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Phytochemical Investigation and HPTLC Screening of Tinospora Cordifolia leaf Extract

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ABSTRACT

Background: *Tinospora cordifolia*, a vital medicinal plant in traditional Indian medicine, possesses immunomodulatory, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic properties. This study aimed to analyze the phytochemical composition and quantify key bioactive compounds in *T. cordifolia* leaf extract using High-Performance Thin-Layer Chromatography (HPTLC).

Methods: Fresh leaves of *T. cordifolia* were collected, authenticated, and subjected to Soxhlet extraction using 70% ethanol. Preliminary phytochemical screening identified carbohydrates, alkaloids, flavonoids, phenols, proteins, and amino acids. HPTLC was performed with a mobile phase of Toluene: Ethyl Acetate: Methanol: Formic Acid (5:6:2:1) and detected at 270 nm. Quantification of Rutin, Gallic Acid, and Quercetin was conducted using calibration curves, with validation as per ICH guidelines.

Results: The ethanolic extract had a 26% yield. HPTLC analysis demonstrated distinct bands for Gallic Acid and Quercetin with Rf values of 0.59 and 0.73, respectively, while Rutin (Rf 0.18) was not detected. Quantitative analysis revealed Gallic Acid content between 1.234% and 1.282%, and Quercetin content from 0.62% to 0.655%. The method showed high linearity ($r^2 > 0.99$), precision, specificity, and robustness.

Conclusion: *T. cordifolia* leaf extract is a valuable source of bioactive compounds, particularly Gallic Acid and Quercetin. The HPTLC method developed is simple, precise, and reliable, suitable for routine analysis of phytoconstituents in herbal formulations. These findings support the therapeutic potential of *T. cordifolia* and its use in developing standardized herbal medicines.

Keywords: *Gallic Acid, HPTLC analysis, Phytochemical screening, Quercetin, Tinospora cordifolia*

1. INTRODUCTION

Natural plant-based products have been integral to healthcare for centuries, with herbal medicine serving as a primary treatment method [1]. The World Health Organization (WHO) reports that 80% of the global population still relies on herbal medicines, contributing to a nearly $60 billion annual market [2]. Medicinal plants have been integral to traditional healthcare systems for centuries, providing a rich source of bioactive compounds with therapeutic potential. Among these, *Tinospora cordifolia*, commonly known as Guduchi, has garnered significant attention for its diverse pharmacological properties, including immunomodulatory, antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic effects [1]. It is a key component of traditional Indian medicine, particularly Ayurveda, where it is utilized in formulations for treating fever, diabetes, and enhancing overall vitality [3].

Phytochemical investigations of *Tinospora cordifolia* have identified a range of bioactive constituents such as alkaloids, glycosides, steroids, terpenoids, and flavonoids, contributing to its medicinal efficacy [4]. These compounds exhibit potent

antioxidant and free radical scavenging activities, which are crucial in mitigating oxidative stress-related diseases [5]. The extraction and standardization of these phytochemicals are essential for ensuring the consistency, safety, and efficacy of herbal formulations [6].

High-Performance Thin Layer Chromatography (HPTLC) is a robust analytical technique widely used for the qualitative and quantitative analysis of phytochemicals in herbal extracts [7]. HPTLC offers distinct advantages such as high resolution, reproducibility, and the ability to analyze multiple samples simultaneously, making it an ideal method for screening bioactive compounds [8]. Previous studies have successfully employed HPTLC for the analysis of various markers in *Tinospora cordifolia*, contributing to the establishment of quality standards for herbal medicines [6].

The present study aims to perform a comprehensive phytochemical investigation of *Tinospora cordifolia* leaf extract and to employ HPTLC screening to identify and quantify key bioactive constituents. This study will contribute to the existing knowledge on Tinospora cordifolia and support its use in the development of standardized herbal formulations. Additionally, the findings of this study could improve the quality control measures for herbal products containing *Tinospora cordifolia* and promote its therapeutic applications in modern medicine.

2. METHODOLOGY

The fresh leaves of *Tinospora cordifolia* were collected from the local areas surrounding Shree Balaji Shikshan Prasarak Mandal's College of B-Pharmacy in Ambajogai, District Beed, Maharashtra, India. The plant material was authenticated by the Botanical Survey of India, Western Regional Centre, Pune, Maharashtra (Authentication Number: [BSI/WRC/IDEN.CER./2024/62]). The collected plant material was thoroughly washed with distilled water to remove dirt, dust, and other impurities. Only healthy leaves were selected for the study. These leaves were shade-dried for two weeks at room temperature (25°C ± 2°C) to preserve the bioactive compounds. After drying, the leaves were ground into a fine powder using a mechanical grinder and stored in airtight containers to maintain their stability until further use.

