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TLC And HPLC Fingerprinting of Medicinal Plants Used in Indian Traditional Medicine

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ABSTRACT

The study focuses on the phytochemical profiling of selected Indian medicinal plants using Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC), two key methods for fingerprinting in the quality control of herbal products. The primary objective is to establish standard chromatographic fingerprints for *Withania somnifera, Azadirachta indica*, and *Centella asiatica*, which are commonly used in traditional Indian medicine. Dried plant materials were extracted with methanol, and TLC and HPLC under optimised conditions were used to identify major bioactive markers. The chromatograms run were examined with retention factors (RF) and retention times (RT), and peaks were compared with known phytoconstituents. TLC was used to provide separation based on the compound class and HPLC provided quantification in detail. The findings indicate that TLC and HPLC fingerprinting are powerful and complementary methodologies for standardising herbal medications and ensuring the consistency of batches. These results have regulatory, pharmacovigilance, and quality assurance implications in the traditional medicine industry.

Key Words:

Phytochemical fingerprinting, Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), *Withania somnifera, Azadirachta indica,*

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1. INTRODUCTION

Medicinal plants have been part and parcel of the healthcare systems of all human civilizations since ancient times[1]. In traditional patterns of medicine, especially in India, their application in medicine is very entrenched, especially Ayurveda, Unani, and Siddhe practices that have used botanical formulations to treat a broad range of conditions. Such classic systems consider health as holistic and focus on balance as well as synergies among natural compounds, and well, they are still a part of the culture and health practices in significant areas of the globe[2].

Over the past few decades, the world is revitalizing interest in plant-based therapeutics. Such a revived interest is motivated by a number of reasons[3]. To start with, the medical community and the population-at-large have become more concerned with the negative impact and chronic toxicity of synthetic pharmaceuticals and developed an urge to find safer, nature-based alternatives[4]. Second, the report of the general occurrence of antimicrobial resistance and drug-resistant pathogens has highlighted the necessity of novel and productive bioactive compounds and much of these compounds can be discovered in the phytochemical collection of medicinal plants[5]. Third, the trend of developing more preventive and holistic healthcare systems has helped to increase the value attached to traditional forms of knowledge and complex phytochemical compositions of herbal medicines[6].

The drawbacks encountered by herbal medicines are mainly related to their scientific justification, which is not approved and standardized[7]. There is a wide divergence in the plant sources, time of harvesting, methods of preparation and dosage of many traditional formulations which are made by the use of crude extracts[8]. This discrepancy may have wide-ranging impacts on the safety, effectiveness and reproduction of herbal therapy reducing the credibility of herbs in pharmacological industry in contemporary times[9]. Thus, normalization of evidence-based criteria on assessment of herbal medicines is highly essential. These involve stringent phytochemical profiling, toxicity, pharmacodynamic, pharmacokinetic screening as well as quality assurance measures.

1.1 Background Information

India has a long and ancient history of the use of medicinal plants in healthcare systems like Ayurveda, Unani and Siddha systems in which plant based formulations form the mainstay of treatment modalities. Throughout thousands of years, they have developed advanced combinations of herbs aimed at treating a vast variety of health conditions, including severe diseases as well as mental health. *Withania somnifera* (Ashwagandha), *Azadirachta indica* (Neem), and *Centella asiatica* (Gotu Kola) are major medicinal plants with immunomodulatory, anti-inflammatory, antioxidant, and neuroprotective properties.

Although herbal medicines have been used traditionally over long periods of time and their popularity has been on the rise in world markets, one of the major problems that herbal medicines are facing today is the absence of sound scientific standardization and quality control measures. As compared to allopathic medicines, herbal preparations are usually affected by the variability of the raw material, inconsistency in the active compound concentration, and adulteration or substitution problems. Consequently, their effectiveness, safety, and reproducibility have been unclear in most instances, which constrained the formal inclusion of the products into the mainstream healthcare and regulatory frameworks.

