Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(3):98-104

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Preliminary phytochemical screening and HPTLC fingerprinting of leaf extracts of *Ficus nervosa Heyne ex Roth*

G. S. Sushma*, B. Archana Devi, CH. Madhulatha, K. Uday Kumar, P. Harathi, N. Siva Subramanian and M. Ramadevi

Teegala Krishna Reddy College of Pharmacy, Hyderabad

ABSTRACT

To establish the fingerprint profile of Ficus nervosa using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. The results of preliminary phytochemical studies confirmed the presence of alkaloid, carbohydrate , glycoside , steroid, protein, tannin, terpenoid, flavonoid and phenol. HPTLC finger printing of chloroform extract of leaf revealed 11 peaks with Rf values in the range of 0.07 to 1; ethyl acetate extract of leaf showed 11 peaks with Rf values in the range of 0.07 to 0.99 and 90% ethanolic extract of leaf revealed 13 peaks with Rf values in the range of 0.03 to 1. It can be concluded that HPTLC fingerprint analysis of leaf of Ficus nervosa can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Key words: *Ficus nervosa* leaf, phytochemical screening, HPTLC fingerprinting.

INTRODUCTION

Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system, (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action , some have been thoroughly investigated and some of are still to be explored.

Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards[1],[2].HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time.[3]

Ficus nervosa belongs to the family Moraceae. It is an monoceious evergreen medium sized trees used traditionally for its curative property in treating diabetis , rheumatism and ulcer disorders[4]. In this present study the Preliminary phytochemical screening of *Ficus nervosa leaf* extraction has been done to identify the chemical constituents and HPTLC fingerprinting of *Ficus nervosa leaf* extract has been performed which may be used as markers for quality evaluation and standardization of the drug.

EXPERIMENTAL SECTION

2.1 Plant material

The plant specimens for the proposed study were collected from Tirumala hills, Tirupathi, India. The plant was authenticated by Dr. Madhava Chetty, Dept. of Botany and specimen herbarium were preserved at Sri Venkateshwara University library.(V. NO-0603).

2.2 Preparation and Extraction of Plant material

The 500 gms of coarsely powdered plant material of leaf of *Ficus nervosa* were defatted with petroleum ether and extracted successively with chloroform, ethyl acetate and 90% ethanol using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by whattman filter paper (no.1). The extract was evaporated under reduced pressure using rotovac evaporator.

2.3 Phytochemical screening

The phytochemical investigation of the different leaf extracts of *Ficus nervosa* was carried out with standard protocol[5] . The extraction of plants material was carried out with petroleum ether, chloroform, ethyl acetate and 90% ethanol. The results were presented in Table 1.

2.4 HPTLC profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne[6] and Wagner[7] *et al.,*

2.4.1 Sample preparation

Chloroform and ethyl acetate and 90% ethanolic extracts obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was redissolved in 1ml of chromatographic grade chloroform, ethyl acetate and 90% ethanol, which was used for sample application on pre-coated silica gel 60F254 aluminium sheets.

2.4.2 Developing solvent system

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent n Hexane: ethyl acetate(3.5:1.5).

2.4.3 Sample application

Application of bands of each extract was carried out(4mm in length and 1ul in concentration for leaf) using spray technique.Sample were applied in duplicate on pre-coated silica gel 60F254 aluminiumsheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.4.4 Development of chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent n-Hexane: ethyl acetate (3.5:1.5) for 15 min.

2.4.5 Detection of spots

The air-dried plates were viewed in ultraviolet radiation to mid day light. (Figure 1)The chromatograms were scanned by densitometer at 420 nm after spraying with anisaldehyde sulphuric acid The Rf values and finger print data were recorded by WIN CATS software.

RESULTS AND DISCUSSION

The phytochemical test on petroleum ether, chloroform, ethylacetate and ethanolic extracts of *Ficus nervosa* leaf showed the presence of various phytoconstituents like alkaloid, carbohydrate , glycoside , steroid, protein, tannin, terpenoid, flavonoid and phenol are present(Table 1).

