

Evaluation of effect of Geographical and Climatic Conditions on Chemical Composition of a Plant Drug by HPTLC

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ABSTRACT

High Performance Thin Layer Chromatography (HPTLC) is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). We planned our work as to investigate the HPTLC fingerprinting profile of alcoholic extracts of stem bark of *F. bengalensis* Lin. Collected from three different geographical regions viz. Sangam Vihar (Delhi), Modasa (Gujarat) and Ramnagar (Uttaranchal) and HPTLC spectra of all the drug extracts were compared to evaluate the effects of geography and climatic conditions on chemical composition of this drug. The code name given for the drugs from Delhi, Gujarat and Uttaranchal are FB/DL, FB/GJ and FB/UA respectively. We found that all the extracts showed presence of different compounds as geography and environmental conditions were changed.

INTRODUCTION

Ficus bengalensis is an indigenous plant belonging to family Moraceae possessing varied pharmacological properties like antidiabetic [1,2,3], antimicrobial [4], antioxidant [5], hypocholesterolemic [6], antiallergic and antistress [7] and also tender ends of hanging roots are prescribed for diarrhea [8]. For pharmaceutical purposes, the quality of medicinal plant must be as high as that of the other medicinal preparations. The quality of a vegetable product depends on the geographical origin, time and stage of growth when collections have been done and post harvest handling [9]. In the present work the stem barks of *F. bengalensis* Linn. Were dried, powdered and defatted by petroleum ether in soxhlet apparatus. The drugs were exhaustively extracted with 95% v/v ethanol and HPTLC fingerprinting was carried out by using "CAMAG LINOVA V" a recent automatic device.

MATERIALS AND METHODS

Plant Material

The stem barks of *F. bengalensis* Linn. Were collected from New Delhi (FB/DL), Gujarat (FB/GJ) and Uttaranchal (FB/UA) in the month of May. The age of plant was found to be in the range of 25–30 years as enquired from the local persons. The specimen of collected bark was given for authentication in Raw Material and Laboratory of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (voucher no. NISCAIR/Consult/RHMD/2008–09/1010/41).

Preparation of the Extracts

Dried barks were coarsely powdered and defatted with petroleum ether by soxhlet apparatus. Defatted drug than exhaustively extracted with 95% v/v ethanol in soxhlet apparatus. The extract was concentrated under reduced pressure to get dark brown mass. The viscous dark brown mass is than dried in air to get dried powered extract [10]. The percentage yield of ethanol extracts of stem barks of FB/DL, FB/GJ and FB/UA was found to be 6.6 %, 9.56 % and 7.0 % respectively. The Phytochemical screening of all the extracts was carried out for Alkaloids, Proteins & Amino acids, Carbohydrates, Flavonoids, Phenolic group, Glycosides, Saponins, Tannins, Steroids,

Triterpenoids [11,12,13,14,15]. It was found that proteins and amino acids, carbohydrates, flavonoids, Phenolic groups, glycosides, saponins, tannins, steroids and triterpenoids were present and alkaloids were absent in all the extracts.

Development of HPTLC Method

The HPTLC was carried out using a Hamilton 100 µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 60F₂₅₄(Merck), 0.2 mm thickness.

Selection of plate and adsorbent

Pre-coated aluminium plates with Silica Gel 60F₂₅₄ (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used for the detection.

Sample solution

50 mg of alcoholic extract of FB/DL, FB/GJ, FB/UA barks were taken, dissolved in methanol and transferred to a 10 ml volumetric flask. The volume was made up to the mark with methanol. This solution was further used for HPTLC finger-printing.

Application of sample

Sample application is the most critical step for obtaining good resolution for quantification in the HPTLC. The automatic application device was used for sample application. The most recent automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm thickness, with 5mg/ml concentration of the sample solution.