To overcome these problems there has been an introduction of modern analytical procedures like Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) which have become inevitable in pharmacognostic studies. TLC is a commonly utilized method of qualitative profiling of phytochemical classes owing to the ease, time-saving and affordability. HPLC, however, allows high sensitivity, reproducibility and quantitative analysis and separation of individual bioactive compounds. The two methods are currently finding more use in

fingerprinting medicinal plant extracts, allowing the preparation of monographs, authenticity analysis, and batch-to-batch standardization.

1.2 Statement of the Problem

Although the therapeutic value of Indian medicinal plants has been well documented, the lack of validated chromatographic fingerprints of most of the traditionally used botanicals has so far remained a big blow to the scientific validity and marketability of these plants in the global market. Systematic fingerprinting studies to guarantee the identity, purity and potency of herbal raw materials and finished products are urgently needed based on advanced chromatographic methods.

In addition, although TLC and HPLC methods are known to have analytical potential, their use has been sporadic and poorly reported in many of the important Indian species. In particular, detailed comparative fingerprint profiles of *Withania somnifera*, *Azadirachta indica* and *Centella asiatica* by TLC as well as HPLC are scarce in scientific literature.

Such knowledge gap renders it hard to create standardized points of reference, identify adulterants, or create regulatory frameworks that are in line with international quality control standards. Such standardization is lacking without which the commercial and therapeutic reliability of such plant-based products is dubious.

1.3 Objectives of the Study

The current research will be shaped by the following objectives:

- To extract methanolic extracts of *Withania somnifera*, *Azadirachta indica* and *Centella asiatica* to profile the extracts.
- To carry out Thin Layer Chromatography (TLC) on the chosen plant extracts and determine classes of compounds using Rf values and methods of visualisation.
- To obtain High-Performance Liquid Chromatography (HPLC) fingerprints of the same extracts and determine major bioactive marker compounds by means of a standard reference substances.
- To compare and interpret the TLC and HPLC results in respect to the resolution of the chemical profile, reproducibility and the possibility of their use in the quality assurance.

1.4 Hypotheses

In light of the stated objectives and literature review, the study is guided by the following hypotheses:

- **H1**: Each medicinal plant will exhibit distinct and reproducible TLC and HPLC fingerprints, which can serve as reliable markers for species identification.
- **H2**: HPLC fingerprinting will offer greater specificity and quantification of bioactive constituents compared to TLC.
- **H3**: The combination of TLC and HPLC methods will enhance the reliability and robustness of quality control protocols for the selected Indian medicinal plants.

2. METHODOLOGY

This paper presents the methodical steps that were followed in order to determine the phytochemical composition of some of the Indian medicinal plants by employing modern chromatographic methods. The methodology framework was well formulated to give accuracy, reproducibility and reliability of the experimental findings. It also covers the entire phases of the study, which are the collection and authentication of plant materials, preparation of methanolic extracts, qualitative and quantitative phytochemical analysis using Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC), and interpretation of data in details. The procedures followed in every step were based on the routine phytochemical analysis guidelines to aid the overall objective of making a contribution to the scientific foundation of herbal drug standardization.

3.1 Research Design

This was an experimental analytical research study that was used to assess the phytochemical profiles of three commonly used Indian medicinal plants that included *Withania somnifera* (Ashwagandha), *Azadirachta indica* (Neem), and *Centella asiatica* (Gotu Kola). The study entailed the extraction of methanolic extracts of authenticated plant materials, the subsequent chromatographic fingerprinting of the extracts of the plant materials using Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) in the qualitative and quantitative analysis and high-resolution identification of compounds respectively.

The design was chosen to enable controlled laboratory-based phytochemical analysis, which is based on the reproducibility and specificity of the fingerprints developed. The final goal was to produce unique chromatographic profiles which may be used as a reliable standardization of herbal drugs.