Chloroform extract of *Ficus nervosa* leaf showed there are eleven polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.07 to 1 in which highest concentration of the phytoconstituents was found to be 18.83% and its corresponding R_f value was found to be 0.83 respectively and was recorded in Table 2. The corresponding

HPTLC chromatogram was presented in Figure 2.

Table 1Preliminary Phytochemical Screening of different extracts of *Ficus nervosa* leaf

Constituents	Test	Pet. Ether Extract	Chloroform Extract	Ethyl acetate Extract	90% ethanolic Extract
Alkaloids	Mayer's reagent	-	+	+	+
	Dragendorff's reagent	-	+	+	+
	Hager's reagent	-	+	+	+
	Wagner's reagent	-	-	+	+
Sugars & Carbohydrates	Molish's reagent	-	-	+	+
	Barfoed's test	-	-	+	+
	Fehling's test	-	+	+	+
	Benedict's test	-	+	+	+
Glycosides	Keller-Killiani test	-	+	+	+
	Borntrager's test	-	+	+	+
	Legal's test	-	+	+	+
	Baljet's test	-	+	+	+
Steroids	Libermann-Burchard test	+	+	-	-
	Salkowski reaction	+	+	-	-
	Libermann's test	+	+	-	-
Tannins	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution	-	-	+	+
	Bromine water	-	-	+	+
Protein	Millon's test	-	-	+	+
	Biuret test	-	-	+	+
	Xanthoprotein test	-	-	+	+
Amino acid	Ninhydrin test	-	-	-	-
Terpenoids	Noller's test	-	-	+	+
Flavonoids	Shinoda test	-	-	+	+
Anthocyanins	Sodium hydroxide test	-	-	-	-
Quinone	Sodium hydroxide test	-	-	-	-
Saponin	Foam test	-	-	-	-
Phenolic compounds	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution	-	-	-	-
Fixed oil and fats	Spot test	-	-	-	-
	Saponification test	-	-	-	-
Gums and mucilage	Swelling test	-	-	-	-
Resins	Turbidity test	-	-	-	-
	Hydrochloric acid test	-	-	-	-

Table 2 HPTLC profile of the chloroform extract of *Ficus nervosa* leaf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.0 AU	0.05 Rf	38.0 AU	5.52 %	0.07 Rf	1.0 AU	487.4 AU	1.90 %
2	0.19 Rf	6.1 AU	0.23 Rf	59.1 AU	8.58 %	0.27 Rf	1.0 AU	1456.1 AU	5.67 %
3	0.27 Rf	0.4 AU	0.29 Rf	12.2 AU	1.77 %	0.30 Rf	8.7 AU	102.4 AU	0.40 %
4	0.35 Rf	4.6 AU	0.39 Rf	17.1 AU	2.48 %	0.42 Rf	2.1 AU	531.8 AU	2.07 %
5	0.45 Rf	0.4 AU	0.51 Rf	35.5 AU	5.15 %	0.56 Rf	8.8 AU	1813.6 AU	7.07 %
6	0.57 Rf	9.6 AU	0.59 Rf	37.1 AU	5.39 %	0.61 Rf	15.2 AU	970.8 AU	3.78 %
7	0.61 Rf	15.3 AU	0.65 Rf	47.4 AU	6.88 %	0.68 Rf	2.5 AU	1485.1 AU	5.79 %
8	0.70 Rf	2.1 AU	0.74 Rf	101.2 AU	14.69 %	0.76 Rf	39.2 AU	2605.2 AU	10.15 %
9	0.76 Rf	69.9 AU	0.80 Rf	129.7 AU	18.83 %	0.83 Rf	74.2 AU	5814.0 AU	22.65 %
10	0.83 Rf	74.6 AU	0.86 Rf	98.6 AU	14.30 %	0.89 Rf	34.8 AU	4439.5 AU	17.30 %
11	0.89 Rf	84.8 AU	0.93 Rf	113.0 AU	16.40 %	1.00 Rf	0.5 AU	5957.4 AU	23.21 %

