Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

HPTLC analysis of *Fumaria parviflora* (Lam.) methanolic extract of whole plant

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Abstract

Background: *Fumaria parviflora* (Lam.), commonly known as "fine-leaved fumitory," is well known for its therapeutic properties in the Indian traditional medicinal system. The presence of important bioactive compounds in plants makes them pharmacologically valuable. Therefore, in the present study, the high-performance thin layer chromatography (HPTLC) analysis of *Fumaria parviflora* (whole plant) methanolic extract was performed for its phytochemical profiling.

Results: The HPTLC densitometric analysis of the methanolic extract of *Fumaria parviflora* (whole plant) was carried out using CAMAG HPTLC system, and the results were obtained in the form of chromatograms (scanned at the wavelength of 254 nm and 366 nm) representing several peaks. The phytochemical profile of the plant was determined and presented in the tables showing the total number of peaks, peak heights, peak area, percent area, and Rf values.

Conclusion: The study concluded that *F. parviflora* methanolic extract of the whole plant contains a rich variety of phytochemicals which might be accountable for its therapeutic value and thus justifies its traditional use in India.

Keywords: *Fumaria parviflora*, HPTLC, Methanolic extract, Densitometry, Chromatogram

Background

Medicinal plants, due to the presence of bioactive phytochemicals, play a very important role in human life for maintaining good health. The use of medicinal herbs in the treatment of infection is an age-old practice, and several natural products are used as phytotherapic for the treatment of many diseases [1]. The search for a newer source of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs [2]. There are thousands of medicinal plants known to have a long history of usage for their curative properties against various diseases and ailments [3]. The use of herbal drugs is once more escalating in the form of Complementary and Alternative Medicine (CAM) [4].

Fumaria parviflora Lam., commonly known as fine-leaved fumitory (in English), Shahatra, Pittapapara, or Pittapapada (in Hindi), belongs to the family Fumariaceae. *Fumaria parviflora* (Fumariaceae) is a pale green, diffuse, much branched annual herb widely used in Ayurvedic medicine as well as in traditional Yunani system of medicine throughout India [5]. The entire herb is traditionally used in leprosy, fever [6], and detoxification and as laxative, diuretic, and diaphoretic [7].

The World Health Organization (WHO) has stressed on the need for scientific validity of herbal drugs and ensuring, devising, and implementing sound science [8]. Several techniques are available for the qualitative and quantitative estimation of phytochemicals present in plants. Nowadays, new technology has made it possible to identify, screen, and isolate these active compounds [9]. The HPTLC (high-performance thin layer chromatography) is an advanced form of TLC as it provides high resolution and much accurate data. It is accepted all over the world as one of the most

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powerful analytical techniques used for phytochemical and biomedical analysis. It is an inexpensive, simple, and rapid method for the estimation of chemical components present in test sample and therefore most widely used by pharmaceutical industries for new drug discovery. The present study was performed for the phytochemical profiling of *Fumaria parviflora* (whole plant) methanolic extract by the HPTLC technique.

Method

Extraction

The plant material was washed and then kept for shade drying for 7 days. The dried plant sample was powdered by mechanical grinder into a fine powder. The air-dried powdered material of the whole plant of *Fumaria parviflora* (100 g) was extracted with hydroalcoholic solvent [methanol and water solvent (1:1 v/v)] using the Soxhletion process with the help of a Soxhlet apparatus. Excess solvent was then evaporated in a water bath at 50–100 °C to obtain the crude and stored in airtight containers.

Instrumentation

A CAMAG HPTLC system equipped with LINOMAT 5 applicator fitted with 100 µl syringe, CAMAG TLC scanner, and winCATS software was used.

Chemicals and solvents

All the solvents used were of chromatography grade, and all the chemicals used were of analytical reagent grade.

Preparation of samples

Dried extract (10 g) of *F. parviflora* was dissolved in 100 ml HPTLC grade methanol and filtered. This solution was used as a test solution for the HPTLC study.