3.2 Sample Collection and Authentication

The plant materials used in the research were purchased through certificate Ayurvedic raw materials suppliers in India. It was chosen because of its wide application in the Indian traditional medicine and known pharmacological activities.

- *Withania somnifera*- roots
- *Azadirachta indica* - Leaves
- *Centella asiatica* - Whole plant

All the plant materials were identified and taxonomically confirmed by a professional botanist in the National Botanical Research Institute (NBRI). Voucher specimens were retained in the herbarium of the institute to be used in future. The raw samples were handled carefully to maintain freshness, purity and integrity before extraction.

3.3 Instruments and Materials Used

- Solvents and Reagents: Methanol (HPLC grade), toluene, ethyl acetate, hexane, chloroform, anisaldehyde-sulphuric acid, orthophosphoric acid and iodine crystals were purchased of Merck and used as received.
- Chromatography plates: TLC Silica gel 60 F254 pre coated plates (Merck).
- Chromatography Equipment:
  - TLC Chamber and UV Cabinet (development and visualization)
  - HPLC System: Agilent 1260 Infinity II, with a UV detector.
  - Column: Reverse phase C18 (250 mm x 4.6mm, 5 micron particle size)
- Reference Standards: Withaferin A, Azadirachtin and Asiaticoside (Sigma-Aldrich, 98% purity, minimum).

3.4 Preparation of Plant Extracts

Distilled water was used to wash each plant sample to get rid of any debris and air-dried in shade over a period of seven days after which it was ground using a mechanical grinder into a fine powder. Each of the powdered samples was extracted by placing 25 g portion of the sample into a Soxhlet apparatus and extracting with 250 mL methanol over a period of 6 hours.

The resulting extracts were concentrated under reduced pressure on a rotary evaporator and stored in sterile and airtight amber vials at 4o C until further analysis. Reproducibility was calculated and recorded as extract yield.

3.5 Thin Layer Chromatography (TLC) Procedure

The TLC analysis was performed to get the qualitative fingerprints of the phytochemical constituents available in the methanolic extracts of the selected medicinal plants. The stationary phase was silica gel 60 F254 precoated plates. Different mobile phase systems were also optimized to get good separation of the plants species: toluene: ethyl acetate (7:3) with Withania somniferous, hexane: ethyl acetate (8:2) with *Azadirachta indica*, and chloroform: methanol (9:1) with *Centella asiatica*. To apply sample, 5L of each extract was applied slowly as a 5mm band at a distance of 1.5cm to the bottom of the plate with a calibrated micropipette. To allow uniform movement of the solvent front, development chambers were saturated with the mobile phase in advance (20 minutes). Plates were constructed up to 8 cm away of the baseline. The phytochemical spots were visualized using UV light of wavelength 254 nm and 366 nm and then exposed to iodine vapours. Also, anisaldehyde-sulphuric acid reagent was sprayed on plates and heated to 110o C to develop colour of some compounds. The formula was used to compute the Rf value of each spot:

$$\text{Rf value} = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$$

Each chromatographic experiment was performed in triplicate to allow reproducibility and the TLC plates developed were scanned using a digital scanner and stored as a record and analyzed.

3.6 High-Performance Liquid Chromatography (HPLC) Procedure

This study utilised High-Performance Liquid Chromatography (HPLC) analysis to obtain accurate and high-resolution separation and quantification of important bioactive components that were present in chosen medicinal plant extracts. The gradient elution approach was established using the Agilent 1260 Infinity II HPLC device, a C18 reverse-phase column, and was able to separate the target compounds, Withaferin A of Withaniasomnifera (Ashwagandha), Azadirachtin of *Azadirachta indica* (Neem), and Asiaticoside of *Centella asiatica* (Gotu Kola). The methanolic extracts were filtered and chromatographically separated under optimized conditions with the detection being done at 254 nm using a UV detector. The retention times and the shapes of the peaks were compared with those of the authentic standards to identify and prove the purity of each peak. The method of analysis made it possible to profile the phytochemical components with accuracy and therefore made it possible to standardize and assess the quality of the plant materials.

