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Preliminary Phytochemical Screening And HPTLC Fingerprint Analysis Of 70% Hydroethanolic Extract Of *Tricholepis Glaberrima*

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Abstract
The aim of the study was to establish the HPTLC fingerprint profile and phytochemical screening of 70% Hydroethanolic extract of Tricholepis glaberrima aerial parts. The solvent system selected for HPTLC was Toluene:Ethyl Acetate:Methanol (7:2:1) v/v, stationary phase was precoated aluminium plate of silica gel 60 F_{254} (10X10cm) with 0.2mm thickness. The separated bands on the HPTLC plates were scanned under UV at 254nm, 366nm and visible 580nm(Iodine vapours). HPTLC fingerprinting analysis of the 70% Hydroethanolic extract of Tricholepis glaberrima aerial parts showed the presence of possible number of components.
Keywords: *70% Hydroethanolic extract, Tricholepis glaberrima, HPTLC fingerprint, Solvent system*

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I. Introduction

India has one of the oldest, richest and most diverse cultural traditions associated with use of medicinal plants. The substances having medical value have been extensively used for treating various disease conditions. Products of primary metabolism such as amino acids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance.[1] The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bioactivities.[2] It is an accepted fact that the qualitative analysis of crude herbal extracts is an important and reliable part of quality control protocol, as any change in the quality of extract directly effects the constituents.[3]

WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards. HPTLC offers a better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time.[4] This study was intended to establish HPTLC fingerprint profile and phytochemical screening of 70% Hydroethanolic extract of aerial parts of *Tricholepis glaberrima*.

Tricholepis glaberrima DC (Asteraceae), commonly known as "Brahmadandi" is an important medicinal plant used in our traditional system of medicine to treat various diseases. It is used in Ayurveda for nervine tonic, aphrodisiac, skin disease and in cough. It is used because of the broad area of biological activities like anti-inflammatory, urinary troubles, antiseptic activities. The plant is rich in many pharmaceutical active ingredients like flavonoids, triterpenoids, saponin glycosides and sterols.[5]

II. Materials and Methods:

Collection and Authentification of Plant Material: The aerial parts of *Tricholepis glaberrima* was collected from Chittoor District and was authenticated by Dr. K. Madhava Chetty, Plant Taxonomist (IAAT:357), Asst. Professor, Department of Botany, Sri Venkateshwara University, Tirupati.

Extraction: 300 gms of dried plant material was extracted with 70% Hydroethanol by maceration. The extract thus obtained was subjected to evaporation on water bath until it becomes semisolid, then was stored in air tight container for further use.

Phytochemical Screening: The 70% Hydroethanolic aerial parts extract of *Tricholepis glaberrima* was tested for the presence of phytochemical constituents using standard methods.[6]

HPTLC Fingerprinting Profile:

70% Hydroethanolic aerial parts extract of *Tricholepis glaberrima* was subjected for HPTLC fingerprint analysis. High performance thin layer chromatography(HPTLC) system is composed of an automatic TLC applicator, basic marathon autosampler, densitometer CD_{60} of DESAGA Sarsedt Gruppe and UV – Visible cabinet for the recognition of the spots. The chromatographic and integrated data were recorded using computer based software DESAGA Pro-Quant 1.6 version.

The extract residue was dissolved in 1 ml of HPLC grade chloroform and was spotted with the help of automatic TLC applicator system of DESAGA Sarsedt Gruppe on precoated silica gel_{60} F_{254}. The mobile phase selected was Toluene:Ethyl Acetate:Methanol (7:2:1) and the chromatogram was developed for 15 min to the maximum height. Then the TLC plate was dried completely and spots were observed. Further it was scanned with densitometer CD_{60} of DESAGA Sarsedt Gruppe system under the UV range of 366nm, 254nm and at Visible range of 580nm (under Iodine vapours) for maximum number of components. A corresponding densitogram was obtained in which peaks were appeared for the spots corresponding to R_f values of each component.[7]

III. Results:

Extraction: The % yield of 70% Hydroethanolic aerial parts extract of *Tricholepis glaberrima* was found to be 2.12%.

