which can be measured in a spectrophotometer. The estimated content of total flavonoids is shown in Table 3. The aerial part (NPD/164A/16) collected from Karaikeni was found rich in flavonoids (1.11%) when compared with the other samples and plant parts. The minimum and maximum percentage of total flavonoids in the whole plant was observed as 0.30% (NPD/152/16) and 0.96% (NPD/265/15) respectively. Both the root samples had shown a negligible quantity of total flavonoids as 0.05%. The total flavonoids content in stem sample were 0.402%, 0.416% and 0.304%.

The analytical data discloses that the aerial parts are likely to have a high content of total flavonoids, while the root contains a negligible quantity. The whole plant and stem comprise a moderate amount of total flavonoids.

Table 3: Content of Total Flavonoids in *Phyllanthus maderaspatensis*

S. No.	Batch No.	Description	Total Flavonoids (% w/w)
1.	NPD/260/15	Whole plant	0.590
2.	NPD/262/15	Whole plant	0.870
3.	NPD/263/15	Whole plant	0.720
4.	NPD/264/15	Whole plant	0.810
5.	NPD/265/15	Whole plant	0.960
6.	NPD/266/15	Whole plant	0.390
7.	NPD267/15	Whole plant	0.750
8.	NPD/268/15	Whole plant	0.330
9.	NPD/269/15	Whole plant	0.880
10.	NPD/270/15	Whole plant	0.560
11.	NPD/271/15	Whole plant	0.660
12.	NPD/272/15	Whole plant	0.310
13.	NPD/274/15	Whole plant	0.550
14.	NPD/275/15	Whole plant	0.500
15.	NPD/276/15	Whole plant	0.510
16.	NPD/278/15	Whole plant	0.440
17.	NPD/154/16	Whole plant	0.396
18.	NPD/155/16	Whole plant	0.592
19.	NPD/814/15	Whole plant	0.493
20.	NPD/410/15	Whole plant	0.667
21.	NPD/156/16	Whole plant	0.403
22.	NPD/222/15	Whole plant	0.363
23.	NPD/189/16	Whole plant	0.665
24.	NPD/160/16	Whole plant	0.661
25.	NPD/411/16	Whole plant	0.545
26.	NPD/196/16	Whole plant	0.639
27.	NPD/153/16	Whole plant	0.461
28.	NPD/813/15	Whole plant	0.618
29.	NPD/412/16	Whole plant	0.518
30.	NPD/178/16	Whole plant	0.752
31.	NPD/159/16	Whole plant	0.514
32.	NPD/164/16	Whole plant	0.571
33.	NPD/166/16	Whole plant	0.498
34.	NPD/168/16	Whole plant	0.569
35.	NPD/169/16	Whole plant	0.452
36.	NPD/537/15	Whole plant	0.396
37.	NPD/152/16	Whole plant	0.304
38.	NPD/152A/16	Aerial parts	0.850
39.	NPD/164A/16	Aerial parts	1.11
40.	NPD/152R/16	Root parts	0.050
41.	NPD/164R/16	Root parts	0.050
42.	NPD/152S/15	Stem	0.405
43.	NPD/164S/16	Stem	0.416
44.	NPD/152S/16	Stem	0.304

3.3 HPLC analysis

The HPLC analysis of *P. maderaspatensis* revealed the presence of phenolic acid and flavonoid as depicted in Table 4. The phenolic acid and flavonoid were quantified as ellagic acid and rutin respectively. The higher content of ellagic acid (0.181%) was observed in the whole plant (NPD/278/15) collected from Ammapatti and lower content (0.013%) was from the whole plant (NPD/222/15) procured from SSK supplier. Among all the plant parts studied, the content of ellagic acid was found maximum in whole plant followed by aerial parts. The amount of ellagic acid observed in aerial parts is 0.13% and 0.090%. The analytical report of *P. maderaspatensis* revealed that stem contains negligible amount of ellagic acid (0.016%, 0.008% and 0.009%). Additionally, the data exposes that the root does not contain ellagic acid.