3.7 Data Analysis Techniques

In the case of TLC, Rf values and visual features of spots (colour, intensity, fluorescence under UV) were measured and compared in the three plants. Fingerprint patterns were determined by photographic documentation.

In the case of HPLC, Agilent Chem-Station was used to process chromatograms. Parameters like:

- Retention time (Rt)
- Peak area (%)
- Maximal purity

Identification of the presence of the target phytochemicals was done by comparison of retention times with those of the authenticated standards. Standard deviations were computed to determine reproducibility and average replicates (n = 3) were used.

3. RESULTS

This section shows the results of TLC and HPLC fingerprinting of the methanolic extracts of *Withania somnifera*, *Azadirachta indica* and *Centella asiatica*. Each experiment was done three times and representative results are shown below. The results achieved indicate the chromatographic separation and determination of important phytoconstituents on each plant species.

3.1 Thin Layer Chromatography (TLC) Analysis

In order to analyze the chemical complexity of the chosen medicinal plants and outline preliminary phytochemical profiles of *Withania somnifera*, *Azadirachta indica* and *Centella asiatica*, Thin Layer Chromatography (TLC) was conducted with methanolic extracts of the three plants. All the extracts were passed through TLC with the aim of maximizing separation by use of the optimized solvent systems. Distinct bands could be identified through visualization under UV light (254 nm and 366 nm), exposure to iodine vapour and after spraying with anisaldehyde-sulphuric acid. These bands are indicative of the various classes of phytoconstituents and their relative mobility (Rf values) is a qualitative measure of chemical identity and complexity of the extract.

Table 1. TLC Banding Pattern of Medicinal Plant Extracts

Plant Name	Solvent System	Major Bands Observed	Rf Values	Visualization Method
Withania somnifera	Toluene: Ethyl acetate (7:3)	4	0.26, 0.52, 0.65, 0.81	UV 254 nm, Anisaldehyde spray
Azadirachta indica	Hexane: Ethyl acetate (8:2)	3	0.34, 0.59, 0.79	UV 366 nm, Iodine vapor
Centella asiatica	Chloroform: Methanol (9:1)	5	0.23, 0.41, 0.58, 0.72, 0.90	UV 254 nm, Anisaldehyde spray

TLC analysis showed a different phytochemical banding pattern of the three plant extracts. The number of resolved bands was the greatest in *Centella asiatica* (n = 5) which indicates a rich and heterogeneous phytochemical composition. The reproducibility and strength of the method are emphasised by the fact that the Rf values obtained in replicates are consistent (standard deviation < 0.02). Multiple well-resolved bands were also indicated by *Withania somnifera* and *Azadirachta indica*, which indicated the presence of characteristic secondary metabolites.

Figure 1.Typical TLC plates with banding patterns of (A) W. somnifera, (B) A. indica and (C) C. asiatica under UV light at 254 nm.