Chromatographic conditions

The HPTLC was performed on 7.0 × 10.0 cm precoated silica gel 60 F 254 HPTLC plate (E. MERCK KGaA). No pre-washing and modification of the plate were done. The sample solution was applied as bands to the plate by CAMAG Linomat applicator fitted with 100 µl syringe (Table 1). The stable application rate was 150 nl/s. The sample loaded plate was kept in automatic development chamber with mobile

Table 1 Tracks representing sample position and volume

Track no.	Appl. position	Appl. volume	Vial #	Sample ID	Active
1	15.0 mm	4.0 µl	1	FI01	Yes
2	35.0 mm	8.0 µl	1	FI01	Yes

Table 2 Parameters used for HPTLC

Parameters	Values
Calibration parameters	
Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak height
Linomat 5 application parameters	
Spray gas	Inert gas
Sample solvent type	Methanol
Dosage speed	150 nl/s
Predosage volume	0.2 µl
Syringe size	100 µl
Application position	8.0 mm
Band length	8.0 mm
Solvent front position	75.0 mm
Detection—CAMAG TLC scanner	
Number of tracks	2
Position of track *X*	15.0 mm
Distance between tracks	20.0 mm
Scan start position *Y*	5.0 mm
Scan end position *Y*	75. 0 mm
Slit dimensions	6.00 × 0.30 mm, micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step
Integration: properties	
Data filtering	Savitsky-Golay 7
Baseline correction	Lowest slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	5.0 mm
Track end position	75.0 mm
Display scaling	Automatic
Measurement	
Wavelength	254 nm and 366 nm
Lamp	D2/Hg
Measurement type	Remission
Measurement mode	Absorption/fluorescence
Optical filter	Second order/K400
Detector mode	Automatic
PM high voltage	181 V

phase—chloroform:ethyl acetate:formic acid (5:4:1 v/v/v). Densitometric scanning was performed with CAMAG TLC scanner-4 equipped with winCATS software. The bands were visualized using CAMAG visualizer, and the images were captured in white light and 254 nm (short UV) and 366 nm (long UV) wavelengths (Table 2). When exposed to short-wave UV light of 254 nm, UV-active compounds will undergo fluorescence quenching and appear as dark spots on a bright background. Conversely, compounds that absorb 366 nm UV light will appear as bright spots on a dark background [10].

Results

The HPTLC analysis of *F. parviflora* Lam. revealed the presence of various phytochemicals as illustrated in the figures and tables below. The chromatograms (Figs. 1, 2, 3, and 4) were obtained upon scanning at UV 254 nm and 366 nm, and peak tables were generated. The Rf values, peak height, peak area, and percent area of the unknown substances are depicted in the tables (Tables 3, 4, 5, and 6).

Discussion

The HPTLC performed on the methanolic extract of *Fumaria parviflora* (Lam.) showed the presence of various phytoconstituents in different concentrations as illustrated in figures and tables. Figure 1 represents the 3-dimensional overlay of the chromatogram of all tracks, at all measured wavelengths. The chromatogram scanned at 254 nm (Fig. 2) represents 11 and 10 peaks for track 1 and track 2, respectively, whereas the chromatogram scanned at 366 nm (Fig. 3) indicates 9 and 12 peaks for track 1 and track 2, respectively. The number of peaks indicates the presence of different phytoconstituents present in the sample. The Rf values (Tables 3, 4, 5, and 6) calculated for the phytoconstituents present in the tested sample would be helpful in the identification of the unknown compounds by comparing them with the reference standards, and from the values of peak area, the

