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Research Article

PHYTOCHEMICAL STUDIES ON THE GLYCOSIDES OF LEAF EXTRACTS OF MEDICINALLY IMPORTANT PLANT *HOLOPTELEA INTEGRIFOLIA* (ROXB.) PLANCH USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Objective: This study was conducted to identify the glycosides 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 carried out 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 computer-aided transcription system software (1.4.6 Camag) with the help of tungstant lamp.

Results: Preliminary phytochemical screening of petroleum 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 glycosides of petroleum ether extract of leaf revealed four polyvalent phytoconstituents (4 peaks) and corresponding ascending order of radio frequency (Rf) values in the range of 0.37-0.82. while methanol extract of leaf showed ten polyvalent phytoconstituents (10 peaks) and corresponding ascending order of Rf values in the range of 0.20-0.83.

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

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

INTRODUCTION

Holoptelea integrifoila belongs to the family ulmaceae commonly called as Indian Elm and 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 [1]. In the traditional system of medicine, bark and leaves of *H. integrifoila* are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [2,3]. The plant *H. integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinalworms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhea and rheumatism [4]. The plant has been reported to possess anti-inflammatory [5], antidiabetic [6], antitumor [7], antidarrheal [8], antioxidant [9], antimicrobial [10], anthelmintic [11,12] activities.

Although the preliminary phytochemical studies revealed the presence of various bioactive compounds other than glycosides, there is no detailed study on phytoprofiling of *H. integrifolia*. In this study, an effort has been made to elucidate the glycosides profile of *H. integrifolia* using high-performance thin layer chromatography (HPTLC) technique. Literature reveals that there are no HPTLC studies performed till so far for qualitative studies of glycosides in extracts of leaves of *H. integrifolia* and hence there was a need for identifying and quantifying glycosides in *H. integrifolia* (Roxb.) planch using validated HPTLC method.

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), Government 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.

Extraction of plant material for 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 glycosides.

Phytochemical screening

The phytochemical investigation of the different leaf extracts of *H. integrifolia* was carried out with standard protocol [13]. The results are presented in Table 1.

HPTLC fingerprinting

HPTLC studies were carried out following the method of Harborne [14] and Wagner and Baldt [15].

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 2 bands was 5 mm. The slit dimension was kept at 6.0 mm × 0.45 mm and 10 mm/s 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 glycosides consisted of ethylacetate:methanol:water in the volume ratio of 20:2.8:2 (v/v) and alcoholic KOH 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 what man 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, 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 computer aided transcription system 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 [16-24].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis 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 (Table 1).

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

Fig. 2 shows fingerprint analysis of glycosides of *H. integrifolia* (Roxb.) planch leaves after derivatisation with alcoholic KOH influorescence at 366 nm.

Fig. 3 shows three dimensional plot of Fingerprint of glycosides in leaf extracts of *H. Integrifolia* (Roxb.) planch.

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*

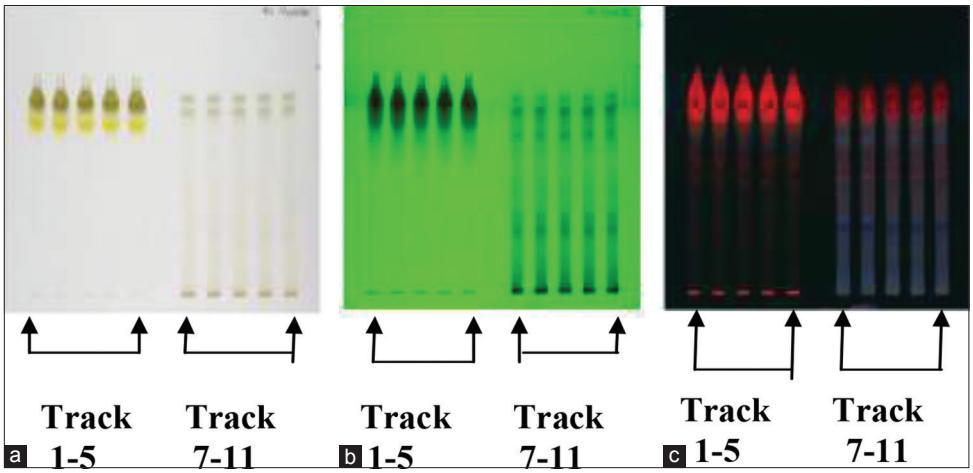

Fig. 1: High performance thin layer chromatography fingerprint profile of glycosides of leaf extracts of *Holoptelea integrifolia* (Roxb.) planch. (a) HPTLC plate seen at visible light (b) HPTLC plate seen at 254 nm (c) HPTLC plate seen at 366 nm. Track 1-5: Petroleum ether extract, Track 7-11: Methanol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

The results from HPTLC fingerprint scanned at wavelength 366 nm for petroleum ether extract of *H. integrifolia* leaf shows that there are four polyvalent phytoconstituents and corresponding ascending order of radio frequency (Rf) values start from 0.37 to 0.82 in which highest concentration of the phytoconstituent was found to be 44.62% and its corresponding Rf value was found to be 0.73, respectively and was recorded in Table 2. The corresponding HPTLC chromatogram was presented in Fig. 4.

