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PHYTOCHEMICAL EVALUATION AND HPTLC FINGER PRINTING PROFILE OF LEAVES OF FILICIUM DECIPIENS

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ABSTRACT

A plant derivative plays a vital role in the modern pharmaceuticals for various ailments. High performance thin layer chromatography (HPTLC) is a valuable technique for the investigation of plant products with respect to different aspects of their quality. The present study was carried out to investigate the secondary metabolites and to develop HPTLC. To study the HPTLC fingerprinting of leaf extract of *Filicium decipiens*, A CAMAG HPTLC system equipped with LINOMAT 5 applicator and WIN CATS-1.3.4 software were used. The Phytochemical tests was done on methanol, ethanol and chloroform extracts. Preliminary Phytochemical studies shows the presence of various phytoconstituents like alkaloids, saponins, flavonoids, phenols and tannins and the three solvent extract were used for the further analysis. HPTLC study revealed the presence of more bands in chloroform than methanol and ethanol extract of *Filicium decipiens*. The HPTLC fingerprinting profile developed for chloroform extract will help in proper identification and quantification of marker compounds. By isolating and identifying marker compounds, it can be used for the formulation of new drugs to treat various diseases.

KEYWORDS: *Filicium decipiens*, HPTLC, fingerprinting, phytochemical screening.

INTRODUCTION

Herbal drug plays an important part in the production of alternative medicines without the adverse effects of the synthetic drugs.[1] Herbal medicines are derived from medicinal plants that in essence, synthesize complex organic constituents with organically active constituents that are often unknown. Most of the herbal medicines are prepared from coarse extracts, not

standardized or evaluated for dynamic ingredient content.[2-3] Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which give health benefits for humans further than those attributed to macronutrients and micronutrients.[4] *Filicium decipiens* (*F.decipiens*) belongs to the Sapindaceae family. It is a large tree up to 25 m tall. It is found in evergreen and semi-evergreen forests, which is native to Sri Lanka, the Western Ghats of southern India, and small highland areas of East Africa. *Filicium decipiens* is traditionally used as anti-diabetic agent in India and Sri Lanka.[5] It also showed a variety of biological activities, such as anti-fungal, anti-bacterial, anti-inflammatory, anti-oxidant and mollusicicidal activities.[6-7] The chemical constituents present in plant such as triterpenoidal saponins, norneohopane caffeate, sitosterol and flavonol glycosides. The four new saponins have been isolated from the stem bark of Filicium decipiens.[8-9] High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of plant materials. HPTLC based strategy is being investigated as a significant tool in routine drug analysis examination. It allows for the analysis of a broad number of compounds both productively and cost viably. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. Additionally, numerous samples are often run during a single analysis thereby dramatically reducing analytical time. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. In HPTLC, a similar examination can be seen utilizing various frequencies of light subsequently giving a more complete profile of the plant than is normally seen with more explicit sorts of investigation.[10-12]

MATERIALS AND METHODS

Collection of the plant material

The fresh plant was collected from Coimbatore district, Tamilnadu. The leaves were washed thoroughly two to three times with running tap water and once with sterile distilled water and immediately sprayed with alcohol. The leaf material was then dehydrated under shade. After complete aeration, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable airtight container for further use.

Preparation of the extracts

Extraction is the general process for separation of active constituents by the use of different solvents. The coarse powders of shade-dried leaves were successively extracted in Soxhlet extractor using the solvents such as methanol, ethanol and chloroform. All three leaf extracts obtained were concentrated and dried under vacuum, packed and stored in refrigerator until further use.

Preliminary phytochemical screening

The extracts obtained were subjected to qualitative tests for the identification of various plant constituents. The preliminary phytochemical screening is a qualitative chemical evaluation which indicates spectrum of chemical constituents in the chosen plant. The chemicals and solvents used throughout the investigation were of analytical grade.

HPTLC fingerprinting profile

HPTLC fingerprint profile of the all leaf extract of *F.decipiens* was carried out. The given plant sample, 20mg was weighed accurately in an electronic balance (Afcoset), dissolved in 250µl of the respective solvent and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis. 2 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed up to 90mm.The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV 366nm. The developed plate was sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version.

