



A Pharmacognostical Study on *Fumaria parviflora* Lamk

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Abstract

Fumaria parviflora Lamk., is a valued herb in Ayurvedic medicine and is used as Parpata by majority of Ayurvedic practitioners amongst the other plant sources mentioned under the same common name. It is found in many parts of India from Indo-Gangetic plain and Nepal down to the Nilgiri Mountains. The whole plant is diuretic, diaphoretic, aperient, laxative and anthelmintic. It is used as antipyretic, blood purifier and in skin disorders. In the present study, physico-chemical parameters were established for identification of the drug. Protopine and β -sitosterol were quantified by validated HPTLC method, developed using precoated silica gel plates as a stationary phase and toluene:ethyl acetate:diethyl amine (7:2:1) and toluene:methanol (9.4:0.6) as a mobile phase respectively. It is a diffuse, annual herb with thin winged stem; alternate leaf finely divided into small, linear lanceolate segments, small white or pink flowers with purplish tips. Microscopically root can be characterized by the presence of centrally located diarch primary xylem encircled by wide secondary xylem occupying major area and a narrow cork; stem by collenchymatous hypodermis, vascular bundle capped with lignified pericyclic fibres and hollow pith; leaf by vascular bundles with groups of sclerenchyma underneath the phloem and narrow spongy parenchymatous lamina. Powder can be typified by xylem vessels with varied thickening, lignified and thick walled testa and spherical pollen grains. The plant was found to be rich in alkaloids. The amount of protopine and β -sitosterol were found to be 0.47–0.50% w/w and 0.23–0.26% w/w. The quality parameters and HPTLC method developed would serve as useful gauge in standardization of *Fumaria parviflora*.

Keywords: *Fumaria parviflora*, HPTLC, Protopine, β -Sitosterol

1. Introduction

Fumaria parviflora Lamk., (Syn. *Fumaria indica* (Haussk.) Pugsley, Fam. Fumariaceae commonly known as Parpata, is a valued herb in Ayurvedic system of medicine but falls under the category of controversial drugs. It is diffuse, annual weed growing through out India from Indo-Gangetic plains to down Nilgiris in South¹.

Entire herb is traditionally used in leprosy, fever², detoxification, and as laxative, diuretic and diaphoretic³.

The plant is shown to possess hypoglycaemic⁴, hepatoprotective⁵, antipyretic⁶, acetylcholinesterase inhibitory⁷, anthelmintic⁸ and antispasmodic⁹ activities. Major chemical constituents reported include alkaloids like protopine^{10–12}, adlumidicine^{12,13}, parfumine^{10–12,14}, berberine¹⁰, palmatine¹², dihydrosanguinarine¹¹, fumaricine¹², 8-oxocoptisine¹³, β -sitosterol, stigmasterol and campesterol¹⁵. The present study aims at establishing quality parameters and HPTLC profile for protopine and β -sitosterol.

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2. Materials and Methods

2.1 Plant Material

Fresh, fully-grown, flowering plants of *F. parviflora* were collected from Jammu in the month of January 2012. The plants collected were authenticated by taxonomist of Gujarat University, Ahmedabad, Gujarat. Voucher specimen sample (LM 632) was deposited at the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat. The plant material was cleaned, dried, powdered to 60 # and used for the present study.

2.2 Chemicals

Standards protopine and β -sitosterol were procured from Extra synthese, France and Natural Remedies, India respectively. All the solvents used were of chromatography grade and other chemicals used were of analytical (AR) grade.

2.3 Pharmacognostical Studies

Whole plant was studied for morphological characters. Microscopical study was performed for both entire (free hand transverse sections) and powdered material. Moisture content¹⁶, ash values and extractive values were determined¹⁷. Phytochemical screening was performed and alkaloids¹⁸, flavanoids and phenolics¹⁹ were estimated.

