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 reveled 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 vaccum 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)

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Methanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentage yield</strong></td>
<td>9%</td>
<td>5%</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td><strong>Consistency</strong></td>
<td>Sticky</td>
<td>Non-greasy</td>
<td>Non-greasy</td>
<td>Non-greasy</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oils&amp;fats</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

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, 5µ, (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
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 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°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 methylene 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 methyleneic part. The corresponding C-C vibration was shown as weak intense band at 1021 cm\(^{-1}\). In \(^1\)H-NMR spectrum, H-3 proton appeared as a triplet of a doublet at 4.336 (J = 4.5 and 1.1 MHz) and H-6 olefinic proton showed a multiplet at S 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 stigmasterol. Six methyl protons also appeared at 0.75, 0.88, 0.925, 0.95, 1.01 and 1.16 (3H each, s, CH\(_3\)). These assignments are in good agreement for the structure of Stigmasterol \(^{(9)}\). But the \(^{13}\)C-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


