

Phytochemical analysis of *Ficus nervosa* heyne ex roth leaves

¹Rama Krishna Reddy P*, ²Himaja Trivedi M, ³Haritha Reddy K,
³Mohana Lakshmi S and ²Venkata Ramana K.

¹Arvindaksha Educational Society's Group of Institutions, Balemla, Suryapet, Nalgonda, Andhra Pradesh, India.

²A.S.N. Pharmacy College, Burrupalem Road, Tenali, Guntur, Andhra Pradesh, India.

³Sree Vidyanikethan College of Pharmacy, A. Rangam peta, Tirupathi, Andhra Pradesh, India.

*Corresponding Author: E-Mail: krishyheart@gmail.com

ABSTRACT

Ficus nervosa Heyne ex Roth is a medium sized evergreen tree belongs to *Moraceae* family. It occurs in streams in valleys of deciduous forests. The present study is aimed to evaluate the phytochemical constituents present in the leaf extract of *Ficus nervosa*. Preliminary phytochemical studies of petroleum ether, chloroform, ethanol and aqueous leaf extract show the presence of alkaloids, steroids, amino acids, tannins, carbohydrates, saponins, fats and oils. TLC profile, Column Chromatography, HPLC, IR and NMR spectroscopy of are petroleum ether extract revealed the presence of the sterol compound. The study will be useful for the identification of leaf of *Ficus nervosa* and will prevent its adulteration. The determination of these characters will aid future investigators in their pharmacological analysis of this species.

Keywords: *Ficus nervosa*, Phytochemicals, Alkaloids, Sterols, TLC, Petroleum ether extract.

1. INTRODUCTION

The herbal products usually symbolize safety in contrast to the synthetic drugs that are considered harmful to human beings and their manufacturing pollutes the environment. Although herbs had been priced for their medicinal and aromatic qualities since time immemorial, the synthetic products of the modern age have surpassed these for a while. Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. The evaluation of a crude drug is necessary because of these main reasons i) bio-chemical variation in the drugs, ii) deterioration due to treatment and storage and iii) substitution and adulteration, a result of carelessness, ignorance or fraud. Phytochemical constituents have played a major role as basic source for the establishment of several pharmaceutical industries. *Ficus nervosa* Heyne ex Roth is a medium sized evergreen tree belongs to *Moraceae* family. It occurs in streams in valleys of deciduous forests. Vernacular names of the tree are Vonjari in Telugu, Neer-al in Tamil, Eechamaram in Malayam and Narrowed leaved fig in English. It occurs in India, Australia, China, Taiwan, Malaysia, rare along streams in valleys of

deciduous forests, Srivarimettu, Talakona, Tirumala, East- Godavari, Visakapatnam in Andhra Pradesh [1,2]. In the present study, an effort has been made to establish the phytochemical analysis of the leaf of *Ficus nervosa* Heyne ex Roth.

2. MATERIALS AND METHODS

2.1. Collection and authentication

The leaves of *Ficus nervosa* Henye ex Roth (*Moraceae*) were collected from Tirumala hills, Tirupathi, India and it was identified and authenticated. The taxonomical identification and authentication of the plant was done by Dr. K. Madhava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupati. The voucher specimen was preserved in our laboratory for further reference.

2.2 Preparation of extraction

About 1 kg of the leaf material was successively extracted with solvents like petroleum ether, chloroform, and methanol in a Soxhlet apparatus. The extract was concentrated and traces of the solvent were completely removed under reduced pressure and stored in vacuum desiccator for further use. Aqueous extract was prepared by macerating the leaf powder in double distilled water. The extract was

Table - 1: Percentage yield and consistency of various extracts of *Ficus nervosa* Heyne ex Roth (Moraceae)

Solvents	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Percentage yield	9%	5%	4%	6%
Consistency	Sticky	Non-greasy	Non-greasy	Non-greasy

Table - 2: Preliminary Phytochemical Analysis of various extracts of *Ficus nervosa* Heyne ex Roth (Moraceae)

Phytochemicals	Petroleum ether	Chloroform	Methanol	Aqueous
Alkaloids	-	+	+	-
Steroids	+	-	-	-
Amino acids	-	-	-	-
Tannins	-	+	+	-
Carbohydrates	-	+	+	+
Saponins	-	+	+	+
Flavonoids	-	-	-	-
Fixed oils&fats	+	+	+	-

concentrated and stored in desiccator. The percentage yield and consistency of various extracts was tabulated (Table 1).

2.3. Phytochemical analysis

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents as per the standard procedures and reported in the table 2 [3,4].

2.4. Thin layer chromatographic characterization of petroleum ether extract of *Ficus nervosa* heyne ex roth (Moraceae) leaves

TLC is performed as per standard procedures [5-7] using Silica gel G as stationary phase in the composition of adsorbent: Water (1:2) and Chloroform: Benzene (9:1) and Benzene: Petroleum ether (1:1) as mobile phase.

2.5. Column chromatographic studies of petroleum ether extract of *Ficus nervosa* heyne ex roth (Moraceae) leaves

Column chromatography is performed by standard procedures by using glass column, Silica gel. 60-120 mesh as stationary phase and Chloroform: Benzene (9: 1) is used as mobile phase. The column is packed by wet packing method [5-8].

