Hptlc Finger Print Profile Of Steroids From Hydroalcohol Extract Of Leaves of *Holoptelea Integrifolia* (Roxb.)

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Abstract

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. High performance thin layer chromatography (HPTLC) is a valuable tool for the investigation of herbal products with respect to different aspects of their quality.

High Performance Thin Layer Chromatography (HPTLC) technique was utilized to find steroids from hydroalcohol exrtact of therapeutically and commercially beneficial leaves of Holoptelea integrifolia Phytochemical screening was performed as per standard procedure followed by HPTLC analysis. HPTLC instrumentation of CAMAG was having Linomat V applicator (Switzerland). CAMAG Thin Layer Chromatography scanner IV with reflectance absorbance mode at 208 nm , Win CATS software (1.4.6 Camag) and tungstant lamp was utilized to perform densitometric scanning. Hydroalcohol extract of Holoptelea integrifolia appeared to give the presence of steroids, tannins, alkaloids, glycosides, flavonoids, phytosterols from preliminary phytochemical analysis. HPTLC studies from hydroalcohol extract of leaves revealed nine multivalent phytochemicals (9 peaks) having Rf values in the increasing range of 0.09 to 0.50 Rf 0.44 has 48.97% concentration.

Preliminary phytochemical analysis and Rf Values authenticated existence of steroids in hydroalcohol extract

Keywords: Holoptelea integrifolia, Hydroalcohol extract, Phytoconstituent, Steroids, HPTLC.

1. 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]. Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folklore medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries[3] 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[4]. 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[5]. 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[6] Plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat contagious diseases[7-9]. 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[10] . Recent approach is the utility of natural products as sources of novel structures of therapeutic value[11]. 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[12]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances[13]. In India tribal people use *Holoptelea integrifolia* for its therapeutic uses. *Holoptelea integrifolia* also called as Indian Elm comes under family ulmaceae. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings[14] In traditional system of medicine, bark and leaves of *Holoptelea*

integrifolia are used as bitter, astringent, acrid, thermogenic, antiinflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism[15,16]. The plant *Holoptelea integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism[17].Our aim is to determine preliminary phytochemical analysis and HPTLC analysis to find markers for quality evaluation and standardization of the drug.

2. MATERIALS AND METHODS

2.1 Plant material collection

Holoptelea integrifolia leaves were assembled in agricultural fields in Tirunelveli district of Tamilnadu. The plant was authenticated by Dr. V. Chelladurai, Research Officer of Botany, Central Council for Research in Ayurvedic Sciences (Retired), Govt. of India, 476 F First South Street Thiyagaraja nagar Tirunelveli, Tamilnadu, Voucher Number 41371 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* Soxhlet Apparatus was filled with powder of leaves of *Holoptelea integrifolia* and extracted using equal volumes of water and ethanol (1:1), once the solvent coming in siphon tube became colourless further extraction was stopped. The extract was collected in an empty beaker and kept in water bath maintained at50 ^{o}C and vaporized to get thick paste, finally to obtain hydro alcohol extract.The extract was finally air dried and it s percentage yield was calculated. The perfect ly dried extract was kept in a refrigerator be low 10^{o}C.

Hydroalcohol extract of *Holoptelea integrif olia* leaves was subjected to the following i nvestigations,

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

2.3 Phytochemical screening

Holoptelea integrifolia hydroalcohol extract was analysed for preliminary screening for presence of phytochemicals by standard procedure[18].

2.4 HPTLC Profile

Harborne[19] and Wagner *et al* [20] method was applied for HPTLC studies

2.4.1 Sample Preparation

Hydroalcohol extract residue was re-dissolved in 5 ml of chromatographic grade Hydroalcohol, which was used to apply sample on pre-coated silica gel 60F254 aluminium sheets.

2.5 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent n-butanol:methanol:water in the volume ratio of 3:1:1 (v/v) and anisaldehyde sulfuric acid was used for derivatization.

2.6 HPTLC instrumentation and Chromatographic conditions

CAMAG microlitre syringe was used to apply sample solutions as band having width 8.0 mm on precoated silica gel aluminium plate 60F254 (20 cm × 10 cm) Plates were activated at 120 ℃ 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 6.0mm×0.45 mm and 10 mm/s scanning speed was employed. Mobile phase consisted of n-butanol:methanol:water in the volume ratio of 3:1:1 (v/v) and anisaldehyde sulfuric acid was used for derivatization.

