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Research Article

FINGERPRINTING ANALYSIS OF THE PHYTOSTEROLS FROM *HOLOPTELEA INTEGRIFOLIA* (ROXB.) PLANCH 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 and methanol extracts of medicinally and economically useful leaves of *Holoptelea* integrifolia (Roxb.) Planch using high-performance thin layer chromatography (HPTLC) technique.

Materials and 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 thin layer chromatography 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.

Results: Preliminary phytochemical screening of petroleum ether extract of *H. integrifolia* showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins, and carbohydrates while methanolic extract of *H. integrifolia* showed the presence of steroids, alkaloids, flavonoids, proteins, and carbohydrates. HPTLC fingerprinting of phytosterols of petroleum ether extract of leaf revealed five polyvalent phytoconstituents (5 peaks) and corresponding ascending order of Rf values in the range of 0.11-0.45. While methanol extract of leaf showed four polyvalent phytoconstituents (4 peaks) and corresponding ascending order of Rf values in the range of 0.13-0.48.

Conclusions: With the results of preliminary phytochemical analysis and above Rf values, we have concluded the presence of phytosterols in both the extracts.

Keywords: High-performance thin layer chromatography fingerprinting, *Holoptelea integrifolia* (Roxb.) planch leaf, Phytochemical screening, Phytosterol.

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].

Holoptelea integrifoila belongs to the family ulmaceae commonly called as Indian Elm and commonly used in India by the tribal people for its medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings [4]. In the traditional system of medicine, bark and leaves of *H. integrifoila* are used as bitter, astringent, acrid, thermogenic, antiinflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent, and in rheumatism [5,6]. The plant *H. integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhea, and rheumatism [7]. In this present study, the preliminary phytochemical screening of *H. integrifolia* leaf extract has been done to identify the phytochemical constituents and HPTLC fingerprinting of phytosterols in *H. integrifolia* extract has been performed which may be used as markers for quality evaluation and standardization of the drug.

MATERIALS AND METHODS

Plant material

Leaves of *H. integrifoila* were collected in the month of August from the agricultural fields of Tirunelveli district, Tamil Nadu. The plant was identified and leaves of *H. integrifolia* were authenticated and confirmed from Dr.V.Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by comparing 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 and methanol extract

The powder of *H. integrifoila* leaves was charged into the thimble of a soxhlet apparatus and extracted using petroleum ether. Appearance of the colorless 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 50°C to get petroleum ether 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 airtight container in a refrigerator below 10°C. After obtaining the petroleum ether extract, the marc was pressed and it is air-dried and again it was extracted using methanol. Appearance of the colorless 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 50°C to get semi solid mass of methanol extract. The extract was stored in an airtight container in a refrigerator below 10°C.

The petroleum ether and methanol extracts of *H. integrifolia* leaves were subjected to the following investigations:

1. Preliminary phytochemical screening
2. HPTLC fingerprinting of phytosterols.

Phytochemical screening

The phytochemical investigation of the different leaf extracts of *H. integrifolia* was carried out with standard protocol [8]. The results are presented in Table 1.

HPTLC fingerprinting

HPTLC studies were carried out following the method of Harborne [9] and Wagner and Baldt [10].

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 [11-19].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of petroleum ether extract of *H. integrifolia* leaves showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins, and carbohydrates while methanolic extract of *H. integrifolia* leaves showed the presence of steroids, alkaloids, flavonoids, proteins, and carbohydrates (Table 1).

The chromatograms shown in Fig. 1 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

Fig.2 shows Fingerprint analysis of phytosterols of Holoptelea integrifolia (Roxb.) Planch leaves after derivatization with anisaldehyde sulphuric acid reagent inflorescence at 366 nm.

Table 1: Preliminary phytochemical screening of petroleum ether and methanol extracts of *H. integrifolia* (Roxb) planch leaves

Plant constituents	Test performed	*H. integrifolia* leaves	
		Petroleum ether extract	Methanolic extract
Test for steroids	Salkowaski reaction	++	+
	Liebermann-buchard reaction	++	+
Test for triterpenoids		++	-
Test for glycosides	Balget's test	++	-
	Keller-Killiani test	+	-
	Legals test	+	+
	Borntrager's test	+	+
Tests for saponin	Foam test	-	-
Tests for carbohydrates	Molisch's test	++	++
	Barfoed's test	++	++
	Fehling's test	++	++
	Benedict's test	++	++
Test for alkaloids	Mayer's reagent	+	-
	Hager's reagent	-	+
	Dragendorff's reagent	+	-
Tests for flavonoids	Ferric-chloride test	++	+
	Shinoda test	++	+
Test for tannins	$FeCl_3$ solution	+	-
	Gelatin test	+	-
Test for proteins	Millon's test	+	+
	Xanthoproteic test	+	+
	Biuret test	+	+
	Ninhydrin test	+	+

++: Higher concentration, +: Present, -: Absent, *H. integrifolia: Holoptelea integrifolia*

