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# Preliminary Phytochemical Screening and HPTLC Fingerprinting of Leaf Extracts of *Pisonea aculeata*

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**Objective:** To establish the fingerprint profile of *Pisonea aculeata* using high performance thin layer chromatography (HPTLC) technique. Methods: 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. **Results:** Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpenes, tannins, saponnins, glycosides, phenolic compounds and flavonoids. HPTLC finger printing of chloroform extract of leaf revealed 14 peaks with Rf values in the range of 0.03 to 0.95; ethyl acetate extract of leaf showed 6 peaks with Rf values in the range of 0.04 to 0.94 and 90% ethanolic extract of leaf revealed 11 peaks with Rf values in the range of 0.03 to 0.93. **Conclusions:** It can be concluded that HPTLC fingerprint analysis of leaf extract of *Pisonea aculeata* 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.

*Keyword:* *Pisonea aculeata* Leaf, Phytochemical Screening, HPTLC Fingerprinting.

## 1. 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].

*Pisonia aculeata* Linn. (Nyctaginaceae) is a large scandent shrub, which holds an important place in folklore medicine. It is extensively used by native medical practitioners and tribes for treating swelling, rheumatic pains, jaundice and tumors[4]

In this present study the Preliminary phytochemical screening of *Pisonea aculeata leaf* extraction has been done to identify the chemical constituents and HPTLC fingerprinting of *Pisonea aculeata* leaf extracts has been performed which may be used as markers for quality evaluation and standardization of the drug.

## 2. Materials and Methods

### 2.1 Plant material

The plant specimens for the proposed study were collected from Tirumala Hills, Tirupathi District, Andhra Pradesh, India and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati Andhra Pradesh, India.

### 2.2 Preparation and Extraction of Plant Material

The leaves of *Pisonea aculeata* were shade dried and powdered coarsely and defatted with petroleum ether and 500 g was packed in a Soxhlet apparatus and extracted successively with chloroform, ethyl acetate and 90% ethanol. 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 *Pisonea aculeata* 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 are 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 re-dissolved 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.

### 2.5 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).

### 2.5.1 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 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

### 2.6 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 minutes.

### 2.6.1 Detection of Spots

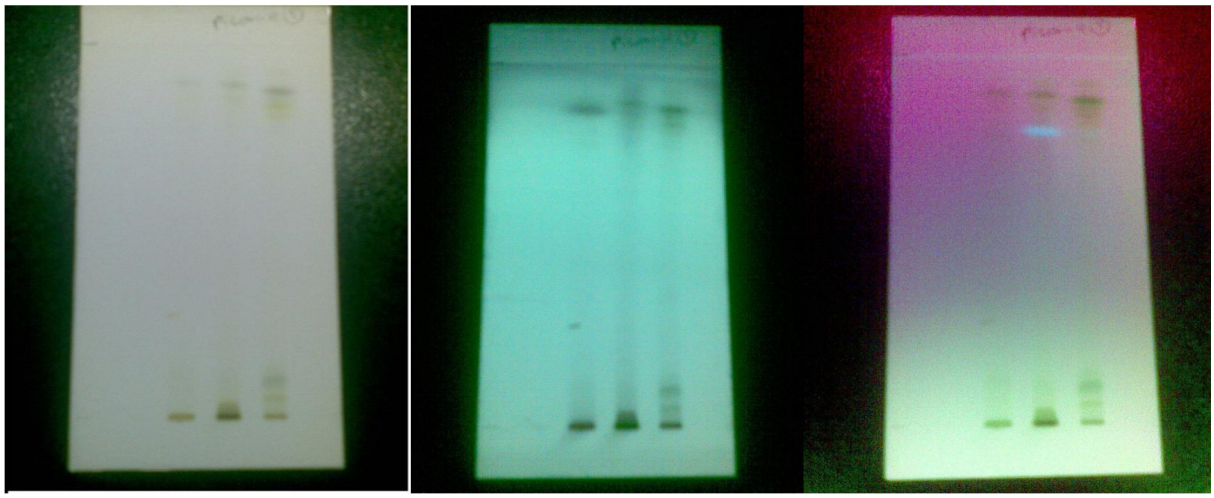
The air-dried plates were viewed in ultraviolet radiation to mid-day light (Figure 1). The chromatograms were scanned by densitometer at 420 nm after spraying with anisaldehyde sulphuric acid. The $R_f$ values and finger print data were recorded by WIN CATS software.