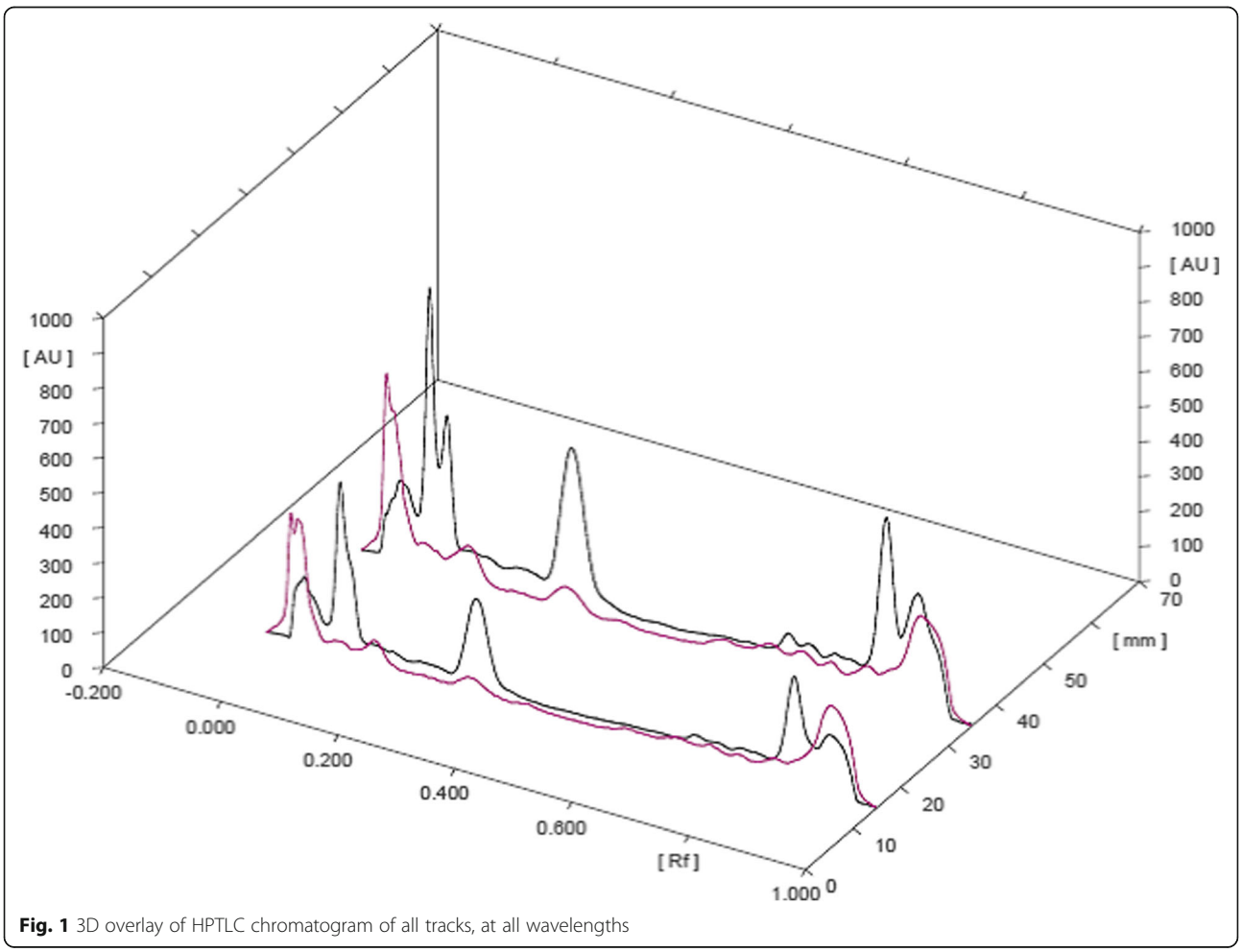
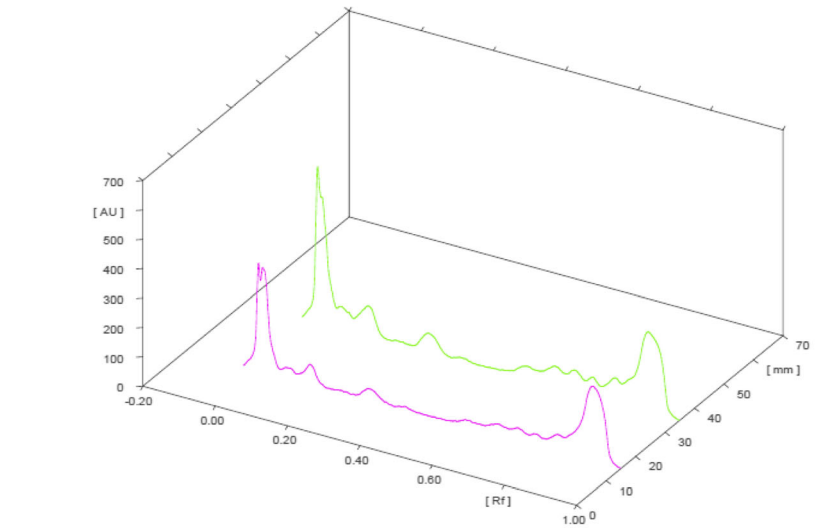


