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Fingerprinting analysis of the phytosterols from *Ailanthus excelsa* (Roxb.) leaves using high-performance thin layer chromatography analysis

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Abstract
Objective: The present study was conducted to identify the phytosterols 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 phytosterols of petroleum ether extract of leaves revealedtwelve polyvalent phytoconstituents (12 peaks) and corresponding ascending order of Rf values in the range of 0.019 to 0.928.
Conclusions: With the results of HPTLC analysis and above R_f values we have concluded the presence of phytosterols in the extract.

Keywords: *Ailanthus excelsa* (Roxb.) leaves, phytosterols, HPTLC fingerprinting

Introduction
Natural remedies from medicinal plants are found to be safe and effective.Many plant species have been used in folklore medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [1]. Standardisation of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardisation of herbals and it's formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and applying suitable standards [2]. High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images [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 phytosterols of petroleum ether extract of leaves of *Ailanthus excelsa* 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 agricultur al fields of Tirunelveli district, Tamilnadu. The plant was identified and leaves of *Ailanthus excelsa* 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 arrangemen, fruit and seed morphology etc.).

The collected plant materialwas 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 excelsa* 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 Phytosterols

HPTLC Fingerprinting

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

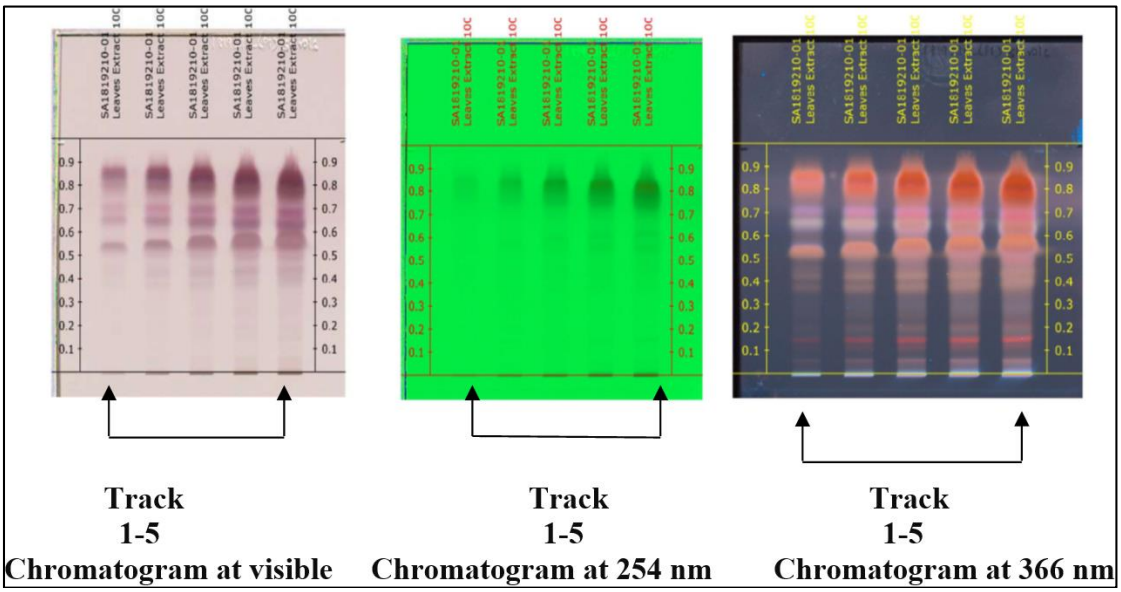
HPTLC instrumentation and Chromatographic conditions

The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microliter syringe on precoated silica gel aluminum plate 60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland). The plates were activated at 120 °C for 20 minutes 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 6.0 mm × 0.45 mm and 10 mm/second 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 phytosterols consisted of chloroform-ethyl acetate in the volume ratio of 4:6 (v/v), and anisaldehyde sulfuric acid was used for derivatization, 20 ml 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 minutes 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; thin layer chromatography (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 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.3 indicate that all sample co nstituents were clearly separated without any tailing and diffuseness.

Phytostrol Confirmation



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

Fig 1: HPTLC fingerprint profile of phytosterols of leaf extract of *Ailanthus excels* Detection of phytosterols in PEAE

