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Fingerprinting of Phytosterols from Hydroalcoholic Extr act of *Ailanthus excelsa* (Roxb.) Leaves using High Performance Thin Layer Chromatography

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Abstract

Objective: 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. Standardisation of plant materials is the need of the day To study flavonoid profile of the medicinal plant. *Ailanthus excelsa* (Roxb.) using High Performance Thin Layer Chromatography (HPTLC) technique.

Methods: The extracts were tested to determine the presence of various phytochmeicals like alkaloids, phenolic compounds, phytosterols, carbohydrates, glycosides, saponins, terpenoids, tannins, fixed oils, fats and protein and aminoacids (Harborne and Harborne, 1998). HPTLC studies were carried out by Harborne and Wagner et al method. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks.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 hydroalcohol extract of leaves revealed nine polyvalent phytoconstituents (09 peaks) and corresponding ascending order of Rf values in the range of 0.010 to 0.826

Conclusion: With the results of HPTLC analysis and R_f values Phytosterols have been concluded in the extract. Hence it w as concluded that the phytosterol compounds present in the Hydroalcohol extract could be responsible for antioxidant activities. Plant derived antioxidants, especially phenols and phytosterols, have been described to have various properties like anticancer, antiaging and prevention of cardiovascular diseases. Furthur, separation and characterization of the bioactive compound from the plant is to be evaluated and reported in near future.

Keywords: HPTLC, *Ailanthus excela* (Roxb.) leaves, Hydroalcohol extract, Phytochemicals,Phytosterols, 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. 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 excels*(Roxb.) (Simaroubaceae) is commonly known as Mahanimba due to its resemblance with the neem tree (Azadirachita indica) and Maharukha due to its large size. Ailanthus is from ailanto which means tree of heaven and is the name for one of the species in the Moluccas, while in Latin excelsa means tall. The plant is known by different names like tree of heaven in English[4]. *Ailanthus excelsa (*Roxb.) a plant used in the Indian school/system of medicine for variety of purposes[5]. *Ailanthus excelsa (*Roxb.) belonging to family Simaroubaceae[6]. In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery,especially when there is a blood in stool[7,8]. *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[9]. The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma[10,11]. The plant shows Antifertility activity, Antifungal activity, Antimalarial activity, Hypoglycemic activity, Antipyretic activity, Antitumor and cytotoxicity, Hepatoprotective activity[12] . Research interest has focused on various herbs that possess hypolipidemic, antiplatlet, antitumour, immunestimulating properties, anti-inflammatory, anti-viral etc. that may be useful adjuncts in reducing the risk of cardiovascular disease, cancer and other diseases. A wide variety of active phytochemicals,including phytosterols, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant phytosterols, curcumins, phthalides, tannins, gallic acid, quercetin, phytophytosterols, alcohols, aldehydes have been identified from medicinal plants[13]. These phytochemicals are estimated by a variety of techniques such as spectroscopy and chromatography. High performance thin layer chromatography (HPTLC) chromatographic fingerprints can be applied for this kind of certification. Fingerprint analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituents profile of the plants with botanical identity and for estimation of chemical and biochemical markers[14-18]. Alkaloids, tannins have been Identified with HPLTC Studies of this Plant[19,20]. but Hydroalcohol extract of this

plant has not been explored for HPTLC Studies so in this present study the HPTLC fingerprinting of Phytosterols of Hydroalcoholic extract of leaves of *Ailanthus excelsa* (Roxb.) 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 agricultural 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 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 Hydroalcohol extract by Soxhlet Extraction Method: 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

1.Preliminary phytochemical screening.

2. HPTLC Fingerprinting of Phytosterols

HPTLC Fingerprinting

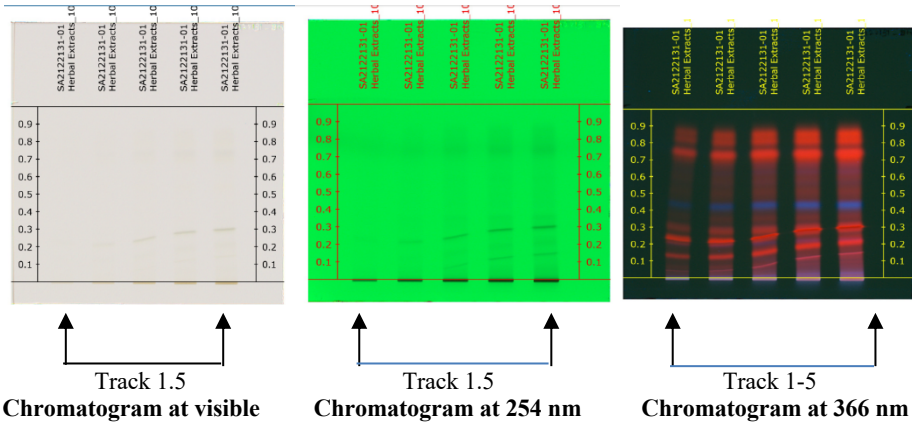
HPTLC studies were carried out following the method of Harborne[21] and Wagner *et al* [22].

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[23-31].

