

**Original Research Article**

## Preliminary Phytochemical Screening and HPTLC Fingerprinting of Leaf Methanolic Extract of *Spermacoce hispida*

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**Abstract:** The main objective of the present work to established the fingerprint profile of *S.hispida* using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening was done and HPTLC studies were carried out. The scanning was performed using 'CAMAG' densitometer scanner 3V equipped with CATS3 software, at a wavelength of 400 and 254 nm using deuterium lamp. Preliminary phytochemical screening of the methanolic extract *S.hispida* showed the presence of alkaloids, carbohydrates, phenols, tannins, flavonoids, gums and mucilage. HPTLC finger printing of *S.hispida* methanolic extract showed best results in Toluene: Ethyl Acetate: Formic acid: 5:4:1 solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and visible light range (400-600nm after spraying with anisaldehyde sulphuric acid reagent) best results were shown at 400nm. The Rf values ranged from 0.06 to 0.99. The result showed that out of 15 components, the component with Rf values 0.95, 0.82 were found to be more predominant as the percentage area is more with 28.85% and 12.91% respectively.

**Keywords:** *S.hispida* leaf, Phytochemical Screening, HPTLC Fingerprinting.

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## INTRODUCTION

The nature has provided an entire warehouse of remedies to heal ailments of mankind. About 80 % of the world's population depends partly or wholly on traditional medicine for its primary health care needs. Botanicals as the key remedy in traditional medical system have been used in medical practice for past epoch and have made a immense association to

maintain human health [1, 2]. *Spermacoce hispida* belong to family Rubiaceae. The Rubiaceae, one of the four largest among the angiosperms, consists of 637 genera and 10,700 species [3]. *S. hispida* was popularly known as "Nattaichuri" in Tamil or "Shaggy button weed" in English [4].



**Fig-1:** *Spermacoce hispida*

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### Ethno-botanical survey of *S. hispida* [5, 6]

<b>BIOLOGICAL NAME</b>	<i>Spermacoce hispida</i>
<b>FAMILY</b>	Rubiaceae
<b>SYNONYM</b>	<i>Spermacoce articularis</i> , <i>Borreria hispida</i> , <i>Borreria articularis</i>
<b>VERNACULAR NAMES</b>	<b>English:</b> Shaggy button weed, Jointed buttonweed; <b>Tamil:</b> Nathaichuri, Cirakkuli, Nathai-choori, Vetuppaccuri; <b>Hindi:</b> Madanaghanti, <b>Sanskrit:</b> Bukah, Madanaghanti, Madanghanta, Vasukah; <b>Malayalam:</b> Kutalccurukki, Nattaccuri, Tardavel, Tartaval, Thartuvvel; <b>Telugu:</b> Madana, Madana budatha kaada, Madana kaada, Madana kattu; <b>Kannada:</b> Daare botu, Daare kaddi, Madana buddame gida, Madana ganti; <b>Marathi:</b> Ghanti-chi-bhaji, Gondi; <b>Oriya:</b> Solaganthi.
<b>HABIT</b>	A procumbent, scabrid, hirsute or hispid herb.
<b>HABITAT</b>	Through India, upto 900m in the hills and on all dry lands, on the bare slopes, along the way side and in thin layer of soil as a weed. It is common along Madurai, Nilgiris, and Coimbatore.
<b>Ethno-Medicinal Importance</b>	<b>Leaves:</b> Leaf juice is used in conjunctivitis. An extract of leaves is given as an astringent in hemorrhoids and gallstones. The leaves are used as haemostatic, in dental carries and tooth ache. <b>Root:</b> The root is used for wound healing, dental problems and as an appetizer. <b>Seed:</b> The seeds are used as a stimulant, demulscent and for the treatment of diarrhoea and dysentery.

## MATERIAL AND METHOD

### Collection and authentication of plant material

The whole plant of *S.hispida* was collected from, India, in the month of December, 2016 and authenticated.

### Preparation of Extract

The fresh leaves of *S.hispida* was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No 40 and stored in an airtight container for further.

