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FINGER PRINTING ANALYSIS OF THE STEROIDS FROM *AILANTHUS EXCELSA* (ROXB.) LEAVES USING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

Amita Tilak*[1], Mudit Kumar[1], Ranjana Sharma[1] and Sudhir Singh Gangwar[1]

[1]Department of Pharmacy, GSVM Medical College, Kanpur, Uttar Pradesh, India.

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***Corresponding Author**
Amita Tilak
Department of Pharmacy,
GSVM Medical College,
Kanpur, Uttar Pradesh, India.

ABSTRACT

Objective: The present study was conducted to identify the steroids from petroleum ether (PEAE) extract of medicinally and economically useful leaves of Ailanthus excelsa (Roxb.) 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 (Switzerland). Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungstant lamp. **Results:** HPTLC finger printing of steroids of petroleum ether extract of leaves revealed six polyvalent phytoconstituents (6 peaks) and corresponding ascending order of R_f values in the range of 0.559 to 0.919. **Conclusions:** With the results of HPTLC analysis and above R_f values we have concluded the presence of steroids in the extract.

KEYWORDS: *Ailanthus excelsa* (Roxb.) leaves, steroids, HPTLC Fingerprinting.

INTRODUCTION

Plants are well-known for the primary and secondary metabolites like carbohydrates, proteins and amino acids and steroids, flavonoids, phenolics, glycosides, saponins, tannins, terpenoids, and coumarins etc. These secondary metabolites impart medicinal properties to the plants.[1] Therefore, it is mandatory to resolve the type of secondary metabolites, their nature and pharmacological, antimicrobial, and clinical research, to reveal their bioactivities, to identify the active components and their side effects, and to enhance the purity of the

pharmacologically important active compounds.[2] These active secondary metabolites are qualitatively and quantitatively estimated by various techniques such as spectroscopy and chromatography. Chromatography techniques are the popular tools for the separation and identification of the bioactive compounds. Thin layer and high performance thin layer chromatography (HPTLC) can be applied for this identification. HPTLC fingerprint analysis helps in the identification of the biochemical constituents of the plant.[3] *Ailanthus excelsa (*Roxb.) a plant used in the Indian school/system of medicine for variety of purposes.[4] *Ailanthus excelsa (*Roxb.) belonging to family Simaroubaceae.[5] In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery, especially when there is a blood in stool.[6,7] *Ailanthus excelsa* is a fast growing tree and is extensively cultivated in many parts of India in the vicinity of villages; it is cultivated as an avenue tree for its deep shade and can be used for ant-erosion purposes.[8] The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma.[9,10] In this present study the HPTLC fingerprinting of petroleum ether extract of leaves of *Ailanthus excels* has been performed which may be used as markers for quality evaluation and standardization of the drug.

MATERIALS AND METHODS

Plant material

Leaves of *Ailanthus excelsa (*Roxb.) were collected in the Month of August from the agricult ural fields of Tirunelveli district, Tamilnadu. The plant was identified and leaves of *Ailanthus excels* were authenticated and confirmed from Dr.V.Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by compairing morphological features (leaf and stem arrangement, flower/inflorescence arrangement, fruit and seed morphology etc.). The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation and Extraction of Plant material

Preparation of Petroleum ether extract by Cold maceration (at room temperature) Method

Cold Maceration Extraction Method: In this process, the coarsely powdered plant material of *Ailanthus excels* leaves is extracted by placing the powder in a stoppered container with the solvent petroleum ether and allowed to stand at room temperature for a different period of time (6h, 12h, 24h, 48h) with frequent agitation until the soluble matter has dissolved. The

mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquid is clarified by filtration or decantation after standing. All the extract was evaporated to dryness, weighed and stored for future use.

