Fingerprinting Of Flavonoids from Hydroalcoholic Extract of *Holoptelea Integrifolia* (Roxb.) Leaves Using Hptlc

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Abstract

Background: *Holoptelea integrifolia* belongs to the family ulmaceae commonly called as Indian Elm and commonly used in India by the tribal people for it's medicinal properties. Plants and plant-based products are the basis of many of the modern pharmaceuticals we use today for various ailments. Authentication of natural products can be done with precious method like High performance thin layer chromatography (HPTLC).
Objective: High Performance Thin Layer Chromatography (HPTLC) technique was utilised to detect flavonoids from hydroalcohol extract of *Holoptelea integrifolia*.
Methods: Phytochemical screening was performed and later HPTLC studies were done. Instrumentation for HPTLC with CAMAG system having Linomat V applicator (Switzerland). Camag TLC scanner IV with reflectance absorbance mode at 277 nm, equipped with Win CATS software (1.4.6 Camag), tungstant lamp were used to perform densitometric scanning.
Results: Steroids, tannins, alkaloids, glycosides, flavonoids, phytosterols were found in *Holoptelea integrifolia* Hydroalcohol extract undergoing preliminary phytochemical analysis.
HPTLC finger printing of Flavonoids of hydroalcohol extract of leaves revealed twelve multivalent phytochemicals having increasing orderof R_f values 0.05- 0.90 R_f 0.90 has 16.17% concentration
Conclusions: Flavonoids in hydroalcohol extract were confimed by preliminary phytochemical and R_f Values

Keywords: *Holoptelea integrifolia* , Hydroalcohol extract, Phytochemical Screening, Flavonoids, HPTLC Fingerprinting

1.INTRODUCTION

Tribal people of India generally use *Holoptelea integrifolia* having family ulmaceae. *Holopelea inegrifolia* is also called as Indian Elm. Rheumatic swellings are treated with boiling mucilaginous barks juices [1] *Holopelea integrifoila* leaves and bark are useful for its property as astringent, bitter, acrid, anti-inflammatory laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [2,3]. Inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism are treated with *Holoptelea integrifolia* [4]. Flavonoids of hydroalcohol extract of leaves of *Holoptelea integrifolia* have been detected with preliminary phytochemical screening and High Performance Thin Layer Chromatography studies for quality evaluation and standardization of the drug.

2.MATERIALS AND METHODS

2.1 Collecion of Plant material

Holoptelea integrifolia leaves were assembled in agricultural fields in Tirunelveli district of Tamilnadu. Dr. V. Chelladurai, Research Officer of Botany, Central Council for Research in Ayurvedic Sciences (Retired), Govt. of India authenticated and confirmed the plant by compairing morphological features (leaf and stem arrangement, flower /inflorescence arrangement, fruit and seed morphology etc.). The plant material was subjected to further extraction process.

2.2 Preparation and Extraction of Plant material for Preparation of Hydroalcohol extract

The powder of *Holoptelea integrifoila* leaves was charged in to the thimble of a Soxhlet Apparatus and extracted using equal volumes of water and ethanol (1:1), when colourless solvent comes in siphon tube which indicates completion of extraction process. Hydroalcohol extract was obtained by evaporating the extract into organ bath maintained at 50º C The extract was finally air dried thoroughly to remove all traces of solvent and its percentage yield was calculated.Hydroalcohol extract of *Holoptel ea integrifolia* leaves was subjected to the fo llowing investigations,

1. Preliminary phytochemical screening.
2. HPTLC Fingerprinting of Flavonoids

2.3 Phytochemical screening

Standardized protocol was utilized for preliminary phytochemical screening of leaves extract of *Holoptelea integrifolia* [5].

2.4 HPTLC Profile

Method of Harborne [6] and Wagner *et al* [7] was applied to do HPTLC Studies

2.4.1 Sample Preparation

Each extract residue was re-dissolved in 5 ml of chromatographic grade Hydroalcohol. Aluminium sheets were utilized to do sample application on pre-coated silica gel

2.5 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent Ethyl acetate: formic acid: Glacial acetic acid: water in the volume ratio of 10: 0.5: 0.5:1.3 (v/v).

2.6 HPTLC instrumentation, Chromatographic conditions and detection of spots

The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F254 (20 cm × 10 cm with 250 µm thickness). The plates were activated at 120°C for 20 min prior to chromatography. Slit dimension with 6.0mm×0.45 mm and 10 mm/s scanning speed and uniform application speed of 1.0 µl/s was applied. The space between 2 bands was 5mm. Ethyl acetate: formic acid: Glacial acetic acid: water in the volume ratio of 10: 0.5: 0.5:1.3 (v/v) and Anisaldehyde Sulphuric acid was used for derivatization of flavonoids. 20 ml of mobile phase was used per chromatography. 20 cm x 10 cm twin trough glass chamber saturated with filter paperwhatman no: 1 in the mobile phase was used for linear ascending development method. Length of chromatogram run was 8.0 cm. Subsequent to the scanning, TLC plates were dried in a current of air using an air dryer. Tungstant lamp was used to do densiomeric scanning with TLC Scanner IV in the reflectance absorbance mode at 540nm having WinCATS software. Subsequent to the development; TLC plate was dipped in Dragendorff reagent followed by drying in oven at 110^{o}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 [8-16].