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

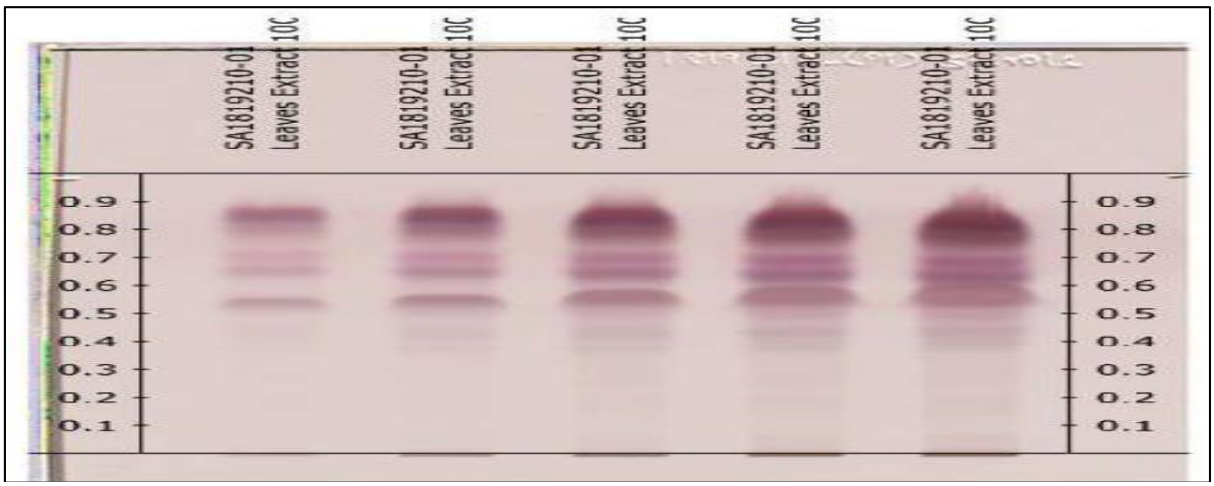


Fig 2: Phytosterols confirmation at visible derivatisation with Anisaldehyde Sulfuric Acid

The purple bands shows the presence of phytosterols in the extract. It was observed that there is a separation of different phytoconstituents, in PEAE.

Table 1: R_f Values for phytosterols 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	0.000	0.0000	0.019	0.3343	11.79	0.048	0.0330	0.00751	5.34	No	
2	0.049	0.0329	0.075	0.1210	4.27	0.101	0.0001	0.00309	2.20	No	
3	0.144	0.0401	0.167	0.1743	6.15	0.197	0.0308	0.00538	3.82	No	
4	0.197	0.0308	0.221	0.0647	2.28	0.256	0.0110	0.00240	1.71	No	
5	0.358	0.0424	0.408	0.1758	6.20	0.425	0.1510	0.00809	5.75	No	
6	0.425	0.1510	0.455	0.2084	7.35	0.495	0.1584	0.01247	8.86	No	
7	0.496	0.1578	0.558	0.2994	10.56	0.581	0.2564	0.02018	14.34	No	
8	0.592	0.2472	0.619	0.2855	10.07	0.630	0.2545	0.01009	7.17	No	
9	0.630	0.2545	0.654	0.3132	11.05	0.679	0.2577	0.01402	9.96	No	
10	0.706	0.2929	0.747	0.3775	13.32	0.810	0.1964	0.03065	21.77	No	
11	0.810	0.1964	0.838	0.2350	8.29	0.871	0.1174	0.01174	8.34	No	
12	0.891	0.1444	0.928	0.2458	8.67	1.000	0.0000	0.01514	10.75	No	

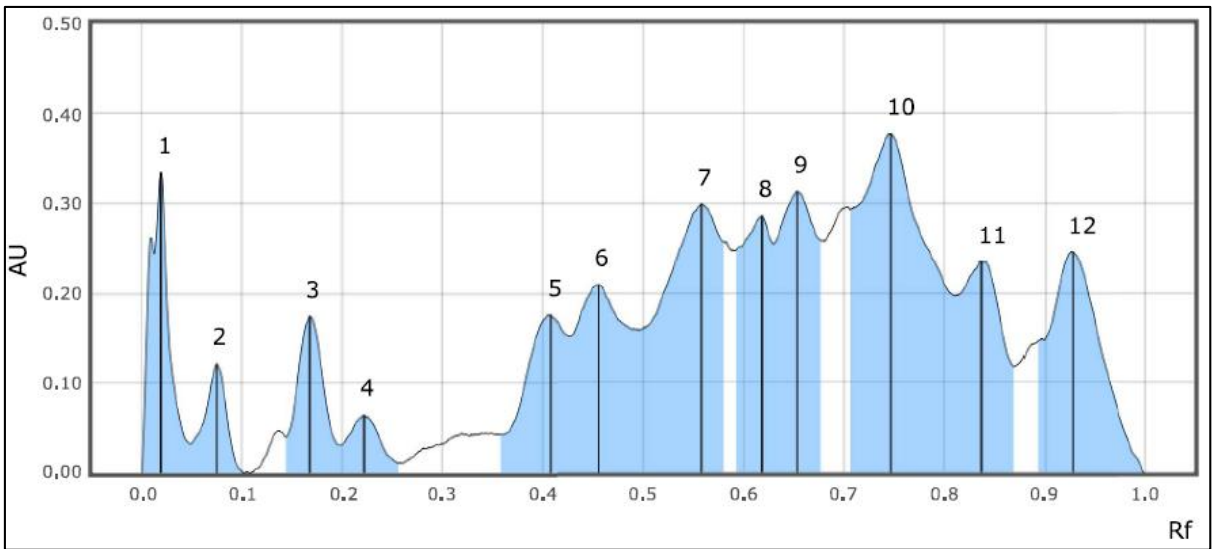


Fig 3: Chromatogram for phytosterols in petroleum ether extract of *Ailanthus excelsa* leaves Fingerprinting study of phytosterols of PEAE at 366 nm

Fingerprinting study of PEAE at 366 nm shows twelve R_f between the range of 0.019- 0.928. R_f 0.747 has 13.32% concentration in Table 1, Figure 3.

Conclusion

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

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