### Extraction procedure

The coarse selected plant material was extracted with 1-1.5 liters of methanol continuous hot percolation using Soxhlet apparatus. After completion of extraction, extract was filtered and the solvent was removed by under reduced pressure. The dried extract was stored in desiccators.

### Qualitative Phytochemical Analysis

The crude methanolic extract of *Spermacoce hispida* (MESH) was analyzed for the presence of various phytoconstituents by following standard phytochemical protocols [7, 8].

### High Performance Thin Layer Chromatography

The methanolic extracts of *S.hispida* and *S.acuta* were used for the HPTLC analysis.

### Development of HPTLC Finger Print

Samples were applied in a concentration of 20µl using CAMAG LINOMAT IV sample applicator on HPTLC precoated Plates Silica Gel Merck 60F 254, 0.2mm layer thickness (20 x 20cm) which was used as a

stationary phase. The volume of the sample loaded was 20 µl. The scanning was performed using 'CAMAG' densitometer scanner 3V equipped with CATS3 software, at a wavelength of 400 and 254 nm using deuterium lamp.

### Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent Acetonitrile: Water: Ethyl acetate: Hexane: Formic acid. (0.5:0.2:5:4.5:0.3) for 15 minutes.

### Detection of Spots

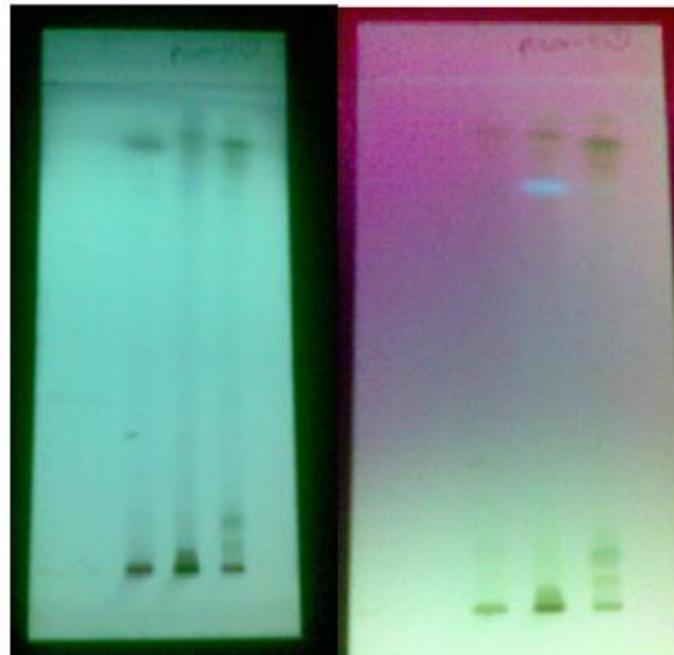
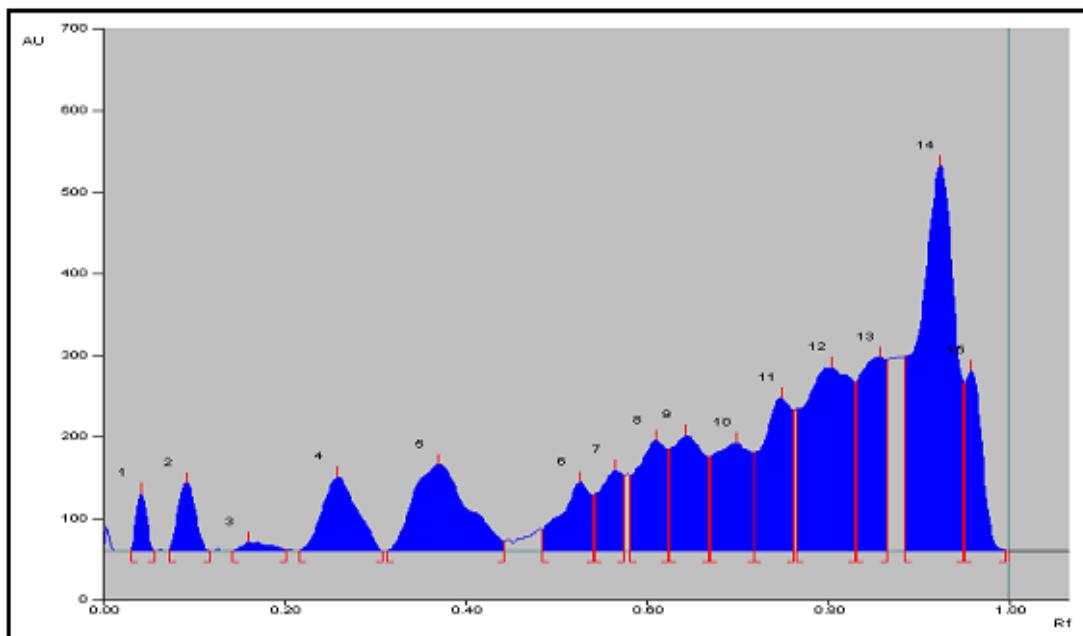
The air-dried plate was viewed in ultraviolet radiation to mid-day light (Fig.2). The chromatograms were scanned by densitometer at 254 nm after spraying with anisaldehyde sulphuric acid. The Rf values and finger print data were recorded by CATS3 software, at a wavelength of 400 and 254 nm using deuterium lamp.

