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HPTLC FINGERPRINTING STUDY OF HYDROALCOHOLIC EXTRACT FOR STEROIDS FROM *Ailanthus excelsa* (Roxb.) LEAVES

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ABSTRACT

Objective: The present study was conducted to identify the steroids from Hydroalcohol extract of medicinally and economically useful leaves of *Ailanthus excels* (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 steroids of Hydroalcohol extract of leaves shows absence of steroids as no purple and maroon bands are seen after derivatisation with Anisaldehyde sulphuric acid. **Conclusions:** With the results of HPTLC analysis we have concluded the absence of steroids in the extract as no purple and maroon bands are seen after derivatisation with Anisaldehyde sulphuric acid.

KEYWORDS: *Ailanthus excelsa* (Roxb.) leaves, steroids, 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 steroids of Hydroalcohol 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

Preparation and Extraction of Plant material

The powder of *Ailanthus excelsa* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using Water and ethanol (1:1) proportion. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50ºC to get Hydroalcohol extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10º C. The Hydroalcohol extract of *Ailanthus excelsa* leaves was subjected to the following investigations.

The Hydroalcohol extract of *Ailanthus excelsa* leaves was subjected to the following investigations

1. Preliminary phytochemical screening.
2. HPTLC Fingerprinting of Steroids

HPTLC Fingerprinting

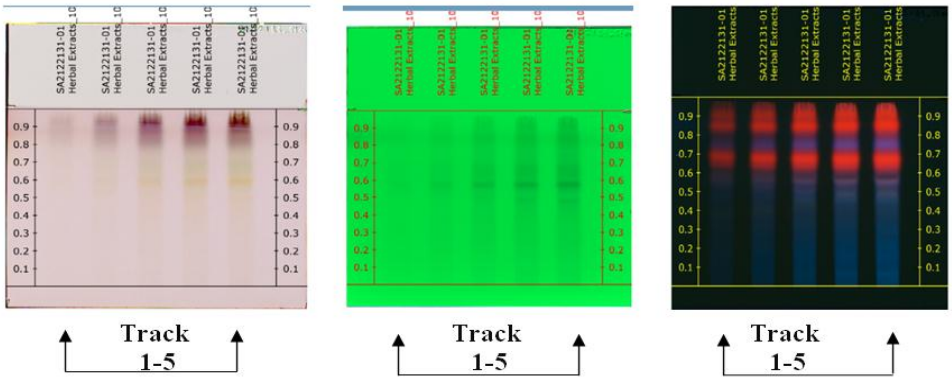
HPTLC studies were carried out following the method of Harborne[11] and Wagner *et al.*[12]

HPTLC instrumentation and Chromatographic conditions

The sample solutions were spotted in the form of bands of width 8.0 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 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 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

Steroid Confirmation



Chromatogram at visible Chromatogram at 254 nm Chromatogram at 366 nm

Track 1-5: Hydroalcohol extract of *Ailanthus excels* leaves.

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

Detection of steroids in Hydroalcohol extract.

It was observed that track 1-5 shows Hydroalcohol extract.

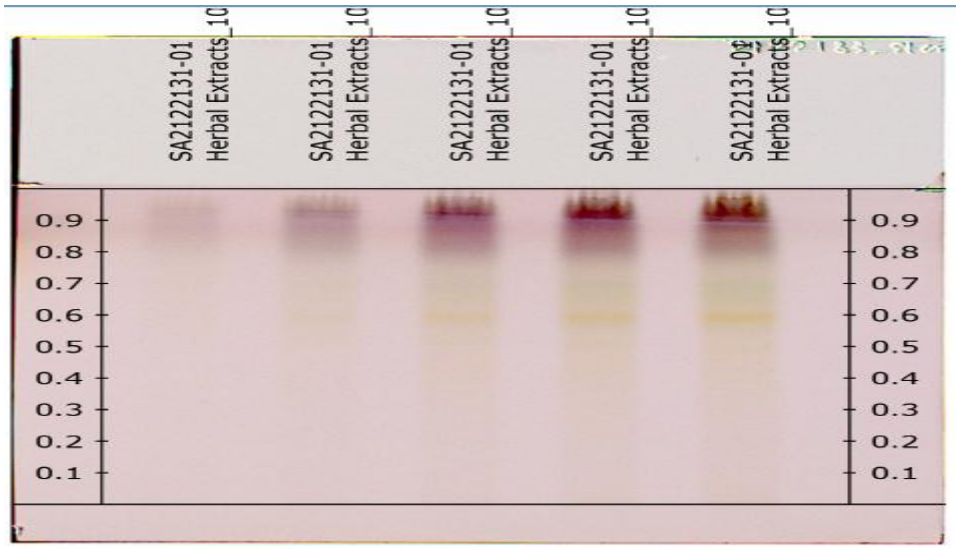


Fig. 2: Steroids confirmation at visible derivatisation with Anisaldehyde sulphuric acid.

There was no appearance of purple and maroon bands are seen after derivatisation with Anisaldehyde sulphuric acid so it shows absence of steroids.

CONCLUSION

It is observed in the above HPTLC studies that, Hydroalcohol extract of *Ailanthus excelsa* (Roxb.) contain a lot of polyvalent chemical constituents with different R_f values like flavonoids and sterols, but absence of steroids.

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