Phytochemical Screening:

The phytochemical tests on 70% Hydroethanolic aerial parts extract of *Tricholepis glaberrima* showed the presence of various phytoconstituents like Alkaloids, Glycosides, Carbohydrates, Fixed oils and fats, Phenolic compounds, Tannins, Phytosterols and Saponins.

HPTLC Fingerprinting Profile: HPTLC analysis of 70% Hydro-ethanolic extract of Tricholepis glaberrima aerial parts at UV 366 nm, 254 nm, Visible 580 nm (Iodine vapor) showed 6 (with highest concentration of 85.6% at R_f value 0.01, 4 (with highest concentration of 42.2% at R_f value 0.86), 8 (with highest concentration of 30.0% at R_f value 0.88) polyvalent phytoconstituents respectively. The results are tabulated in table 1 to 3 with their respective densitograms shown in graphs 1 to 3 respectively.

Table 1: Peak list of 70% Hydroethanolic extract of *Tricholepis glaberrima* aerial parts at UV 366nm with R_f values of the spots

Peak No.	Y-Pos (mm)	Area	Area (%)	Height	R_f values
1	9.9	1818.86	85.6	628.42	0.01
2	17.9	7.98	0.4	5.84	0.12
3	47.7	9.11	0.4	8.15	0.54
4	54.6	38.45	1.8	7.90	0.63
5	72.7	155.82	7.3	38.15	0.88
6	79.8	93.65	4.4	50.43	0.98

Graph 1: Densitogram showing the separation of peaks in 70% Hydro-Ethanolic aerial parts extract of *Tricholepis glaberrima* at UV 366 nm

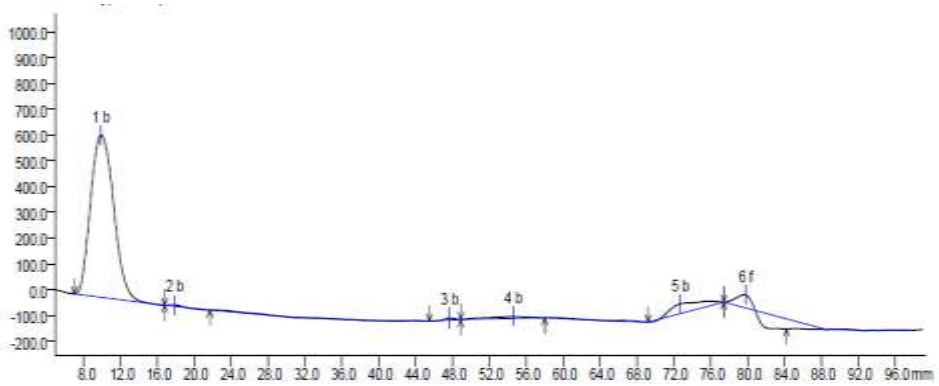


Table 2: Peak list of 70% Hydroethanolic extract of *Tricholepis glaberrima* aerial parts at UV 254nm with R_f values of the spots

Peak No.	Y-Pos (mm)	Area	Area (%)	Height	R_f values
1	9.9	2178.65	19.9	824.66	0.01
2	57.2	42.31	0.4	15.69	0.67
3	71.0	4628.32	42.2	1098.25	0.86
4	80.0	4114.01	37.5	855.40	0.99

Graph 2: Densitogram showing the separation of peaks in 70% Hydro-Ethanolic aerial parts extract of *Tricholepis glaberrima* at UV 254 nm

