Research Article
CODEN (USA): IJPLCP

[Devi *et al.*, 4(3): March., 2013]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Phytochemical screening and HPTLC fingerprint analysis of bark extracts of *Ficus nervosa Heyne Ex Roth*

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Abstract

This research analyzed major chemical components present in fresh extracts of stem bark of *Ficus nervosa.* Bark of *Ficus nervosa* was collected, powdered and defatted with petroleum ether and then extracted successively with chloroform, ethyl acetate and 90% alcohol. Preliminary phytochemical screening of the bark extracts of *Ficus nervosa* showed the presence of chemical constituents like alkaloids, glycosides, sugars and carbohydrates, flavonoids, tannins, phenolic compounds, etc. HPTLC finger print analysis of the *Ficus nervosa* bark extracts showed the presence of possible number of components. HPTLC finger print study demonstrated the consistent quality of chemical constituents. The experimental conditions as well as general comments on the application of chromatographic fingerprint analysis are discussed.

Key-Words: *Ficus nervosa*, Phytochemical constituents, HPTLC

Introduction

Herbal medicine is the oldest form of healthcare known to mankind. Herbs have been used by all cultures throughout history. Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. Plants have been known to relieve various diseases in Ayurveda. Natural herbs are used as remedies for human ailments as they contain components of therapeutic values. The goals of using plants as sources of therapeutic agents are to isolate bioactive compounds for direct use as drugs, to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, to use agents as pharmacological tools, to use the whole plant or part of it as a herbal remedy[1].

Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles[2].

The herb, *Ficus nervosa* belongs to the family, Moraceae[3]. It is an evergreen tree distributed in China, India, Malaysia and throughout Taiwan. *Ficus* species (Moraceae) are plants well known all over the world as "fig plant".

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Recently, over 1000 Formosan plants have been screened for *in vitro* antimycobacterial activities, and *Ficus nervosa* has been found to be one of the active species.

Material and Methods

Plant material

The plant specimens for the proposed study were collected from Tirumala hills, Tirupati. The plant was authenticated by Dr. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, India, and specimen was preserved at University herbarium, Voucher number 0603. The bark was separated from other parts, washed, cleaned and dried for further use.

Preparation of the Extract

The coarsely powdered plant material of *Ficus nervosa* stem bark was defatted with petroleum ether and extracted successively with chloroform, ethyl acetate and 90% ethanol using Soxhlet apparatus. 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.

Preliminary Phytochemical Screening

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids etc. The preliminary phytochemical screening of all the

extracts of *Ficus nervosa* bark was carried out as per the standard procedure[4].

High Performance Thin Layer Chromatography

HPTLC[5] is a sophisticated and automated form of TLC. HPTLC is the fastest of all chromatographic methods. HPTLC precoated, silica gel G 60 F25 (Merck, Germany) plates were used for the application of sample. A small quantity of extracts was dissolved in respective solvents. Sample was applied on precoated plate with the help of Linomat 5 applicator. Solvent system optimized for TLC study was chosen for HPTLC study.

The details of HPTLC were as follows:-

Plate : Aluminium plate precoated with silica gel GF254
Thickness : 250µm
Plate size : 5 × 10 cm
Sample application : 10 µl
Solvent system : n-hexane : ethyl acetate (8.5 : 1.5 v/v)
Detection : U.V. (Visible light, 254nm, 366 nm)
Instrument : CAMAG TLC Scanner and densitometric evaluation with WINCATS software.

Results and Discussion

Preliminary phytochemical screening of the bark extracts of *Ficus nervosa* showed the presence of chemical constituents like alkaloids, glycosides, sugars and carbohydrates, flavonoids, tannins, phenolic compounds, etc. The results are given in Table: 1.

HPTLC shows separation of components present in the chloroform, ethyl acetate and 90% alcoholic extract of the stem bark of *Ficus nervosa.* The method may be applied to identify the plant of *Ficus nervosa* from other species. HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species.