The Petroleum ether extract of *Ailanthus excelsa* (PEAE) leaves was subjected to the following investigation,

1. HPTLC Fingerprinting of Steroids

HPTLC Fingerprinting

HPTLC studies were carried out following the method of Harborne[11] and Wagner *et al.*[12]

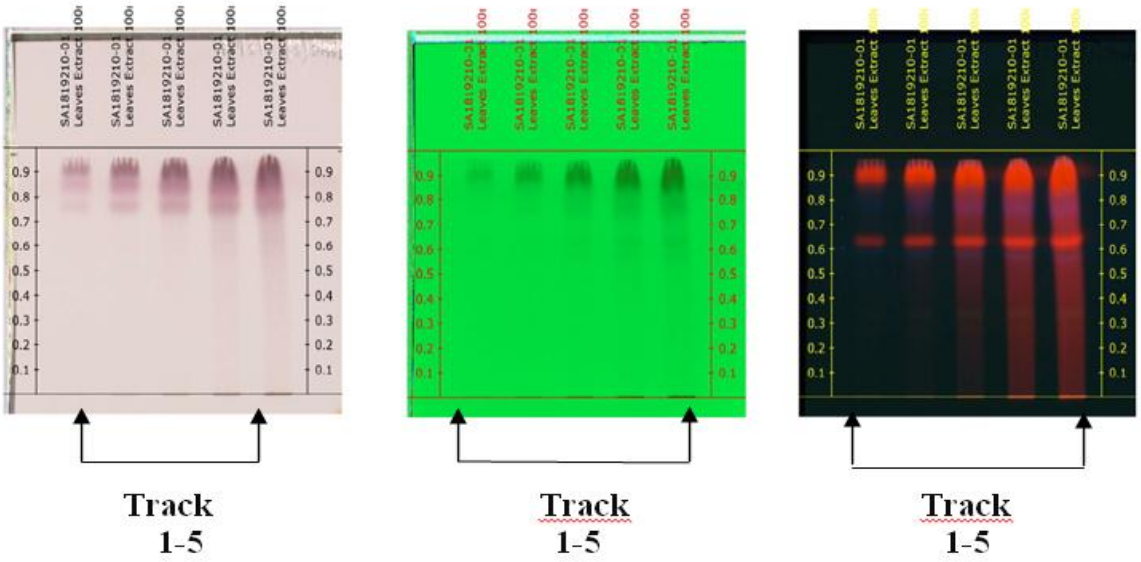
HPTLC instrumentation and Chromatographic conditions

The sample solutions were spotted in the form of bands of width 8.0mm with a Camag microliter syringe on precoated silica gel aluminum plate 60F254 (20cm×10cm with 250µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland). The plates were activated at 120°C for 20min prior to chromatography. A constant application rate of 1.0 µL/s was employed, and space between two bands was 5 mm. The slit dimension was kept at 6.0mm × 0.45mm and 10 mm/s scanning speed was employed. The slit bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase for fingerprinting of steroids consisted of n-butanol:methanol:water in the volume ratio of 3:1:1 (v/v) and anisaldehyde sulfuric acid was used for derivatization and 20mL of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with filter paper whatman no:1 in the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25°C ± 2) at relative humidity of 60% ± 5. The length of the chromatogram run was 8.0 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed with Camag TLC Scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungsten lamp. Subsequent to the development; TLC plate was dipped in anisaldehyde sulfuric acid reagent (ASR) followed by drying in the oven at 110°C. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression.[13-21]

RESULTS AND DISCUSSION

The chromatograms shown in fig.1 indicate that all sample constituents were clearly separate d without any tailing and diffuseness.

Steroids Confirmation



Chromatogram at visible **Chromatogram at 254 nm** **Chromatogram at 366 nm**

Track 1-5: Petroleum ether extract of *Ailanthus excelsa* leaves

Fig. 1: HPTLC fingerprint profile of steroids of leaf extract of *Ailanthus excels*

Detection of steroids in PEAE

It was observed that track 1-5 shows petroleum ether extract. The chromatogram in Fig. 3 shows separation of constituents.