**Table-1: Qualitative phytochemical analysis of methanolic extract of *S.hispida***

Type of Constituent	Methanol
Alkaloids	-
Phenolic compounds/Tannins	+++
Flavonoids	+
Saponins	+
Steroids	+++
Triterpenoids	+++
Proteins	++
Carbohydrates	+
Glycosides	++

**Table-2: HPTLC finger print analysis of methanolic extract of *S. hispida***

<b>Extract</b>	<b>Solvent system</b>	<b>No. of peak</b>	<b>Rf values</b>	<b>Percent Area (%)</b>
Methanol extract	Toluene: Ethyl Acetate: Formic acid 5:4:1	15	0.06, 0.11, 0.20, 0.31, 0.44, 0.53, 0.56, 0.61, 0.65, 0.70, 0.75, 0.82, 0.86, 0.95, 0.99	1.14, 1.80, 0.39, 3.86, 7.05, 3.37, 2.84, 5.60, 4.87, 6.18, 8.00, 12.91, 8.36, 28.85, 4.79

**Fig-2: HPTLC Plate at 254nm & 366****Fig-3: HPTLC Chromatogram of methanolic extract of *S.hispida***

## RESULT AND DISCUSSION

Preliminary phytochemical screening was done and HPTLC studies were carried out. The scanning was performed using 'CAMAG' densitometer scanner 3V equipped with CATS3 software, at a wavelength of 400

and 254 nm using deuterium lamp. Preliminary phytochemical screening of the methanolic extract *S.hispida* showed the presence of alkaloids, carbohydrates, phenols, tannins, flavonoids, gums and mucilage. The result was tabulated in table 1. HPTLC finger printing of *S.hispida* methanolic extract showed

best results in Toluene: Ethyl Acetate: Formic acid: 5:4:1 solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and visible light range (400-600nm after spraying with anisaldehyde sulphuric acid reagent) best results were shown at 400nm (Table 2 & Fig. 2-3). The R<sub>f</sub> values ranged from 0.06 to 0.99. The result showed that out of 15 components, the component with R<sub>f</sub> values 0.95, 0.82 were found to be more predominant as the percentage area is more with 28.85% and 12.91% respectively.

## CONCLUSION

At the present time, the curiosity in study of natural products is growing quickly, especially as a part of drug discovery programs. In the ethnobotanical survey we found that the *S.hispida* was used in conjunctivitis, hemorrhoids, gall stone, wound healing, dental problems and appetizers. The initial study was carried out with HPTLC and the results showed that there are many compounds in *S.hispida*. From the HPTLC studies, it has been found that methanolic leaf extract 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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