The results from HPTLC finger print scanned at wavelength 420 nm for chloroform extract of *Ficus nervosa* bark. There are six polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.07 to 0.94 in which highest concentration of the phytoconstituents was found to be 27.75% and its corresponding R_f value was found to be 0.57.

The results from HPTLC finger print scanned at wavelength 420 nm for Ethylacetate extract of *Ficus nervosa* bark. There are fourteen polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.10 to 0.94 in which highest concentration of the phytoconstituents was found to be 16.64% and its corresponding R_f value was found to be 0.10.

The results from HPTLC finger print scanned at wavelength 420 nm for Alcoholic extract of *Ficus nervosa* bark showed that there are eight polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.09 to 0.92 in which highest concentration of the phytoconstituents was found to be 47.48% and its corresponding R_f value was found to be 0.92.

The R_f values for different extracts were given in Table: 2, Table: 3 and Table: 4 respectively.

The chromatograms of different extracts were given in Fig: 2, Fig: 3 and Fig: 4 respectively.

Conclusion

Secondary metabolites are present and they are responsible for therapeutic effects.

HPTLC study as recommended in this study provides a chromatographic fingerprint of phytochemicals and is suitable for confirming the identity and purity of medicinal plant raw material.

HPTLC pre-coated plates with the mobile phase n–hexane : ethyl acetate developed chromatograms which showed distinct phytochemical variations in chloroform, ethyl acetate and hydro alcoholic extracts.

Acknowledgement

My sincere thanks to the principal and the management, Teegala Krishna Reddy College of Pharmacy, Meerpet, Hyderabad, for providing necessary facilities to carry out the research work.

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Table: 1 Preliminary Phytochemical Studies of Extracts of FNB

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	L- Burchard test	+	+	-	-
	Salkowski test	+	+	-	-
	Libermann's test	+	+	-	-
Tannins	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution test	-	-	+	+
	Bromine water	-	-	+	+
	Potassium dichromate test	-	-	+	+
Protein	Million'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, mucilage	Swelling test	-	-	-	-
Resins	Turbidity test	-	-	-	-
	Hydrochloric acid test	-	-	-	-

Where: + Present, - Absent

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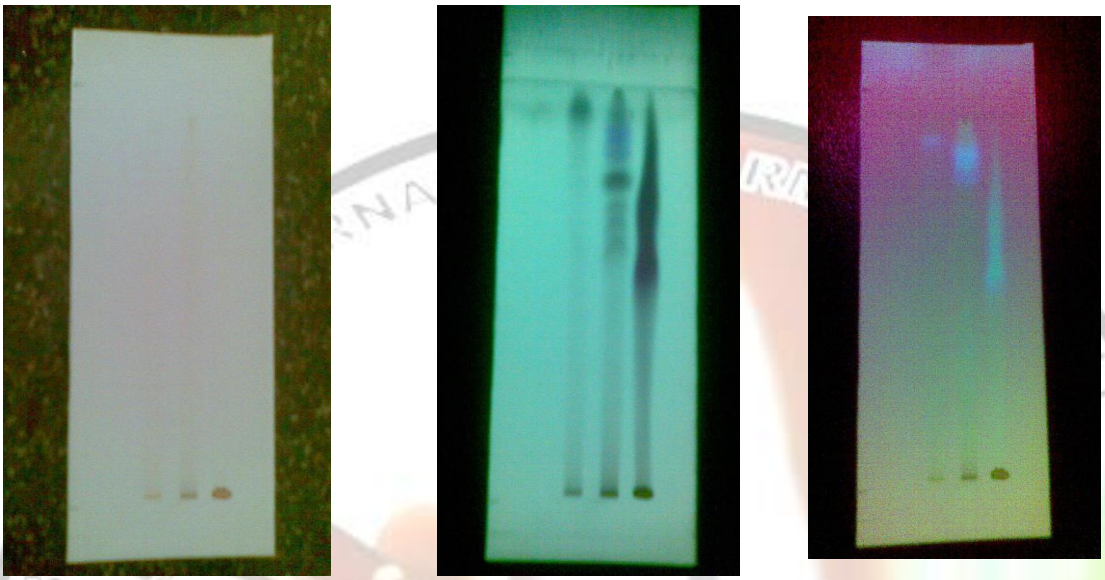