3.RESULTS AND DISCUSSION

Preliminary phytochemical analysis of *Holopelea inegrifolia* hydroalcohol given the presence of phytosterols,flavonoids, steroids, tannins, alkaloids, glycosides,

The chromatograms shown in fig.1 indicate that all sample co nstituents were clearly separated without any tailing and diffuseness.

3.1 Flavonoid Confirmation

3.2 Detection of Flavonoids in hydroalcohol extract at 299 nm

3D plot of Phytosterols of *Holoptelea integrifolia* leaf and the chromatograms in Fig. 1 shows separation of constituents.

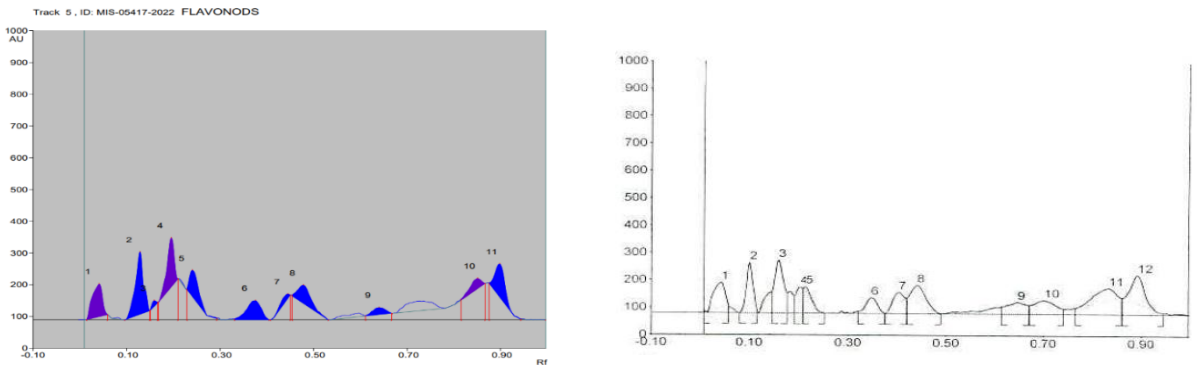

Fig 1: 3-D Plot and HPTLC chromatogram, 299 nm, showing different peaks (bands) of Flavonoids of *Holoptelea integrifolia* leaf

Table 1: Peak table with Rf values, height and area of Flavonoids.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1m	0.01	0.0	0.05	92.8	11.91	0.06	28.0	2044.4	13.49	unknown *
2	0.08	0.1	0.10	184.7	23.70	0.12	3.5	2350.2	15.50	unknown *
3m	0.15	78.2	0.16	112.9	14.48	0.18	74.7	1391.2	9.18	unknown *
4	0.19	63.9	0.21	16.3	2.08	0.21	91.6	112.4	0.74	unknown *
5	0.21	92.2	0.22	20.2	2.59	0.26	0.2	243.0	1.60	unknown *
6m	0.32	8.2	0.35	57.3	7.35	0.38	0.4	1292.4	8.53	unknown *
7	0.38	0.5	0.41	44.2	5.66	0.42	57.7	769.3	5.07	unknown *
8	0.42	58.2	0.44	63.8	8.19	0.49	0.4	1617.9	10.67	unknown *
9m	0.62	26.5	0.65	13.0	1.67	0.67	32.6	303.6	2.00	unknown *
10	0.67	33.0	0.71	24.8	3.19	0.75	19.5	723.4	4.77	unknown *
11	0.77	23.7	0.84	46.1	5.92	0.86	62.0	1860.6	12.27	unknown *
12	0.87	62.1	0.90	103.4	13.26	0.95	5.3	2450.6	16.17	unknown *

3.3 Fingerprinting study of Flavonoids of Hydroalcohol extract at 299 nm

Fingerprinting study of flavonoids shows twelve Rf Between the range of 0.05- 0.90 Rf 0.90 has 16.17% concentration in Figure 1, Table 1.

4. CONCLUSION

A novel method for HPTLC analysis of Hydroalcohol extract of *Holoptelea integrifolia* has been presented along with results show the presence of Flavonoids. The essences of these metabolites are beneficial for maintenance of human health and chronic degenerative diseases. The developed fingerprint analysis will help to isolate and identify new Phytosterols, which will offer a possibility to discover a lead molecule for drug development.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No human or animals were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data associated with this research pape r is available in Dept.of Pharmacology Sanjivani College of Pharmaceutical Educ ation and Research, Kopargaon, Maharasht ra, India The data will be shared upon request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest/competing interest, financial or otherwise.

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 Dr. Gowtham and Mr. Kashid contributed to the final manuscript.

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