Fig. 1 3D overlay of HPTLC chromatogram of all tracks, at all wavelengths



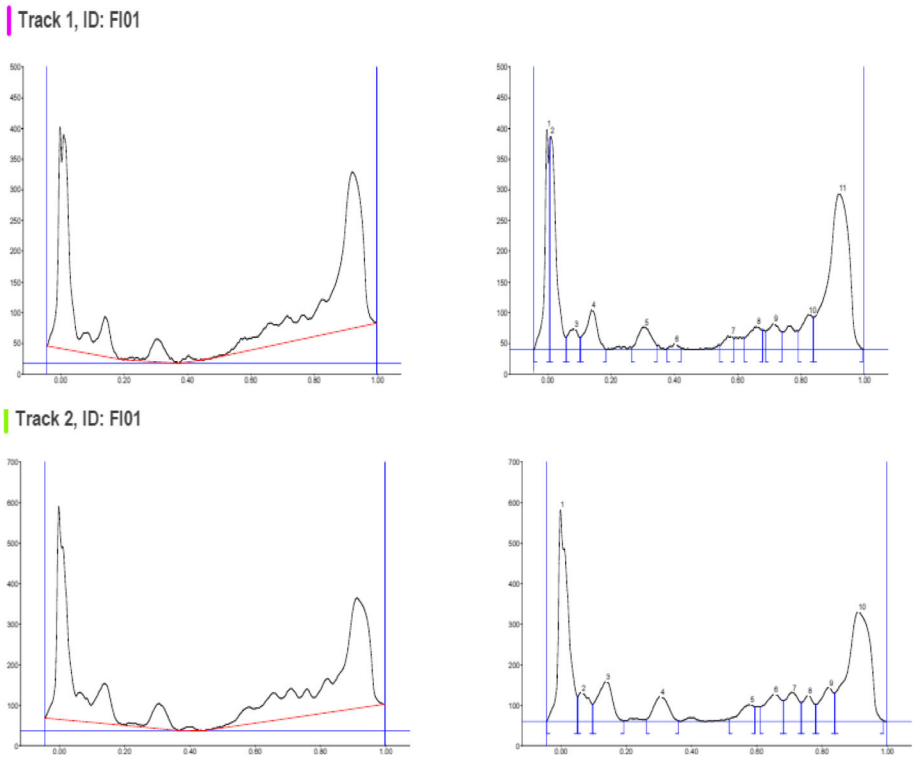
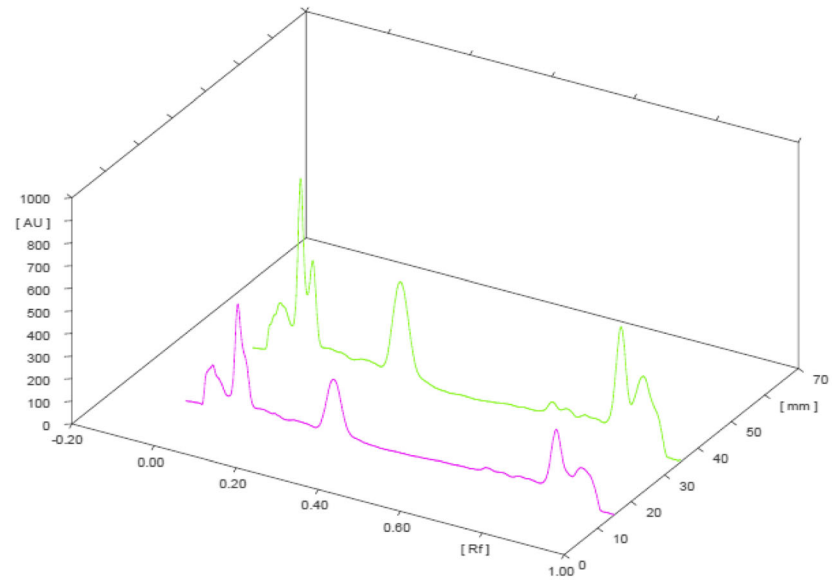
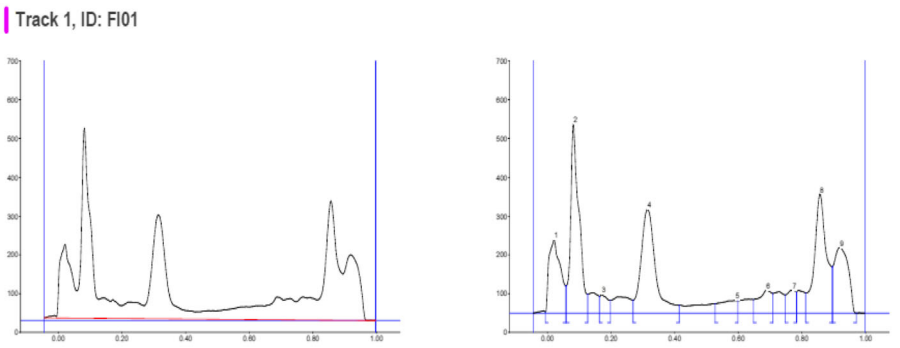