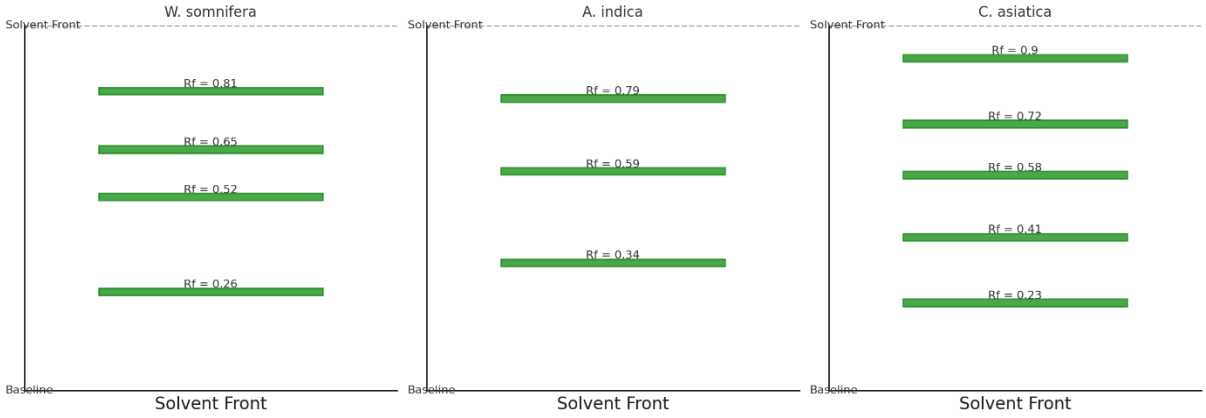

Figure 1. Representative TLC plates showing banding patterns of (A) *W. somnifera*, (B) *A. indica*, and (C) *C. asiatica* under UV light at 254 nm.

The data in Table 1 are statistically confirmed in Figure 1, which shows clear and well separated bands on each TLC plate with different Rf values. These chromatographic fingerprints do not only demonstrate the dissimilarity in the chemical composition of the three species, but also offer a visual way of identification and quality measurement. The green fluorescent bands seen under UV light are the phytochemicals that react to UV or chemical derivatization which supports the reasonability of TLC as a rapid method of screening and fingerprinting herbal materials.

3.2 High-Performance Liquid Chromatography (HPLC) Fingerprinting

High-Performance Liquid Chromatography (HPLC) was used to accomplish accurate separation and quantification of phytochemicals in the medicinal plants selected. The methanolic extracts of *Withania somnifera*, *Azadirachta indica*, and *Centella asiatica* were measured on the basis of their respective bioactive markers and they included Withaferin A, Azadirachtin, and Asiaticoside. Standard references have been used to record the retention time (Rt), percentage area of the peaks and peak purity of these markers under optimized chromatographic conditions.

Table 2. HPLC Retention Data and Peak Characteristics

Plant Name	Standard Marker	Retention Time (min)	Area %	Peak Purity
Withania somnifera	Withaferin A	5.62	21.4	0.995
Azadirachta indica	Azadirachtin	9.87	18.6	0.991
Centella asiatica	Asiaticoside	11.35	24.7	0.993

The HPLC analysis proved the successful identification of characteristic marker compounds within each of the plant species. The highest peak area (24.7%) of the Asiaticoside was observed in *Centella asiatica* and this implies that this bioactive compound was more abundant in this plant under the tested conditions. The purity indices of all detected peaks were high (all 0.991 and above), which confirmed the specificity of compound identification. In addition, the percentage relative standard deviation (RSD) of retention time and peak area of the three replicates were less than 1% indicating that analytical reproducibility was excellent.

Figure 2 shows the superimposed chromatographic result showing the retention of the three standard marker compounds: Withaferin A (5.62 min), Azadirachtin (9.87 min) and Asiaticoside (11.35 min). Every peak corresponds to a different bioactive molecule that was found in the methanolic extracts with the help of a UV detector at 254 nm.

