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

Anjali Bhargava\(^*\), Pragya Shrivastava\(^1\) and Anita Tilwari\(^2\)

**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 phytotherapeutic 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 Soxhlet 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      | F01       | Yes    |
| 2         | 35.0 mm        | 8.0 μl       | 1      | F01       | 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](image-url)
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*          |
The 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 4** HPTLC peak table of methanolic extract of *F. parviflora* Lam. (at 254 nm, track 2)

| Peak | Rf  | 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       | 1897.1 | 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 *         |

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

| Peak | Rf  | 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 *         |
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)

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**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.

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**Availability of data and materials**

All data and material are available upon request.

**Ethics approval and consent to participate**

Not applicable.

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

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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