Fig. 2 HPTLC chromatograms scanned at 254 nm





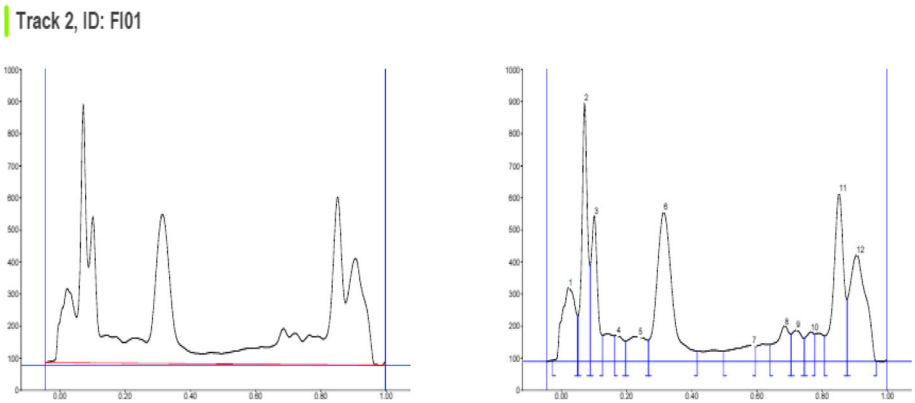


Fig. 3 HPTLC chromatograms scanned at 366 nm

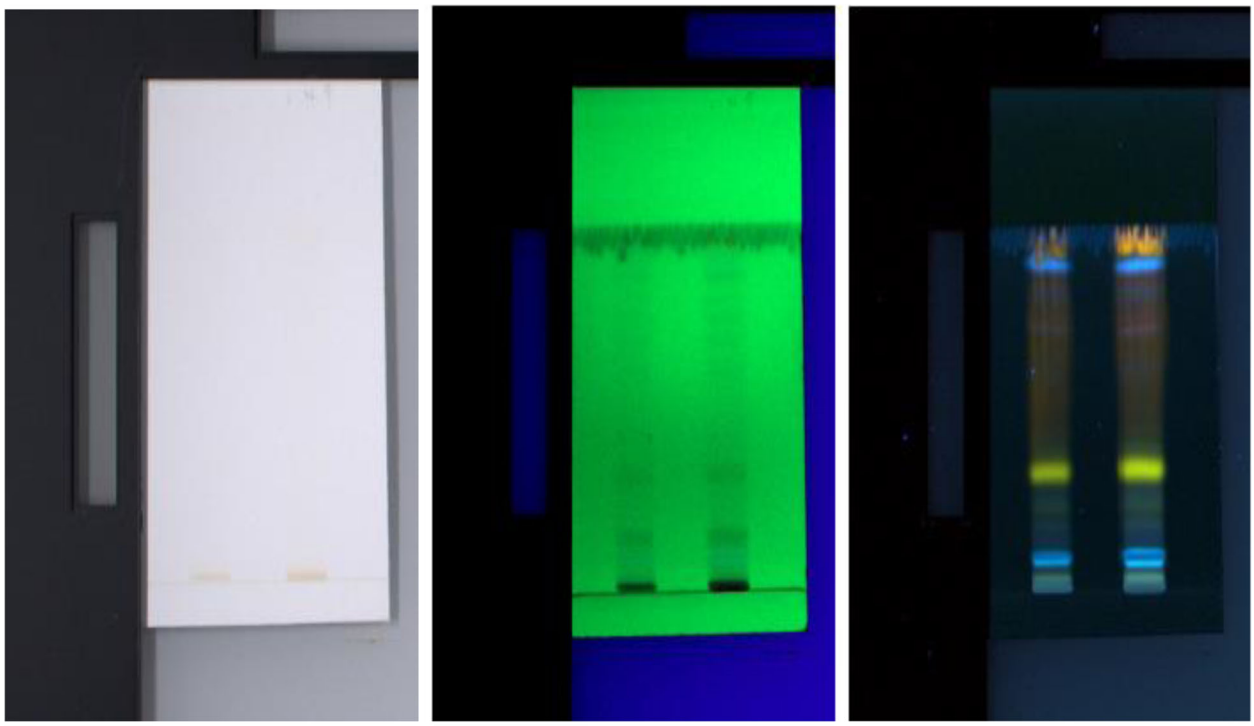


Fig. 4 HPTLC chromatograms visualized under **a** white light, **b** UV 254 nm, and **c** UV 366 nm

Table 3 HPTLC peak table of methanolic extract of *F. parviflora* Lam. (at 254 nm, track 1)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	0.3	-0.00	360.5	28.35	0.01	328.5	3987.7	12.95	unknown *
2	0.01	343.7	0.01	349.3	27.47	0.06	20.8	5327.5	17.30	unknown *
3	0.06	21.1	0.09	33.9	2.67	0.10	20.5	848.8	2.76	unknown *
4	0.10	20.7	0.14	64.9	5.11	0.19	0.2	1761.9	5.72	unknown *
5	0.27	3.2	0.31	37.3	2.93	0.35	6.3	1208.9	3.92	unknown *
6	0.38	1.8	0.40	10.4	0.81	0.42	2.6	185.9	0.60	unknown *
7	0.54	7.6	0.58	22.5	1.77	0.59	18.8	527.0	1.71	unknown *
8	0.62	19.2	0.66	37.6	2.95	0.68	32.3	1227.2	3.98	unknown *
9	0.69	30.3	0.72	43.2	3.39	0.74	29.4	1291.5	4.19	unknown *
10	0.79	30.3	0.83	57.5	4.52	0.84	52.8	1553.6	5.04	unknown *