20 ml of mobile phase was used per chromatography. 20 cm x 10 cm twin trough glass chamber saturated with filter paper whatman no: 1 in the mobile phase were utilized for linear ascending method. The mobile phase was subjected to chamber saturation time of 20 min at room temperature (25 ^{0}C ± 2) with relative humidity of 60% ± 5. Chromatographic length was 8.0 cm. An air dryer was utilized for drying TLC Plates after scanning. CAMAG Thin Layer Chromatography scanner IV with reflectance absorbance mode at 290 nm , Win CATS software (1.4.6 Camag) and tungstant lamp was utilized to perform densitometric scanning 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. Comparing peak areas with linear regression method was applied for evaluation[21-29].

3. RESULTS AND DISCUSSION

The plant is a biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, saponins, steroids, resins, tannins, flavanoids, sesquiterpene lactones which exert physiological and therapeutic effect. The compounds present in plant that are responsible for medicinal property are usually secondary metabolites which are having definite chemical structure[30]. Among all these compounds, steroids have the fundamental structure of four carbon rings called the steroid nucleus. The addition of different chemical groups at different positions on backbone leads to the formation of many different types of steroidal compounds including sex hormones progesterone and testosterone, the antiinflammatory steroids like corticosteroids, cardiac steroids digoxin and digitoxin, animal steroid like cholesterol, steroidal glycosides[31,32]. 7,8 Plant steroids synthesized by cyclisation of 2,3-epoxysqualene into cycloartenol are further metabolized owing to the enzymatic conversion to produce biologically active steroids[33]. 5 Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity[34].

Plant derived bioactive compounds are attractive candidates for drug development[35,36].

Steroids comprise a large group of substances that mediate a very varied set of biological responses. The most widespread in the body is cholesterol, an essential component of cell membranes and the starting point for the

synthesis of other steroids - sex hormones, adrenal cortical hormones and the bile salts. Steroids (e.g., glucocorticoids, mineralocorticoids, androgens, estrogens and progestagens) have major responsibilities as hormones, controlling metabolism, salt balance and the development and function of the sexual organs. Steroids in the form of bile salts (e.g., salts of cholic and deoxycholic acid and their glycine and taurine conjugates) assist in digestive processes, while another steroid is a vitamin (calcitriol) that takes part in calcium control. Steroids (naturally occurring or synthetic) such as methylprednisolone, hydrocortisone, glucocortisteroids, corticosteroids, squalamine, oestrogens and androgens are also used for the treatment of various diseases such as allergic reactions, arthritis, some malignancies and diseases resulting from hormone deficiencies or abnormal production[37]. Steroids may serve as an intermediate in the biosynthesis of downstream secondary natural products. and it is believed to be a biosynthetic precursor for cardenolides in plants[38]. Independent of their function, the presence of steroids in practically every organism suggests that they have a powerful role in chemosystematics[39].

Since in previous studies on Petroleum ether and Methanol extracts of *leaves of Holoptelea integrifolia* (Roxb.) were analysed for HPTLC Studies for fingerprinting of Flavonoids, alkaloids, steroids, phytosterols, phenols, glycosides[40-45]. Leaves of *Holoptelea integrifolia* (Roxb.) were not studied for HPTLC Studies till so far involving Hydroalcohol extract so Hydroalcohol extract of Leaves of *Holoptelea integrifolia* (Roxb.) was analysed for phytochemical profiling using high performan ce thin layer chromatography (HPTLC).
Preliminary phytochemical analysis revealed appearance of alkaloids, flavonoids, steroids, tannins, phytosterols, glycosides.
The chromatograms shown in fig.1 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