2.4 Estimation of Protopine and β -sitosterol in *F. parviflora* by HPTLC Method

2.4.1 Chromatographic Conditions

HPTLC was performed on 20 cm \times 20 cm precoated silica gel 60 F254 plates (E. Merck, Germany). Before chromatography the plates were pre-washed by methanol and activated at 60°C for 5 min. Samples were applied to the plates as bands 6 mm wide and 12.2 mm apart using Camag Linomat IV applicator (Muttenez, Switzerland) fitted with a 100 microlitre syringe (Camag, Switzerland). Linear ascending development was performed in twin-trough glass chamber with mobile phase vapour {[toluene:ethyl acetate:diethyl amine, 7:2:1 for protopine] and [toluene: methanol, 9.4:0.6 for β -sitosterol]} at room temperature (25°C). Densitometric scanning was

performed with a Camag TLC Scanner III operated by CATS software.

The method was validated in terms of linearity, interday precision, intraday precision, repeatability, accuracy, specificity, limit of detection and limit of quantification.

2.4.2 Calibration Curve

A stock solution (1 mg/ml) was prepared by dissolving accurately weighed each of 5 mg of protopine and β -sitosterol in 5 ml methanol in a volumetric flask separately. Standard solutions for calibration were prepared by dilution of the stock solution with methanol; the concentrations were such that amounts of protopine between 1000-5000 ng and that of β -sitosterol 500-2500 ng. The correlation coefficient, slope intercepts and regression equation were also calculated to provide mathematical estimate degree of linearity. A calibration curve was derived by plotting peak area (Y axis) versus concentration (X axis).

2.4.3 Quantification of Protopine and β -sitosterol in Extract

5 g crude drug powder was exhaustively extracted by refluxing with ethanol (3x50ml). Extract was passed through charcoal, concentrated and vacuum dried to yield 7.52%w/w of residue. It was dissolved in methanol in a volumetric flask to get the test solution of 5 mg/ml. 10 μ l of this solution was used for protopine estimation. After developing the plates, densitometric scanning was done at 293 nm and visualization of spots was achieved using dragendorff's reagent for photodocumentation. β -sitosterol was quantified using 5 μ l of the test solution and densitometric scanning was performed at 545 nm after derivatizing with anisaldehyde sulphuric acid reagent followed by heating at 110°C. The peak area values of standard and sample were used to calculate the amount of protopine and β -sitosterol present in extract.

3. Results and Discussion

3.1 Macroscopical Evaluation

F. parviflora is prostrate, annual weed about 60 cm in height, with cylindrical, tapering root measuring about 10 to 15 cm in length and 0.4 to 0.8 mm in diameter,

bearing lateral wiry rootlets; erect, longitudinally wrinkled, often branched 20-30 cm long stem with 4 to 5 winged projections; alternate, exstipulate leaf that is finely divided into narrow flat segments, each segment being broad oblong or linear lanceolate, 2 to 3 cm in length and 1 to 2 mm in width with acute or sub-acute apex, 2 to 4 cm long twisted petiole, sheathing at base; small white or pink flowers with purplish tips, in terminal inflorescence; indehiscent, tiny, sub-globose and externally faintly rugose fruits and globose minute seeds.

3.2 Microscopical Evaluation

Transverse section of the root is almost circular with slightly irregular margin, shows a diarch centrally



Fig. 1. Herb of *Fumaria parviflora* Lamk.

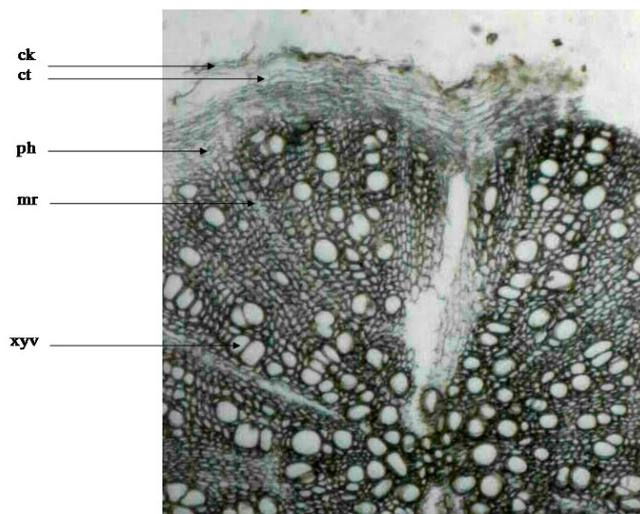


Fig. 2. TS of *F. parviflora* root.

located primary xylem in the centre encircled by wide secondary xylem composed of wide lumened xylem vessels (xyv) radially running at places and associated with tracheids, fibres and parenchyma that are traversed with multiseriate medullary rays (mr); narrow band of parenchymatous phloem (ph) and cortex (ct) encircled by cork (ck).