2.6. High performance liquid chromatographic analysis of isolated compound from petroleum ether extract of *Ficus nervosa* heyne ex roth (Moraceae) leaves

HPLC is performed by standard procedures given by Ravi Sankar, 2006; Gurudeep

Chatwal *et al.*, 2004; Sharma, 2006. In this column is Phenomenex Luna C18, 5 μ , (4.6 X 250 mm) at the wave length 272 nm, flow rate is 1.0 ml/minute. 0.1% v/v phosphoric acid in water (A); Acetonitrile (B) is used as mobile phase and methanol is used as the solvent (figure 1)

2.7. Infrared spectroscopic analysis of isolated compound from petroleum ether extract of *Ficus nervosa* heyne ex roth (Moraceae) leaves

The infra red spectroscopy of the isolated steroidal compound was obtained on a Perkin – Elemer BXF1 FT-IR Spectrophotometer (LIRC, Vijayawada) with samples prepared as potassium bromide (KBr) pellets [5-7].

2.8. Proton magnetic resonance spectroscopic analysis of isolated compound from petroleum ether extract of *Ficus nervosa* heyne ex roth (Moraceae) leaves

Proton magnetic resonance of isolated compound was recorded in CDCl₃ solution on the Bruker DPX-400 NMT Spectrometer in 400 MHz (LIRC, Vijayawada) using TMS (Tetra Methyl Saline) as internal standard.

3. RESULTS AND DISCUSSION

The chief phytochemicals present in the different extracts of *Ficus nervosa* Heyne ex Roth (Moraceae) are alkaloids, steroids, tannins, carbohydrates, saponins and fixed oils. Petroleum ether was used for extraction for further studies because percentage yield of petroleum ether extract was more compared to other solvents. Thin layer chromatography was performed for the extract using different mobile phases. One spot

Table - 3: TLC with different mobile phases

Mobile Phase	Observation		Spraying agent	Colour observed
	UV	Visible		
Chloroform: Benzene (9:1)	Fluorescent green	Pink	Carr Price reagent	Blue
Benzene: Petroleum ether (1:1)	Fluorescent green	Pink	Carr Price reagent	Blue

was obtained with the mobile phase Chloroform: Benzene (9:1) with R_f value 0.638. The solvent system Benzene: Petroleum ether (1:1) eluted one spot of R_f value 0.5. The bands appeared was first in light orange colour followed by bands of orange, light yellow, pale yellow finally colourless were collected from the column. The first fraction gave positive results for steroid. Thin layer chromatography was performed for the fraction using Chloroform: Benzene in the ratio 9:1 as mobile phase and the R_f value was 0.53. After development, the plate was observed under UV light and the spot appeared in pink colour at visible light and in fluorescent green colour at UV light (Table 3). The spot appeared in blue colour after the application of Carr Price reagent.

The column chromatographic separation of petroleum ether extract of *Ficus nervosa* on silica gel yielded, which is a crystalline powder with melting point of 162^o c. It gave a positive test for Libermann - Buchard reagent for steroidal nucleus. In IR spectrum, a very intensely broad band at 3430 cm^{-1} and moderately intense band at 1096 and 723 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at 804 cm^{-1} . The corresponding C=C vibrations was shown around 1734 cm^{-1} as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band 2918 cm^{-1} and medium intensity band at 1463 cm^{-1} . The vibration of the methylenic part was shown by the band at 2849 cm^{-1} and the medium band at 1450 cm^{-1} . The moderately intense band at 723 cm^{-1} was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at 1021 cm^{-1} . In ¹H-NMR spectrum, H-3 proton appeared as a triplet of a double doublet at 4.336 (J = 4.5 and 1.1 MHz) and H-6 olefinic proton showed a multiplet at δ 5.221. Two olefinic protons appeared downfield at 4.33 (m) and 4.43 (m) which were identical with the chemical shift of H-22 and H-23, respectively of stigmaterol. Six methyl protons also appeared at 0.75, 0.88, 0.925, 0.95, 1.01 and 1.16 (3H each, s, CH₃). These assignments are in good agreement for the structure of Stigmaterol⁽⁹⁾. But the ¹³C-NMR studies, DEPT analysis, Mass spectroscopy and X-ray crystallographic studies has to be

carried out to confirm the exact structure of isolated steroidal compound.

5. REFERENCES

1. Madhava Chetty K, Sivaji K and Tulasi Rao K. **Flowering plants of Chittoor District, Andhra Pradesh, India, Students Offset Printers, Tirupathi, 333-334.**
2. Gamble JS. Flora of Madras, **Botanical Survey of India, Calcutta, 1967, 3rd ed., 954.**
3. Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy, **Nirali Prakashan, Pune, 2004.**
4. Evans WC, Trease and Evans. Pharmacognosy, 5th edition, Elsevier, a division of Reed Elsevier **India Private Limited, Noida, 2008.**
5. Ravi Sankar S. Text Book of Pharmaceutical Analysis, **RX Publications, Tirunelveli, 3rd Edition, 2006.**
6. Gurudeep Chatwal R and Sham Anand K. Instrumental Methods of Chemical Analysis, **Himalaya Publishing House, 5th Edition, Mumbai, 2004.**
7. Sharma BK. Instrumental Methods of Chemical Analysis, **Krishna Prakashan Media (P) Ltd, Delhi, Twenty 5th Edition, 2006.**
8. Agarwal SS and Paridhavi M. **Herbal Drug Technology, Universities Press (India) Private Limited, Hyderabad, 2007.**
9. Jain PS and Bari SB. Isolation of Lupeol, Stigmaterol and Campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*, **Asain Journal of Plant Sciences, 2010; 9(3): 163-167.**