RESULTS AND DISCUSSION

Phytosterol Confirmation



Track 1-6: Hydroalcohol extract of *Ailanthus excelsa* leaves

Fig. 1 : HPTLC fingerprint profile of Phytosterols of leaf extract of *Ailanthus excelsa* Detection of Phytosterols in Hydroalcohol extract

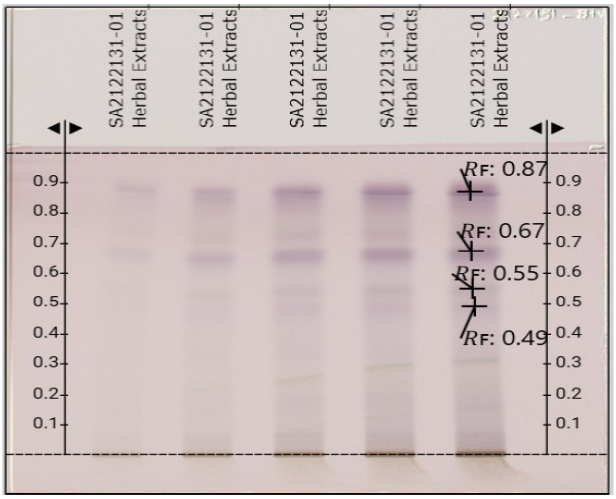


Fig. 2: Phytosterols confirmation at visible derivatisation with Anisaldehyde Sulphuric acid reagent

Table 1: Rf Values for phytosterols in Hydroalcohol extract of Ailanthus excelsa leaves

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%		
1	0.000	0.0000	0.010	0.2086	17.81	0.021	0.0000	0.00234	5.79	No	
2	0.060	0.0000	0.079	0.0110	0.94	0.116	0.0000	0.00041	1.01	No	
3	0.121	0.0000	0.160	0.0875	7.47	0.181	0.0116	0.00159	3.95	No	
4	0.182	0.0115	0.226	0.1137	9.71	0.273	0.0073	0.00455	11.27	No	
5	0.273	0.0073	0.315	0.3992	34.09	0.356	0.0072	0.00945	23.40	No	
6	0.410	0.0000	0.435	0.0106	0.90	0.484	0.0000	0.00041	1.02	No	
7	0.485	0.0000	0.600	0.0415	3.54	0.652	0.0184	0.00377	9.34	No	
8	0.660	0.0179	0.750	0.2011	17.17	0.792	0.0642	0.01286	31.85	No	
9	0.794	0.0640	0.826	0.0980	8.37	0.852	0.0854	0.00499	12.36	No	

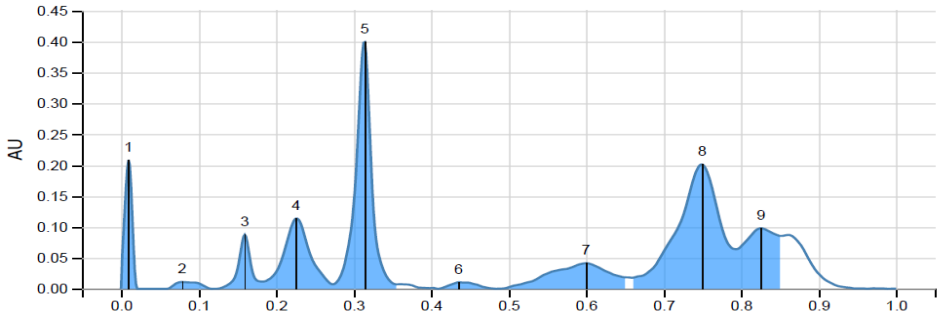


Fig 3: Chromatogram for phytosterols in Hydroalcohol extract of *Ailanthus excelsa* leaves

It was observed that track 1-5 (Figure 1) shows Hyderoalcohol extract. Figure 2, the fluorescence shows purple bands which concludes the presence of Phytosterols in the extract. It was observed that there is a separation of different phytoconstituents, in Hydroalcohol extract

Fingerprinting study of Hydroalcohol extract at 366 nm shows nine (09 Peaks) Rf between the range of 0.010-0.826. Rf 0.315 has maximum 34.09 % concentration in Table 1, Figure 3 shows R_f Values for Phytosterols in Hydroalcohol extract of *Ailanthus excelsa* leaves.

The evaluation of crude extract is an integral part of correct identity. HPTLC is useful as a phytochemical marker[32, 33]. and more effective in the field of plant taxonomy also for the identification of plants through secondary metabolites[34]. HPTLC fingerprinting is proved to be a linear, precise, and accurate method for herbal identification[35]. Such finger printing is useful in quality control of herbal products and checking for the adulterants[36]. Therefore, it can be useful for the evaluation of different marketed pharmaceutical preparations [37–39].

Due to the adverse effects of synthetic drugs, in recent years, scientists are on the search for alternative medicine. There are some diseases which are chronic and need a long duration of medication, plant-based drugs are less toxic and have no side effects. We have got positive results for antiarthritic activity of this plant in our previous studies. Furthermore, the literature survey of phytosterols has shown potent antiarthritic, antiinflammatory, immunosuppressant activity[40-44]. In recent years, phytosterols like beta sitosterol have shown central inhibitory and neuromodulatory functions which claims its use as an anxiolytic, sedative, anticonvulsant, antidepressant activities in our studies[45].

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. 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 a lead molecule for drug development.

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AUTHOR CONTRIBUTIONS

Dr. Ravindra C. Sutar conceptualized and designed the study, curated the data and prepared the original draft, discussed the methodology and analysed the data, prepared results and contributed to the final manuscript.

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