Transverse section of the stem is pentagonal to irregularly circular in outline and shows epidermis (e) covered with thick cuticle; 3 to 4 rows of collenchymatous hypodermis (hyp); narrow parenchymatous cortex (ct); indistinct endodermis; wedge shaped collateral vascular bundle (vb) arranged at the ridges and capped with lignified pericyclic fibres (per) and wide centrally hollow pith (pi).

Transverse section of pinnule passing through the midrib is triangular, conically protruding at the lower side and flat or somewhat depressed on the upper side and shows sinuously running cells of the upper (up)

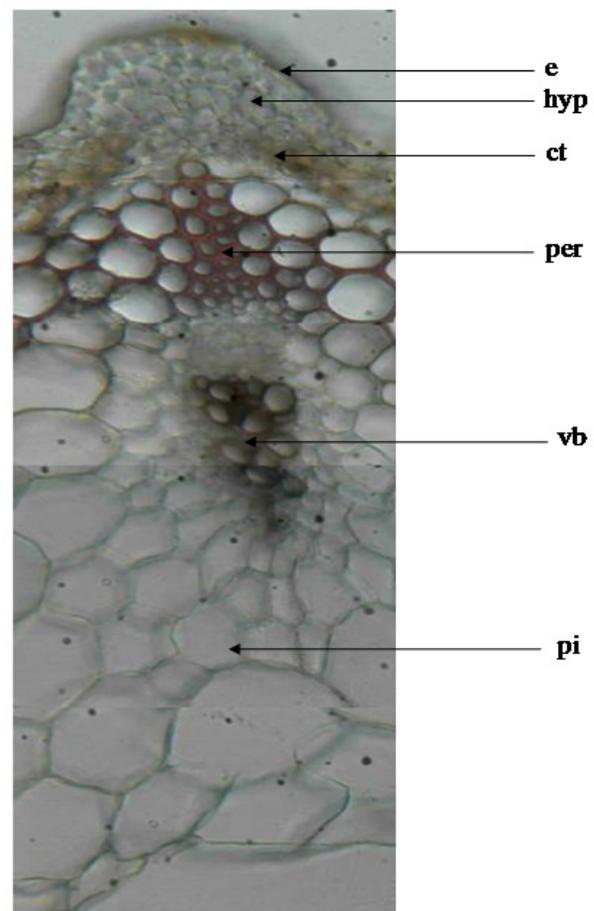


Fig. 3. TS of *F. parviflora* stem.

and lower epidermis (le) covered with thin cuticle; three conjoint collateral vascular bundles (vb) in the midrib the central one being biggest in size and with groups of sclerenchyma (scl) underneath the phloem and narrow lamina enclosing 5 to 7 rows of spongy parenchyma, embedded with rows of small sized meristeles.

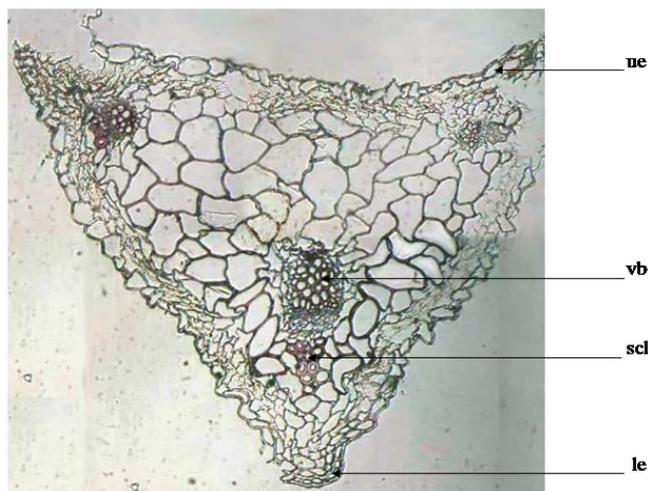


Fig. 4. TS of *F. parviflora* pinnule.