11	0.84	53.0	0.92	254.7	20.03	1.00	1.5	12880.6	41.82	unknown *

Table 4 HPTLC peak table of methanolic extract of *F. parviflora* Lam. (at 254 nm, track 2)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	2.4	-0.00	525.4	38.55	0.05	61.7	12155.7	27.16	unknown *
2	0.05	62.1	0.06	71.5	5.25	0.10	42.4	1887.1	4.22	unknown *
3	0.10	42.7	0.14	99.1	7.27	0.19	1.5	3453.6	7.72	unknown *
4	0.26	6.0	0.31	62.6	4.59	0.36	1.7	2182.4	4.88	unknown *
5	0.52	6.4	0.58	41.9	3.07	0.60	36.1	1419.3	3.17	unknown *
6	0.61	36.4	0.66	68.2	5.00	0.68	51.9	2608.7	5.83	unknown *
7	0.68	52.2	0.71	72.7	5.34	0.74	47.3	2259.6	5.05	unknown *
8	0.74	47.4	0.76	65.6	4.82	0.78	42.2	1624.1	3.63	unknown *
9	0.78	42.2	0.82	84.2	6.18	0.84	69.7	2589.7	5.79	unknown *
10	0.84	70.3	0.91	271.6	19.93	0.99	3.2	14578.9	32.57	unknown *

concentration of the compounds can be determined. The bands of separated compounds can be seen (Fig. 4) on the TLC plates visualized under white light and UV of wavelengths 254 nm and 366 nm.