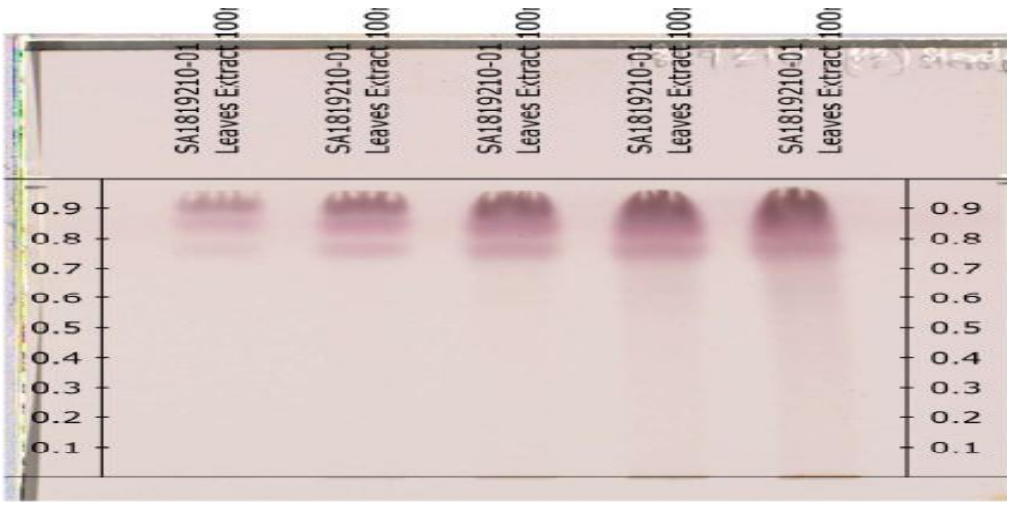


Fig. 2: Steroid confirmation after derivatisation with anisaldehyde sulfuric acid.

It was observed that there is a separation of different phytoconstituents, in PEAE

Table 1: R_f Values for steroids in petroleum ether extract of *Ailanthus excelsa* leaf.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.531	0.0000	0.559	0.0291	2.37	0.569	0.0079	0.00031	0.40	No	
2	0.569	0.0079	0.594	0.0239	1.94	0.606	0.0133	0.00064	0.84	No	
3	0.606	0.0133	0.640	0.0570	4.64	0.677	0.0032	0.00202	2.66	No	
4	0.696	0.0070	0.780	0.2021	16.45	0.810	0.1586	0.01252	16.45	No	
5	0.810	0.1586	0.868	0.4420	35.97	0.888	0.4050	0.02530	33.24	No	
6	0.888	0.4050	0.919	0.4746	38.63	0.996	0.0033	0.03532	46.41	No	

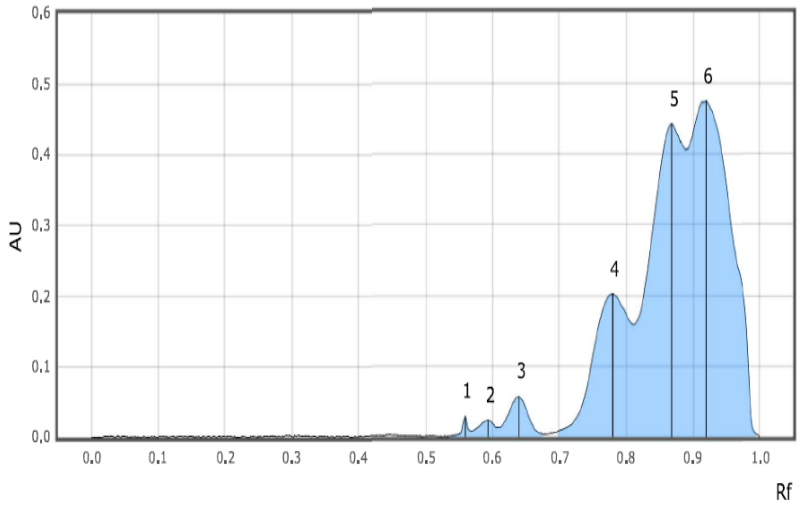


Fig 3: Chromatogram for steroids in petroleum ether extract of *Ailanthus excelsa* leaves Fingerprinting study of steroids of PEAE at 366 nm.

Fingerprinting study of PEAE at 366 nm shows six R_f between the range of 0.559 - 0.919. R_f 0.919 has 38.63% concentration in Table 1 , Figure 3.

CONCLUSION

It is observed in the above HPTLC studies that, PEAE contain a lot of polyvalent chemical constituents with (Roxb.) different R_f values. The developed fingerprint analysis of leaf extract of *Ailanthus excels* will help to isolate and identify new steroids which will offer a possibility to discover lead a molecule for drug development.

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