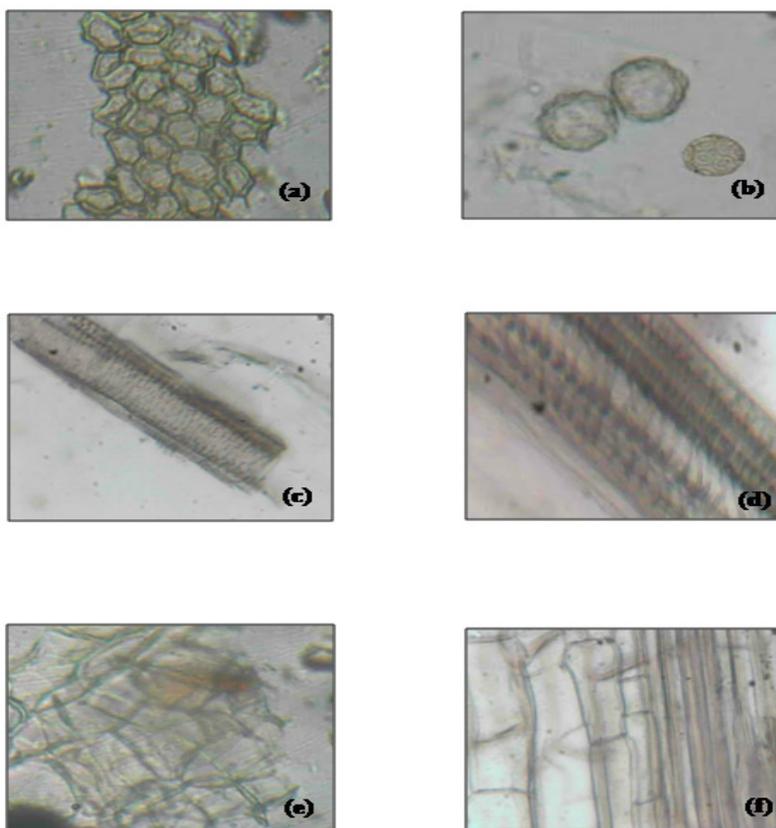


Fig. 5. Powder characters of *F. parviflora* whole plant.

3.2.1 Powder

It shows testa in surface view (a); large, spherical pollen grains having more than six pores with raised areas on the exine (b); longitudinally cut fragments of bordered pitted (c), annular (d) and spiral xylem vessels; polygonal cells of cork in surface view (e) and fibres with phloem parenchyma (f).

3.3 Physicochemical Evaluation

Data of Physico-chemical parameters including moisture content, ash and extractive values are given in Table 1. The plant was found to be rich in alkaloids and phenolics (Table 2).

3.4 Estimation of Protopine and β -Sitosterol by HPTLC Method

TLC studies of extract indicated presence of both protopine and β -sitosterol at R_f 0.88 and R_f 0.39 respectively exactly matching with reference standards. The content of protopine and β -sitosterol were found to be 0.47 – 0.50% w/w and 0.23 – 0.26% w/w.

Table 1: Physico-chemical parameters

Particulars	% w/w ± SD
Loss on Drying	78.3 ± 0.12
Total ash	25.63 ± 0.75
Water soluble ash	8.63 ± 0.44
Acid insoluble ash	4.75 ± 0.26
Water soluble extractive value	34.0 ± 0.19
Alcohol soluble extractive value	7.5 ± 0.35

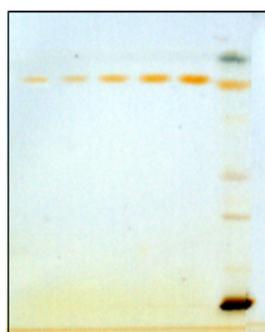
SD=standard deviation, Number of readings=3

Table 2: Content of phytoconstituents

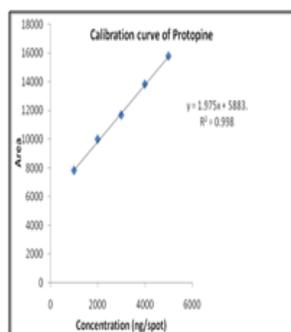
Sr. No	Phytoconstituents	% w/w
1	Alkaloids	6.21 ± 0.13
2	Phenolics	6.15 ± 0.28
3	Flavanoids	3.64 ± 0.35