The maximum (0.49%) and minimum (0.018%) amount of rutin was found in the aerial parts (NPD/164A/16) and roots (NPD/164R/16) sample collected from Karaikudi. In the whole plant, minimum rutin content (0.040%) was found in the plant (NPD/272/15) collected from S.P. Natham and maximum (0.448%) was shown in the plant (NPD/196/16) procured from Poonum herbs. In stem, content of rutin observed was 0.129%, 0.106% and 0.158%. In root, amount of rutin observed was 0.020% and 0.018%.

The HPLC analysis furnishes the evidence that the content of rutin is higher than ellagic acid, irrespective of plant parts. The study also reveals that rutin and ellagic acid is abundant in aerial parts and whole plant respectively. The stem part of *P. maderaspatensis* has shown the moderate quantity of both rutin and ellagic acid. The result revealed that root does not contain ellagic acid and rutin and in some of the root sample analyzed has shown the traces of ellagic acid and rutin.

Table 4: HPLC analysis data of *Phyllanthus maderaspatensis*

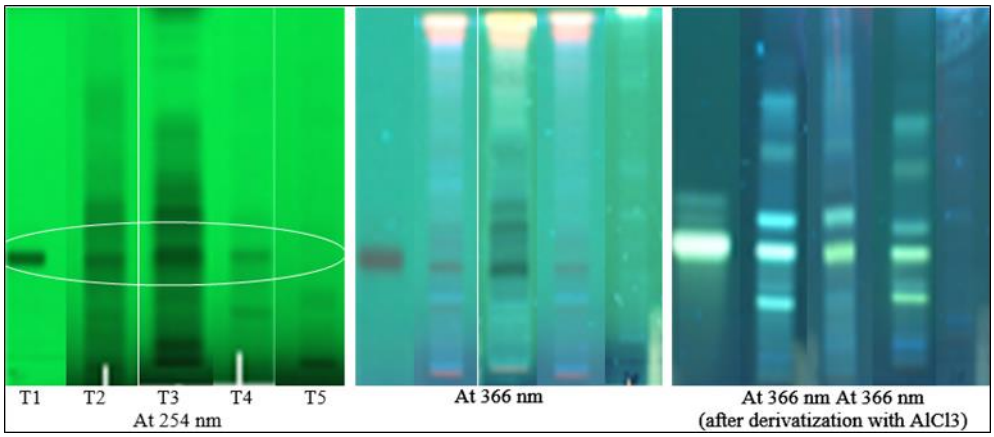
S. No.	Batch no	Description	Ellagic acid (%)	Rutin (%)
1.	NPD/260/15	Whole plant	0.042	0.134
2.	NPD/262/15	Whole plant	0.061	0.328
3.	NPD/263/15	Whole plant	0.060	0.316
4.	NPD/264/15	Whole plant	0.059	0.168
5.	NPD/265/15	Whole plant	0.067	0.198
6.	NPD/266/15	Whole plant	0.019	0.087
7.	NPD267/15	Whole plant	0.037	0.126
8.	NPD/268/15	Whole plant	0.029	0.195
9.	NPD/269/15	Whole plant	0.046	0.146
10.	NPD/270/15	Whole plant	0.027	0.075
11.	NPD/271/15	Whole plant	0.083	0.108
12.	NPD/272/15	Whole plant	0.092	0.040
13.	NPD/274/15	Whole plant	0.046	0.091
14.	NPD/275/15	Whole plant	0.053	0.151
15.	NPD/276/15	Whole plant	0.104	0.066
16.	NPD/278/15	Whole plant	0.181	0.062
17.	NPD/154/16	Whole plant	0.028	0.151
18.	NPD/155/16	Whole plant	0.026	0.257
19.	NPD/814/15	Whole plant	0.032	0.293
20.	NPD/410/15	Whole plant	0.057	0.304
21.	NPD/156/16	Whole plant	0.023	0.203
22.	NPD/222/15	Whole plant	0.013	0.096
23.	NPD/189/16	Whole plant	0.045	0.317
24.	NPD/160/16	Whole plant	0.031	0.256
25.	NPD/411/16	Whole plant	0.025	0.190
26.	NPD/196/16	Whole plant	0.059	0.448
27.	NPD/153/16	Whole plant	0.036	0.233
28.	NPD/813/15	Whole plant	0.058	0.285
29.	NPD/412/16	Whole plant	0.022	0.154
30.	NPD/178/16	Whole plant	0.078	0.415
31.	NPD/159/16	Whole plant	0.045	0.298
32.	NPD/164/16	Whole plant	0.030	0.245
33.	NPD/166/16	Whole plant	0.022	0.224
34.	NPD/168/16	Whole plant	0.024	0.233
35.	NPD/169/16	Whole plant	0.021	0.194
36.	NPD/537/15	Whole plant	0.014	0.152
37.	NPD/152/16	Whole plant	0.019	0.116
38.	NPD/152A/16	Aerial parts	0.13	0.480
39.	NPD/164A/16	Aerial parts	0.090	0.490
40.	NPD/152R/16	Root parts	0.000	0.020
41.	NPD/164R/16	Root parts	0.000	0.018
42.	NPD/152S/15	Stem	0.016	0.129
43.	NPD/164S/16	Stem	0.008	0.106
44.	NPD/152S/16	Stem	0.009	0.158

