HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY ANALYSIS OF *SPERMACOCE HISPIDA*

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Summary

Spermacoce hispida is used as traditional herbal medicine in India to treat various disorders. A rapid, accurate and simple high performance thin layer chromatography method for quantitative estimation of *Spermacoce hispida* is described here. The result showed that *Spermacoce hispida* showed the presence of alkaloids, flavonoids, alkaloids, phenolics, steroids, tannins and terpenoids.

Keywords:*Spermacoce hispida,* HPTLC, Phytochemicals

Introduction

Spermacoce hispida Linn belongs to the family Rubiaceae, was popularly known as "Nattaiccuri" in Tamil or "Shaggy button weed" in English [1]. It is widely present in the Western Ghats of Kerala [2] and in Maruthamalai forest [3] in Tamil Nadu. The seed- of the plant has been used for the treatment of internal injuries of nerves and kidney. It is suggested that it remove signs of old age, purify blood and improve vitality, and has been used by the tribals living in the forest regions in the Western ghats of Kerala since ancient times [2]. It has been also reported that *S. hispida* is an effective natural drug for the treatment of hypertension [4]. *S. hispida* was one of the five plants which contained the maximum amount of flavonoids among 25 plants analyzed [3]. It has been reported that methanolic extract of this whole plant extract exhibited strong antioxidant activity [5]. The objective of the present investigation was to determine the presence of phytochemical constituents by HPTLC.

Materials and Methods

Plant Collection and Extraction:
Spermacoce hispida was collected from Coimbatore, Tamilnadu, India. The plant specimen was authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SC/5/23/08-09/Tech.1784). The voucher specimen was deposited at the herbarium of Karpagam University, Coimbatore. The whole plants of *Spermacoce hispida* were washed thoroughly in tap water, shade dried and powdered. The powder (100 gms) was eshaustively extracted with ethanol in the ratio of 1:5 (w/v) for 24h by using soxhlet apparatus. The extract was completely evaporated to dryness using rotary flash evaporator (Buchi type).

Mobile phase:

Flavonoids	:	Ethyl acetate-formic acid-glacial acetic acid-water (10:1.1:1.1:2.6)
Alkaloid	:	n-Butanol - Acetic acid – water (4 : 4 : 1)
Polyphenolics	:	Toluene-acetone-formic acid (4.5: 4.5: 1)
Sterols	:	Ethyl acetate-chloroform-water (9:1:0.1)
Tannins	:	Isobutanol-acetic acid-water (14:1:3.5)
Terpenoids	:	n-hexane - ethyl acetate (1: 1)

Spray reagent

Flavonoids	:	1% Ethanolic aluminium chloride reagent.
Alkaloid	:	Dragendorff reagent followed with 10% Ethanolic sulphuric acid reagent
Polyphenolics	:	Fast blue B reagent
Sterols	:	Libermann-Burchard reagent.
Tannins	:	5% Ferric chloride reagent
Terpenoids	:	Libermann-Burchard reagent.

Test solution preparation

The given aqueous extract 100 mg was dissolved in 5 ml of water, centrifuged and collected the supernatant liquid. This portion was used as test solution for HPTLC analysis.

Sample and reference standard preparations

5 µl of each test solutions and reference standard were loaded as 8 mm band length in the 5x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Results and Discussion

HPTLC chromatogram of alkaloids

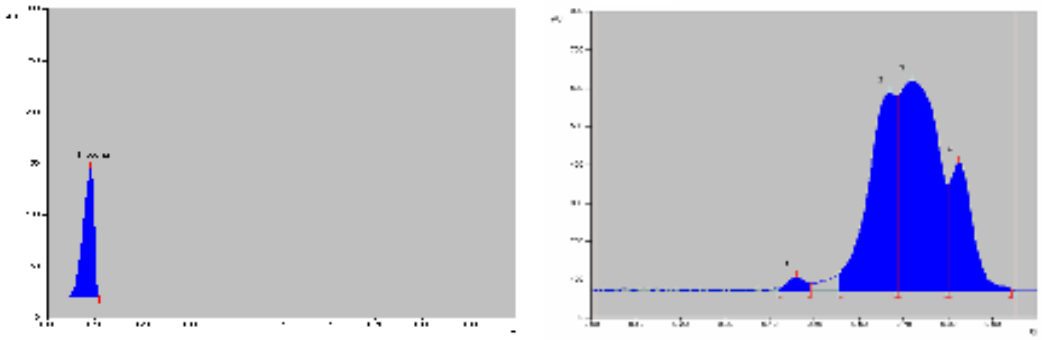
Table 1 and figure 1 showed the alkaloid profile of *Spermacoce hispida*. Here nicotine was used as standard which developed with Rf value 0.09. About two alkaloids were present in *Spermacoce hispida* with the Rf values of 0.67 and 0.82 respectively.

