ORIGINAL ARTICLE

High-performance Thin-layer Chromatography Analysis for the Identification of Marker Compounds from Selected Medicinal Plants: *Terminalia coriacea, Hydrolea zeylanica, Cytisus capitatus,* and *Dorycnium pentaphyllum*

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Abstract

The research sought to examine the marker chemicals in the extracts of four medicinal plants: *Terminalia coriacea*, *Hydrolea zeylanica*, *Cytisus capitatus*, and *Dorycnium pentaphyllum* utilizing high-performance thin-layer chromatography (HPTLC). The selection of these plants was based on their historical medicinal applications, with the study concentrating on identifying essential bioactive components, including flavonoids and tannins that enhance their therapeutic efficacy. Plant extracts were obtained by a solvent-based extraction process and thereafter analyzed with HPTLC under UV illumination at 254 nm and 366 nm for visualization. The research found Rutin as the marker compound for flavonoids and Gallic acid as the marker compound for tannins in plants. Rutin was identified in *T. coriacea* and *C. capitatus,* but Gallic acid was recognized in *D. pentaphyllum* and *H. zeylanica*. These compounds' retention factor (Rf) values were documented and compared with reference standards for identification purposes. Moreover, a quantitative study of these marker compounds indicated that *T. coriacea* possesses the highest rutin content (3.2 mg/g), whereas *D. pentaphyllum* exhibits the highest gallic acid concentration (4.5 mg/g). The findings demonstrate that these plants include substantial concentrations of bioactive chemicals, warranting further investigation into their pharmacological potential. The research illustrates the efficacy of HPTLC as a method for the standardization, quality control, and verification of medicinal plants. It underscores the significance of chemical profiling in guaranteeing the therapeutic efficacy and safety of Phyto therapeutic agents.

Key words: *Cytisus capitatus*, *Dorycnium pentaphyllum*, finger printing, high-performance thin-layer chromatography analysis, *Hydrolea zeylanica*, retention factor, *Terminalia coriacea*

INTRODUCTION

Medicinal plants have played an important role from ancient times forward healing in Ayurveda, Chinese, and Indigenous healing practices. Secondary metabolites such as alkaloids, glycosides, tannins, resin, and phenolic compounds are the sources of healing. These natural contents have already shown various therapeutic efficacies such as antimicrobial, anti-inflammatory, and antioxidant activity for modern drug developments. Most of the therapeutic agents being used are from natural resources. These constituents play an important role in managing chronic illnesses such as cancer, diabetes, and heart-related dysfunction.[1] In addition, Medicinal plants play a significant role in cultural significance and sustainability, mapping the gap between modern scientific advancement and traditional wisdom. Their huge

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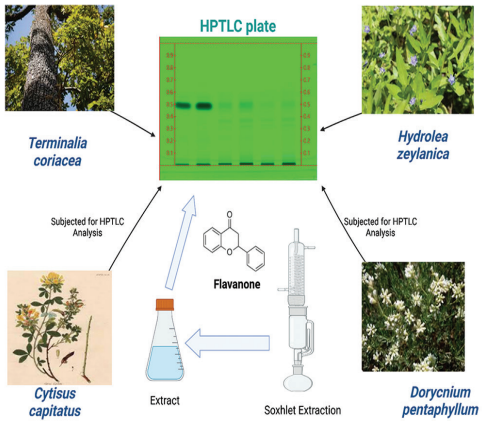
Graphical Abstract



constitute suggests future drug discovery and developments. Standardization, high-performance thin-layer chromatography (HPTLC) fingerprinting, and quality control ensure their safe and effective use worldwide.[2] Nanotechnology plays an important role in drug delivery for different therapeutic activities. When the phytoconstituents are loaded in nanoformulation, they enhance absorption and efficacy.[3]

There is a need to ensure the safety, efficacy, and trust of herbal drugs for their therapeutic use, which is possible only when we standardize them. The bioactive constituents may vary depending on the plant source, harvesting technique, growing condition, and processing techniques. Standardization ensures purity, identification, marker compound availability, and potency consistency. Quality control, including high-performance liquid chromatography and other analytical tools, is crucial in identifying pollutants such as pesticides, microorganisms, and heavy metals. The regulatory standards are another part of enduring their approval through quality testing. In addition, the significant validations allow us to develop trust among the user to ensure better therapeutic efficacy.[4]

To ensure herbal medicines are widely accepted, it's essential to standardize them. This will help in their unification into evidence-based medicine and the enhancement of pharmaceuticals.