RESULT AND DISCUSSION

Phytochemical screening

The Phytochemical tests on methanol, ethanol and chloroform extracts of *F.decipiens* leaves showed the presence of various phytoconstituents like alkaloids, saponins, flavonoids, phenols and tannins (Table 1).

Table 1: Preliminary phytochemical screening of different extracts of *F. decipiens* plant extract

Test	M	E	C
Alkaloid	+	+	+
Flavonoid	+	+	+
Phenol & Tannins	+	+	+
Saponin	+	_	+

M- methanol extract, E-Ethanol extract, C-Chloroform extract, "+" Presence, "-"Absence

HPTLC Profile

The densitogram shown in Fig. 1 *F.decipiens* at 254 nm indicate that all sample constituents were separated. It is evident from Table 2 i.e. peak list and Rf values of the densitogram of *F.decipiens* 2 µl methanol extract at 366 nm found 1 spot respectively. The following Max Rf 1.22 which was shown in Fig 2. Fig. 3 indicating Rf values 0.02, 0.10 and 1.19 were found to be more predominant as the percentage area was more with 0.95%, 3.30% and 95.75%, respectively. Table 3 peak list and Rf values of the densitogram of *F.decipiens* 2 µl Ethanol extract shown in Fig. 3.

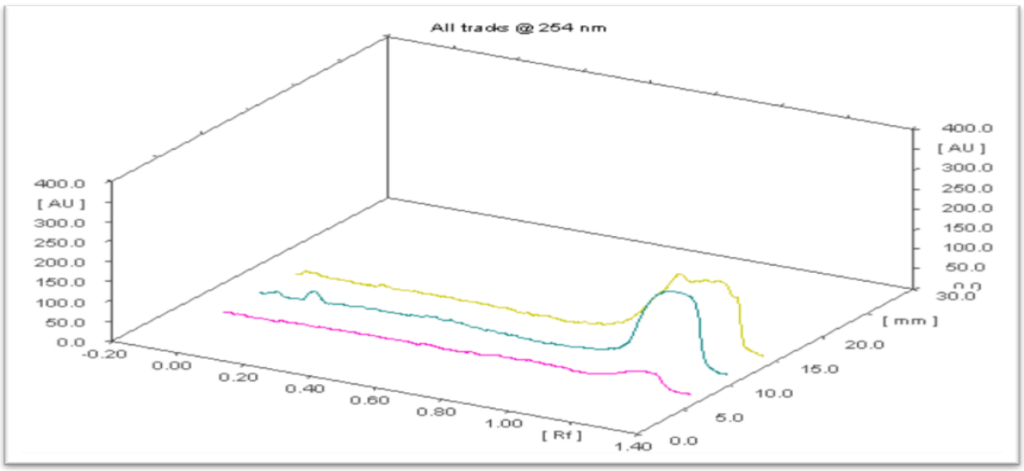

Fig. 1: Three dimensional representation of HPTLC chromatogram of *Filicium decipiens* methanol, ethanol and chloroform extract measured at 254 nm.

Table 2: Peak list and rf values of the densitogram of 2 µl methanol extract of *f. Decipiens*, at 366 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	1.06 Rf	10.9 AU	1.22 Rf	37.7 AU	100.00 %	1.32 Rf	0.4 AU	2387.4 AU	100.00 %