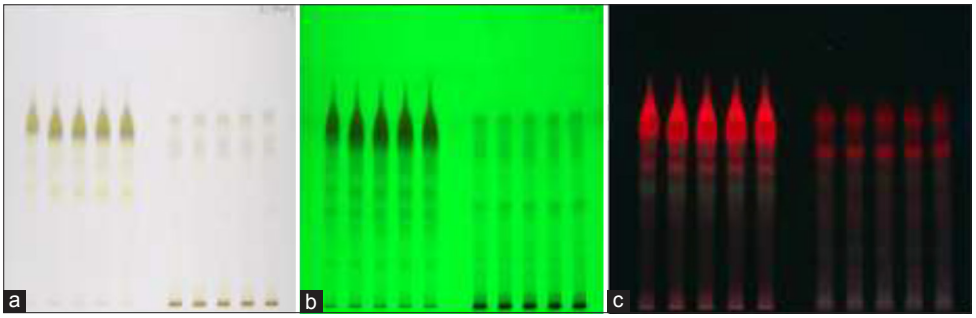

Fig. 1: High-performance thin layer chromatography fingerprint profile of phytosterols of leaf extract of *Holoptelea integrifolia* (Roxb.) Planch (Track 1-5: Petroleum ether extract, Track 7-11: Methanol extract, Note: There were no data available for track 6). (a) HPTLC plate seen at visible light. (b) HPTLC plate seen at 254 nm. (c) HPTLC plate seen at 366 nm

Fig. 3 shows Three-dimensional plot of fingerprint of phytosterols of *Holoptelea integrifolia* leaf.

The results from HPTLC fingerprint of phytosterols scanned at wavelength 540 nm for petroleum ether extract of *H. integrifolia* leaf shows that there are five polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.11 to 0.45 in which highest Concentration of the phytoconstituents was found to be 31.27%, and its corresponding Rf value was found to be 0.35, respectively, and was recorded in Table 2. The corresponding HPTLC chromatogram was presented in Fig. 4.

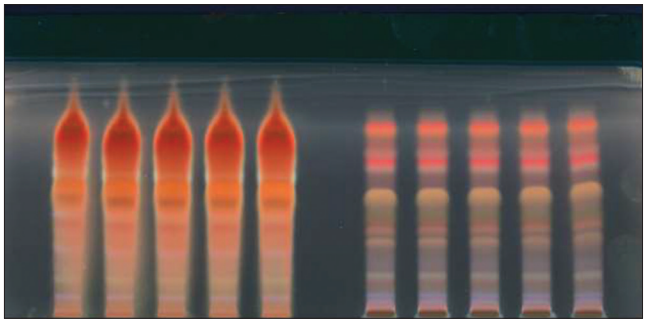

Fig. 2: Fingerprint analysis of phytosterols of *Holoptelea integrifolia* (Roxb.) Planch leaves after derivatization with anisaldehyde sulfuric acid reagent inflorescence at 366 nm

The results from HPTLC fingerprint of phytosterols scanned at wavelength 540 nm for the methanol extract of *H. integrifolia* leaf shows that there are four polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.13 to 0.48 in which the highest concentration of the phytoconstituents was found to be 39.84% and its corresponding Rf value was found to be 0.48, respectively, and was recorded in Table 3. The corresponding HPTLC chromatogram was presented in Fig. 5 [20,21].

CONCLUSION

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

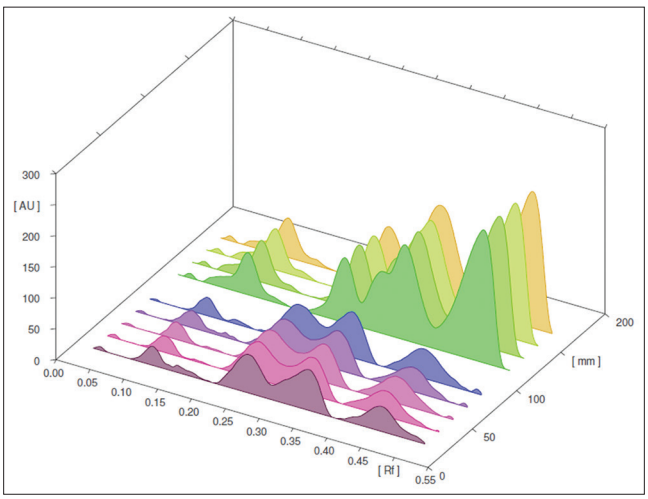

Fig. 3: Three-dimensional plot of fingerprint of phytosterols of *Holoptelea integrifolia* leaf