HPTLC serves as a precious analytical tool for identifying, separating, and measuring phytochemicals found in herbal content. It provides significant fingerprint profiles, helping to trace marker substances and manage quality control. HPTLC ensures that medicinal plants are authentic, standardized, and safe, making it convenient for researchers, drug developers, and regulators to do their important investigations.[5]

HPTLC is a cost-effective analytical tool frequently used for profiling phytochemicals and recognizing bioactive compounds in medicinal plants. The capability to separate complex mixtures into one individual on a stationary phase allows for a significant investigation and comparison of phytochemicals. HPTLC is a valuable tool for pinpointing major chemicals that reveal the quality of herbal content, authenticity, and medicinal benefits. This tool is incredibly adaptable, using different mobile states and detection tests, like UV light at 254 nm and 366 nm, to enhance the visibility of compounds. The ability of HPTLC to analyze multiple samples ensures both reliability and efficiency, making it a great selection during research. This tool plays a crucial role in ensuring consistency and quality. It provides reliable fingerprint patterns that confirm the authenticity of plant materials, identify any adulterants, and validate herbal formulations for regulatory and research purposes.[6]

This study targets identifying marker compounds in *Terminalia coriacea*, *Hydrolea zeylanica*, *Cytisus capitatus,* and *Dorycnium pentaphyllum* using the HPTLC tool. By looking at plant extracts under UV light at 254 nm and 366 nm, we can identify unique band patterns and determine their retention factor (R_f) values. Marker chemicals such as tannins, flavonoids, alkaloids, and saponins are recognized, aiding in therapeutic standardization, quality control, and pharmacological potential.[7]

MATERIALS AND METHODS

Plant material collection and authentication

The plant materials have been selected from the different regions. *Terminalia* covariance and *H. zeylanica* were collected from Tirupati Tirumala hills and *C. capitatus*, and *D. pentaphyllum* was collected from Wardha district, Maharashtra, India. Pharmacognosist Dr. Shamim Qureshi, a professor at Anwarul Uloom College of Pharmacy, new Mallypally, Hyderabad, has authenticated these plants. A copy of the specimen authentication letter has been submitted to the Department of Pharmacognosy, Datta Meghe College of Pharmacy, DMIHER, deemed a university in Wardha, Maharashtra.

Preparation of plant extracts

The fresh leaves were collected from the source and dried in the shade to remove sufficient moisture. After drying, it was subjected to size reduction for extraction. The dried powder of leaves was passed through 80 mesh size. The sonication (ultrasonic) method was employed for extraction. The remaining 5 g powder was mixed separately in 50 mL of ethanol and water medium and sonicated for up to 15 min. The extract was filtered using Whatman filter paper (No. 1). The solvent was evaporated to make the final 100 mg/mL concentration for investigation.[8]

HPTLC analysis

HPTLC is a robust analytical method employed to separate, identify, and quantify bioactive chemicals in plant extracts, facilitating their standardization, authentication, and quality control.[9]

Instrument and reagents

The HPTLC analysis was conducted with a CAMAG HPTLC system, which included a Linomat V automatic sample applicator and a TLC scanner 3. The reagents employed for the analysis comprised Ethyl acetate, Formic acid, Glacial acetic acid, and Water for Rutin detection, as well as toluene, ethyl acetate, and Formic acid for Gallic acid detection. All reagents were of analytical quality.[10]

Sample preparation and application

The plant extracts were produced by dissolving 100 mg of the concentrated ethanolic extract in 1 mL of methanol. Samples were filtrated using a 0.45 µm membrane filter to eliminate particle contaminants. Ten microliters of each sample were put as bands on pre-coated silica gel 60 F254 plates using an HPTLC applicator. The accurate application guaranteed consistency for the next chromatographic development and analysis using UV detection.[11]

