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PHYTOCHEMICAL STUDIES ON THE STEROLS OF METHANOLIC LEAF EXTRACTS OF MEDICINALLY IMPORTANT PLANT *AILANTHUS EXCELSA* (ROXB.) USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Objective: The present study was conducted to identify the sterols from methanol extract of medicinally and economically useful leaves of *Ailanthus exc elsa* (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 (S witzerland). 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 sterols of methanol extract of leaves revealed at Rf 0.59 maroon colour hence sterols are detected. **Conclusions:** With the results of HPTLC analysis and above Rf value we have concluded the presence of sterols in the extract.

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

INTRODUCTION

*Ailanthus excelsa (*Roxb.) a plant used in the Indian school/system of medicine for variety of purposes.[1] *Ailanthus excelsa (*Roxb.) belonging to family Simaroubaceae.[2] In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery, especially when there is a blood in stool.[3,4] *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.[5] The bark has been used in

Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma.[6,7] In this present study the HPTLC fingerprinting of sterols of methanol 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. 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 Methanol 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 methanol 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 Methanol extract of *Ailanthus excelsa* leaves was subjected to the following investigation.

1. HPTLC Fingerprinting of Sterols

HPTLC Fingerprinting

HPTLC studies were carried out following the method of Harborne[8] and Wagner *et al.*[9]

HPTLC instrumentation and Chromatographic conditions

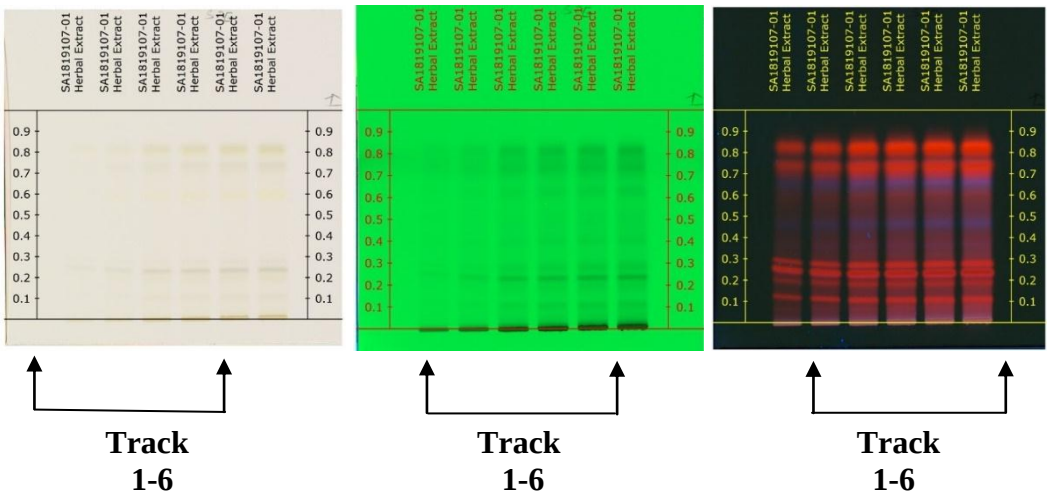
The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microliter syringe on precoated silica gel aluminum plate 60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland). The 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 ration 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.[10-18]

RESULTS AND DISCUSSION

The fig.3 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

Sterols Confirmation



Chromatogram at visible Chromatogram at 254 nm Chromatogram at 366 nm

Track 1-5: Methanol extract of *Ailanthus excelsa* leaves.
Fig. 1: HPTLC fingerprint profile of sterols of leaf extract of *Ailanthus excelsa*
Detection of sterols in methanol extract.

It was observed that track 1-6 shows methanol extract. The Fig. 3 shows separation of constituents.

Fig. 2: Sterols confirmation at visible derivatisation with Anisaldehyde sulfuric acid reagent.

The maroon bands shows the presence of sterols in the extract. It was observed that there is a separation of different phytoconstituents, in methanol extract.

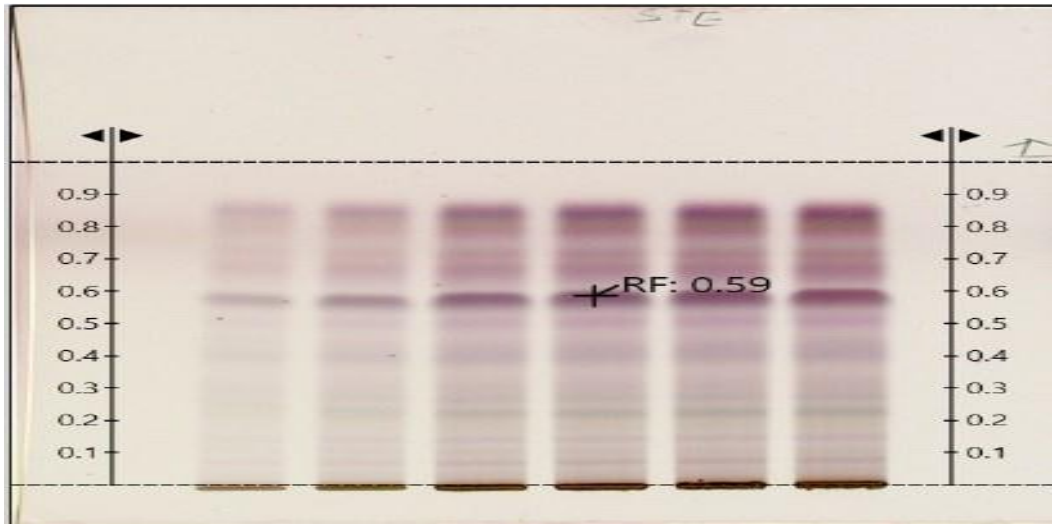


Fig 3: R_f Value for sterols in methanol extract of *Ailanthus excelsa* leaf.

CONCLUSION

It is observed in the above HPTLC studies that, methanol 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 sterols which will offer a possibility to discover lead a molecule for drug development.

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