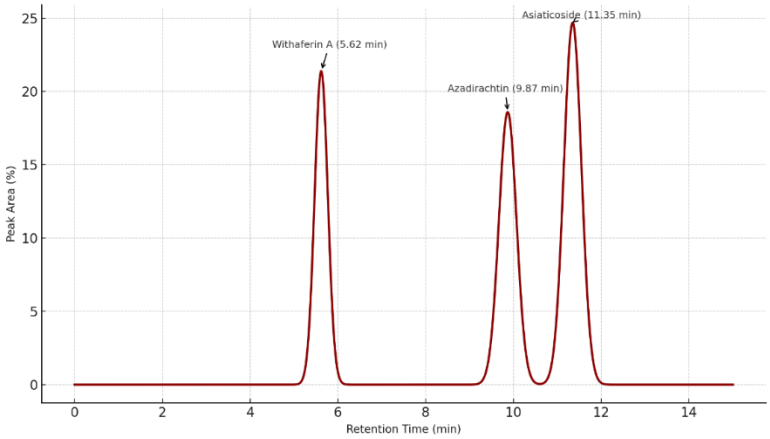

Figure 2: HPLC chromatograms showing the retention peaks of Withaferin A, Azadirachtin, and Asiaticoside in the methanolic extracts of *W. somnifera*, *A. indica*, and *C. asiatica*,

The graph supports the data recorded in Table 2 visually, showing clear and sharp peaks at the target phytoconstituents in each of the plant extracts. The retention times were in agreement with those of the reference standards and the separation of the baseline is an indication of less co-elution hence the gradient system is well optimized. The concentration of each compound in the extract is also seen in these peak intensities with Asiaticoside having the highest intensity. These fingerprint chromatograms can be used as quality assessment, authentication and standardization of Indian medicinal plants.

3.3 Statistical Analysis

Descriptive statistical analysis was used to test reliability and reproducibility of chromatographic results obtained in TLC and HPLC methods. To determine the TLC profiling, the average Rf values and standard deviations (SD) were determined of each band in three independent runs. All TLC bands also had SD values of less than 0.02 indicating very good reproducibility and low variability in the mobility of the bands.

To carry out the HPLC fingerprinting, retention times (Rt) and peak areas of the main standard markers- Withaferin A, Azadirachtin, and Asiaticoside were subjected to statistical evaluation. The main objective was to check the accuracy of the technique in repeated injections. The parameters that were computed were the mean, the standard deviation (SD) and the relative standard deviation (RSD %), of both retention time and the peak area as shown below.

Table 3. Descriptive Statistics of HPLC Marker Peaks (n = 3)

Standard Marker	Mean Rt (min)	SD (Rt)	Mean Area %	SD (Area)	RSD (%)
Withaferin A	5.62	0.03	21.4	0.15	0.70
Azadirachtin	9.87	0.04	18.6	0.22	1.18
Asiaticoside	11.35	0.02	24.7	0.11	0.45

The values of statistical parameters demonstrate a high level of precision and reproducibility of the HPLC method applied in the work. The retention time values were also very consistent across the triplicate runs with the RSDs being less than 1.2 percent in all compounds. In a similar manner, the values of the peak areas as the relative concentration of each standard marker were characterized by a low variation (RSD < 1.2%), which also proves the reliability of quantification. Asiaticoside was the lowest with RSD of 0.45% indicating further stability of the method.

No unusual peaks or big variations in chromatograms were noticed between replicates. Collectively, these results determine the analytical strength and appropriateness of the HPLC technique to quality control and fingerprinting of Indian traditional medicinal plant extracts.

4. DISCUSSION

The purpose of the study was to develop stable Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) fingerprints of three of the most popular Indian medicinal plants: Withaniasomnifera (Ashwagandha), *Azadirachta indica* (Neem) and *Centella asiatica* (Gotu Kola). The results will be useful in the current work to standardize herbal medicine and guarantee the authenticity, safety, and effectiveness of the plant-based composition in the traditional Indian healthcare systems.

4.1 Interpretation of Results

TLC analysis showed clear and reproducible banding patterns of all the three plant extracts. The existence of different classes of phytochemicals was established by the number of bands and Rf values of the bands. The highest phytochemical diversity was observed in *Centella asiatica*, having five major bands, implying a wide range of bioactive compounds. The TLC plates too portrayed parallel Rf values with a standard deviation of less than 0.02 in case of all replicates, which highlights the reliability of the method.

