

Chromatographic Fingerprint Analysis of Hydro-alcoholic extract of Medicinally Important plant *Elephantopus scaber* L. Using HPTLC Technique

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ABSTRACT

Objective: To develop chemical fingerprint profile of secondary metabolites of therapeutically important plant *Elephantopus scaber* L. using HPTLC technique. **Method:** Preliminary phytochemical investigation was performed for the detection of secondary metabolites. HPTLC fingerprint profile of hydro-alcoholic extract of *E. scaber* was developed for alkaloids, flavonoids and saponins. Toluene: Ethyl acetate: Diethyl amine (7:2:1), Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:0.5:0.5:1.3) and Chloroform: Acetic acid: Methanol: Water (6.4:3.2:1.2:0.8) were the solvent systems developed. **Results:** The HPTLC chromatogram of hydro-alcoholic extract of *E. scaber* resolved 12 alkaloids, 25 flavonoids and 26 saponins with different Rf values including some unknown. **Conclusion:** Chemical profiling of selected plant is suggestive of several known and unknown bioactive molecule having potential therapeutic efficacy. The unknown alkaloids, flavonoids and saponins separated needed to be elucidated further. It can be concluded that the HPTLC fingerprint analysis is useful in exact identification of plant material. Also, it serves as a significant tool in differentiating adulterants and biomarker in herbal drug industry.

Key words: HPTLC Fingerprinting, *E. Scaber*, Chromatograms, Biomarker, Rf value.

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INTRODUCTION

Herbal medicines have been in use since ancient days for treatment and management of human ailments. Traditional system of medicine over the years has gained a wide acceptance worldwide. Associated undesired effects and economic constraints with the modern medicine drive us for search of potent drug from natural resources. Therapeutic efficacy of drugs of plant origin solely depends upon the authenticity and purity of plant material. A major key factor in efficacy of herbal products resides in assurance of safety and quality. Hence, phytochemical standardization and evaluation is need of the hour.

Fingerprinting (FP) approach has been recently recognized and accepted for quality assessment of traditional medicines. The concept of phyto equivalence was developed to ensure consistency of herbal products. According to this concept, a chemical profile, such as chromatographic fingerprint, for herbal product, should be constructed and compared with the profile of a clinically proven reference product.¹

Different methods are used for FP which may be roughly categorized as (i) Low resolution techniques like TLC or IR-based methods (ii) High resolution techniques like GC, HPLC, MS, NMR or hyphenated techniques and are commonly accepted as well

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suiting for FP aiming at a comprehensive extract characterization.²

Fingerprint analysis by HPTLC is useful as phytochemical markers and also as good estimators of genetic variability in plant populations. It is becoming routine analytical technique because of the advantages of small amount of mobile phase needed, speed, and simultaneous estimation of several samples unlike HPLC.³ HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of medicinally important plants.⁴ Significant application of HPTLC was found to be useful in determination of adulteration in raw material as well as finished goods, identification of substitutions of botanicals in place of official ones and in comparing the same extracts with different batches and same species with varied populations collected from different demographic regions. Due to advancement in technology HPTLC is the preferred tool in analysis of various natural products.⁵

Elephantopus scaber A perennial rigid herb found in dry localities. Plant pacifies vitiated vata, pitta, toxins, cough, and cardiac ailments. As per traditional claims, the roots are used as an antipyretic, cardio tonic and diuretic.⁶ Decoction of the roots and leaves is used as emollient and given in dysuria, diarrhea, dysentery and stomach pain.⁷ Aqueous extract of leaves is applied externally to treat eczema and ulcers.⁸ Roots are used in the treatment of Kidney stone.⁹ In the present study fingerprint analysis of hydro-alcoholic extract of *E. scaber* was carried out for chemical profiling of the secondary metabolites present in it.

MATERIALS AND METHODS

Collection of plant material

The selected Indian medicinal plant *Elephantopus scaber* Linn was collected from the forest Jamboti, 40 km away from Belagavi, Karnataka, India and authenticated by Dr. Harsha Hegde, Scientist B, RMRC, ICMR, Belagavi. The herbaria was prepared and stored (Voucher Specimen Numbers RMRC-914) at RMRC, ICMR, Belgaum, Karnataka.