## 3. Results and Discussion

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

**Table 1:** Preliminary Phytochemical Screening of different extracts of *Pisonea aculeata* 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 | - | - | - | - |



1 2 3 1 2 3 1 2 3

**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 *Pisonea aculeata*

The results from HPTLC finger print scanned at wavelength 420 nm for alcoholic extract of *Pisonia aculeata* leaf. There are eleven polyvalent phytoconstituents and corresponding ascending order of $R_f$ values start from 0.03 to 0.93 in which highest concentration of the phytoconstituents was found to be 32.60% and its corresponding $R_f$ value was found to be 0.03 respectively and was recorded in Table 2. The corresponding HPTLC is presented in Figure 2

**Table 2:** Rf Values for Alcoholic Extract

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 246.5 AU | 0.01 Rf | 281.4 AU | 32.60 % | 0.03 Rf | 0.4 AU | 2596.0 AU | 17.61 % |
| 2 | 0.04 Rf | 0.6 AU | 0.07 Rf | 49.0 AU | 5.68 % | 0.09 Rf | 44.1 AU | 1282.7 AU | 8.70 % |
| 3 | 0.09 Rf | 44.5 AU | 0.11 Rf | 87.6 AU | 10.15 % | 0.17 Rf | 0.4 AU | 2613.7 AU | 17.73 % |
| 4 | 0.24 Rf | 0.2 AU | 0.27 Rf | 150.9 AU | 17.49 % | 0.30 Rf | 1.0 AU | 1442.9 AU | 9.79 % |
| 5 | 0.49 Rf | 7.4 AU | 0.51 Rf | 11.8 AU | 1.37 % | 0.56 Rf | 1.9 AU | 388.5 AU | 2.63 % |
| 6 | 0.59 Rf | 4.2 AU | 0.63 Rf | 14.1 AU | 1.63 % | 0.64 Rf | 3.9 AU | 408.1 AU | 2.77 % |
| 7 | 0.67 Rf | 8.4 AU | 0.69 Rf | 18.9 AU | 2.18 % | 0.73 Rf | 0.3 AU | 587.3 AU | 3.98 % |
| 8 | 0.74 Rf | 0.2 AU | 0.78 Rf | 41.9 AU | 4.86 % | 0.80 Rf | 14.0 AU | 1100.9 AU | 7.47 % |
| 9 | 0.80 Rf | 14.3 AU | 0.81 Rf | 27.1 AU | 3.14 % | 0.83 Rf | 9.6 AU | 388.6 AU | 2.64 % |
| 10 | 0.84 Rf | 6.5 AU | 0.85 Rf | 40.5 AU | 4.69 % | 0.86 Rf | 34.4 AU | 487.9 AU | 3.31 % |
| 11 | 0.86 Rf | 34.7 AU | 0.90 Rf | 139.9 AU | 16.21 % | 0.93 Rf | 0.1 AU | 3449.2 AU | 23.39 % |

