Hptlc Fingerprint Profile of Glycosides 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 Glycosides 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 Glycosides, tannins, alkaloids, glycosides, flavonoids, phytosterols from preliminary phytochemical analysis. Our aim is to do HPTLC studies from hydroalcohol extract of leaves of *Holoptelea integrifolia.* Objective that were assessed are hydroalcohol extract of leaves of *Holoptelea integrifolia*. HPTLC studies revealed eleven multivalent phytochemicals (11 peaks) having Rf values in the increasing range of 0.13 to 0.90 Rf 0.90 has 35.67% concentration. Preliminary phytochemical analysis and Rf Values authenticated existence of Glycosides in hydroalcohol extract

Keywords: *Holoptelea integrifolia*, Hydroalcohol extract, Phytoconstituent, Glycosides, 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].Hydroalcohol extract of leaves of *Holoptelea integrifolia* was subjected to 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 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. Reagents and chemicals

All chemicals used in this study were of analytical grade.

2.3 Organoleptic evaluation

The sensory characteristics, i.e. the appearance, odour, taste and touch define the macroscopy of the plant crude drug. The macroscopical characterisation of *Holoptelea integrifolia* was done. Special structural features were perceived using a simple microscope of 10Xmagnification.

2.4Preparation 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 hydroalcohol extract. The extract was finally air dried and its percentage

yield was calculated. The perfectly dried extract w as kept in a refrigerator below 10**º**C.

Hydroalcohol extract of *Holoptelea integrifolia* l eaves was subjected to the following investigatio ns,

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

Phytochemical screening

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

2.5 HPTLC Profile

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

2.5.1 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.

2.5.2 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent ethylacetate:methanol:water in the volume ratio of 20:2.8:2 (v/v) and alcoholic KOH was used for derivatization

2.5.3 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 °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 6.0mm×0.45 mm and 10 mm/s scanning speed was employed. 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. 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].

Table 1: Optimized Chromatographic conditions for HPTLC Fingerprinting for Tannins of *Holoptelea integrifolia*

PARAMETERS	DESCRIPTION
Stationary phase	Silica gel 60F254 pre-coated on aluminium she
Mobile phase	Ethylacetate:Methanol:Water in the volume rati 20:2.8:2 (v/v)
Prewashing of the plate	Activated at 120ºC for 20minute prior to Chromatography
Development of the chamber	CAMAG Twin Trough Chamber
Chamber saturation	20 min
Sample applicator	CAMAG LINOMAT V
Band width	8.0mm
Development distance	80 mm
Derivatizing reagent	Alcoholic KOH
Drying of plate	At 110ºC for 5 min
Densitometric scanner	CAMAG TLC scanner IV
Lamp	Tungsten
Wavelength	290 nm
Chromatographic evaluation	CAMAG TLC software Win cats1.4.6

RESULTS AND DISCUSSION

Plant derived bioactive compounds are attractive candidates for drug development[30,31]
Medicinal plants and their endophytes are important resources for the discovery of clinically-relevant natural products[32,33,34]. They are also incorporated into ancient folk medicine of virtually all human cultures. Additionally, these plants are a rich source of secondary metabolites with interesting biological activities; these secondary metabolites have a variety of structural arrangements and properties[35]. Glycosides are organic compounds derived or extracted from plant or animal sources. Upon enzymatic or acid hydrolysis, these compounds give one or more sugar moieties along with a non-sugar residue. The sugar moiety is described as a glycone, whereas the non-sugar part is called aglycone or genin. Chemically, these glycosides are acetals or sugar ethers formed by the interaction of hydroxyl groups of the non-sugar and sugar moieties with a loss of a water molecule[36,37]. There are four glycosidic linkages including S-, N-, C-, and O-glycosidic bonds indicating connecting atoms between anomeric carbon of glycone and that of aglycone. Among them, C-glycosyl structures are usually more resistant to hydrolysis. It is well-known that sugars are available in two acyclic and cyclic forms leading to the formation of L/D and α/β

stereoisomers, respectively. L and D configurations are described by the position of hydroxyl group connected to the asymmetric carbon furthest from carbonyl group in the Fischer projection in which OH is placed on the left or right side, respectively. α/β anomers are determined by the position of substituents connected to anomeric carbon in the cyclized form. In this regard, glycosides are categorized into α - glycosides and β-glycosides depending on the position of glycosidic bond whether it is positioned below or above the plane of glycone[38]. The biological activity of glycosides are directly affected by their stereochemistry; hence, their stereoselective preparation is highly in demand[39]. Most of the naturally occurring glycosides such as digoxin and digitoxin possess β-D stereochemistry. However, there are a few exceptions, such as ouabain having α -L stereochemistry which is very potent cardiac glycoside. It is worth mentioning that appropriate stereoisomers of cardiac glycosides play a remarkable role in binding to the Na+ , K+ -ATPase receptor to promote cardiac muscle contraction[40,41] . In this respect, digitoxin, digoxin and ouabain have shown desirable biological activities[40]. There are different types of glycosides, such as triterpene, β -sitosterol, flavonoid, iridoid, phenylpropanoid, anthraquinone, kaempferol, and saponine glycosides. In the saponine glycoside, the aglycone part is referred to as a sapogenin, whereas the glycone parts are generally oligosaccharides[42,43]. Oligosaccharides may be linked to sapogenin via an ether or ester linkage at one or two glycosylation sites, giving the corresponding monodesmosidic or bidesmosidic saponins, respectively. However, attachment of the glycone to three sites (tridesmosidic) in a sapogenin is rare[44,45] .