Extraction

The shade dried plant was processed for size reduction and sieved through sieve no.40 to get a coarse powder. Further, extraction was carried out by continuous hot extraction process using ethanol and water (70:30) as solvent for 8-10 hrs. The extract was concentrated using rotary evaporation technique (IKA Rotary Evaporator) at

40°C to give a syrupy mass. This was stored in a closed container and used for analysis.

Preliminary Phytochemical Investigation

Preliminary phytochemical analysis for the determination of presence of alkaloids, flavonoids, saponins, tannins, triterpenoids, carbohydrates, glycosides and steroids was carried out.¹⁰⁻¹³

Solvent System (Mobile Phases) Development

Confirmation of presence of these phyto constituents was done by developing a thin layer chromatographic profile. Different solvent systems with varying polarity and proportions were tried for better separation and resolution.

HPTLC analysis

HPTLC fingerprinting was carried out using pre-coated silica gel aluminum plates (*E. Merck*, Darmstadt, Germany). Thus developed spots were identified by spraying various indicator reagents. HPTLC test solution was prepared by dissolving 100 mg of hydro-alcoholic extract of plant material in 5 mL of methanol, centrifuged (Eppendorf, Minispin Plus) at 3000 rpm (493 x g) for 8 min.^{14,15}

Instrumentation

Stationary phase	: Plate size (X x Y) 20.0 x 10.0 cm Precoated silica gel aluminum plates 60F-254
Instrument	: CAMAG Linomat 5 "Linomat5_080222" S/N 080222(1.00.12)

Linomat 5 application parameters

Spray gas	: Inert gas
Sample solvent type	: Methanol
Dosage Speed	: 150 nL / s
Pre-dosage Volume	: 0.2 µL

Sequence	
Syringe size	: 100 µL
Number of tracks	: 1
Band length	: 8.0 mm
Application Volume	: 5.0 µL

Measurement Table

Wavelength	: 254nm / 366nm / 540 nm
Lamp	: D2
Detector Mode	: Automatic

Development of chromatogram and detection of spots

Chromatogram was developed after application of the spots, in a twin trough glass TLC chamber previously

Table 1: Preliminary phytochemical investigations of hydro-alcoholic extract of *E. scaber*

Sr. No.	Chemical component	Name of the chemical test	Results (Plant)
			<i>Elephantopus scaber</i> L.
1.	Carbohydrates	Molisch's test	+
		Benedict's test	+
		Fehling's test	-
2.	Alkaloids	Mayer's test	+
		Wagner's test	+
		Dragendroff's test	+
		Hager's test	-
		Tannic acid test	-
3.	Phytosterols	Salkowski test	+
		Liebermann Burchard test	+
4.	Glycosides	Legal's test	-
		Liebermann's test	-
		Keller killiani test	-
		Xanthyrol test	-
5.	Tannins and Phenols	Ferric chloride test	+
6.	Proteins	Millon's test	+
		Ninhydrin test	+
7.	Amino acids	Ninhydrin test	+
		Test for Tyrosine	+
		Test for Tryptophan	-
8.	Flavonoids	Shinoda test	+
		Lead acetate test	+
		Alkaline solution test	-
		Ferric chloride test	+
9.	Triterpenoids	Salkowaski test	+
		Liebermann Burchardt test	+
10.	Saponins	Frothing Test	+

saturated with respective solvent systems for 30 min. The HPTLC plates were dried and photo documentation was done at UV 254, 366, 540 nm and daylight with Camag Repro star chamber; scanning was done and images were captured. Using win CATS Planar Chromatography Manager software; Rf values at fingerprint data were recorded.

RESULTS

Preliminary phytochemical investigations reveal the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, and tannins in the hydro-alcoholic extract of *E. scaber* (Table 1).

