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Research Article

PHYTOCHEMICAL PROFILE STUDIES ON THE STEROIDS OF METHANOLIC LEAF EXTRACT OF MEDICINALLY IMPORTANT PLANT *HOLOPTELEA INTEGRIFOLIA* (ROXB.) PLANCH USING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Objective: The present study was conducted to identify the steroids from the methanol extract 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 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.

Results: Preliminary phytochemical screening of methanolic extract of *H. integrifolia* showed the presence of steroids, alkaloids, flavonoids, proteins, and carbohydrates. HPTLC fingerprinting of steroids of methanol extract of leaf showed seven polyvalent phytoconstituents (7 peaks) and corresponding ascending order of retention factor (Rf) values in the range of 0.17-0.64.

Conclusions: With the above Rf values and preliminary phytochemical analysis, we have concluded the presence of steroids in methanol extract.

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

INTRODUCTION

Plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat contagious diseases [1-3]. They are a source of active secondary metabolites which prove to be invaluable for the management of such diseases. In much of the developing world, 70-95% of the population relies on these traditional medicines for primary care [4].

Recent approach is the utility of natural products as sources of novel structures of therapeutic value [5]. Plants have developed chemical defenses over millions of years against environmental threats such as ultraviolet radiation, reactive oxygen species, and microbial attacks. Therefore, phytochemicals are less toxic and biologically active [6]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances [7].

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 [8]. 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, de purative, repulsive, urinary astringent, and in rheumatism [9,10]. 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 [11]. In spite of its abundant uses, the chromatographic fingerprint profile for determination of steroids in *H. integrifolia* leaves have not been reported yet due to this reason in this present study the preliminary phytochemical screening of *H. integrifolia* leaf extract has been done to identify the chemical constituents and high-performance thin layer chromatography (HPTLC) fingerprinting of steroids in *H. integrifolia* methanolic leaf extract has been performed which may be used as markers for quality evaluation and standardization of the drug. The present study may serve as a basis for their use in medicinal preparations.

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.

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 methanol extract of *H. integrifolia* leaves was subjected to the following investigations:

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

Phytochemical screening

The phytochemical investigation of the different leaf extracts of *H. integrifolia* was carried out with standard protocol [12]. The results are presented in Table 1.

HPTLC fingerprinting

HPTLC studies were carried out following the method of Harborne [13] and Wagner *et al.* [14].

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 min 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/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 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 min 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 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 (ASR) 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 [15-23].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of methanolic extract of *H. integrifolia* leaves showed the presence of steroids, alkaloids, flavonoids, proteins, and carbohydrates.

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 steroids in methanol extract of *H. integrifolia* (Roxb.). Planch leaves after derivatization with ASR.

Fig. 3 shows three-dimensional plot of fingerprint of steroids in methanol extract of leaf of *H. integrifolia* (Roxb.) planch.

The results from HPTLC fingerprint scanned at wavelength 540 nm for the methanol extract of *H. integrifolia* leaf shows that there are seven

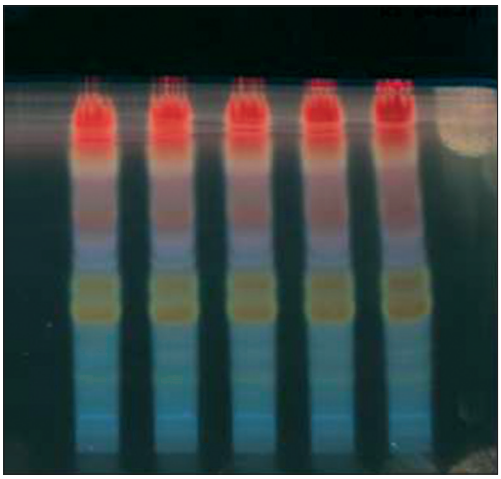

Fig. 2: Fingerprint analysis of steroids in methanol extract of *Holoptelea integrifolia* (Roxb.) planch leaves after derivatization with anisaldehyde sulfuric acid reagent in fluorescence at 366 nm

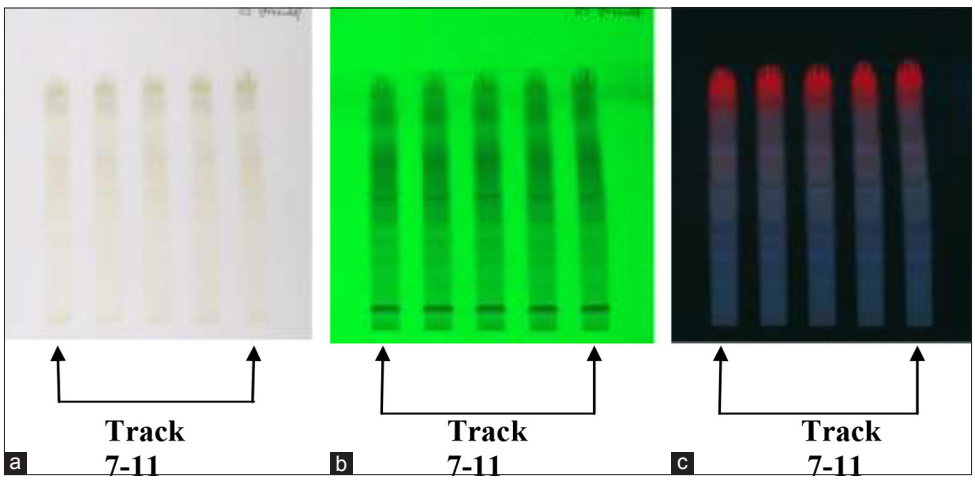

Fig. 1: High-performance thin layer chromatography (HPTLC) fingerprint profile of methanol extract of steroids of leaf extract of *Holoptelea Integrifolia* (Roxb.) planch. (a) HPTLC plate seen at visible light (b) HPTLC plate seen at 254 nm (c) HPTLC plate seen at 366 nm . Track 7-11: Methanol extract.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

polyvalent phytoconstituents and corresponding ascending order of retention factor (Rf) values start from 0.17 to 0.64 in which the highest concentration of the phytoconstituent was found to be 35.95% 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 [24,25].