Table 3 HPTLC profile of the ethyl acetate extract of *Ficus nervosa* leaf

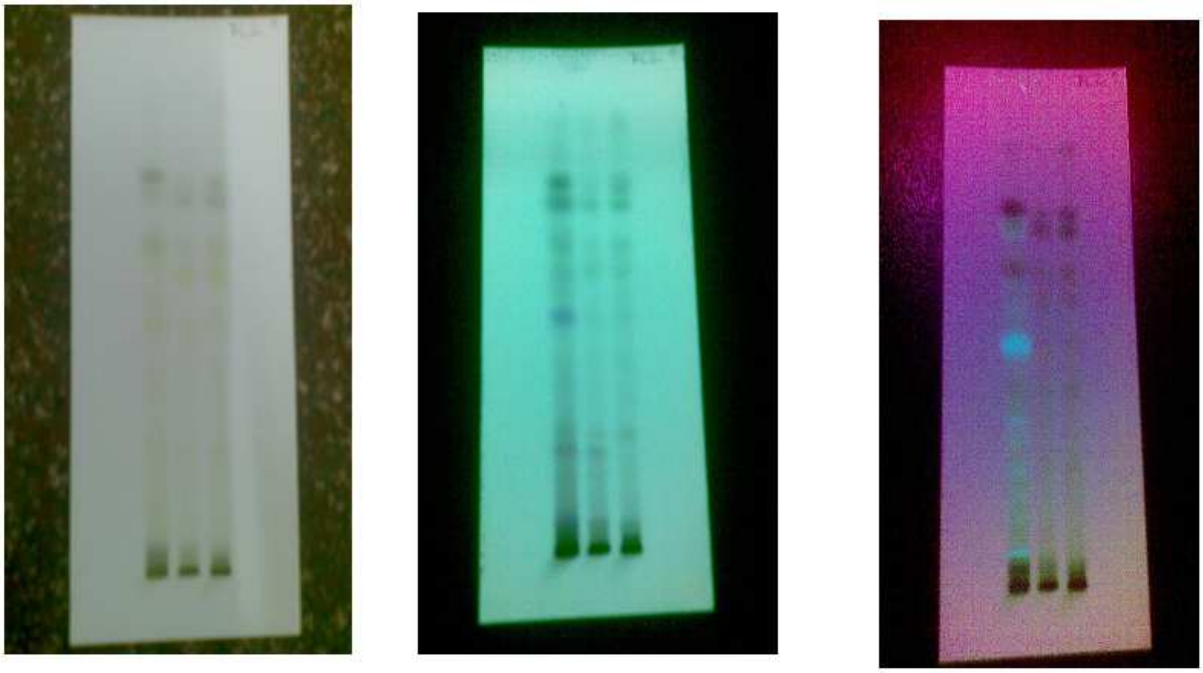
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.4 AU	0.06 Rf	52.6 AU	7.19 %	0.07 Rf	0.5 AU	739.4 AU	3.05 %
2	0.13 Rf	0.4 AU	0.16 Rf	35.4 AU	4.84 %	0.17 Rf	22.3 AU	753.6 AU	3.11 %
3	0.17 Rf	22.3 AU	0.20 Rf	91.9 AU	12.57 %	0.22 Rf	17.5 AU	2023.1 AU	8.35 %
4	0.22 Rf	17.8 AU	0.24 Rf	51.4 AU	7.02 %	0.26 Rf	2.3 AU	766.6 AU	3.16 %
5	0.44 Rf	0.6 AU	0.49 Rf	28.7 AU	3.92 %	0.53 Rf	7.7 AU	1006.9 AU	4.16 %
6	0.56 Rf	6.3 AU	0.59 Rf	42.4 AU	5.79 %	0.62 Rf	13.9 AU	1341.7 AU	5.54 %
7	0.63 Rf	14.0 AU	0.65 Rf	31.7 AU	4.33 %	0.68 Rf	7.3 AU	945.6 AU	3.90 %
8	0.70 Rf	6.9 AU	0.75 Rf	116.5 AU	15.93 %	0.77 Rf	70.1 AU	3532.6 AU	14.58 %
9	0.77 Rf	70.3 AU	0.80 Rf	85.8 AU	11.73 %	0.82 Rf	70.3 AU	3498.8 AU	14.44 %
10	0.82 Rf	70.4 AU	0.86 Rf	100.5 AU	13.75 %	0.90 Rf	78.2 AU	5321.9 AU	21.96 %
11	0.90 Rf	78.3 AU	0.93 Rf	94.6 AU	12.93 %	0.99 Rf	2.8 AU	4302.3 AU	17.75 %

