Journal of Science Research International (JSRI)
ISSN: 2456-6365 (Online)

OPEN ACCESS

CHROMATOGRAPHIC FINGERPRINT ANALYSIS OF *PAVETTA CRASSICAULIS* BREMEK. METHANOLIC LEAF EXTRACT

Arun Patil*[1] and Varsha Jadhav[2]

[1]*Department of Botany, Yashawantrao Chavan College, Halkarni, Kolhapur (M.S.), India*

[2]*Department of Botany, Shivaji University, Kolhapur (M.S.), India*

**Corresponding author E-mail: arunpatil545@gmail.com*

Received: 19 January 2025	Revised: 15 February 2026	Accepted: 04 March 2026	Published: 27 March 2026

DOI: **https://doi.org/10.5281/zenodo.19247692**

Abstract:

Pavetta crassicaulis Bremek. belongs to family Rubiaceae and commonly called as Papat. It is bushy shrub and leaves variable in size and shape. Ethnomedicinal studies of Kolhapur district revealed that traditionally leaves of *P. crassicaulis* were used to treat piles and bone fracture. The HPTLC fingerprint analyses were carried out as Harborne and Wagner et al described. The Toluene: Chloroform: Ethyl alcohol (4:4:1) was employed as mobile phase. The HPTLC fingerprinting of the leaf extracts showed several peaks with different Rf values. *Pavetta crassicaulis* methanol leaf extract shows eleven peaks indicating presence of eleven compounds. Among them peak number one is found as flavonoid with Rf value 0.08, peak number six and seven is found as essential oils with Rf value 0.38 and 0.46, peak number eight and nine are found as polyphenols with Rf value 0.50 and 0.55 and peak number ten and eleven are found as alkaloids with Rf value 0.57 and 0.67. Remaining peaks in the track are unknown. The HPTLC fingerprint profile is used in differentiation of the species from the adulterant and act as biochemical markers for this medicinally important plant in the pharmaceutical industry and plant systematic studies.

Keywords: *Pavetta crassicaulis, Leaf, Medicinal Plant, HPTLC Fingerprint, Piles, Bone Fracture, Phytocomponents, Identification, Authentication.*

Introduction

Natural remedies are found to be safe and effective. Many plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries (1). Higher plants as a source of bioactive compounds continue to play a dominant role in the maintenance of human health (6). Plants are rich source of secondary metabolites with interesting biological activities.

Pavetta crassicaulis Bremek. belongs to family Rubiaceae and commonly called as Papat. It is bushy shrub and leaves variable in size and shape. Traditionally leaves of *P. crassicaulis* were used to treat piles and bone fracture. Standardization of the plant material is a need of the day. With increasing demand for herbal products as medicines, there is an urgent need for standardization of plant products. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (Indian Traditional Medicine) and CTHM (Chinese Traditional Herbal Medicine). It can serve as a tool for identification, authentication and quality control of herbal drug. The HPTLC fingerprint profile is used in differentiation of the species from the adulterant and act as biochemical markers for this medicinally important plant in the pharmaceutical industry and plant systematic studies (3).

Material and Methods

HPTLC fingerprint analysis

HPTLC fingerprint analysis was carried out by following method of Harborne (1994) and Wagner *et al.* (1993).

1. Sample preparation

The shade dried powdered sample was extracted (sonicated) with respective solvents (methanol and water) of 25ml for thirty minutes. The extracts obtained were evaporated to dryness in evaporating dish on water bath to get the residue. Each extract residue was redissolved in 1ml HPTLC grade solvent solution which was used for sample application on precoated silica gel 60F 254 aluminium sheets.

2. Developing solvent system

A number of solvent systems were tried, but satisfactory resolution was obtained in the solvent Toluene: Chloroform: Ethyl alcohol (4:4:1).

3. Sample application

The sample of different volumes (2µl, 4µl, 6µl, 8µl, 10µl) were spotted in the form of bands of width 8mm with a 100µl sample using a Hamilton syringe on silica gel which was precoated on aluminium plate 60F 254 (20cm x 10cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system which was programmed through WIN CATS software 4.1.7 version.

4. Development of chromatogram

The mobile phase consisted of Toluene: Chloroform: Ethyl alcohol (4:4:1) and 15ml of mobile phase was run per chromatography. The linear ascending development was carried out in a twin through glass chamber (20cm x 10cm) saturated with the mobile phase.

5. Detection of spots after derivatization

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was dipping with anisaldehyde sulphuric acid reagent and dried at 110^0C in hot air oven for 3 min. The plate was kept in photo documentation chamber (CAMAG TLC Visualizer) and captured the images under UV light at 254nm, 366nm and visible after derivatization. The Rf values and finger print data were recorded by WIN CATS software.