The results from HPTLC fingerprint scanned at wavelength 366 nm for the methanol extract of *H. integrifolia* leaf. There are ten polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.20 to 0.83 in which highest Conc. The phytoconstituents was found to be 19.94%, and its corresponding Rf value was found to be 0.28, respectively and was recorded in Table 3. The corresponding HPTLC chromatogram was presented in Fig. 5 [25,26].

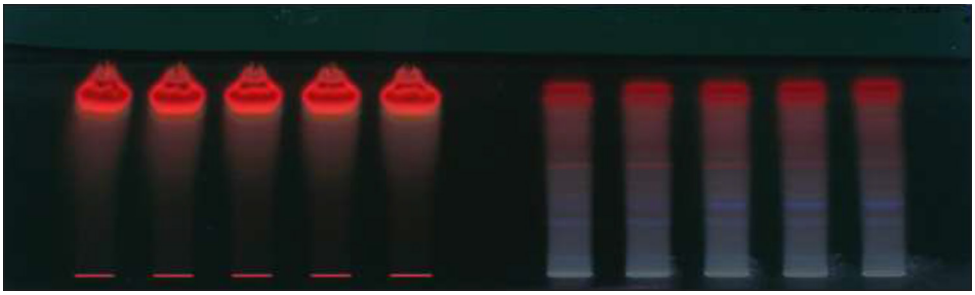

Fig. 2: Fingerprint analysis of glycosides of *Holoptelea integrifolia* (Roxb.) planch leaves after derivatization with alcoholic KOH nfluorescence at 366 nm

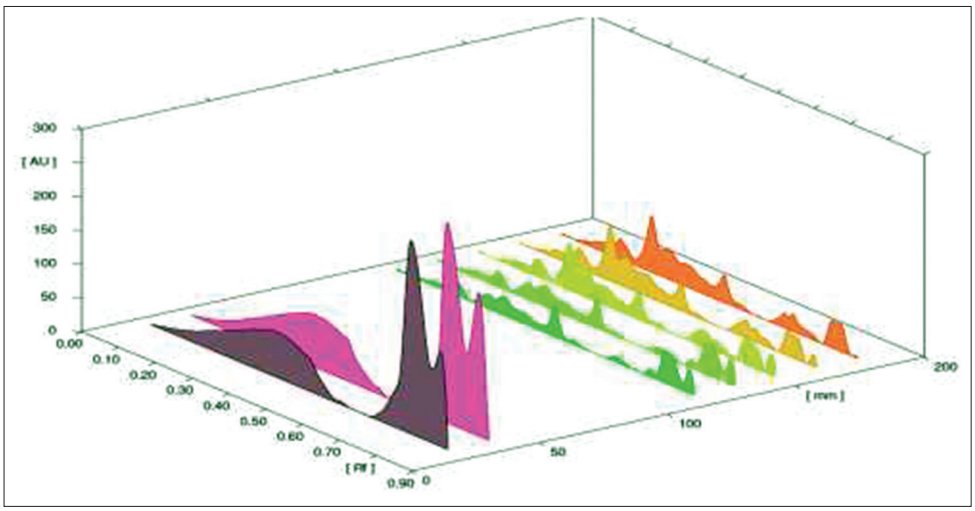

Fig. 3: Three-dimensional plot of fingerprint of glycosides in leaf extracts of *Holoptelea integrifolia* (Roxb.) planch

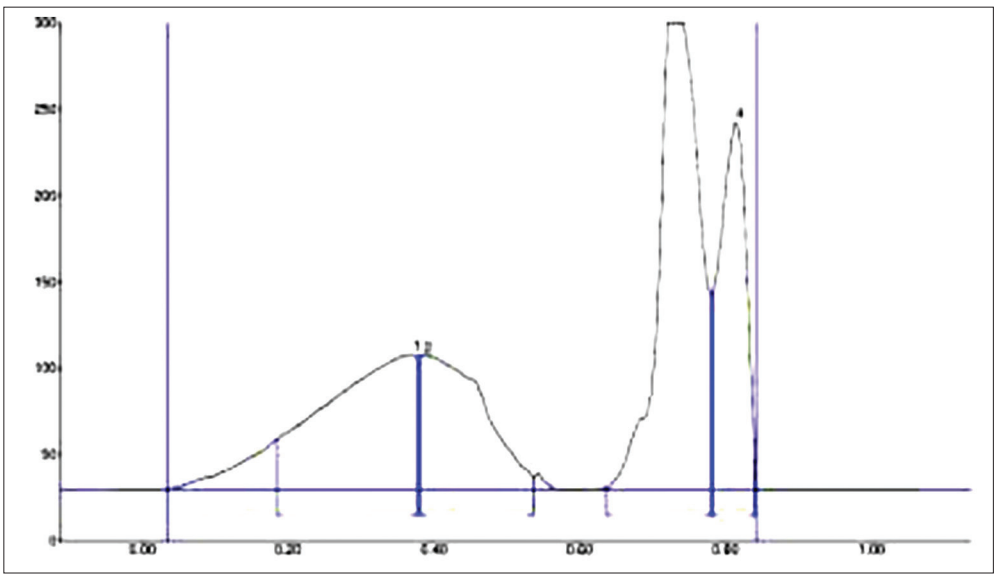

Fig. 4: Chromatogram of glycosides in petroleum ether extract of *Holoptelea integrifolia* leaf