Hptlc Fingerprint Profile Of Tannins 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 Tannins 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 324 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 presenceof Tannins, steroids, alkaloids, glycosides, flavonoids, phytosterols from preliminary phytochemical analysis. HPTLC studies from hydroalcohol extract of leaves revealed eleven multivalent phytochemicals (11 peaks) having Rf values in the increasing range of 0.11 to 0.78 Rf 0.18 has 38.06% concentration. Preliminary phytochemical analysis and Rf Values authenticated existence of Tannins in hydroalcohol extract

Keywords: Holoptelea integrifolia, Hydroalcohol extract, Phytoconstituent, Tannins, 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

During the month of august leaves of *Holoptelea integrifolia* were collected from the agricultural fields of Tirunelveli district, Tamilnadu. Dr. V. Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India authenticated and confirmed the leaves of *Holoptelea inegrifolia* by identifying morphological features (leaf and stem arrangement, flower /inflorescence arrangement, fruit and seed morphology etc.).

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 at 50 ^{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 was kept in a refrigerator below 10^{o}C. Hydroalcohol extract of *Holoptelea integrifolia* leaves was subjected to the following investigations,

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

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 toluene-ethyl acetate-formic acid in the volume ratio of 6:4:0.3 (v/v).

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 tannins consisted of toluene-ethyl acetate-formic acid in the volume ratio of 6:4:0.3 (v/v) and FeCl3 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 110ºC. 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

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

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

3.1 Tannin Confirmation

3.2 Confirmation of Tannins in hydroalcohol extract at 324 nm

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

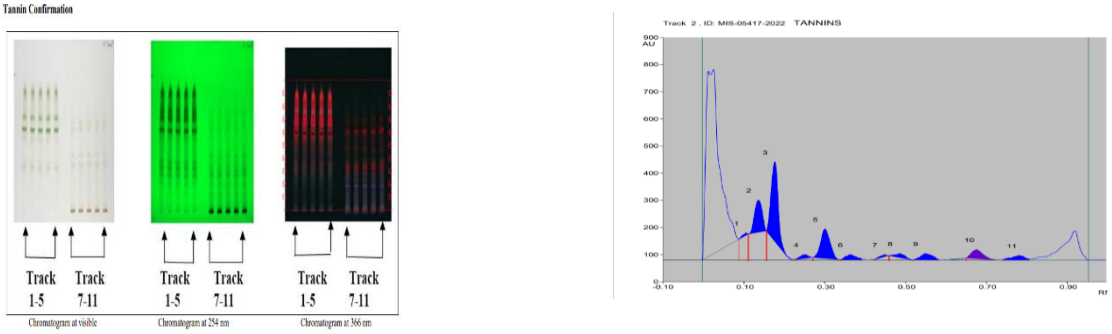

Fig 1: HPTLC fingerprint profile , 3-D Plot and HPTLC chromatogram showing different peaks (bands) of Tannins of *Holoptelea integrifolia* leaf

Fig: 2 Tannins confirmation at visible derivatization

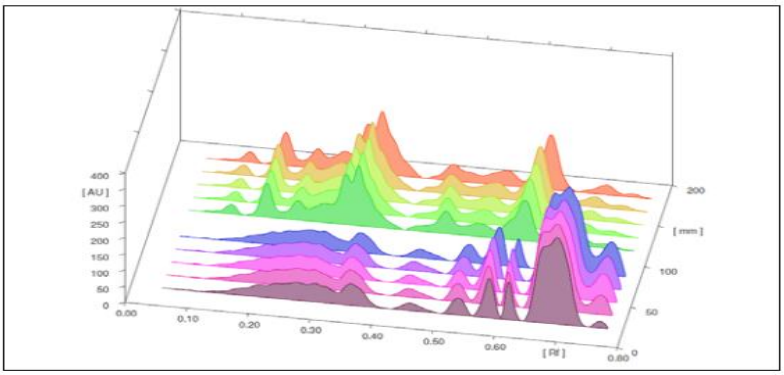

Fig 3: 3-D plot of Fingerprint of tannins

Table 1: Rf values, height and area of Tannins

Peak	Start Rf	Start Height	MaxRf	Max Height	Max %	End Rf	End Height	Area	Area%	Assigned Substance
1	0.09	78.3	0.11	8.4	1.28	0.11	96.2	100.1	0.80	Unknown
2	0.11	96.2	0.14	118.1	18.08	0.16	107.8	2071.1	16.61	Unknown
3	0.16	110.7	0.18	292.0	44.69	0.22	1.0	4747.1	38.06	Unknown
4	0.23	3.9	0.26	13.6	2.08	0.27	9.7	259.3	2.08	Unknown
5	0.27	9.7	0.30	108.0	16.53	0.34	2.9	2332.6	18.70	Unknown
6	0.34	1.8	0.37	18.7	2.86	0.40	4.0	460.1	3.69	Unknown
7	0.43	4.1	0.45	7.1	1.09	0.46	16.6	116.9	0.94	Unknown
8	0.46	16.7	0.49	14.7	2.26	0.52	0.8	330.9	2.65	Unknown
9	0.52	1.1	0.55	23.5	3.60	0.58	3.4	661.4	5.30	Unknown
10	0.65	7.5	0.68	32.8	5.02	0.73	1.3	875.9	7.02	Unknown
11	0.75	2.0	0.78	16.4	2.51	0.81	3.9	516.9	4.14	Unknown

3.3 Fingerprinting study of Tannins of Hydroalcohol extract at 324 nm

Fingerprinting study of Hydroalcohol extract at 324 nm shows eleven Rf Between the range of 0.11- 0.78. Rf 0.18 has 38.06% 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 Tannins. 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 Tannins, 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 available in Dept.of Pharmacology Sanjivani College of Pharmaceutical Educati on and Research, Kopargaon, Maharashtra, I ndia 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.