Table 1 HPTLC – Peak table for alkaloid profile for *Spermacoce hispida*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.09	133.6	2636.7	Nicotine standard
S.hispida	1	0.46	36.4	1224.9	Unknown
S.hispida	2	0.67	516.9	26390.7	Alkaloid 1
S.hispida	3	0.72	548.8	37543.4	Unknown
S.hispida	4	0.82	335.6	13871.6	Alkaloid 2

Figure 1 Peak densitogram displayed for alkaloids

Nicotine standard *Spermacoce hispida*



HPTLC chromatogram of flavonoids

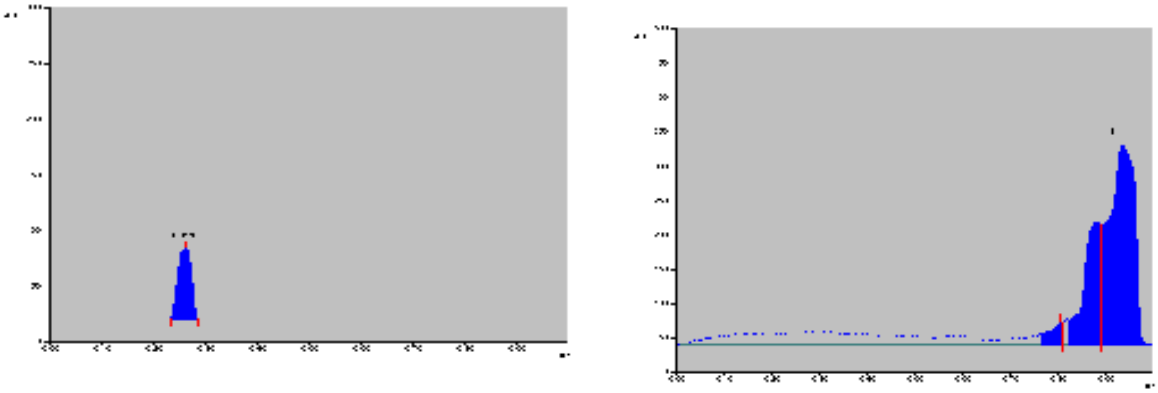
Table 2 and figure 2 showed the flavonoid profile for *Spermacoce hispida*. Here rutin was used as standard, which developed with the Rf value 0.26. In this profile, one flavonoid compound was present in *Spermacocoe hispida* with the Rf value of 0.88.

Table 2 HPTLC - Peak table for flavonoid profile for *Spermacoce hispida*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.26	82.6	2435.3	Rutin standard
S.hispida	1	0.80	34.5	856.9	Unknown
S.hispida	2	0.88	178.4	5708.8	Flavonoid 1
S.hispida	3	0.94	290.6	14695.5	Unknown

Figure 2 Peak densitogram displayed for flavonoids

Rutin standard *Spermacoce hispida*



HPTLC chromatogram of phenolics

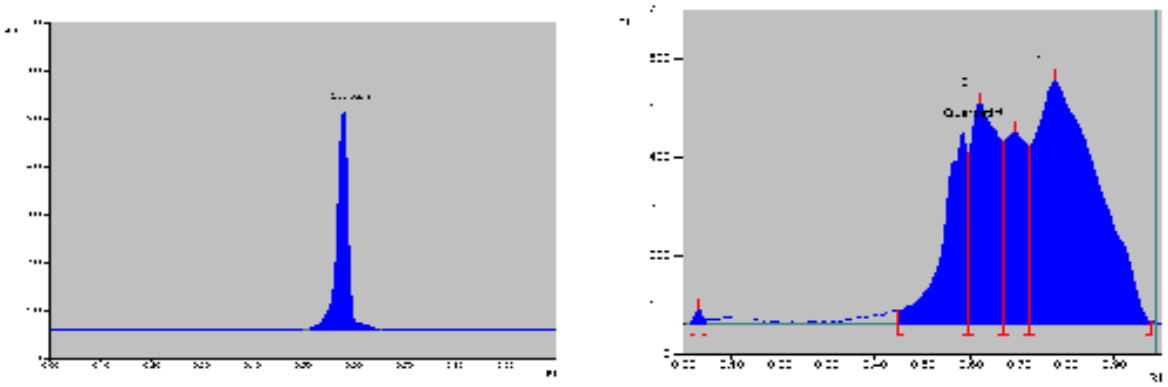
Table 3 and figure 3 showed the phenolics profile for *Spermacoce.* Here quercetin was used as standard, which developed with the Rf value 0.58. In this profile two phenolic compounds were present with the Rf value of 0.58 and 0.62. A compound with similar Rf value was also obtained in the chromatogram reveled the presence of quercetin in *Spermacoce hispida*.