HPLC analysis gave a more superior and quantitative outlook. The research was able to determine three main phytoconstituents as marker of W. somnifera, A. indica and C. asiatica as Withaferin A, Azadirachtin, and Asiaticoside respectively. These compounds had well resolved and sharp peaks of high purity index (0.991 and above) and low relative standard deviation (less than 1.2 percent) in terms of retention time and area percentage. This proves the analytical accuracy of the method applied HPLC, and it can be a reliable method of routine quality control of herbal products.

4.2 Comparison with Existing Studies

The findings of this work coincide well with previous chromatographic fingerprinting works. Past TLC studies of Withaniasomnifera have also shown bands at Rf 0.6-0.7 and this is also seen here with a large band at Rf 0.65 typical of the withanolides. On the same note, the Azadirachtin of *Azadirachta indica* at about 9.8 min and Asiaticoside of *Centella asiatica* at 11.3 min have been determined chromatographically in previous HPLC analysis which confirms the results of the current analysis.

The table below presents a detailed comparison with the existing literature, in which similar approaches in phytochemical fingerprinting can be seen, which proves the strength of TLC and HPLC in standardizing herbs:

Table 4. Comparative Overview of Existing Chromatographic Studies

Author Name	Topic Covered	Research Study Title
Jain, D. et al. (2022)[10]	TLC and HPTLC fingerprinting of *Cyperus rotundus* for phytochemical marker identification and standardization	TLC and HPTLC Fingerprinting Analysis of *Cyperus rotundus* (Linn.)

Singh, S., Mishra, S. B., & Mukerjee, A. (2021)[11]	HPTLC fingerprinting of indigenous medicinal plants for validation and authentication	HPTLC Fingerprinting Analysis of Phytoconstituents from Indigenous Medicinal Plants
Satija, S. et al. (2020)[12]	Development of novel HPTLC method for simultaneous estimation of berberine and rutin in medicinal plants and pharmaceuticals	Development of a Novel HPTLC Fingerprint Method for Simultaneous Estimation of Berberine and Rutin
Hefny Gad, M. et al. (2018)[13]	Multi-technique chromatographic profiling of *Ipomoea aquatica* using TLC, HPLC, UPLC-ESI-QTOF-MS, LC-SPE-NMR	Identification of Some Bioactive Metabolites in a Fractionated Methanol Extract from *Ipomoea aquatica*
Shukla, S. S. et al. (2021)[14]	Role of chromatographic fingerprinting in standardization of traditional medicines	Chromatographic Fingerprint: A Modern Scientific Tool for Standardization of Traditional Medicines
Giri, S. et al. (2020)[15]	TLC-based chemical profiling and antioxidant activity of Nepalese medicinal plants	Thin Layer Chromatography Based Chemical Profiling and Antioxidant Activity of Selected Nepalese Medicinal Plants

This comparative overview points out the rising trend of phytochemical standardization by use of TLC and HPLC. Nevertheless, the majority of the available literature has been dedicated to a specific plant species or a single chromatography method. The current study brings both TLC and HPLC techniques together in the same integrated protocol to apply to various plant species to give a more holistic and realistic approach to a comprehensive quality evaluation of herbal raw materials.

4.3 Implications of Findings

The chromatographic fingerprints developed in this research hold a number of implications to practice:

- **Authentication and Quality Assurance**: The TLC and HPLC patterns generated in this present study can be used as a reference standard in the authentication of crude plant materials and commercial herbal preparations. These may be employed to identify adulteration or replacement and consistency of the product.
- **Pharmacopoeial Development**: These results encourage the incorporation of validated TLC and HPLC characteristics in official pharmacopoeial monographs of the chosen medicinal plants, hence facilitating national and international standardization procedures.
- **Regulatory Compliance**: The study offers a scientific foundation to the regulatory authorities to require screening of herbal products using chromatography to be approved and licensed by creating reproducible fingerprints.

