Research Article

HPTLC Fingerprints and Spectra Comparison of Alcoholic Extracts of Stem Bark of *Ficus bengalensis* lin

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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.

Keywords: *Ficus bengalensis*, Stem bark, HPTLC, FB/DL, FB/GJ, FB/UA.

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 diarrhoea[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 LINOVAT V” a recent automatic device.

MATERIAL 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 authentification 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, Triterpinoids.[11-15] 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 Hemilton 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 $60F_{254}$(Merck), 0.2 mm thickness.

Selection of plate and adsorbent

Precoated aluminium plates with Silica Gel $60F_{254}$ (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	
	Rf	Peak Area	Rf	Peak Area	Rf	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	-	-	-	-

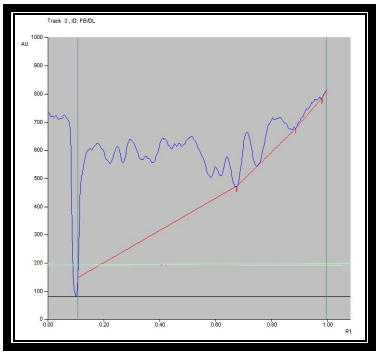

Fig. 1a: HPTLC of FB/DL

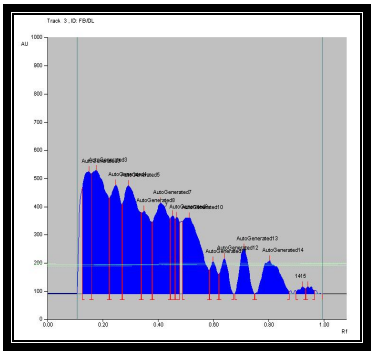

Fig. 1b: HPTLC of FB/DL

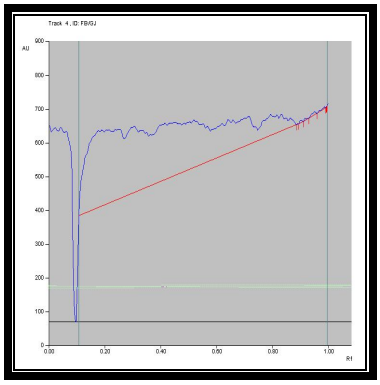

Fig. 1c: HPTLC of FB/GJ

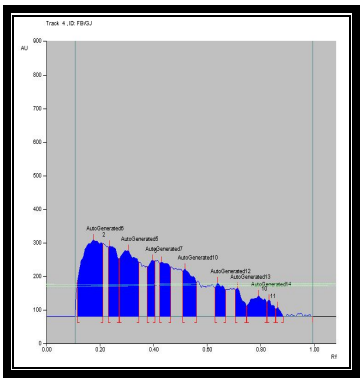

Fig. 1d: HPTLC of FB/GJ

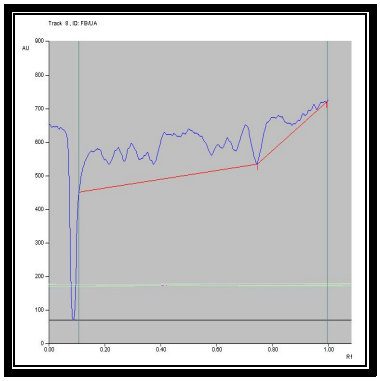

Fig. 1e: HPTLC of FB/UA

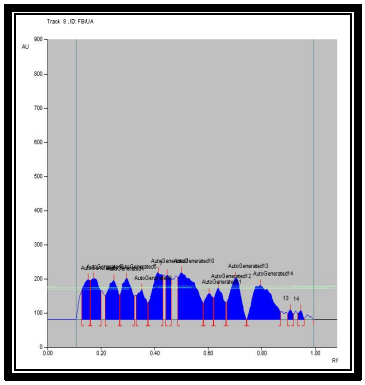

Fig. 1f: HPTLC of FB/UA

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

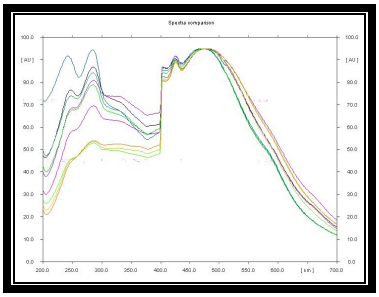

Fig. 2a: Spectra of Compound 1 in all the extracts

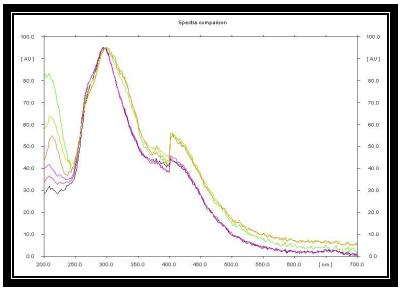

Fig. 2b: Spectra of autogenerated Compound 4 in FB/DL and FB/UA

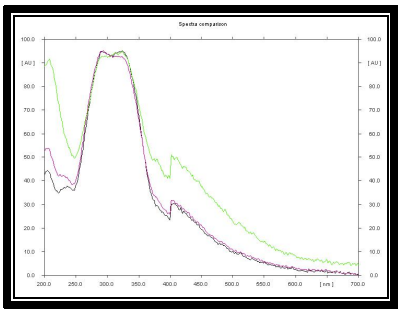

Fig. 2c: Spectra of autogenerated Compound 6 in FB/DL and FB/UA

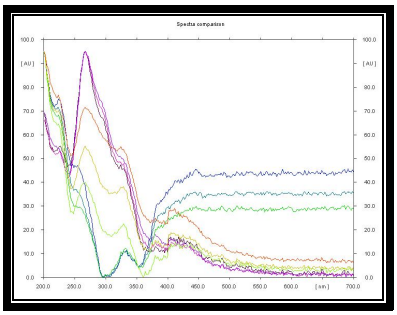

Fig. 2d: Spectra of Compound 7 in all the extracts

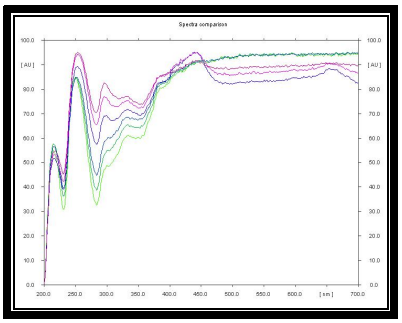

Fig. 2f: Spectra of autogenerated Compound 14 in FB/DL and FB/GJ

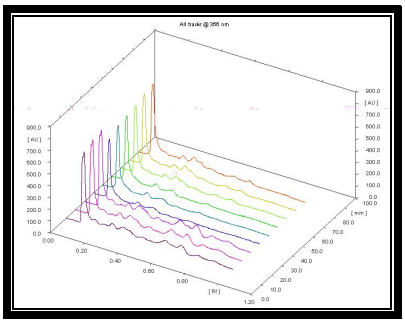

Fig. 2g: All the tracks in 3d

Fig. 2: Spectra comparison of different extracts

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REFERENCES

1. Edwin E, Sheeja E, Chaturvedi M, Sharma S and Gupta VB. A Comparative study on anti hyperglycaemic activity of Ficus bengalensis, Linn aerial roots and barks. Phcog Mag. 2008;4(13):97.
2. Gupta S, Shukla R, Prabhu KM, Aggrawal S, Rysia U and Murthy PS. Acute and chronic toxicity studies on partially purified hypoglycemic preparation from water extract of bark of Ficus bengalensis. Indian Journal of clinical Biochemistry. 2002;17(1):58-63.