It has been reported from the previous studies that a wide range of bioactive compounds of medicinal significance are present in various species of *Fumaria*. The HPTLC study conducted on *Fumaria vaillantii* showed the presence of protopine and rutin in methanol extract of the whole plant at Rf 0.51 and 0.26, respectively [11]. Some of the *Fumaria* species are known to exhibit antifungal [12], antibacterial [13], and anti-inflammatory [14] activities due to the presence of bioactive phytochemicals such as alkaloids, polyphenols, and flavonoids. Thus, from the earlier researches, it is evident that various species of *Fumaria* contain some bioactive compounds important for pharmaceutical industries.

The findings of the present study are limited to the HPTLC analysis of *Fumaria parviflora* methanolic extract to estimate the presence of different phytochemicals from the chromatogram peaks and obtain the peak tables; however, the identification of the unknown phytochemicals is not done.

Conclusion

The present study revealed the presence of several phytochemicals in *F. parviflora* which might be the cause for its healing properties and thus justifies its usage as a remedy in various ailments. New drug formulations require the isolation and identification of

Table 5 HPTLC peak table of methanolic extract of *F. parviflora* Lam. (at 366 nm, track 1)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	3.3	0.02	190.0	11.70	0.06	70.5	5384.8	11.83	unknown *
2	0.06	71.0	0.08	489.0	30.12	0.13	49.0	9923.0	21.80	unknown *
3	0.16	43.7	0.17	48.6	2.99	0.20	33.1	987.9	2.17	unknown *
4	0.27	33.8	0.31	268.0	16.51	0.41	20.7	9536.9	20.95	unknown *
5	0.53	24.8	0.59	32.9	2.02	0.60	32.4	1455.0	3.20	unknown *
6	0.65	35.4	0.69	58.6	3.61	0.71	51.2	1992.6	4.38	unknown *
7	0.75	47.1	0.77	59.5	3.66	0.79	56.8	1382.1	3.04	unknown *
8	0.81	52.4	0.86	307.8	18.96	0.90	119.7	9028.8	19.83	unknown *
9	0.90	120.6	0.92	169.5	10.44	0.97	0.0	5834.2	12.82	unknown *

Table 6 HPTLC peak table of methanolic extract of *F. parviflora* Lam. (at 366 nm, track 2)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	4.1	0.02	230.4	6.93	0.05	142.8	7276.0	8.67	unknown *
2	0.05	143.3	0.07	804.9	24.20	0.09	298.2	11006.8	13.11	unknown *
3	0.09	300.6	0.10	455.1	13.68	0.13	82.6	6932.7	8.26	unknown *
4	0.16	81.0	0.17	82.4	2.48	0.20	64.4	1728.2	2.06	unknown *
5	0.20	64.4	0.24	79.3	2.39	0.27	66.4	3441.3	4.10	unknown *
6	0.27	67.0	0.31	465.3	13.99	0.42	32.9	17611.8	20.98	unknown *
7	0.50	32.6	0.59	51.0	1.53	0.60	49.7	2854.5	3.40	unknown *
8	0.64	53.3	0.69	111.7	3.36	0.71	85.8	3529.3	4.20	unknown *
9	0.71	86.3	0.72	98.3	2.96	0.75	72.5	2395.1	2.85	unknown *
10	0.75	73.2	0.77	92.8	2.79	0.78	87.1	1888.6	2.25	unknown *
11	0.81	80.7	0.85	523.2	15.73	0.88	192.9	12671.9	15.09	unknown *
12	0.88	194.0	0.91	331.5	9.97	0.97	1.3	12611.5	15.02	unknown *

important phyto-compounds possessing pharmacological properties. The HPTLC study carried out for *F. parviflora* chemical profiling will be helpful in the identification of bioactive compounds and markers, by comparing the Rf values of the compounds with the reference standards.