Development of chromatographic conditions

The HPTLC plates were constructed using mobile phases tailored for particular marker chemicals. To identify flavonoids (Rutin), use ethyl acetate and formic acid. Glacial acetic acid: A solvent mixture of Water (10:1.1:1.1:2.6) was utilized; however, for tannins (Gallic acid), a combination of Toluene: Ethyl acetate: Formic acid (6:4:0.8) was applied. Plates were fabricated in a twin-trough chamber, pre-saturated with the mobile phase to ensure uniform migration.[12]

Detection and visualization

Detection and visualization were conducted under UV light at 254 nm and 366 nm to identify marker chemicals according to their fluorescence and absorption characteristics. Following development, plates were derivatized using an anisaldehyde-sulfuric acid reagent for flavonoids and vanillin-sulfuric acid for tannins to improve visibility. The derivatized plates were heated at 110°C for 5 min, resulting in discrete bands corresponding to Rf values, facilitating compound identification and standardization.[13]

Marker compound identification

Marker compound identification was achieved by comparing sample bands' Rf values and spectral profiles with reference standards. Rutin (flavonoid) and Gallic acid (tannin) were markers. The Rf values of the sample bands were matched against the standards, with spectral confirmation using densitometric scanning. Distinct Rf values confirmed the presence of Rutin in *T. coriacea* and *C. capitatus*, and Gallic acid in *D. pentaphyllum*, validating their phytochemical profiles.[14]

RESULTS

Phytochemical profile of extracts

Initial phytochemical analysis indicated the existence of significant secondary metabolites in the chosen plant extracts. *T. coriacea*, *H. zeylanica*, and *D. pentaphyllum* demonstrated substantial quantities of tannins and flavonoids, but *C. capitatus* primarily displayed flavonoids. Alkaloids, saponins, and phenolic substances were identified in differing concentrations throughout the extracts. These results correspond with the HPTLC findings, validating the phytochemical diversity of the plants and endorsing their medicinal potential for subsequent pharmacological research.[15]

HPTLC Fingerprinting of selected plants

Figures 1 and 2 illustrate the HPTLC chromatograms for detecting flavonoid (Rutin) and tannin (Gallic acid) in the crude extracts of the chosen plants. Each chromatogram displays unique bands that correlate to particular Rf values. Significant bands for Rutin were detected at Rf 0.65 in *T. coriacea* and *C. capitatus*. Clear bands for Gallic acid were seen with Rf around 0.32 in *D. pentaphyllum* and *H. zeylanica*. The fingerprint profiles validate the existence of marker chemicals, offering a qualitative and quantitative benchmark for phytochemical standardization and guaranteeing the consistency and legitimacy of these medicinal plants for therapeutic use.[16]

Identification of marker compounds

The identification of marker chemicals was validated by comparing spectral data with reference standards. The UV-Vis spectra of the sample bands corresponded with the characteristic absorption peaks of Rutin (flavonoid) and Gallic

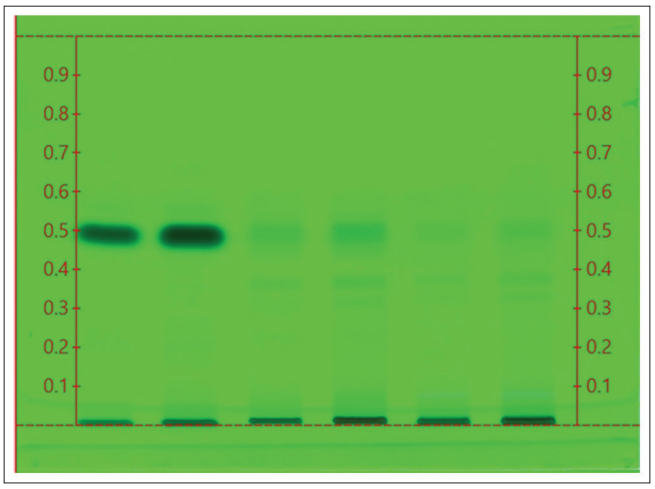


Figure 1: High-performance thin-layer chromatography chromatogram for flavonoid (rutin) detection of different plant crude extracts