To achieve reproducible peaks with high resolutions various combinations of different solvents in different proportions were tested. Satisfactory results were obtained with Toluene: Ethyl acetate: Diethyl amine

(7:2:1), Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:0.5:0.5:1.3) and Chloroform: Acetic acid: Methanol: Water (6.4:3.2:1.2:0.8) for alkaloids, flavonoids and saponins respectively. The hydro-alcoholic extract of *E. scaber* showed presence of 12 alkaloids, 25 flavonoids and 26 saponins with different Rf values when scanned at 254, 366 and 540 nm wavelength (Table 2).

The HPTLC chromatogram showed 12 bands of alkaloids (Figure 1). Major alkaloids separated were observed at Rf value 0.06, 0.11, 0.13, 0.18, 0.32, 0.41, 0.48, 0.57 at 254 nm; 0.19, 0.73 at 366 nm and 0.23, 0.71 at 540 nm. One unknown alkaloid each at 366 nm (Rf- 0.73) and at 540 nm (Rf- 0.23) was detected (Figure 2 and 3).

Total 25 flavonoids (Figure 1) were resolved satisfactorily with Rf values 0.17, 0.23, 0.30, 0.33, 0.40, 0.47, 0.61, 0.66, 0.73, 0.78 at 254 nm; 0.15, 0.20, 0.26, 0.71, 0.74,

Table 2: HPTLC Fingerprint Analysis data of hydro-alcoholic extract *E. scaber*:

Peak #	Wavelength 254		Wavelength 366		Wavelength 540	
	Identified	Unknown	Identified	Unknown	Identified	Unknown
Alkaloids	8	0	2	1	2	1
Flavonoids	10	0	6	2	9	4
Saponins	9	0	6	2	11	2

0.81 at 366 nm and 0.15, 0.23, 0.25, 0.49, 0.58, 0.62, 0.74, 0.76, 0.80 at 540 nm. Two unknown flavonoids at 366 nm (Rf- 0.15 and 0.74) and four at 540 nm (Rf- 0.23, 0.58, 0.74 and 0.76) were detected (Figure 4 and 5). HPTLC chromatogram for saponins (Figure 1) exhibited 26 bands with the Rf values 0.10, 0.14, 0.21, 0.30, 0.35, 0.46, 0.55, 0.68, 0.78 at 254 nm; 0.10, 0.23, 0.36, 0.47, 0.65, 0.76 at 366 nm and 0.12, 0.15, 0.16, 0.23, 0.27, 0.33, 0.38, 0.43, 0.50, 0.59, 0.71 at 540 nm. Total four saponins i.e two at 366 nm (Rf- 0.47 and 0.65) and 540 nm (Rf- 0.12 and 0.33) each was detected (Figure 6 and 7).

DISCUSSION

Natural products are proven to be the major source of bioactive compounds and served as a significant tool in discovery of many lead compounds for management and treatment of human ailment. They truly play a vital role in field of drug discovery and development.¹⁶ Screening and evaluation of herbal drugs becomes an integral part towards their exact identification and standardization. Numerous analytical tools and techniques were developed for quality control of plants and plant products. HPTLC fingerprinting has potential to verify and determine the reliability of secondary metabolites in natural resources and their formulations as well.¹⁷ HPTLC Fingerprint analysis facilitates the assessment of quality and stability of plant material and their products by visual and standardized fingerprint profile comparison. Present study was intended to screen hydro-alcoholic extract of *E. scaber* for the presence of secondary metabolites and development of fingerprints by HPTLC Technique. Alkaloids, flavonoids and saponins are the bioactive constituents having significant therapeutic value and pharmacological properties. The quality and quantity of these secondary metabolites in plant resources varies depending upon the region, demographic location and time of collection of the plant material as well as plant part or tissue used.¹⁸ In present study, the hydro-alcoholic extract of *E. scaber* showed presence of different alkaloids, flavonoids and saponins with different Rf values, area, intensity and