Development

The plate was developed in CAMAG glass twin-through chamber (1010 cm) previously saturated with the solvent for 60 min (temperature 25.2 °C, relative humidity 40%). The development distance was 8 cm. subsequent to the scanning. The modified mobile system as **Toluene: Ethyl acetate: Formic acid (10:6:0.2)** [16] was developed for establishing the TLC pattern of alcoholic extracts of FB/DL, FB/GJ and FB/UA. Various visualising techniques were used for best HPTLC fingerprinting [17].

Detection

The plate was scanned at UV 366 nm using CAMAG TLC Scanner-3. R_f value of each compound which were separated on plate and data of peak area of each band was recorded.

RESULT AND DISCUSSION

HPTLC fingerprinting of FB/DL, FB/GJ and FB/UA showed the presence of 14, 8 and 12 compounds respectively. The spectra of HPTLC showed that some compounds were found to be present in all the extract of the *Ficus bengalensis* Linn. Though the geography is different. On matching the spectra of FB/DL, FB/GJ and FB/UA it was found that Compound 1 and 7 were present in all the extracts. Compound 4 and 6 were present in FB/DL and FB/UA both but absent in FB/GJ. Compound 14 was present in FB/DL and FB/UA but absent in FB/UA. Results of HPTLC spectra showed that maximum compounds were present in FB/DL.

Results and data of R_f value and peak area are mentioned in table no. 1 and figure no. 1 (a-f)-2 (a-f)

Table 1: HPTLC fingerprinting of FB/DL, FB/GJ, FB/UA at UV 366nm

| Peak | FB/DL | | FB/GJ | | FB/UA | |
|------|----------------|-----------|----------------|-----------|----------------|-----------|
| | R _f | Peak Area | R _f | Peak Area | R _f | Peak Area |
| 1 | 0.16 | 482.8 | 0.23 | 467.8 | 0.22 | 374.6 |
| 2 | 0.22 | 389.9 | 0.36 | 364.3 | 0.27 | 344.8 |
| 3 | 0.27 | 362.4 | 0.55 | 264.6 | 0.33 | 307.6 |
| 4 | 0.34 | 324.9 | 0.71 | 169.4 | 0.37 | 261.6 |
| 5 | 0.38 | 288.0 | 0.78 | 116.3 | 0.46 | 281.6 |
| 6 | 0.44 | 291.4 | 0.84 | 71.9 | 0.58 | 140.3 |
| 7 | 0.48 | 275.7 | 0.93 | 11.3 | 0.62 | 135.0 |
| 8 | 0.59 | 94.4 | 0.97 | 2.9 | 0.67 | 92.0 |
| 9 | 0.62 | 76.9 | - | - | 0.74 | 1.0 |
| 10 | 0.67 | 0.0 | - | - | 0.87 | 22.8 |
| 11 | 0.75 | 0.5 | - | - | 0.92 | 16.6 |
| 12 | 0.87 | 8.0 | - | - | 0.96 | 3.4 |
| 13 | 0.93 | 19.0 | - | - | - | - |
| 14 | 0.97 | 12.1 | - | - | - | - |