Result and Discussion

HPTLC fingerprint analysis of methanolic leaf extract of *Pavetta crassicaulis* shows several peaks indicating presence of several components and was recorded in Table 1 - A to E. Photo documentation of HPTLC profile under

UV 366 light was presented in the fig. 1. The corresponding HPTLC chromatograms were presented in figure 2(A to E).

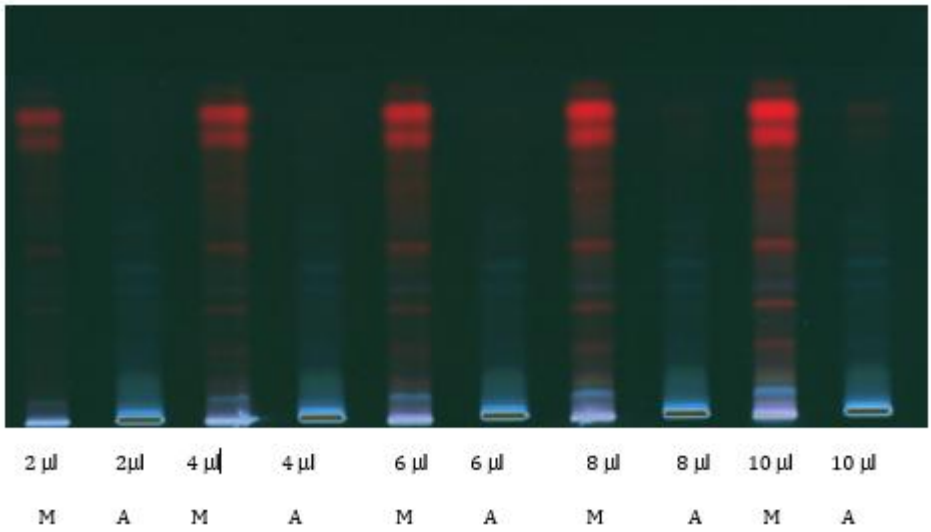


Image under UV 366 nm [M - Methanol, A - Aqueous]

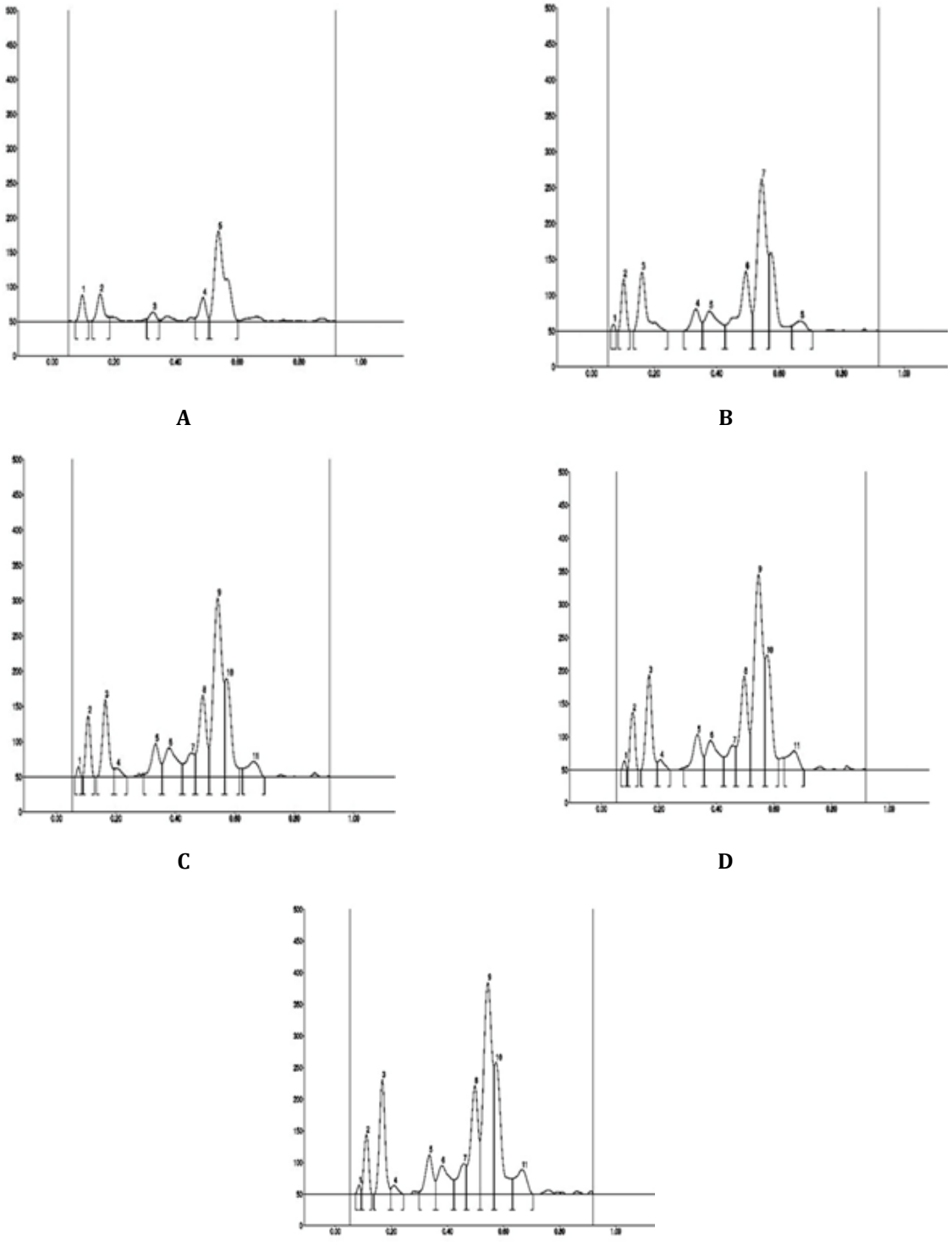
Figure 1: Photo documentation of HPTLC profile of leaf extract of *Pavetta crassicaulis* Bremek. **under UV 366 nm**