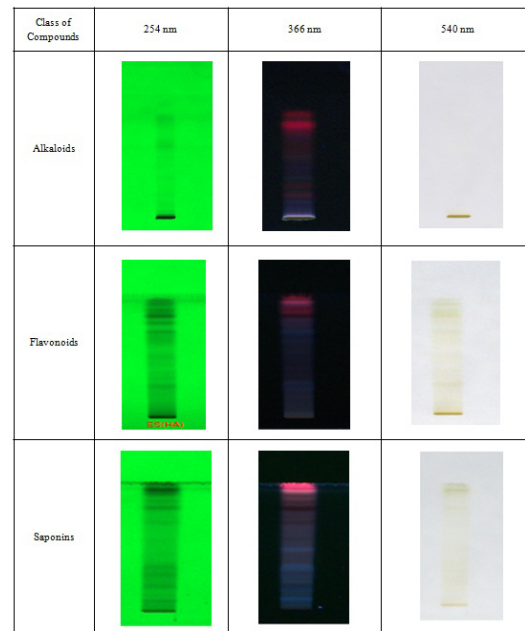


Figure 1 : Images of HPTLC chromatograms of hydro-alcoholic extracts of *E. scaber*.

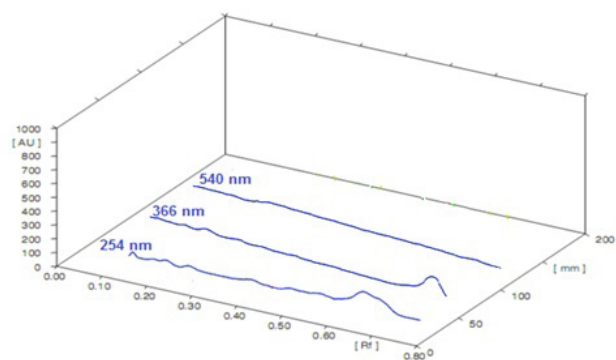


Figure 2: Overlay of 3D Display of chromatograms at 254 nm, 366 nm and 540 nm for Alkaloids.

height at 254 nm, 366 nm and 540 nm. Two unknown alkaloids, six unknown flavonoids and four unknown saponins were detected at different wavelengths having different Rf values. Those need to be studied further for their structure and characteristics. Most suitable

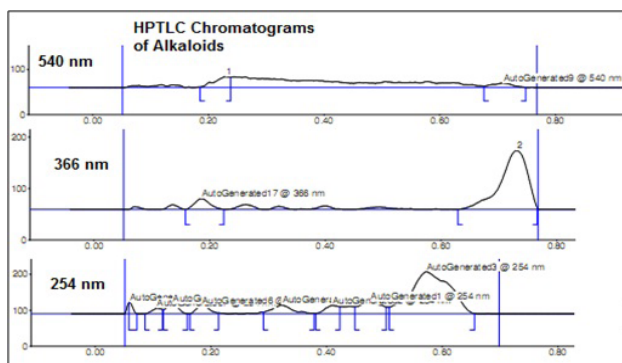


Figure 3: HPTLC Chromatograms of hydro-alcoholic extract of *E. scaber* at 254 nm, 366 nm and 540 nm for Alkaloids.

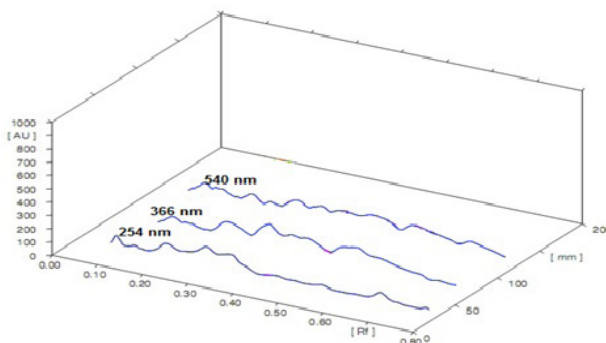


Figure 6: Overlay of 3D Display of chromatograms at 254 nm, 366 nm and 540 nm for Saponins.

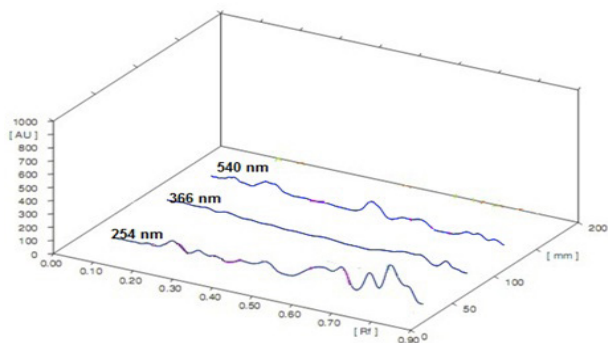


Figure 4: Overlay of 3D Display of chromatograms at 254nm, 366nm and 540 nm for Flavonoids.