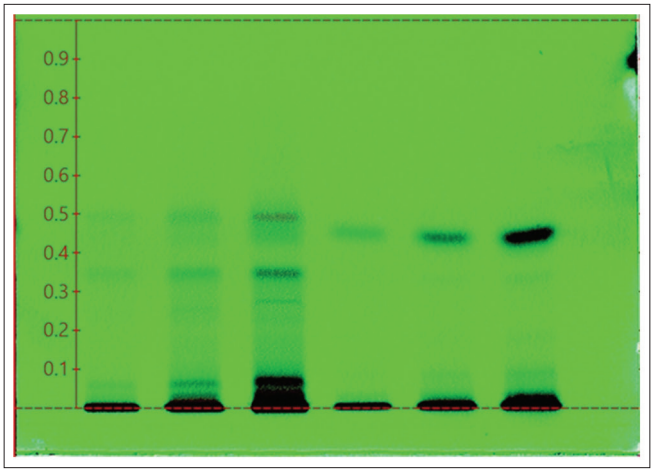


Figure 2: High-performance thin-layer chromatography chromatogram for tannin (Gallic acid) detection of different plant crude extracts

Table 1: Quantitative analysis of marker compounds in selected medicinal plants

Plant NAME	Marker compound	Rf value	Concentration (mg/g)
Terminalia coriacea	Rutin (Flavonoid)	0.65	3.2
Cytisus capitatus	Rutin (Flavonoid)	0.65	2.8
Dorycnium pentaphyllum	Gallic acid (Tannin)	0.32	4.5
Hydrolea zeylanica	Gallic acid (Tannin)	0.32	3.9

acid (tannin), confirming their existence in the respective extracts. Rf values and spectrum profiles identified Rutin in *T. coriacea* and *C. capitatus*, but gallic acid was observed in *D. pentaphyllum* and *H. zeylanica*. These findings underscore the dependability of HPTLC for detecting marker compounds and the standardization of phytochemicals [Table 1].[16]

Quantitative analysis

Quantitative analysis ascertained the amounts of marker chemicals in the plant extracts. Rutin was measured at 3.2 mg/g in *T. coriacea* and 2.8 mg/g in *C. capitatus*, whilst Gallic acid was quantified at 4.5 mg/g in *D. pentaphyllum* and 3.9 mg/g in *H. zeylanica*. The data obtained from densitometric analysis indicate the concentration of bioactive chemicals in the chosen plants. The results highlight the possible therapeutic uses and the effectiveness of HPTLC for precise phytochemical measurement.[17]

DISCUSSION

HPTLC to identify and quantify marker chemicals in *T. coriacea, H. zeylanica, C. capitatus*, and *D. pentaphyllum* extracts. These plants were chosen for their alleged medical capabilities, and their phytochemical profiles were analyzed by identifying specific bioactive components, such as flavonoids and tannins.[18-20]

The HPTLC examination demonstrated unique chromatographic profiles for each plant, exhibiting considerable differences in the presence of the marker chemicals, Rutin (flavonoid) and Gallic acid (tannin). The identification of these compounds relied on their distinctive Rf values and UV spectra, which were compared to reference standards. Rutin was identified in *T. coriacea* and *C. capitatus*, but Gallic acid was observed in *D. pentaphyllum* and *H. zeylanica*. Identifying these bioactive components corresponds with the conventional medical applications of these plants, wherein flavonoids are frequently associated with antioxidant and anti-inflammatory attributes. At the same time, tannins are connected to antibacterial and astringent actions.[21]

Densitometric quantitative research revealed that *T. coriacea* possesses the greatest rutin concentration at 3.2 mg/g. *D. pentaphyllum* demonstrated the greatest quantity of gallic acid at 4.5 mg/g. The findings underscore the medicinal potential of these plants, with *T. coriacea* and *D. pentaphyllum* exhibiting significant quantities of bioactive chemicals that warrant exploration for drug development.[22]

The HPTLC fingerprinting technology demonstrated efficacy in identifying and standardizing medicinal plants, thereby ensuring their authenticity, purity, and therapeutic efficacy.[23]

CONCLUSION

The HPTLC study effectively detected the major marker components, Rutin and Gallic acid, in the medicinal plants *T. coriacea*, *H. zeylanica, C. capitatus*, and *D. pentaphyllum*. The unique chromatographic profiles and quantitative findings validated the existence and concentration of these beneficial substances, corroborating the historic usage of these plants in therapy. The results highlight the efficacy of HPTLC as a valuable instrument for plant standardization, quality assurance, and the identification of bioactive chemicals, facilitating further research and development of these plants in pharmaceutical and therapeutic applications.

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