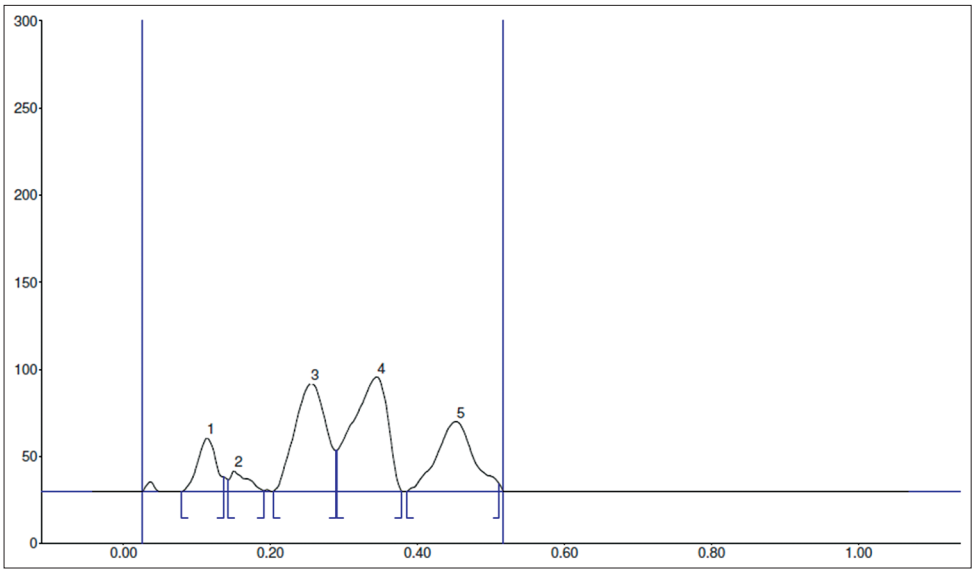

Fig. 4: Chromatogram for phytosterols of petroleum ether extract of *Holoptelea integrifolia* leaf

Table 2: Rf values for phytosterols of petroleum ether extract of *H. integrifolia* leaf

Peak	winCATS planar chromatography manager									
	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Assigned substance
1	0.08	0.0	0.11	30.8	14.60	0.14	8.6	638.3	8.59	Unknown*
2	0.14	6.8	0.15	11.9	5.65	0.19	0.7	239.3	3.22	Unknown*
3	0.20	0.2	0.26	62.0	29.38	0.29	23.6	2234.3	30.08	Unknown*
4	0.29	23.9	0.35	66.0	31.27	0.38	0.3	2625.2	35.34	Unknown*
5	0.39	0.1	0.45	40.3	19.10	0.51	4.4	1690.8	22.76	Unknown*

H. integrifolia: Holoptelea integrifolia

Table 3: Rf values for phytosterols in methanol extract of *Holoptelea integrifolia* leaf

Peak	winCATS planar chromatography manager									
	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Assigned substance
1	0.06	0.5	0.13	67.9	12.43	0.17	8.7	2038.2	9.71	Unknown*
2	0.20	0.0	0.28	105.6	19.34	0.30	40.0	2991.8	14.25	Unknown*
3	0.30	40.9	0.36	155.0	28.38	0.41	30.6	7356.6	35.04	Unknown*
4	0.41	30.9	0.48	217.6	39.84	0.51	0.2	8607.6	41.00	Unknown”

H. integrifolia: Holoptelea integrifolia

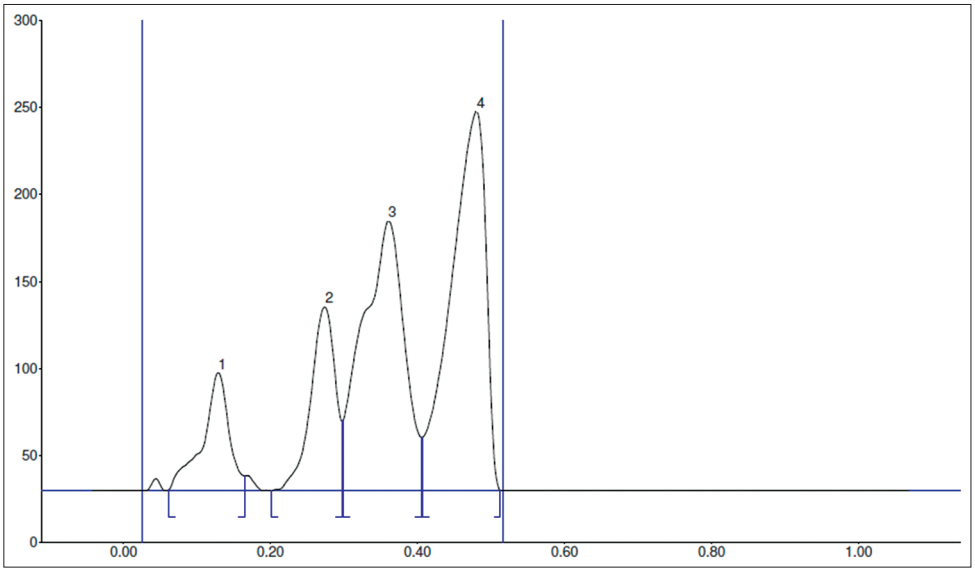

Fig. 5: Chromatogram for phytosterols in methanol extract of *Holoptelea integrifolia* leaf

shown potent antiarthritic, antiinflammatory, immunosuppressant activity [22-26]. 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 [27]. The phytosterol isolated from the *H. integrifolia* has shown prominent antiarthritic activity in our studies which will offer the possibility to discover a lead molecule for drug development.

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