Table 3 HPTLC - Peak table for Phenolics profile for *Spermacoce hispida*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.58	500.8	13306.6	Quercetin standard
S.hispida	1	0.03	31.1	498.7	Unknown
S.hispida	2	0.58	387.6	18346.5	Quercetin
S.hispida	3	0.62	449.6	23888.5	Phenolics 1
S.hispida	4	0.69	388.4	15833.9	Unknown
S.hispida	5	0.78	494.6	60092.6	Unknown

Figure 3 Peak densitogram displayed phenolics

Quercetin *Spermacocce hispida*



HPTLC chromatogram of steroid

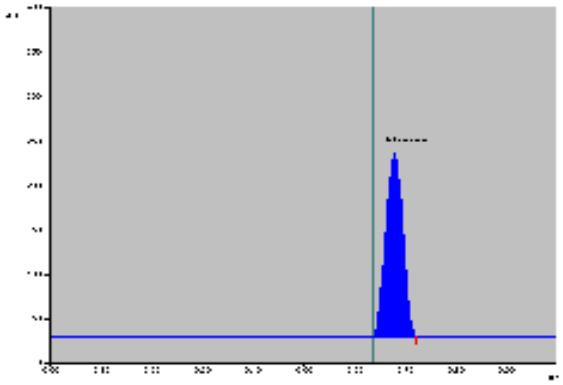
Table 4 and figure 4 showed the steroid profile for *Spermacoce hispida*. The standard solasodine produced a clear zone with Rf value 0.68. Three steroid compounds were present in *Spermacoce hispida* with the Rf value of 0.17, 0.59 and 0.63 respectively.

Table 4 HPTLC - Peak table for steroid profile for *Spermacoce hispida*

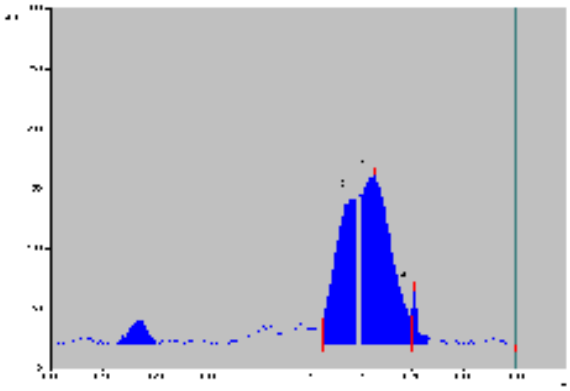
Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.68	248.1	9725.5	Solasodine standard
S.hispida	1	0.17	20.5	652.8	Steroid 1
S.hispida	2	0.59	121.7	4487.8	Steroid 2
S.hispida	3	0.63	140.9	7113.3	Steroid 3
S.hispida	4	0.70	45.4	465.3	Unknown

Figure 4 Peak densitogram displayed steroids

Solasodine



Spermacocce hispida



HPTLC chromatogram of tannins

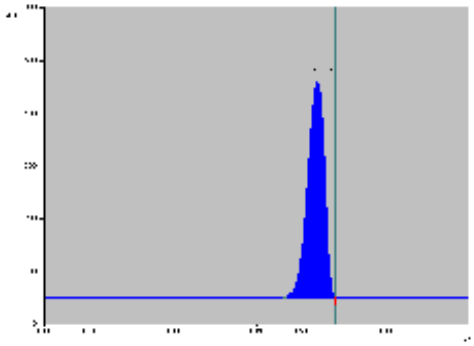
Table 5 and figure 5 showed the tannin profile for *Spermacoce hispida*. The standard gallic acid produced a prominent spot with Rf value 0.64. Two tannin compounds were developed in *Spermacoce hispida* with the Rf value of 0.71 and 0.80.