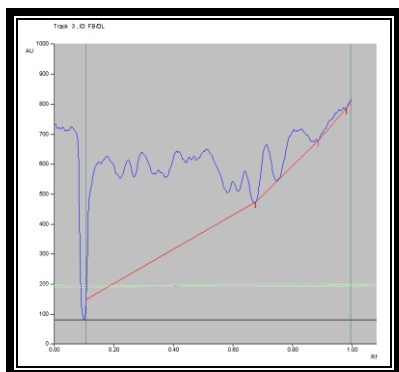


Fig 1a: HPTLC of FB/DL

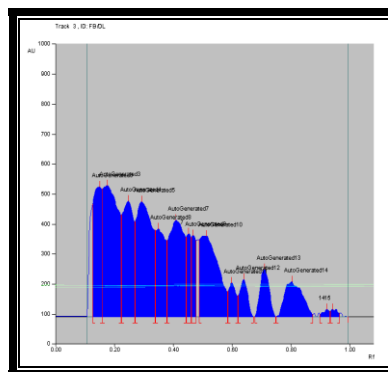


Fig 1b: HPTLC of FB/DL

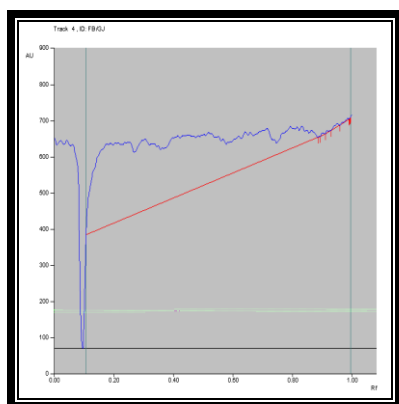


Fig 1c: HPTLC of FB/GJ

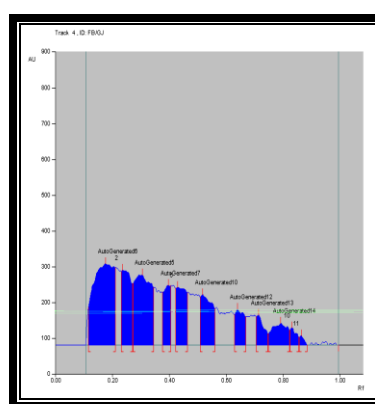


Fig 1d: HPTLC of FB/GJ

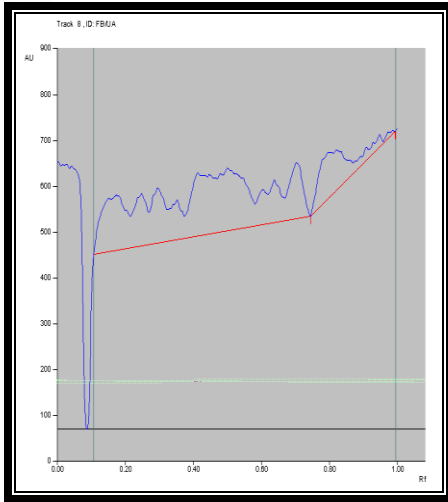


Fig 1e: HPTLC of FB/UA

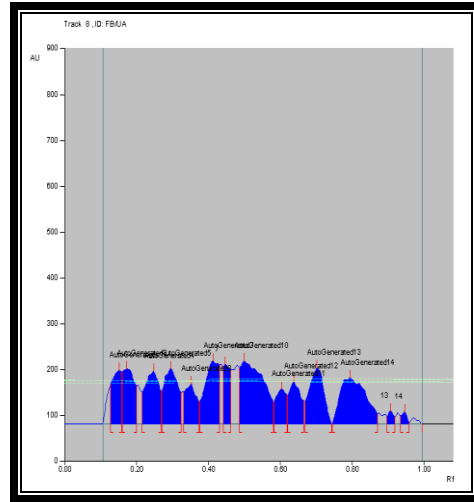


Fig 1f: HPTLC of FB/UA

Figure 1: HPTLC finger printing of FB/DL, FBGJ and FB/UA

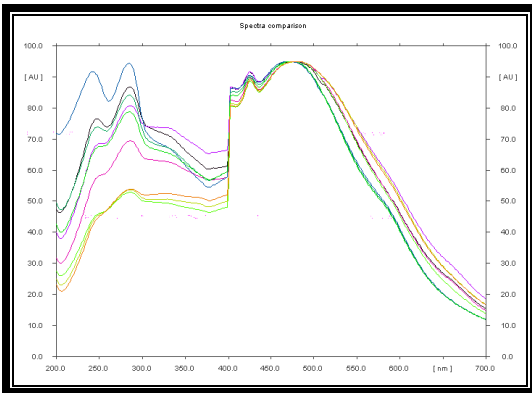


Fig 2a: Spectra of Compound 1 in all the extracts