Abbreviations

Lam: Lamarck; Rf: Retention factor; HPTLC: High-performance thin layer chromatography; UV: Ultraviolet; D2: Deuterium; Hg: Hydrargyrum (mercury)

Acknowledgements

The authors are thankful to Vindhya Herbal Testing & Research Laboratory, Bhopal, Madhya Pradesh, for providing technical facilities and assistance required for this work.

Collection, identification, and authentication of plant

The plant material was collected in the month of July 2020 and identified taxonomically by Dr. Suman Mishra, Consultant Taxonomist, Xcellventure Institute of Fundamental Research Pvt. Ltd., Bhopal (MP). She is also a botany scientist in MFP-PARC, Barkheda Pathani, Bhopal. The plant was identified and authenticated as *Fumaria parviflora* Lam. belonging to the family Fumariaceae by its macroscopic, microscopic, and powder microscopic examination.

Authors' contributions

AB executed the work and prepared the manuscript. PS planned the work and provided proper guidance for the research. AT contributed to the research design and edited the manuscript. All the authors have read and approved the manuscript.

Funding

No funding was received for this research.

Availability of data and materials

All data and material are available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 23 September 2020 Accepted: 30 November 2020
Published online: 04 January 2021

References

1. Sisodiya D, Shrivastava P (2018) Antimicrobial activity of *Euphorbia thymifolia* (L.) and *Manilkara hexandra* (Roxb.). Int J Curr Adv Res 7(2):9660–9663
2. Latha SP, Kannabiran K (2006) Antimicrobial activity and phytochemicals of *Solanum trilobatum* Linn. Afr J Biotechnol 5:2402–2404
3. Sisodiya D, Shrivastava P (2018) Phytochemical screening, thin layer chromatography and quantitative estimation of bioactive constituents in aqueous extract of *Manilkara hexandra* (Roxb.) dubard. Int J Recent Sci Res 9(1):23083–23086
4. Cooper LN, Blais BS (2004) Theory of cortical plasticity. World scientific publishing, Singapore
5. Kumar S, Sharma AK, Kamboj A (2017) *Fumaria parviflora* Lam. (Fumitory): a traditional herbal medicine with modern evidence. Asian J Pharm Pharmacol 3(6):200–207
6. Anonymous (2004) The ayurvedic pharmacopoeia of India, 1 Ministry of Health and Family Welfare, New Delhi, Government of India, 84–86.
7. Anonymous (2007) *Fumaria parviflora* Lam. In: Khare CP (ed) Indian medicinal plants. Springer, Heidelberg, p 275
8. Tilburt JC, Kaptchuk TJ (2008) Herbal medicine research and global health: an ethical analysis. Bull World Health Organ 86:594–599
9. Sisodiya D, Shrivastava P (2017) Qualitative and quantitative estimation of bioactive compounds of *Euphorbia thymifolia* L. Asian J Pharm Edu Res 6(3): 34–43
10. Thin-layer chromatography evaluation. https://www.merckmillipore.com. Accessed 6 Nov 2020.

11. Upadhye AS, Rajopadhye AA (2011) Botanical and phytochemical standardization of *Fumaria vaillantii* Loisel. Indian J Nat Prod Resour 2(3): 369–374
12. Moghtader M (2013) In vitro antifungal effects of *Fumaria vaillantii* Loisel. essential oil on *Aspergillus flavus*. J Yeast Fungal Res 4:21–25
13. Khamtache-Abderrahima S, Lequart-Pillonb M, Gontierb E, Gaillardb I, Pilardb S et al (2016) Isoquinoline alkaloid fractions of *Fumaria officinalis*: characterization and evaluation of their antioxidant and antibacterial activities. Ind Crops Prod 94:1001–1008
14. Bribi N, Algieri F, Rodriguez-Nogales A, Vezza T, Garrido-Mesa J et al (2016) Intestinal anti-inflammatory effects of total alkaloid extract from *Fumaria capreolata* in the DNBS model of mice colitis and intestinal epithelial CMT93 cells. Phytomedicine 23:901–913

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