Table 4 HPTLC profile of the 90% ethanolic extract of *Ficus nervosa* leaf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	20.2 AU	0.01 Rf	20.2 AU	1.35 %	0.03 Rf	0.0 AU	99.9 AU	0.18 %
2	0.04 Rf	1.0 AU	0.06 Rf	56.7 AU	3.77 %	0.08 Rf	0.1 AU	944.4 AU	1.73 %
3	0.14 Rf	1.5 AU	0.16 Rf	29.1 AU	1.94 %	0.17 Rf	17.6 AU	535.0 AU	0.98 %
4	0.17 Rf	18.0 AU	0.20 Rf	108.5 AU	7.22 %	0.23 Rf	33.6 AU	2574.3 AU	4.72 %
5	0.23 Rf	34.6 AU	0.24 Rf	62.4 AU	4.15 %	0.29 Rf	2.0 AU	1392.6 AU	2.55 %
6	0.30 Rf	1.9 AU	0.34 Rf	32.2 AU	2.14 %	0.38 Rf	7.8 AU	1183.9 AU	2.17 %
7	0.44 Rf	4.3 AU	0.49 Rf	141.4 AU	9.41 %	0.52 Rf	33.8 AU	4580.7 AU	8.40 %
8	0.52 Rf	34.1 AU	0.58 Rf	89.0 AU	5.92 %	0.62 Rf	47.2 AU	4807.3 AU	8.82 %
9	0.62 Rf	47.4 AU	0.66 Rf	103.9 AU	6.91 %	0.68 Rf	39.6 AU	4314.4 AU	7.92 %
10	0.70 Rf	72.6 AU	0.75 Rf	312.4 AU	20.78 %	0.77 Rf	36.7 AU	12163.1 AU	22.32 %
11	0.77 Rf	207.8 AU	0.80 Rf	291.2 AU	19.37 %	0.83 Rf	39.2 AU	10501.4 AU	19.27 %
12	0.83 Rf	109.6 AU	0.85 Rf	143.1 AU	9.52 %	0.90 Rf	30.5 AU	6394.7 AU	11.73 %
13	0.90 Rf	100.7 AU	0.93 Rf	113.3 AU	7.53 %	1.00 Rf	0.2 AU	5014.1 AU	9.20 %

Ethylacetate extract of *Ficus nervosa* leaf showed eleven polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.07 to 0.99 in which highest concentration of the phytoconstituents was found to be 15.93% and its corresponding R_f value was found to be 0.77 respectively and was recorded in Table 3. The corresponding HPTLC chromatogram was presented in Figure 3.

90% Ethanolic extract of *Ficus nervosa* leaf showed thirteen polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.03 to 1 in which highest concentration of the phytoconstituents was found to be 20.78% and its corresponding R_f value was found to be 0.77 respectively and was recorded in Table 4. The corresponding HPTLC chromatogram was presented in Figure 4.