3.1 Steroid Confirmation

Confirmation of steroids in hydroalcohol extract at 208 nm

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

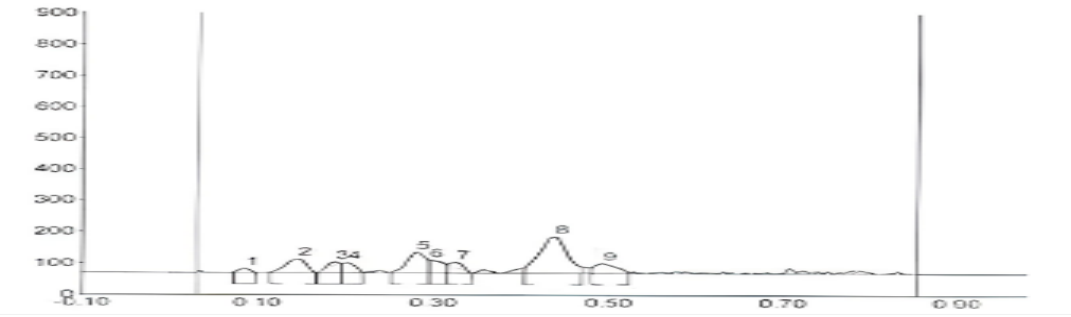

Fig 1: HPTLC chromatogram of extract at 208 nm, showing different peaks (bands) unknown steroids of *Holoptelea integrifol*

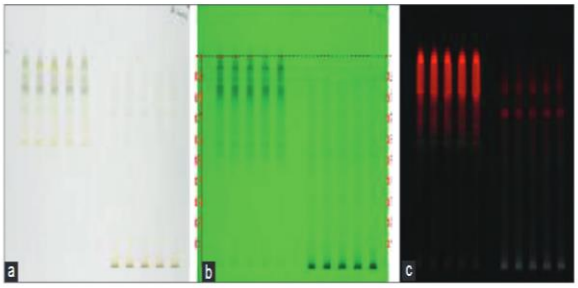

Fig:2 High-Performance Thin Layer Chromatography fingerprint profile of steroids of leaf extract of Holoptelea Integrifolia (Roxb) planch (a) HPTLC Plate seen at visible light b) HPTLC Plate seen at 254nm c) HPTLC Plate seen at 366nm)

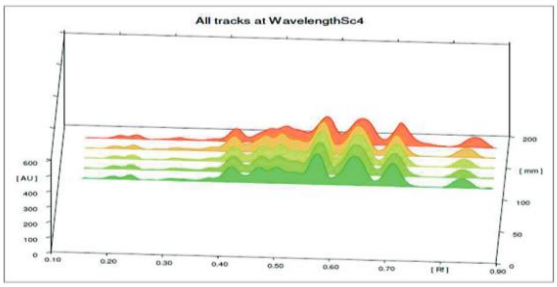

Fig :3-D plot of Fingerprint

Table1: Rf values, height and area of steroids

Peak	Start Rf	Start Height	MaxRf	Max Height	Max%	End Rf	End Height	Area	Area%	Assigned Substance
1	0.08	0.5	0.09	13.1	5.16	0.10	0.6	159.0	3.17	Unknown
2	0.12	0.3	0.15	42.7	16.78	0.17	0.9	935.2	18.63	Unknown
3	0.17	1.5	0.19	14.0	5.50	0.20	29.7	143.2	2.85	Unknown
4	0.20	30.0	0.20	11.3	4.46	0.22	1.7	129.9	2.59	Unknown
5	0.25	1.1	0.28	36.5	14.36	0.30	41.5	566.4	11.28	Unknown
6	0.30	39.9	0.30	6.1	2.42	0.32	27.8	68.2	1.36	Unknown
7	0.32	28.1	0.33	19.5	7.65	0.35	1.8	245.0	4.88	Unknown
8	0.41	17.4	0.44	95.8	37.65	0.47	20.8	2457.6	48.97	Unknown
9	0.48	19.1	0.50	15.3	6.03	0.53	4.8	314.3	6.26	Unknown

3.2 Fingerprinting study of Steroids of Hydroalcohol extract at 208 nm

Fingerprinting study of Hydroalcohol extract at 208 nm shows nine Rf Between the range of 0.09- 0.50. Rf 0.44 has 48.97% concentration in Figure 1 Table 1.

4. CONCLUSION

A novel method for HPTLC analysis of Hydroalcohol extract of *Holoptelea integrifoli*a has been presented along with results which shows the presence of steroids. 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 steroids, 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 paper i s available in Dept.of Pharmacology Sanjivani College of Pharmaceutical Edu cation and Research, Kopargaon, Mahar ashtra, 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.

Funding

We acknowledge the resource support for the study was provided by the Hon'ble Management of Sanjivani Group of Institutes, Sanjivani College of Pharmaceutical Education and Research, Kopargaon.

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