1 2 3 1 2 3 1 2 3

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

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

Fig. 1: HPTLC plates of bark extracts of *Ficus nervosa*

Table 2: R_f Values for Chloroform Extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.9 AU	0.03 Rf	590.7 AU	24.24 %	0.07 Rf	39.0 AU	16376.3 AU	7.43 %
2	0.09 Rf	202.8 AU	0.16 Rf	218.9 AU	8.98 %	0.16 Rf	14.7 AU	12418.6 AU	5.64 %
3	0.34 Rf	319.7 AU	0.50 Rf	676.4 AU	27.75 %	0.57 Rf	36.4 AU	94263.2 AU	42.78 %
4	0.57 Rf	566.5 AU	0.64 Rf	626.5 AU	25.70 %	0.80 Rf	73.1 AU	83737.2 AU	38.00 %
5	0.82 Rf	167.1 AU	0.86 Rf	196.8 AU	8.08 %	0.90 Rf	38.7 AU	10976.7 AU	4.98 %
6	0.90 Rf	109.0 AU	0.92 Rf	128.1 AU	5.26 %	0.94 Rf	0.2 AU	2580.2 AU	1.17 %

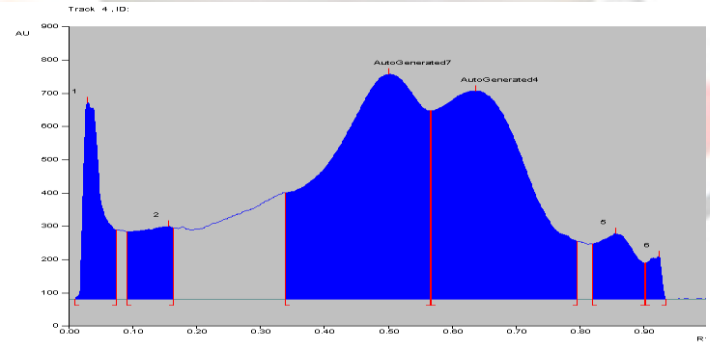

Fig. 2: Chromatogram of Chloroform Extract

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Table 3: R_f values for Ethyl acetate Extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.7 AU	0.03 Rf	385.6 AU	16.64 %	0.10 Rf	35.9 AU	11012.9 AU	13.93 %
2	0.10 Rf	60.9 AU	0.11 Rf	73.5 AU	3.17 %	0.14 Rf	31.5 AU	1245.3 AU	1.58 %
3	0.14 Rf	29.3 AU	0.15 Rf	33.4 AU	1.44 %	0.18 Rf	17.7 AU	939.3 AU	1.19 %
4	0.21 Rf	17.7 AU	0.22 Rf	20.5 AU	0.88 %	0.24 Rf	2.5 AU	366.9 AU	0.46 %
5	0.35 Rf	0.1 AU	0.38 Rf	23.2 AU	1.00 %	0.39 Rf	8.2 AU	251.6 AU	0.32 %
6	0.41 Rf	9.4 AU	0.44 Rf	20.0 AU	0.86 %	0.46 Rf	8.0 AU	621.7 AU	0.79 %
7	0.47 Rf	8.2 AU	0.55 Rf	186.7 AU	8.06 %	0.57 Rf	53.1 AU	8731.0 AU	11.04 %
8	0.58 Rf	151.8 AU	0.60 Rf	172.9 AU	7.46 %	0.63 Rf	36.5 AU	5570.5 AU	7.05 %
9	0.63 Rf	136.5 AU	0.64 Rf	149.9 AU	6.47 %	0.67 Rf	37.1 AU	4254.3 AU	5.38 %
10	0.67 Rf	97.8 AU	0.71 Rf	378.1 AU	16.32 %	0.74 Rf	30.3 AU	14756.3 AU	18.66 %
11	0.74 Rf	161.2 AU	0.77 Rf	246.2 AU	10.63 %	0.79 Rf	38.5 AU	8763.5 AU	11.08 %
12	0.79 Rf	238.7 AU	0.82 Rf	261.0 AU	11.27 %	0.84 Rf	38.8 AU	11521.1 AU	14.57 %
13	0.85 Rf	238.8 AU	0.86 Rf	243.8 AU	10.52 %	0.92 Rf	34.0 AU	9842.2 AU	12.45 %
14	0.92 Rf	105.1 AU	0.93 Rf	122.0 AU	5.27 %	0.94 Rf	1.4 AU	1188.9 AU	1.50 %