2 µl (track -1) leaf extract shows 5 peaks (fig. 2 - A) with Rf value in the range of 0.10 to 0.54 (table 1- A) indicating presence of 5 different component. Out of five components, the component with Rf value 0.54 was found to be predominant as the percentage area was more with 64.62%. The remaining components were found to be in the percentage area range of 4.24% to 12.15%.

Table 1- B indicates that 4 µl (track-3) leaf extract shows 8 peaks (fig. 2 - B) at the following Rf value 0.07, 0.10, 0.16, 0.33, 0.38, 0.49, 0.55 and 0.69 indicating occurrence of 8 different components. Out of eight different components, the components with Rf value 0.16, 0.49 and 0.55 were found to be predominant as the percentage area is more with 14.43%, 17.99% and 42.49% respectively. Remaining components were found to be less in quantity as the percentage area for all the peaks were less than 7.47%. Component with Rf value 0.07 was very less in quantity as the percentage area was very less with 0.69%.

Table 1 - C indicates that 6 µl (track-5) leaf extract shows 11 peaks (fig. 2 - C) indicating occurrence of 11 different components with Rf value in the range of 0.07 to 0.66. Out of 11 different components, the components with Rf value 0.45, 0.49 and 0.54 were found to be predominant as the percentage area was more with 13.280%, 32.28%, and 13.70% respectively. The remaining components were found to less in quantity. Components with Rf values 0.07 and 0.20 were very less in quantity as the percentage area was very less with 0.70% and 1.31 respectively.

8µl (track-7) leaf extract shows 11 peaks (fig. 2 - D) with Rf value in the range of 0.08 to 0.67 indicating presence of at least 11 different components (Table -1- D). Out of 11 components, the components with Rf value 0.50, 0.55 and 0.58 were found to be predominant as the percentage area was more with 13.93%, 33.00% and 14.11% respectively. The components with Rf value 0.08 and 0.21 were very less in quantity as the percentage area was very less with 0.53% and 1.30% respectively.



A **B**

C **D**

E

Figure 2: A, B, C, D and E - HPTLC chromatograms of methanolic leaf extract of *Pavetta crassicaulis* showing different peaks of phytoconstituents

10 µl (track - 9) of leaf extract revealed 11 peaks (fig. 2 - E) with Rf value in the range of 0.08 to 0.67 (Table 1 - E) indicating presence of 11 different components. Out of 11 different components, the components with Rf value 0.17, 0.50, and 0.55 were found to be predominant as the percentage area was more with 14.37%, 31.82% and 15.88% respectively. The remaining components were found to be less in quantity as the percentage area for all the peaks were less than 5.70%.

Pavetta crassicaulis methanol leaf extract shows eleven peaks indicating presence of eleven compounds. Among them peak number one is found as flavonoid with Rf value 0.08, peak number six and seven is found as essential oils with Rf value 0.38 and 0.46, peak number eight and nine are found as polyphenols with Rf value 0.50 and 0.55 and peak number ten and eleven are found as alkaloids with Rf value 0.57 and 0.67. Remaining peaks in the track are unknown (Table 2).

Table 1 - A, B, C, D and E – Peak list and Rf value of the chromatograms of track -1, 3, 5, 7 and 9 of methanolic leaf extract of *Pavetta crassicaulis* Bremek.