3. Sgrawat H, Sharma S, Chaturvedi M, Edein E and Shukla S. Evaluation of the phytochemicals and antidiabetic activity of Ficus bengalensis. Int J Diab Dev Ctries. 2007;27(2):56-59.
4. Mousa O, Vuorela P, Kiviranta J, Wahab SA, Hiltunen R and Vuorela H. Bioactivity of certain Egyptian *Ficus* species. Journal of Ethnopharmacology. 1994; 41(1-2): 71-76.
5. Augusti KT, Anuradha SP, Smith KB, Sudheesh M and Joseph MC. Natraceutical effects of garlic oil, its nonpolar fraction and a Ficus flavonoid as compared to vitamine E in CCl4 induced liver damage in rats. Indian Journal of Experimental Biology. 2005;43(5):437-444.
6. Shukla R, Anand K, Prabhu KM and Murthy PS. Hypocholestrolemic effect of water extract of bark of banyan tree, *Ficus bengalensis.* Indian journal of clinical biochemistry. 1995;10:119-121.
7. Taur DJ, Nirmal SA, Patil RY and Kharya MD. Antistress and antiallergic effects of *Ficus bengalensis* bark in asthma. Natural Product Research, 2007; 21(14):1266-1270.
8. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M and Saha BP. Screening of anti-diarrhoeal profile of some plant extract of a specific region of West Bengal, India. Journal of Ethnopharmacology, 1998;60(1):85-89.
9. Sarine YK. Quality control of herbal drugs, Indian Journal of Natural Products, 1993;10:35.
10. Sgrawat, H, Sharma S, Chaturvedi M, Edein E and Shukla S. Evaluation of the phytochemicals and antidiabetic activity of Ficus bengalensis. Int J Diab Dev Ctries, 2007;27(2): 56–59.
11. Cromwell BT, Peach K and Tracey MV. Modern Methods of Plant Analysis.In: alkaloids, (Berlin, Springer Verlag) 1955;3:373–374.
12. Kokate CK, Purohit AP and Gokhale SB, Pharmacognosy, 42nd Edition, 2008 Nirali Prakashan, Pune, 447.
13. Finar IL. Organic chemistry Stereochemistry and Chemistry of Natural Products. Volume II (Longman Scientific and Technical press, London) 1975;518.
14. Peach K, Tracey M V. Modern Methods of Plant Analysis Vol. 4. Berlin: Springer Verlag, 1955, 68.
15. Trease GE and Evans WC. Pharmacognosy. In: Phenols and Phenolic Glycosides, ELBS, London, 1989;223–224, 246–249.
16. The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare Department of Indian System of Medicine and Homoeopathy, New Delhi, Part-I, 1st Edition, Vol-V, 2006; 110-114, 119-124.
17. Geinssman TA. Flavonoids. In: Peach, K, Tracey MV editors. Modern Methods of Plant Analysis. Berlin Springer Verlag: 1955;3:34-36.