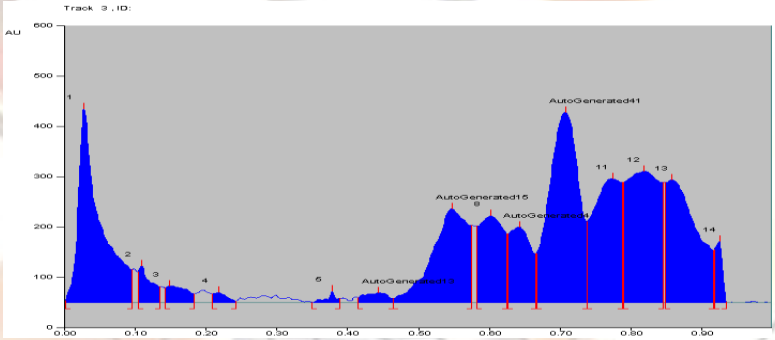

Fig. 3: Chromatogram of Ethyl acetate Extract

Table 4: R_f values for 90% Alcoholic Extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.5 AU	0.03 Rf	308.6 AU	30.57 %	0.09 Rf	46.8 AU	7043.9 AU	15.14 %
2	0.09 Rf	47.1 AU	0.09 Rf	51.4 AU	5.09 %	0.15 Rf	19.4 AU	1744.1 AU	3.75 %
3	0.32 Rf	3.6 AU	0.33 Rf	17.6 AU	1.74 %	0.35 Rf	1.2 AU	190.8 AU	0.41 %
4	0.53 Rf	0.5 AU	0.54 Rf	10.4 AU	1.03 %	0.55 Rf	5.6 AU	98.2 AU	0.21 %
5	0.55 Rf	5.9 AU	0.59 Rf	22.0 AU	2.18 %	0.63 Rf	3.8 AU	939.0 AU	2.02 %
6	0.66 Rf	4.4 AU	0.70 Rf	42.6 AU	4.22 %	0.72 Rf	26.8 AU	1254.6 AU	2.70 %
7	0.72 Rf	27.4 AU	0.75 Rf	77.5 AU	7.68 %	0.76 Rf	76.7 AU	1660.6 AU	3.57 %
8	0.76 Rf	76.9 AU	0.86 Rf	479.2 AU	47.48 %	0.92 Rf	26.3 AU	33599.3 AU	72.21 %

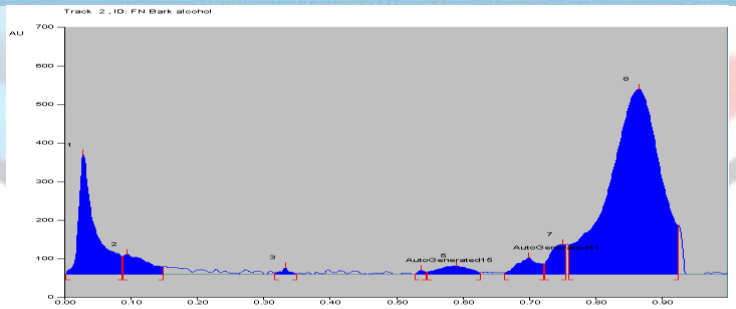

Fig. 4: Chromatogram of 90% Alcoholic Extract