HPTLC plate Seen at Visible light | **HPTLC plate seen at 254nm** | **HPTLC plate seen at 366nm**

Track 1: Alcoholic extract
Track 2: Ethyl Acetate extract
Track 3: Chloroform extract

Figure 1. HPTLC profile of leaf extract of *Ficus nervosa*

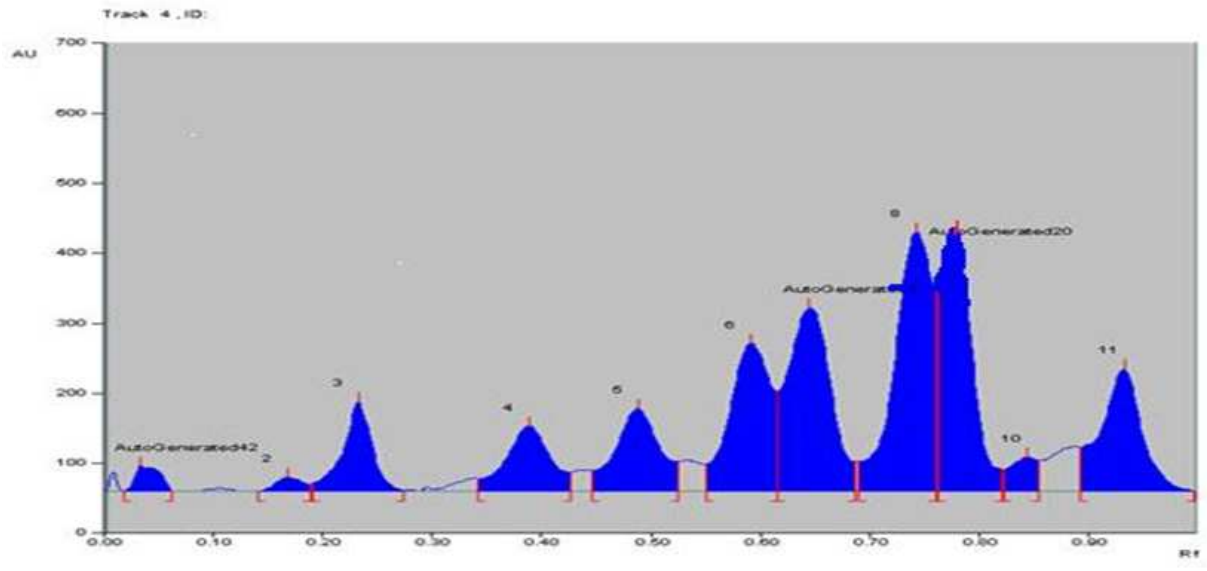


Figure 2.HPTLC chromatogram of *Ficus nervosa* chloroform leaf extract showing different peaks of phytoconstituents.

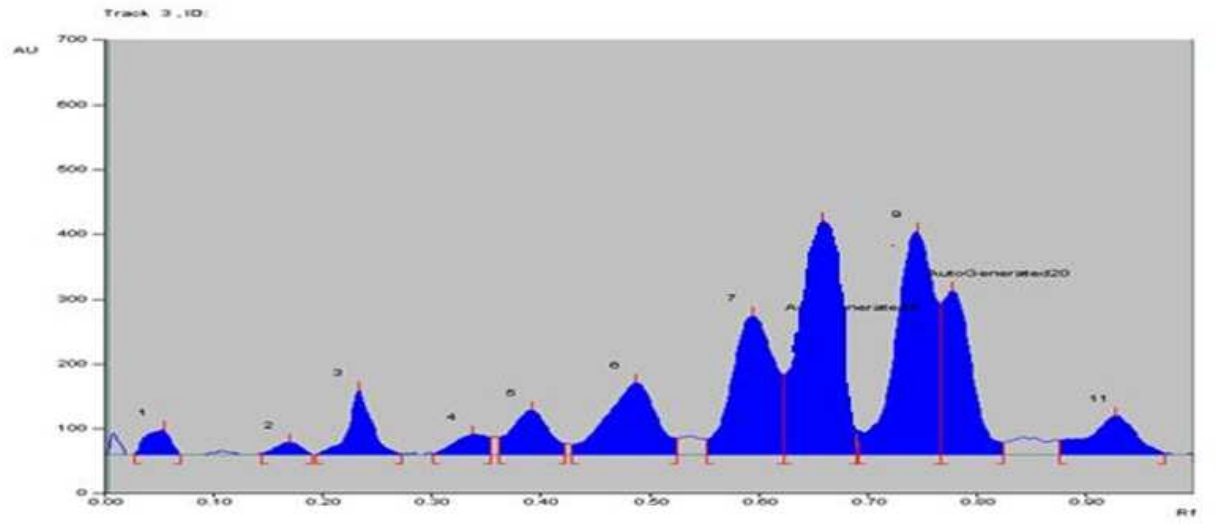


Figure 3 HPTLC chromatogram of *Ficus nervosa* ethyl acetate leaf extract showing different peaks of phytoconstituents.