Table 3: Validation parameters of protopine

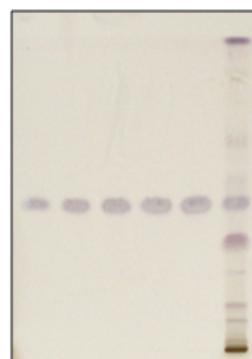
Parameters	Results
Linearity	0.998
Precision (% C.V.)	
Repeatability of Measurement	0.341
Repeatability of Application	1.941
Interday	1.46-2.58 %
Intraday	1.60-2.06 %
Range	1000-5000 ng band ⁻¹
Limit of Detection	417.78 ng band ⁻¹
Limit of Quantification	1265.99 ng band ⁻¹
Accuracy	99.52 – 101.28 %
Specificity	Specific



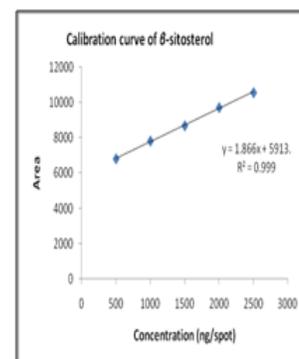
HPTLC chromatogram



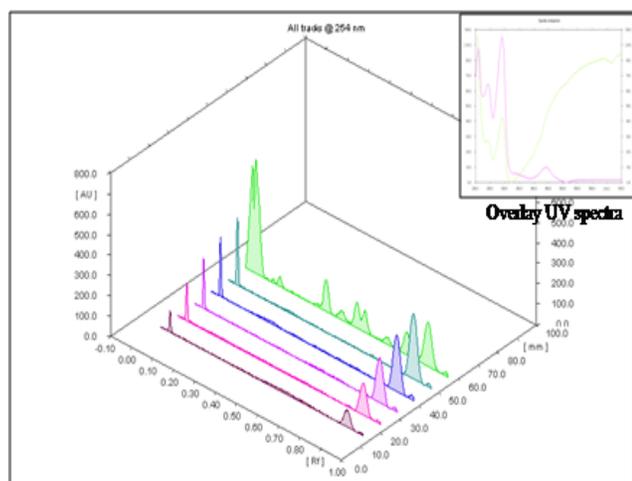
Calibration curve of protopine



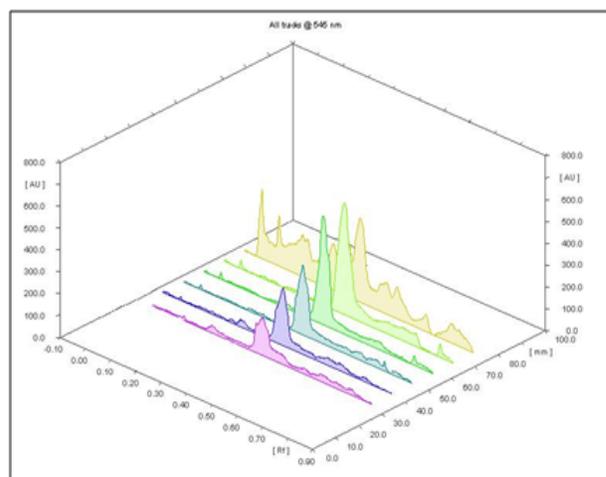
HPTLC chromatogram



Calibration curve of beta sitosterol



HPTLC densitometric chromatogram scanned at 293 nm



HPTLC densitometric chromatogram scanned at 545 nm

Fig. 6. HPTLC study of protopine.

Fig. 7. HPTLC study of beta-sitosterol.

Table 4: Validation parameters of β -sitosterol

Parameters	Results
Linearity	0.999
Precision (% C.V.)	
Repeatability of Measurement	0.302
Repeatability of Application	0.687
Interday	1.19-2.98%
Intraday	1.10-1.93%
Range	500-2500 ng band ⁻¹
Limit of Detection	183.53 ng band ⁻¹
Limit of Quantification	556.16 ng band ⁻¹
Accuracy	99.04 – 99.97 %
Specificity	Specific

4. Acknowledgement

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