REFERENCES

1. Chaudhary RR. Herbal Medicine for Human Health. New Delhi: Regional Publication, SEARO, 2. No. 20, W.T.O; 1992. p. 1-80. 2.

2. World Health Organization. Quality Control Method for Medicinal Plant Materials. Geneva: WHO; 1989. p. 1-15.

3. Bobbarala V, Bramhachari PV, Ravichand J, Reddy YHK, Kotresha D, Chaitanya KV. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of Aegle marmelos (L.) Correa extracts. J Pharm Res 2011; 4(1):252-255.

4. Prabavathy D, Valli Nachiyar C. Antimicrobial and antidiabetic activity of an

endophytic fungi isolated from Adathoda beddomei. Int J Pharm Pharm Sci 2013;5(3):???. 2.
5. el-Mousallamy AM. Leaf flavonoids of Albizia lebbeck. Phytochemistry 1998;48(4):759-61. 3.
6. Nazneen Bobby MD, Wesely EG, Johnson M. High performance thin layer chromatography profile studies on the alkaloids of Albizia lebbeck. Asian Pac J Trop Biomed 2012;2(1):S1-6.
7. Kiruba S, Mahesh M, Nisha SR, Miller Paul Z, Jeeva S. Phytochemical analysis of the flower extracts of Rhododendron arboretum Sm. Ssp. Nilagiricum (Zenker) Tagg. Asian Pac J Trop Biomed 2011;???:284-6
8. Tirupathi RG, Suresh BK, Ujwal KJ, Sujana P, Raoa AV, Sreedhar AS. Anti-microbial principles of selected remedial plants from Southern India. Asian Pac J Trop Biomed 2011;1(4):298-305
9. Raja RD, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac J Trop Med 2011;4(5):375-8
10.Robonson MM, Zhang X. The World Medicine Situation, Traditional Medicine: Global Situation, Issues and Challenges. 3rd ed. Geneva: WHO; 2011
11.Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012;75(3):311-35. 6.
12.Osbourn AE, Lanzotti V. Plant Derived Natural Products. Synthesis Function and Application; New York: Springer Science; 2009. 7.
13. Parekh J, Chanda S. Phytochemical Screenig of some plants from western region of India. Plant Arch 2008;8(2):657-62
14. Nadkarni KM. Indian Materia Medica. Popular Prakashan Pvt Ltd. Mumbai, India. 1976, 651-652.
15. Kirtikar KR, Basu BD. Indian Medicinal Plants. Edn 3, Sri Satguru Publications, New Delhi, India, 2000; 3:2292- 2294.
16. Prajapati ND, Purohit SS, Sharma AK. A Handbook of Medicinal Plants a Complete Source Book. Agrobias. Jodhpur, India, 2003, 273.
17. Warrier PK, Nambiar VPK, Ramakutty C. Indian Medicinal Plants a compendium of 500 species, Orient longman private Limited, 1995, 3:162.
18. Khandelwal K R. Practical Pharmacognosy. Techniques and Experiments. 10th ed. Nirali Prakashan, Pune, India. 2006, 149-156.
19. Harborne JB. *Phytochemical methods;* 3rd edition, London: Chapman and Hall, 1998.
20. Wagner H, Baldt S. *Plant drug analysis;* Berlin: Springer; 1996. R.P.W. Scott, Encyclopedia of Chromatography, 10th edn, Marcel Dekker, USA, 2001, 252-254.
21. ICH/CPMP Guidelines Q2B, Validation of Analytical Procedures– Methodology, 1996.
22. Cazes J, Scott RPW. Chromatography Theory, Marcel Decker, NY, 2002, 443-454.
23. Reviewer Guidance, Validation of Chromatographic Methods, 1994.
24. Sethi PD. HPTLC: Quantitative Analysis of Pharmaceutical Formulations, CBS Publications, New Delhi, 1996, 162-165.
25. Heftman E. Chromatography Fundamentals and Applications of

Chromatography and Related Differential Migration Methods. 6th edn, Elsevier, Amsterdam. 2004; 69A:253-291.
26. British Pharmacopoeia, International edn, HMSO, Cambridge, 2002; II, Appendix 112 (IB).
27. Sherma J. Encyclopedia of Pharmaceutical Technology, 2nd edn, Marcel Dekker, USA, 2001, 252-254.
28. ICH/CPMP guidelines Q2A, Text on Validation of Analytical Procedures, 1994.
29. USP 23, NF 19, Asian edn, United States Pharmacopeial Convention, Rockville, M.D., 982, 1225.