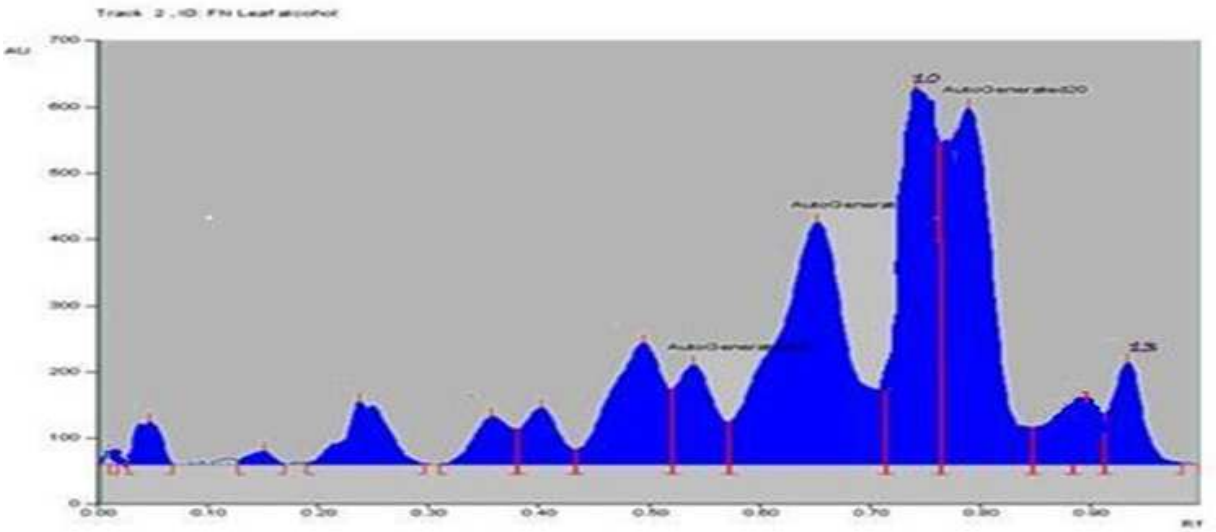


Figure 4. HPTLC chromatogram of *Ficus nervosa* 90% ethanolic leaf extract showing different peaks of phytoconstituents.

CONCLUSION

HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant.

It is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations.Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies.

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials, and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is

more versatile than ordinary TLC methods, as the spots were well resolved. Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant.

Acknowledgement
I wish to express my sincere gratitude to Principal and Manager, TKR College Of Pharmacy for providing me an opportunity to do my project work. I am short of words to thank Nishka laboratoratories helped me to carry out the analytical results.

REFERENCES

[1]. R Chaudhay Ranjit , *Herbal Medicine for Human Health*; Regional Publication, SEARO, No. 20, W.T.O, New Delhi; **1992,** 1-80.
[2]. *Quality Control Method for Medicinal Plant Materials*; W.H.O., Geneva; **1989**, 1-15.
[3]. P D Sethi, *High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations*; CBS Publishers and Distributers, New Delhi; **1996**, 10-60.
[4].K Madhava chetty , *Book of Flowering plants of chittoor district*; AP, India; **2008**, 333- 334.
[5].KR Khandelwal , *Techniques and Experiments,Practical Pharmacognosy* ;17th edition, Nirali Prakashan, Pune, **2007**, 149- 156.
[6]. JB Harborne , *Phytochemical methods;* 3rd edition, London: Chapman and Hall; **1998**.
[7]. H Wagner ,S Baldt , *Plant drug analysis;* Berlin: Springer; **1996**.