Table 5 HPTLC - Peak table for tannins profile for *Spermacoce hispida*

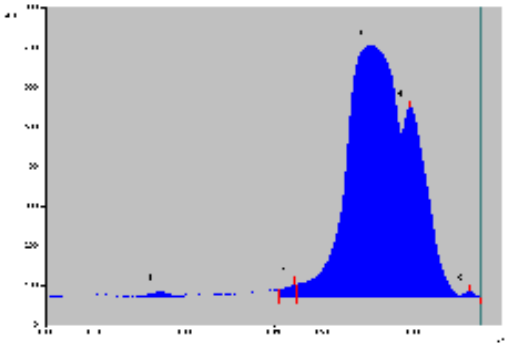
Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.64	437.8	18474.8	Gallic acid standard
S.hispida	1	0.25	15.5	569.9	Unknown
S.hispida	2	0.54	34.0	815.9	Unknown
S.hispida	3	0.71	634.8	63272.9	Tannin 1
S.hispida	4	0.80	480.0	23731.2	Tannin 2
S.hispida	5	0.93	16.1	313.2	Unknown

Figure 5 Peak densitogram displayed tannin

Galic acid



Spermacocce hispida



HPTLC chromatogram of terpenoids

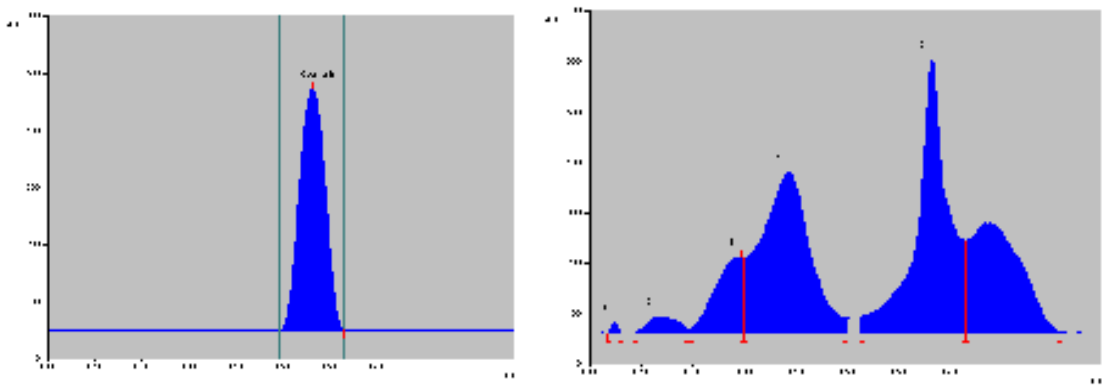
Table 6 and figure 6 showed the terpenoid profile for *Spermacoce hispida*. The coumarin was used as a standard which developed with the Rf value 0.57. Five terpenoid compounds were present in *Spermacoce hispida* with the Rf value of 0.05, 0.14, 0.30,0.67 and 0.78 respectively.

Table 6 HPTLC - Peak table for terpenoids profile for *Spermacoce hispida*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.57	435.5	20529.2	Coumarin standard
S.hispida	1	0.05	24.1	260.8	Terpenoid 1
S.hispida	2	0.14	34.5	1746.8	Terpenoid 2
S.hispida	3	0.30	152.7	6920.3	Terpenoid 3
S.hispida	4	0.39	320.8	25142.2	Unknown
S.hispida	5	0.67	543.5	27405.6	Terpenoid 4
S.hispida	6	0.78	220.3	17992.1	Terpenoid 5

Figure 5 Peak densitogram displayed terpenoid

Coumarin *Spermacocce hispida*



Chromatographic fingerprint is a holistic, valid and rapid method. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved. In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. ROS produced in vivo include superoxide radical (O_2 .•-), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). H_2O_2 and O_2 .•- can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical (•OH) [6]. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [7,8]. Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, *etc*. [9].

Conclusion

The reported HPTLC method was found to be rapid, simple and accurate for quantitative estimation of phytochemicals in ethanolic extract of whole plant of *Spermacoce hispida.*The result showed that *Spermacoce hispida* showed the presence of alkaloids, flavonoids, alkaloids, phenolics, steroids, tannins and terpenoids. Quercetin was present in *Spermacoce hispida.*Thus spermacoce hispida has high medicinal properties for various diseases.

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