Table 1 - A: Track 1

Peak	Max Rf	Max Height	Area	Area %
1	0.10	38.6	516.9	8.64
2	0.16	40.2	727.2	12.15
3	0.33	14.0	254.0	4.24
4	0.49	34.9	619.2	10.35
5	0.54	130.7	3866.9	64.62

Table 1 - B: Track 3

Peak	Max Rf	Max Height	Area	Area %
1	0.07	10.2	83.5	0.69
2	0.10	72.8	902.8	7.47
3	0.16	82.9	1744.7	14.43
4	0.33	31.1	687.8	5.69
5	0.38	28.5	923.1	7.63
6	0.49	83.3	2175.5	17.99
7	0.55	211.9	5136.9	42.49
8	0.69	14.9	436.7	3.61

Table 1 - C: Track 5

Peak	Max Rf	Max Height	Area	Area %
1	0.07	15.0	130.9	0.70
2	0.11	87.3	1141.1	6.08
3	0.16	109.7	1865.3	9.95
4	0.20	12.7	246.6	1.31
5	0.33	47.5	1055.3	5.63
6	0.38	41.5 4	1454.7	7.76
7	0.45	34.2	865.8	4.62
8	0.49	116.1	2490.8	13.28
9	0.54	254.2	6165.8	32.87
10	0.57	140.1	2570.0	13.70
11	0.66	22.0	769.2	4.10

Table 1 - D: Track 7

Peak	Max Rf	Max Height	Area	Area %
1	0.08	14.4	120.2	0.53
2	0.11	87.0	1198.8	5.32
3	0.17	143.7	2447.0	10.86
4	0.21	16.0	293.1	1.30
5	0.33	53.9	1306.5	5.80
6	0.38	44.9	1531.0	6.80
7	0.46	37.5	917.	4.07
8	0.50	142.0	3137.2	13.93
9	0.55	295.7	7433.0	33.00
10	0.58	173.6	3177.5	14.11
11	0.67	29.1	963.2	4.28

Table 1– E: Track 9

Peak	Max Rf	Max Height	Area	Area %
1	0.08	15.0	135.3	0.51
2	0.11	94.6	1321.0	4.93
3	0.17	181.3	3015.3	11.26
4	0.21	14.1	264.7	0.99
5	0.34	62.8	1382.8	5.16
6	0.38	45.9	1524.8	5.70
7	0.46	48.8	1200.8	4.49
8	0.50	172.0	3848.3	14.37
9	0.55	335.0	8520.1	31.82
10	0.57	208.5	4251.2	15.88
11	0.67	39.1	1308.4	4.89

Table 2: HPTLC fingerprint profile of methanol leaf extract of *Pavetta crassicaulis* Bremek.

Peak	Rf	Assigned substance
1	0.08	Flavonoid
2	0.11	Unknown
3	0.17	Unknown
4	0.21	Unknown
5	0.34	Unknown
6	0.38	Essential oil 1
7	0.46	Essential oil 2
8	0.50	Polyphenol 1
9	0.55	Polyphenol 2
10	0.57	Alkaloid 1
11	0.67	Alkaloid 2

Conclusion

HPTLC fingerprint profile of methanol leaf extract of *Casearia championii*_showed the presence of various phytoconstitutents. The results of the present study also supplement the folkloric usage of the studied plant which possesses several known and unknown bioactive compounds with bio-activity. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various ailments. In the present study we observed Flavonoid, Polyphenol, and different alkaloid profiles with various Rf values, intensity, area, and height. This can be used in the pharmaceutical industry as a pharmacogenetic tool to identify this medicinally important plant. In addition, it can be adopted as a chemo-taxonomical tool in the plant systematic.

References

1. Bobbarala, V., Bramhachari, P. V., Ravichand, J., Reddy, Y. H. K., Kotresha, D., & Chaitanya, K. V. (2011). Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of *Aegle marmelos* (L.) Correa extracts. *Journal of Pharmaceutical Research, 4*(1), 252–255.
2. Harborne, J. B. (1994). *Phytochemical methods* (3rd ed.). Chapman and Hall.
3. Johnson, M., Mariswamy, Y., & Gnaraj, W. E. (2011). Chromatographic fingerprint analysis of steroids in *Aerva lanata* L by HPTLC technique. *Asian Pacific Journal of Tropical Biomedicine, 1*, 428–433.
4. Sermakkani, M., & Thangapandian, V. (2012). GC–MS analysis of *Cassia italica* methanol leaf extract. *Asian Journal of Pharmaceutical and Clinical Research, 5*(2), 90–94.
5. Wagner, H., & Bladt, S. (1993). *Plant drug analysis*. Springer.