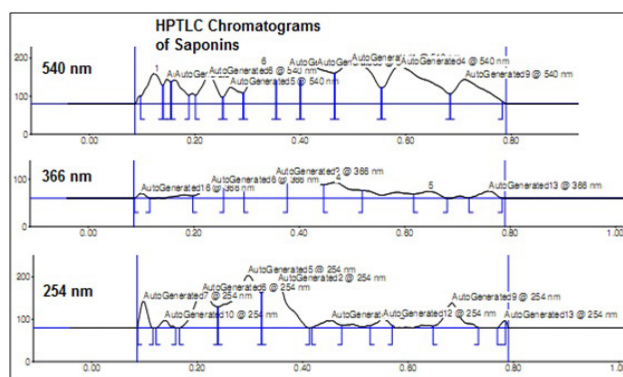


Figure 7: HPTLC Chromatograms of hydro-alcoholic extract of *E. scaber* at 254 nm, 366 nm and 540 nm for Saponins.

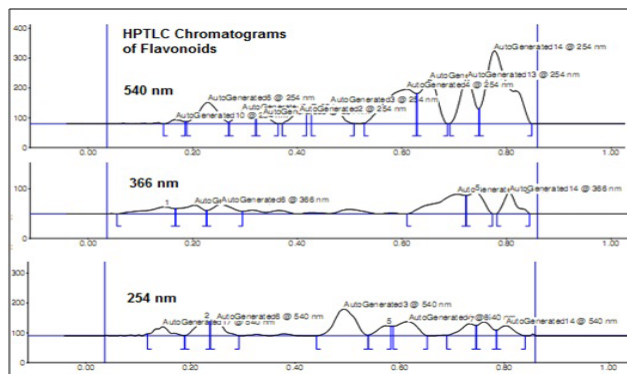


Figure 5: HPTLC Chromatograms of hydro-alcoholic extract of *E. scaber* at 254 nm, 366 nm and 540 nm for Flavonoids.

solvent system with which satisfactory separation was observed was Toluene: Ethyl acetate: Diethyl amine (7:2:1), Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:0.5:0.5:1.3) and Chloroform: Acetic acid: Methanol: Water (6.4:3.2:1.2:0.8) for alkaloids, flavonoids and saponins respectively. Outcome of the present study supports the traditional claims regarding usage of selected plant due to presence of various bioactive

compounds. Separation and identification of these compounds maybe used for development and formulations of new drugs for human benefit. HPTLC fingerprint profiling serves as an important phyto chemical marker and a valid estimation of genetic variability in plants. HPTLC technique was found to be simple, precise, economic and valuable tool for accurate identification and standardization of natural resources and natural products. The HPTLC fingerprint images can be stored and used as reference for further study of this plant.¹⁹

CONCLUSION

From the study carried out and adaption of HPTLC techniques by various pharmacopoeias such as USP-2007, Chinese Pharmacopoeia approves a clear recognition of this method in drug profiling, it can be concluded that HPTLC is the technique of choice for undertaking analysis of numerous complex mixtures of natural product extract as well as finished goods. It will be

useful in determining the usefulness of natural product and quality control of the same.

CONFLICTS OF INTEREST

We wish to declare that there are no conflicts of interest associated with this publication.

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SUMMARY

- HPTLC fingerprint profile of hydro-alcoholic extract of *E. scaber* was developed.
- The HPTLC chromatogram of hydro-alcoholic extract of *E. scaber* resolved 12 alkaloids, 25 flavonoids and 26 saponins with different Rf values including some unknown.
- Chemical profiling of selected plant is suggestive of several known and unknown bioactive molecule having potential therapeutic efficacy.
- HPTLC fingerprint analysis is useful in exact identification of plant material.
- It serves as a significant tool in differentiating adulterants and biomarker in herbal drug industry.

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