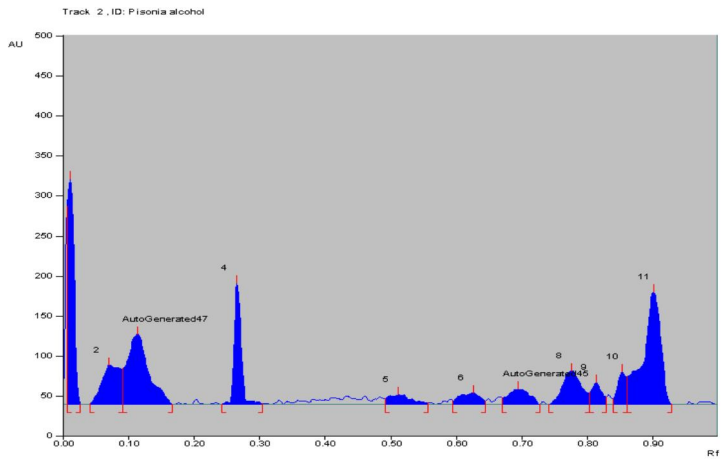



**Figure 2**: Chromatogram of Alcoholic Extract of *Pisonea aculeata* Leaf.

The results from HPTLC finger print scanned at wavelength 420 nm for ethyl acetate extract of *Pisonia aculeata* leaf showed six polyvalent phytoconstituents and corresponding ascending order of $R_f$ values start from 0.04 to 0.94 in which highest conc. of the phytoconstituents was found to be 39.00% and its corresponding $R_f$ value was found to be 0.04 respectively and was recorded in Table 3. The corresponding HPTLC chromatogram was presented in Figure 3.

**Table 3:** Rf Values for Ethyl acetate Extract

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 163.0 AU | 0.01 Rf | 185.5 AU | 39.00 % | 0.04 Rf | 39.8 AU | 3033.3 AU | 34.52 % |
| 2 | 0.07 Rf | 0.5 AU | 0.11 Rf | 42.5 AU | 8.94 % | 0.14 Rf | 1.0 AU | 1213.8 AU | 13.81 % |
| 3 | 0.76 Rf | 2.4 AU | 0.79 Rf | 46.1 AU | 9.69 % | 0.81 Rf | 10.8 AU | 954.7 AU | 10.87 % |
| 4 | 0.84 Rf | 4.2 AU | 0.86 Rf | 29.5 AU | 6.20 % | 0.88 Rf | 16.7 AU | 571.4 AU | 6.50 % |
| 5 | 0.88 Rf | 17.2 AU | 0.90 Rf | 161.9 AU | 34.03 % | 0.92 Rf | 7.5 AU | 2860.6 AU | 32.56 % |
| 6 | 0.92 Rf | 8.0 AU | 0.93 Rf | 10.2 AU | 2.15 % | 0.94 Rf | 6.0 AU | 152.7 AU | 1.74 % |

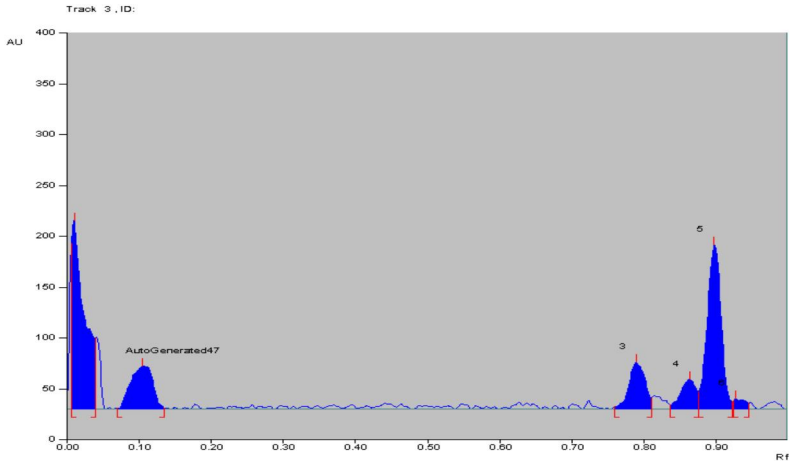



**Figure 3**: Chromatogram of Ethyl acetate Extract of *Pisonea aculeata* Leaf.

The results from HPTLC finger print scanned at wavelength 420 nm for chloroform extract of *Pisonia aculeata* leaf. There are fourteen polyvalent phytoconstituents and corresponding ascending order of $R_f$ values start from 0.03 to 0.95 in which highest Conc. the phytoconstituents was found to be 18.90% and its corresponding $R_f$ value was found to be 0.03 respectively and was recorded in Table 4. The corresponding HPTLC chromatogram was presented in Figure 4.