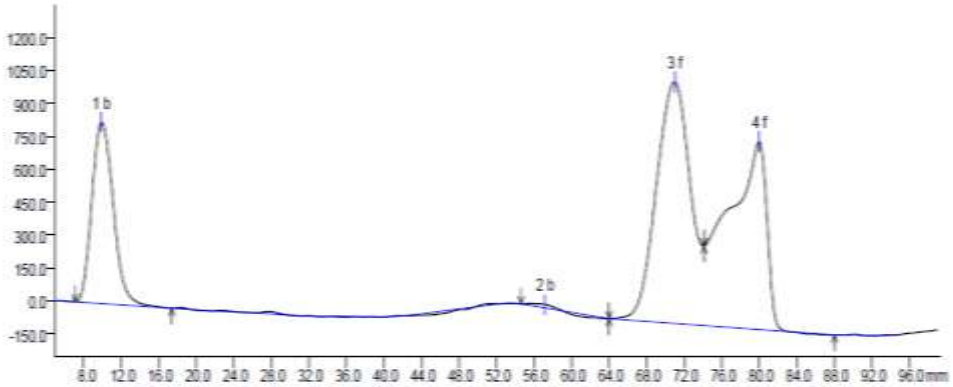
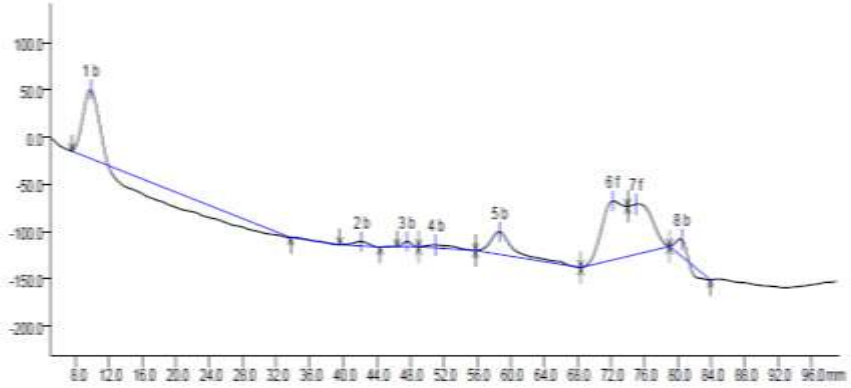


Table 3: Peak list of 70% Hydro-Ethanolic aerial parts extract of *Tricholepis glaberrima* at Visible 580 nm with R_f values of the spots

Peak No.	Y-Pos (mm)	Area	Area (%)	Height	R_f values
1	9.8	159.58	24.5	72.61	0.01
2	42.2	10.00	1.5	4.78	0.46
3	47.6	6.29	1.0	5.55	0.54
4	51.1	12.33	1.9	3.12	0.58
5	58.7	77.02	11.8	24.01	0.69
6	72.2	195.36	30.0	62.32	0.88
7	75.0	166.65	25.6	52.88	0.92
8	80.5	23.89	3.7	17.89	0.99

Graph 3: Densitogram showing the separation of peaks in 70% Hydro-Ethanolic aerial parts extract of *Tricholepis glaberrima* at Visible 580 nm



IV. Discussion:

A simple and accurate HPTLC analysis was done for 70% Hydroethanolic extract of *Tricholepis glaberrima* aerial parts. The mobile phase was Toluene:Ethyl Acetate:Methanol (7:2:1) v/v gave good resolution and well defined spots were obtained. At UV 366nm, 254nm and visible 580nm (Iodine vapour), extract showed 6 (R_f values: 0.01, 0.12, 0.54, 0.63, 0.88, 0.98), 4 (R_f values: 0.01, 0.67, 0.86, 0.99) and 8 (R_f values: 0.01, 0.46, 0.54, 0.58, 0.69, 0.88, 0.92, 0.99) polyvalent compounds respectively. The differences in the R_f values of most of the peaks that appeared, reflected the qualitative differences in the phytocompounds. This HPTLC densitometry fingerprint profile can be used as a marker for extract quality assessment and standardization. Thus, HPTLC fingerprint profile along with their R_f values of the extract were recorded, which would serve as a reference standard for future studies of the plant.

V. Conclusion:

Modern technique of HPTLC analysis was used so as to standardize and to separate the compound which can be isolated for further studies. It can be concluded that HPTLC fingerprint analysis of 70% Hydroethanolic extract of *Tricholepis glaberrima* aerial parts can be used for characterization and correct identification of the plant and also as a phytochemical marker.

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