3.4 TLC fingerprint

Thin layer chromatography (TLC) fingerprinting technique is an internationally accepted and popular tool used for the identification of herbs and herbal products. Each plant has a unique set of phytoconstituents and these compounds can be identified by TLC using a stationary phase and suitable solvent system (mobile phase).

The quality of the herbal materials can be assessed by the TLC fingerprint by finding out the variation in the phytoconstituents in the different batches of the plant material. In this study the variation of the phytoconstituents in the whole plant, aerial parts, stem and root was evaluated using rutin as a reference analytical marker.

The TLC fingerprint of whole plant, aerial parts, stem and root have compared and found that intensity of rutin was maximum in aerial parts followed by whole plant, stem and root which is depicted in Figure-11.



T1: Standard Rutin.
T2: Whole plant of *Phyllanthus maderaspatensis*.
T3: Aerial parts of *Phyllanthus maderaspatensis*.
T4: Stem of *Phyllanthus maderaspatensis*.
T5: Root of *Phyllanthus maderaspatensis*.

Fig 11: TLC fingerprint of *Phyllanthus maderaspatensis* showing the presence of rutin

4. Conclusion

Evaluation of medicinal plants through phytochemical analysis is the preliminary step for the authenticity and quality of every crude drug. The analytical data can provide important information about the raw material collection and development of herbal product.

The present study revealed that the different parts of plants studied such as whole plant, aerial parts, stem, root and also plant collected from different locations of Tamil Nadu vary in the phytoconstituents which in turn affects the pharmacological activity of the plant. The samples of *P. maderaspatensis* were extensively studied for the physico-chemical parameters like loss on drying, total ash, acid insoluble ash, TLC fingerprint, alcohol extractive value and water extractive value. The physico-chemical properties and the unique TLC fingerprints are considered as important parameters for the quality control of crude drugs. The percentage of water and alcohol extractive value provided the yield of active principle in the respective solvents. The extractive value data revealed that the phytoconstituents are more soluble in water than alcohol. The amount of inorganic material in the form of sand and other adulterant along with the plant was determined by ash and acid insoluble ash. The maximum and minimum amount of ash value was observed in whole plant and stem respectively. The estimation of moisture content in the sample is a significant tool for determining the possibility of degradation due to high moisture content. The data revealed that the percentage of moisture content was minimum in root sample and maximum in the whole plant.

