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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY FINGERPRINT PROFILE OF LEAF EXTRACTS OF *HOLOPTELEA INTEGRIFOLIA* (ROXB.) PLANCH

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ABSTRACT

Objective: The objective was to develop the fingerprint profile 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 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, whereas methanolic extract of *H. integrifolia* showed the presence of steroids, alkaloids, flavonoids, proteins and carbohydrates. HPTLC fingerprinting of petroleum ether extract of leaf revealed 9 polyvalent phytoconstituents (9 peaks) and corresponding ascending order of Rf values in the range of 0.44-0.81. While methanol extract of leaf showed 10 polyvalent phytoconstituents (10 peaks) and corresponding ascending order of Rf values in the range of 0.18-0.80.

Conclusions: It can be concluded that HPTLC fingerprint analysis of leaf extract of *H. integrifolia* (Roxb) Planch can be used as a diagnostic tool for the correct identification and authentication of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

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

INTRODUCTION

Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system, (ayurveda) and proposed for their interesting multilevel activities. Among the medicinal plants used in ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some of are still to be explored. Standardization 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 standardization of herbals and its formulations. Furthermore, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [1,2]. High-performance thin layer chromatography (HPTLC) offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [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, anti-inflammatory, 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 the present study, the Preliminary phytochemical screening of *H. integrifolia* leaf extract has been done to identify the chemical constituents and HPTLC fingerprinting of *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), Government of India by comparing morphological features (lea 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 is fingerprinting.

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 [10] *et al.*

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, Maharashtra, India) 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.

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*

The slit bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase for fingerprinting consisted of toluene-chloroform-ethanol in the volume ratio of 4:4:1 (v/v) and anisaldehyde sulphuric 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, 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 tungstant lamp. Subsequent to the development; TLC plate was dipped in anisaldehyde sulphuric 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 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 shown in Fig. 1 indicates that all sample constituents were clearly separated without any tailing and diffuseness.

The results from HPTLC fingerprint scanned at wavelength 254 nm for petroleum ether extract of *H. integrifolia* leaf shows that there are 9 polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.44 to 0.81 in which highest Concentration of the phytoconstituents was found to be 28.73%, and its corresponding Rf value was found to be 0.59 respectively and was recorded in Table 2. The corresponding HPTLC chromatogram was presented in Fig. 4.

The results from HPTLC fingerprint scanned at wavelength 254 nm for the methanol extract of *H. integrifolia* leaf. There are 10 polyvalent phytoconstituents, and corresponding ascending order of Rf values start from 0.18 to 0.80 in which highest concentration. The phytoconstituents was found to be 30.15%, and its corresponding Rf value was found to be 0.54 respectively and was recorded in Table 3. The corresponding HPTLC chromatogram was presented in Fig 5 [20,21].

CONCLUSION

HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. It is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of a chemical constituent has been found useful in the placement of the plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such fingerprinting is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies.

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials, and it allows for the more versatile than ordinary TLC methods, as the spots were well resolved. Though further work to