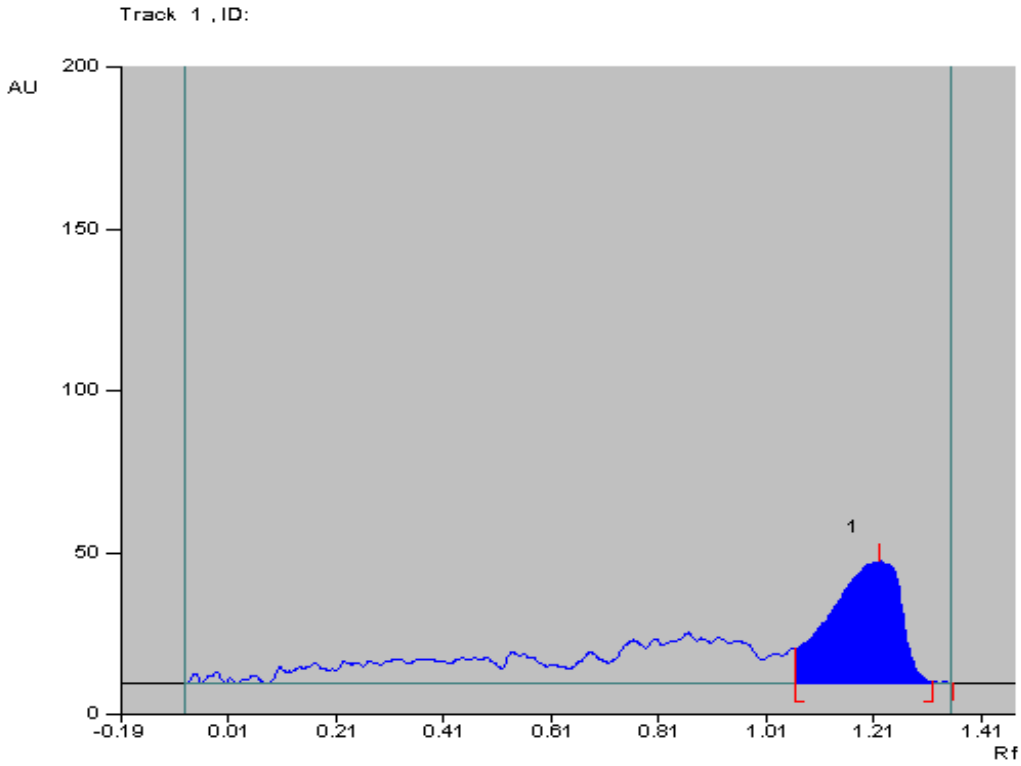

Fig. 2: Chromatogram of Methanol extract of *F. decipiens* at 366 nm.

Table 3: Peak list and rf values of the densitogram of 2 µl ethanol extract of *f. decipiens*, at 366 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.07 Rf	0.3 AU	-0.02 Rf	11.3 AU	4.99 %	0.00 Rf	0.2 AU	150.5 AU	0.95 %
2	0.05 Rf	1.9 AU	0.10 Rf	28.6 AU	12.65 %	0.13 Rf	3.3 AU	521.8 AU	3.30 %
3	0.95 Rf	7.9 AU	1.19 Rf	186.5 AU	82.37 %	1.33 Rf	0.8 AU	15148.4 AU	95.75 %

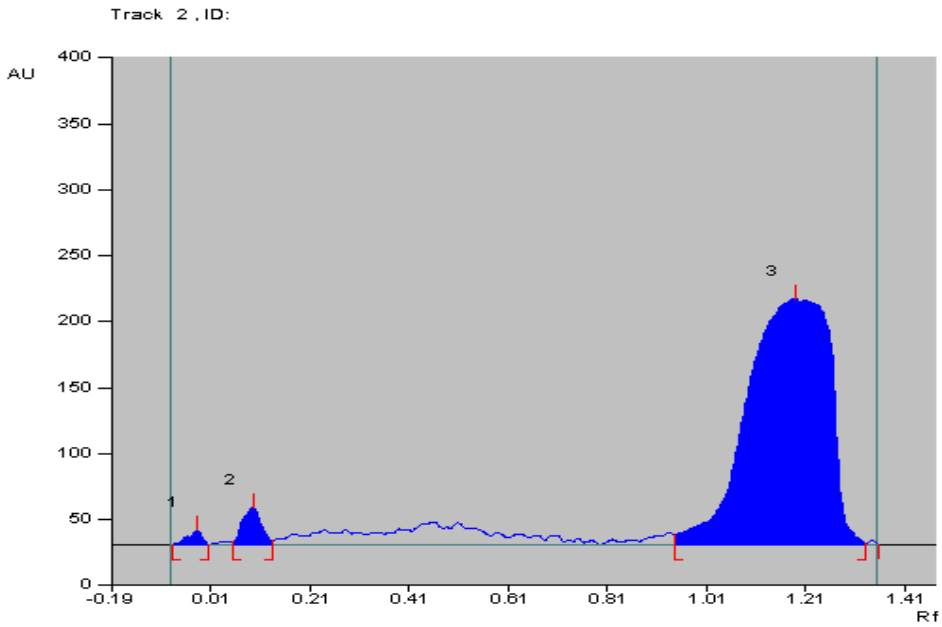

Fig. 3: Chromatogram of Ethanol extract of *Filicium decipiens* at 366 nm.