The physico-chemical study revealed that the content of total flavonoids, ellagic acid and rutin exhibit a wide range of variation in different parts of *P. maderaspatensis* collected from southern region of Tamil Nadu. The maximum content of flavonoids and rutin was found in the aerial parts followed by whole plant, stem and root. The data revealed that content of flavonoid and rutin was negligible in root. The higher amount of ellagic acid was estimated in the whole plant followed by aerial parts, stem and it was not detected in root samples. The variations of phytoconstituents was observed in different parts of the plants collected, which indicate that the plant physiology plays a vital role in the content of phyto constituents. The study helps out in the screening of active principles in *P. maderaspatensis* and also provides an idea about the phytoconstituents present in different parts of plant.

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6. Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

7. References

1. Singh A. A note on variation of active principles in Indian Medicinal Plants and TIM formulations. Ethno Botanical Leaflets 2008;12:603-606.
2. Cuneyt C, Jolita R, Liudas I, Valdas J, Necdet Ç. Changes in the content of bioactive substances among *Hypericum montbretii* populations from Turkey. Rev Bras Farmacogn 2014; 24:20-24.
3. Alok M, Versha P, Deepak K. Quantitative estimation of Berberine content of *Berberis asiatica* from different altitude of Garhwal Himalaya. Asian Journal of Pharmaceutical and Clinical Research 2014;7(1):165-167.
4. Asha VV, Akhila S, Wills PJ, Subramoniam A. Further studies on the antihepatotoxic Activity of *Phyllanthus maderaspatensis* Linn. Journal of Ethnopharmacology 2004;92(1):67-70.
5. Sayyada K, Vartika R, Ajay Kumar S, Rawat Shanta M. Comparative pharmacognostic studies of three *Phyllanthus* species, Journal of Ethnopharmacology. 2006; 104(I):79-86.
6. Swarupa Rani S, Venkata Raju RR. Antimicrobial studies of *Phyllanthus maderaspatensis* and *Celosia argentea*, The International Journal of Engineering and Science 2014;3(3):35-38.
7. Baue AW, MDK, Sherris JC, Truck M. Antibiotic susceptibility testing by standard single disc diffusion method, Amer. J Cli. Pathol 1966;45:493-496.
8. Karthikeyan M, Jeethu Anu M, Annamalai A. Antibacterial activity of *Phyllanthus maderaspatensis*, Pharma Science Monitor. 2012;3(3):1-10.
9. Sada A, Abdur R, Sambul R, Mohd. Zakir S. Physico chemical standardization and Safety study of Kanocha (*Phyllanthus maderaspatensis*). A less investigated Unani drug 2019;6(1):290-300.

10. Surendra KS, Sheela MA, Deepak HB, Amit A, Chandrasekaran CV. Hepatoprotective activity of *Phyllanthus* species on tert-butyl hydroperoxide (t-BH)-induced cytotoxicity in Hep G2 cells 2011;7(27):229-233.
11. Rajkappor B, Venugopal Y, Anbu J, Harikrishnan N, Gobinath M, Ravichandran V. Protective effect of *Phyllanthus polyphyllus*. On acetaminophen induced hepatotoxicity in rats. Pak J Pharm Sci 2008;21(1):57-62.
12. Srirama R, Deepak HB, Senthilkumar U, Ravikanth G, Gurumurthy BR, Shivanna MB *et al*. Hepatoprotective activity of Indian *Phyllanthus*, Pharmaceutical Biology 2012;50(8):948-953.
13. Muhammad SA, Ahmad MA, Mumtaz H, Muhammad YA. Seasonal variation in some medicinal and biochemical ingredients in *Mentha longifolia*. Pak J Bot 2011;43:69-77.
14. Anna S, Cezary P, Max H. Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews 2011;10(4):471-491.
15. Ravikanth G, Srirama R, Senthilkumar U, Ganeshaiah KN, Uma Shaanker R. Genetic resources of *Phyllanthus* in Southern India: Identification of geographic and genetic hot-spots and its implications for conservation. Scientific Evaluation and Medicinal Applications 2011, 97-118.
16. Waltor HF. Principle and methods of chemical analysis. Edn 3, Prentice-HALL of India Pvt. Ltd, New Delhi 1964;16:460.