Leaves of *Holoptelea integrifolia* (Roxb.) were subjected for preparation of Hydroalcohol extract which was analysed for phytochemical profiling using high-performance thin layer chromatography (HPTLC). For Fingerprinting of Glycosides

Preliminary phytochemical analysis revealed appearance of alkaloids, flavonoids, Glycosides, tannins, phytosterols, glycosides.

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

2.6 Glycosides Confirmation

2.7 Confirmation of Glycosides in hydroalcohol extract at 254 nm

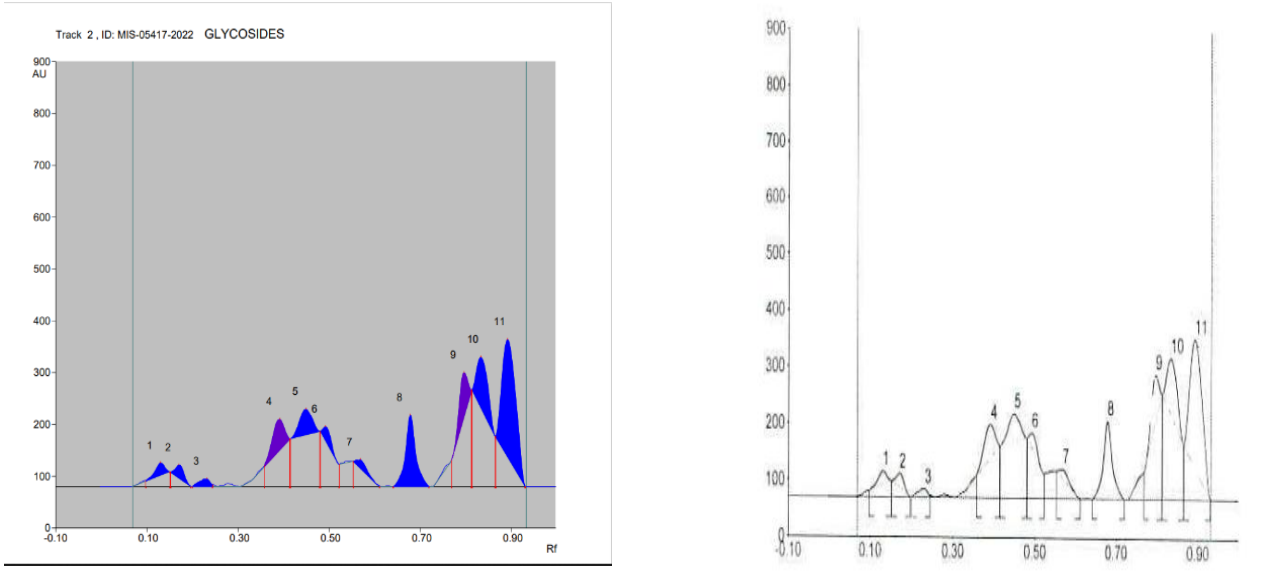

Fig 1: 3-D Plot and HPTLC chromatogram showing different peaks (bands) of Glycosides of *Holoptelea integrifolia* leaf

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

Table 2: Rf values, height and area of Glycosides

Peak	Start R	Start Hei	MaxRf	Max Hei	Max%	End R	End Heig	Area	Area%	Assigned Substance
1	0.10	11.9	0.13	23.9	3.08	0.15	28.6	463.5	2.57	Unknown
2	0.15	28.4	0.17	26.5	3.41	0.20	0.2	457.5	2.53	Unknown
3	0.20	0.1	0.24	14.5	1.87	0.25	3.6	334.8	1.85	Unknown
4	0.36	40.8	0.40	61.6	7.93	0.42	92.5	1524.1	8.44	Unknown
5	0.42	92.6	0.45	49.1	6.32	0.48	106.4	1284.4	7.11	Unknown
6	0.48	106.8	0.50	30.3	3.90	0.53	43.0	469.9	2.60	Unknown
7	0.56	48.9	0.57	17.3	2.23	0.61	0.6	348.2	1.93	Unknown
8	0.64	0.1	0.68	137.8	17.73	0.72	0.3	2938.2	16.26	Unknown
9	0.77	50.6	0.80	89.6	11.52	0.82	186.3	1488.3	8.24	Unknown
10	0.82	187.1	0.84	99.2	12.77	0.87	97.2	2312.8	12.80	Unknown
11	0.87	97.5	0.90	227.4	29.25	0.93	0.2	6443.5	35.67	Unknown

Fingerprinting study of Hydroalcohol extract at 254 nm shows eleven Rf Between the range of 0.13- 0.90. Rf 0.90 has 35.67% concentration (Figure 1, Table 1.)

3 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 Glycosides. 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 Glycosides, 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 is ava ilable in Dept.of Pharmacology
Sanjivani College of Pharmaceutical Education an d Research, Kopargaon, Maharashtra, 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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