Table 4 peak list and Rf values of the densitogram of *F.decipiens*. 2µl Chloroform extract shown in Fig. 4. *F.decipiens* at 366 nm found 4 spots respectively. The following Max Rf 0.04, 1.10, 1.18 and 1.22. Fig. 4 indicating Rf values 0.04, 1.10, 1.18 and 1.22 were found to be more predominant as the percentage area was more with 0.58%, 46.08%, 21.69%and 31.65%, respectively. From the results we can say that the chloroform extract of *F.decipiens* has been thoroughly investigated by HPTLC method and better separation was achieved. The visualization reagents enable to see the spots efficiently and the densitometry will able to quantify the constituents.

Table 4: Peak list and rf values of the densitogram of 2 µl chloroform extract of *f. decipiens*, at 366 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.06 Rf	0.3 AU	-0.04 Rf	11.0 AU	2.16 %	-0.03 Rf	8.4 AU	106.9 AU	0.58 %
2	0.92 Rf	20.2 AU	1.10 Rf	167.4 AU	32.78 %	1.12 Rf	154.0 AU	8488.7 AU	46.08 %
3	1.14 Rf	152.9 AU	1.18 Rf	165.0 AU	32.31 %	1.19 Rf	161.3 AU	3995.8 AU	21.69 %
4	1.20 Rf	161.5 AU	1.22 Rf	167.3 AU	32.75 %	1.34 Rf	0.3 AU	5829.4 AU	31.65 %

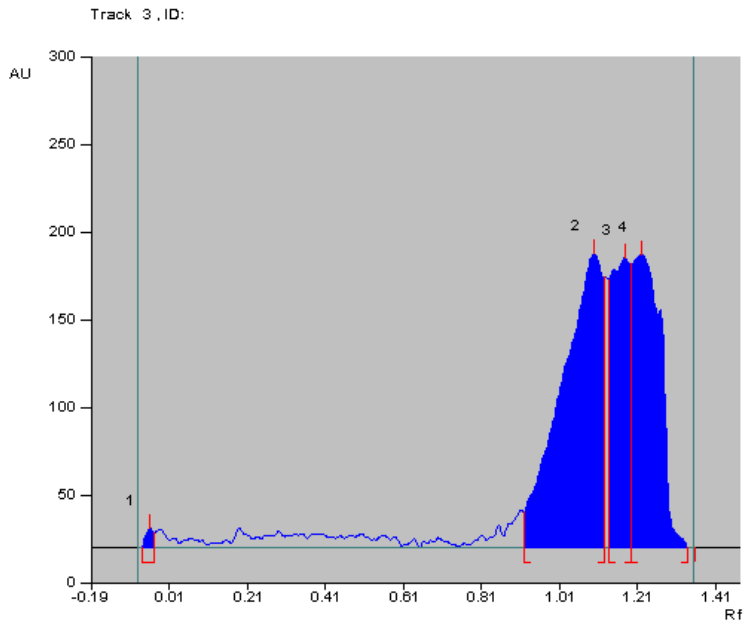

Fig. 4: Chromatogram of chloroform extract of *Filicium decipiens* at 366 nm.

CONCLUSION

Chromatography is essentially a group of techniques used for separation of the constituents of mixture by continuous distribution or adsorption of analyte between two phases. Among various chromatographic analytical techniques HPTLC has a firm place as a reliable method for analysing several samples of divergent nature and composition at the same time. HPTLC is a valuable tool for dependable identification, it gives chromatographic fingerprints that can be visualized and stored as electronic images which can be utilized several times without any errors and change. The chromatographic fingerprint, therefore is suitable for monitoring the identity and purity profile of a plant extract

The result of HPTLC analysis of *F.decipiens* leaf extracts shows that there are more bands. The maximum number of chemical constituents present in chloroform extract of *F.decipiens* in comparison to methanol and ethanol leaf extract of *F.decipiens*. Further, bioactivity guided fractionation and analysis of isolated chemical entity can reveal the active constituents in the different leaf extract of *F.decipiens*.

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