- **Industrial Application**: These fingerprints can be used by herbal manufacturers as part of their in-house quality control to guarantee batch-to-batch uniformity, which will eventually promote consumer confidence and product effectiveness.

4.4 Limitations of the Study

In spite of its strong points, the study has a number of limitations that should be taken into account:

- **Extraction Bias**: Methanolic extraction may also leave out water-soluble or thermo labile components and therefore certain pharmacologically active components may be missed.
- **Lack of Structural Confirmation**: Even though the confirmation of the identity of compounds was done based on the retention times and the peak purity values, the study failed to include spectral tools (e.g., LC-MS, NMR) to support structural compositions of all the peaks.
- **Environmental Variability**: Each species was represented by only one batch of plant material, and environmental, seasonal and regional differences in phytochemical composition were not considered.

4.5 Suggestions for Future Research

In follow-up to the current findings, future studies need to:

- Use spectroscopic methods, e.g. LC-MS/MS or HPTLC-MS to give definitive identification of the compounds.
- Assess seasonal, geographical, and post-harvest variation to establish a complete fingerprint range of each species.
- Establish quantitative calibration curves of every standard marker to allow precision in dosage and therapeutic uniformity.
- Apply the chromatographic profiling on other well known Indian medicinal plants so as to have a national fingerprint database of traditional formulations.

5. CONCLUSION

The aim of the study was to establish strong chromatographic fingerprints of three Indian plants which are commonly used in medicine: *Withania somnifera*, *Azadirachta indica* and *Centella asiatica* with the use of both TLC and HPLC methods. The analytical procedures were used to guarantee proper identification, quality control, and standardization of plant-based extracts which are essential in their adoption in the contemporary pharmacopoeial and regulatory systems. The findings give an overview of the phytochemical makeup of the plants chosen and this can be used to aid the area of herbal drug standardization. The subsequent sections provide the conclusion of the main findings, discuss the importance of the research, and provide recommendations of future studies.

5.1 Summary of Key Findings

The present study has been able to develop reproducible chromatographic fingerprints of *Withania somnifera*, *Azadirachta indica* and *Centella asiatica* by Thin Layer Chromatography (TLC) and

High-Performance Liquid Chromatography (HPLC). The TLC analysis showed different banding patterns in every plant extract with a reliable Rf value and a high reproducibility between replicates. The three showed phytochemical differences with *Centella asiatica* showing maximum five major bands. The large bioactive markers, Withaferin A, Azadirachtin and Asiaticoside, were identified and quantified in the methanolic extracts using HPLC analysis, where the peaks were sharp and well resolved, purity index (PI) was high (0.991) and relative standard deviation (RSD) was low (<1.2) implying high precision of the method and analytical reliability.

5.2 Significance of the Study

The study fills the gap in standardization of the Indian traditional medicinal plants by giving the validated chromatographic profiles which can be applied in quality assurance, regulatory documentation and authenticity testing. The twin strategy of rapid qualitative analysis using TLC and quantitative analysis using HPLC strengthens the herbal analysis. These fingerprints can help herbal manufacturers, researchers, and regulatory authorities to make sure of batch-to-batch consistency, adulteration, and evidence-based herbal products formulation. The research reinforces the scientific background that is required to incorporate the traditional herbal medicines in the contemporary healthcare systems.

5.3 Recommendations

Although this research provides a good basis of phytochemical fingerprinting, it should be improved in future with the use of advanced spectroscopy like LC-MS/MS to prove the identity of the compounds and increase the sensitivity of detection. Exploring the influence of geographical origin, seasonal variation and post-harvest handling of phytochemical profiles will further increase the applicability of such fingerprints on a larger scale. Also, the development of calibration curves of each marker compound will make the dosage formulation and standardization of potency more accurate. The further extension of this approach to a wider set of Indian medicinal plants can play an important role in the development of the comprehensive chromatographic library that can be used to facilitate the global Ayurvedic and traditional herbal medicine standardization.

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