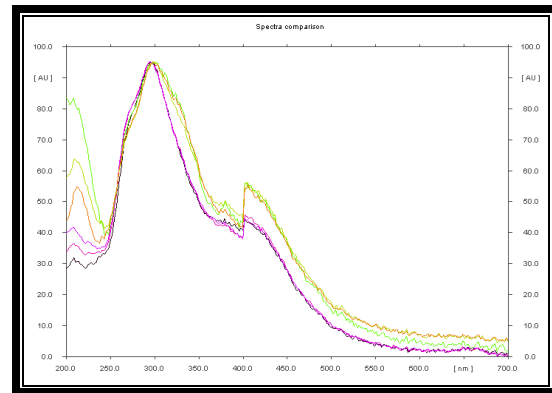


Fig 2b: Spectra of autogenerated Compound 4 in FB/DL and FB/UA

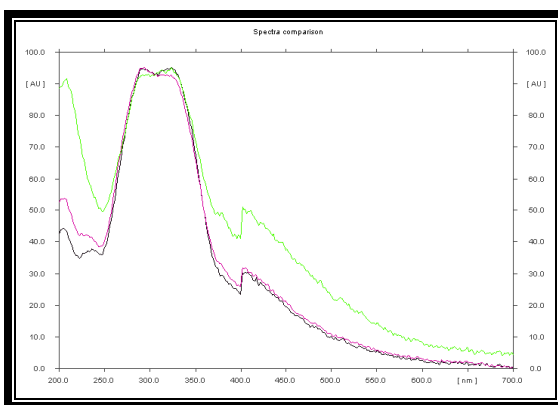


Fig 2c: Spectra of autogenerated Compound 6 in FB/DL and FB/UA

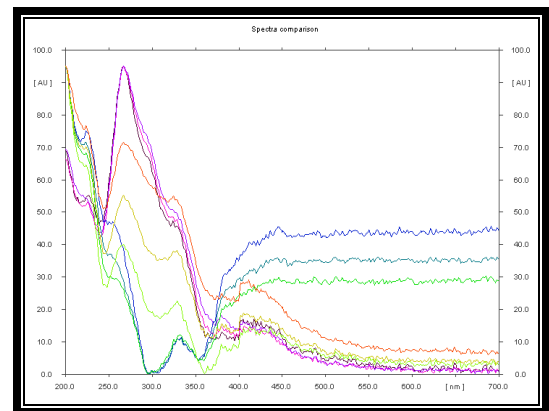


Fig 2d: Spectra of Compound 7 in all the extracts

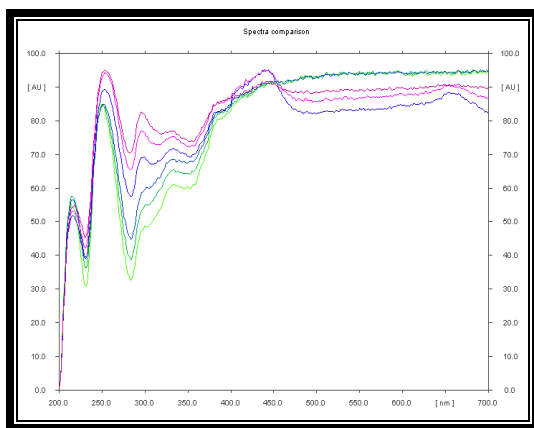


Fig 2f: Spectra of autogenerated Compound 14 in FB/DL and FB/GJ

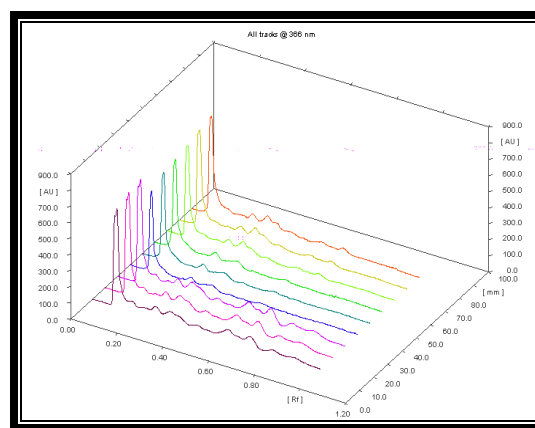


Fig 2g: All the tracks in 3d

Figure 2: Spectra comparison of different extracts

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