CONCLUSION

The results obtained from a qualitative evaluation of HPTLC fingerprint images for steroids from the methanol extract of leaves of *H. integrifolia* will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of *H. integrifolia* leaves

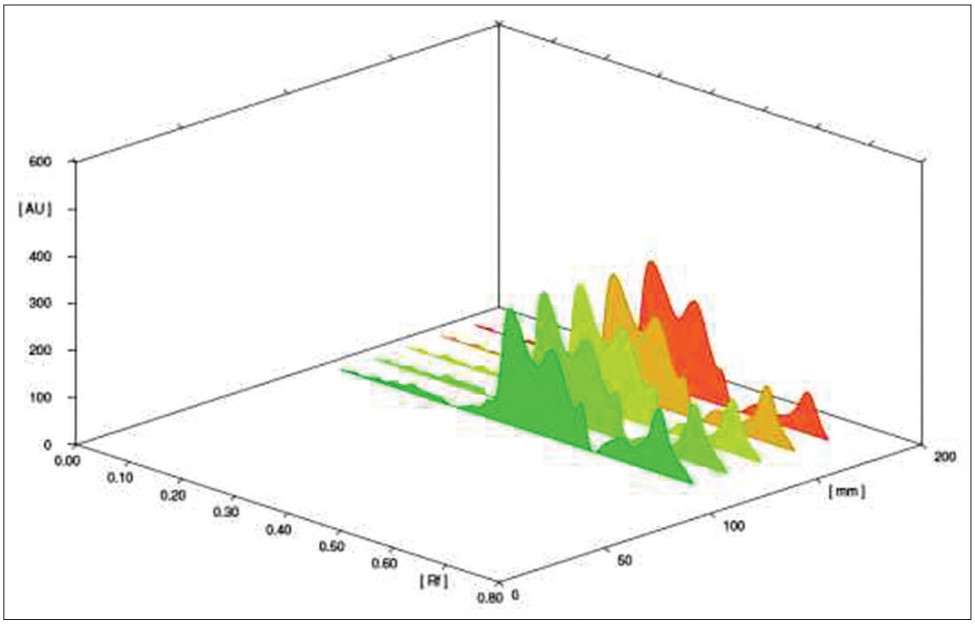

Fig. 3: Three-dimensional plot of fingerprint of steroids in methanol extract of leaf of *Holoptelea integrifolia* (Roxb.) planch

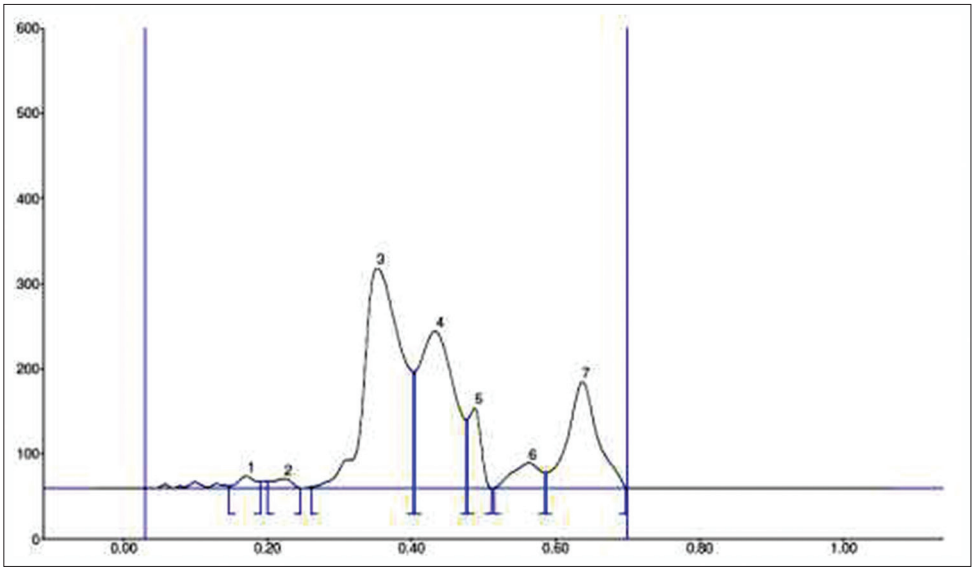

Fig. 4: Chromatogram of steroids in methanol extract of *Holoptelea integrifolia* leaf

Table 2: Rf Values for steroids in methanol extract

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Assigned substance
1	0.15	3.1	0.17	14.9	2.07	0.19	8.3	300.7	1.14	Unknown*
2	0.20	8.8	0.22	11.7	1.63	0.25	0.0	277.7	1.05	Unknown*
3	0.26	2.1	0.35	259.1	35.95	0.40	137.1	11405.8	43.33	Unknown*
4	0.41	137.5	0.43	184.5	25.60	0.48	81.3	7606.8	28.90	Unknown*
5	0.48	81.9	0.49	94.8	13.15	0.51	0.2	1334.6	5.07	Unknown*
6	0.52	0.4	0.56	30.2	4.19	0.59	18.4	986.7	3.75	Unknown*
7	0.59	18.5	0.64	125.5	17.41	0.70	1.7	4410.4	16.75	Unknown*

H. integrifolia: Holoptelea integrifolia, Rf: Retention factor

can provide standard fingerprints for steroids which can be used as a reference for the identification and quality control of the drug.

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