**Table 4:** Rf Values for Chloroform Extract

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 255.9 AU | 0.01 Rf | 275.0 AU | 18.90 % | 0.03 Rf | 47.2 AU | 2853.3 AU | 9.55 % |
| 2 | 0.03 Rf | 47.3 AU | 0.05 Rf | 158.9 AU | 10.93 % | 0.07 Rf | 37.6 AU | 2881.4 AU | 9.65 % |
| 3 | 0.07 Rf | 69.8 AU | 0.09 Rf | 235.1 AU | 16.16 % | 0.15 Rf | 0.2 AU | 8209.2 AU | 27.48 % |
| 4 | 0.31 Rf | 5.2 AU | 0.35 Rf | 28.2 AU | 1.94 % | 0.38 Rf | 19.0 AU | 1116.7 AU | 3.74 % |
| 5 | 0.38 Rf | 19.2 AU | 0.43 Rf | 74.4 AU | 5.11 % | 0.49 Rf | 8.1 AU | 3472.2 AU | 11.62 % |
| 6 | 0.60 Rf | 4.4 AU | 0.64 Rf | 28.5 AU | 1.96 % | 0.67 Rf | 0.3 AU | 940.6 AU | 3.15 % |
| 7 | 0.67 Rf | 0.3 AU | 0.69 Rf | 16.2 AU | 1.12 % | 0.71 Rf | 0.3 AU | 174.1 AU | 0.58 % |
| 8 | 0.75 Rf | 0.2 AU | 0.77 Rf | 35.3 AU | 2.43 % | 0.78 Rf | 30.9 AU | 549.1 AU | 1.84 % |
| 9 | 0.78 Rf | 31.9 AU | 0.78 Rf | 44.7 AU | 3.08 % | 0.79 Rf | 21.5 AU | 479.7 AU | 1.61 % |
| 10 | 0.79 Rf | 22.0 AU | 0.81 Rf | 80.3 AU | 5.52 % | 0.82 Rf | 47.0 AU | 1305.0 AU | 4.37 % |
| 11 | 0.82 Rf | 48.0 AU | 0.84 Rf | 123.9 AU | 8.52 % | 0.85 Rf | 31.5 AU | 2030.5 AU | 6.80 % |
| 12 | 0.85 Rf | 101.6 AU | 0.86 Rf | 112.9 AU | 7.76 % | 0.86 Rf | 96.4 AU | 1380.3 AU | 4.62 % |
| 13 | 0.86 Rf | 96.9 AU | 0.88 Rf | 219.3 AU | 15.08 % | 0.91 Rf | 0.0 AU | 4114.4 AU | 13.77 % |
| 14 | 0.92 Rf | 0.3 AU | 0.93 Rf | 21.9 AU | 1.50 % | 0.95 Rf | 0.1 AU | 364.3 AU | 1.22 % |

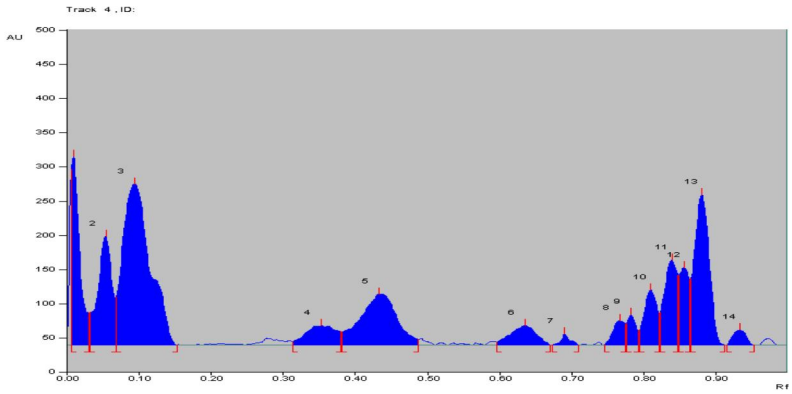



**Figure 4:** Chromatogram of Ethyl acetate Extract of *Pisonea aculeata* Leaf.

## 4. Conclusion

Nowadays, the interest in study of natural products is growing rapidly, especially as a part of drug discovery programs. In our previous study, we have proved that the anti-ulcer activity is associated with the active constituents of *Pisonea aculeata*.

In continuation to the previous study, we have shown interest to isolate the pure constituents responsible for the above mentioned pharmacological action. The initial study was carried out with HPTLC and the results showed that there are many compounds in *Pisonea aculeata*. From the HPTLC studies, it has been found that chloroform, ethylacetate and ethanol extracts contain not a single compound but a mixture of compounds and so it is established that the pharmacological activity shown by them are due to the cumulative effect of all the compounds in composite.

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