The extraction of bioactive compounds from the leaves of *Tinospora cordifolia* was carried out using the Soxhlet extraction method with 70% ethanol as the solvent. To begin, 25 grams of the powdered leaf material were packed into a thimble made of Whatman filter paper. This thimble was then placed into a Soxhlet apparatus, and 70% ethanol was used as the extraction solvent. The extraction process was conducted continuously for 48 hours until the solvent in the siphon tube became colorless, indicating complete extraction of the phytochemicals. Following the extraction, the mixture was filtered through Whatman No. 1 filter paper to remove particulate matter. The solvent was subsequently evaporated using a rotary evaporator at 40°C under reduced pressure to obtain the dry extract. The yield of the extract was calculated using the formula:

$$Percent\ Yield = \left(\frac{W_1}{W_2}\right) X\ 100$$

where W1 represents the weight of the crude extract (6.5 grams) and W2 is the initial weight of the powdered plant material (25 grams). The calculated yield of the ethanolic extract was 26%, indicating a good efficiency of the extraction process.

The preliminary phytochemical screening of the ethanolic extract of *Tinospora cordifolia* was conducted to identify the presence of various primary and secondary metabolites. Standard qualitative methods were employed to detect carbohydrates, alkaloids, flavonoids, saponins, phenols, proteins, amino acids, steroids, and tannins. Each test followed specific procedures and led to characteristic colour changes or precipitate formation, confirming the presence or absence of each compound. Notably, the extract tested positive for carbohydrates, flavonoids, phenols, proteins, alkaloids, and amino acids, while glycosides, steroids, and tannins were not detected. The presence of these bioactive compounds suggests potential therapeutic applications of *Tinospora cordifolia*.

High-Performance Thin-Layer Chromatography (HPTLC) was employed to analyze the secondary metabolites present in the *Tinospora cordifolia* leaf extract, specifically focusing on Rutin, Quercetin, and Gallic acid. The standards for these compounds were prepared by dissolving 10 mg of each in 10 ml of methanol to achieve a stock concentration of 1000 µg/ml. These solutions were further diluted to obtain a working concentration of 250 µg/ml. The herbal extract was also prepared by dissolving 10 mg of the dry extract in 10 ml of methanol, followed by sonication for 20 minutes. Supernatant solutions were then applied to the TLC plate in volumes of 10 µl and 20 µl. For the chromatographic analysis, precoated silica gel 60 F254 TLC aluminium plates (10 cm × 10 cm, 250 µm thickness, E. MERCK, Darmstadt, Germany) were used as the stationary phase. The mobile phase consisted of Toluene: Ethyl Acetate: Methanol: Formic Acid in the ratio of 5:6:2:1. The samples were applied using a CAMAG Linomat 5 sample applicator with a 100 µL syringe, maintaining a band width of 6 mm and a 5 mm gap between bands. The plates were developed in a twin trough glass chamber (CAMAG, Muttenz, Switzerland) with a chamber saturation time of 10 minutes. The densitometric scanning of the developed plates was performed using a CAMAG TLC scanner at a wavelength of 270 nm, operated by WINCATS software (Version 1.4.3).

The statistical analysis in this study involved calculating the percent yield of the *Tinospora cordifolia* extract and performing quantitative analysis of Gallic Acid and Quercetin using HPTLC. Calibration curves with linear regression were used to determine the accuracy, precision, and quantification of the bioactive compounds.

3. RESULTS

The HPTLC method was validated as per International Council for Harmonisation (ICH) guidelines. Validation parameters included linearity, precision, specificity, accuracy, and robustness. The linearity of the method was assessed by constructing calibration curves for Rutin, Quercetin, and Gallic acid, showing good correlation coefficients ($r^2 > 0.99$). The method demonstrated repeatable and reliable results, with the retention factor (Rf) values determined as 0.18 for Rutin, 0.59 for Gallic Acid, and 0.73 for Quercetin. Specificity tests indicated clear separation of these analytes from other matrix components, while accuracy was confirmed through recovery studies, where spiking known amounts of standards into the extract yielded satisfactory results. The method was robust, showing consistent performance even with slight changes in chromatographic conditions.

Manual Trials

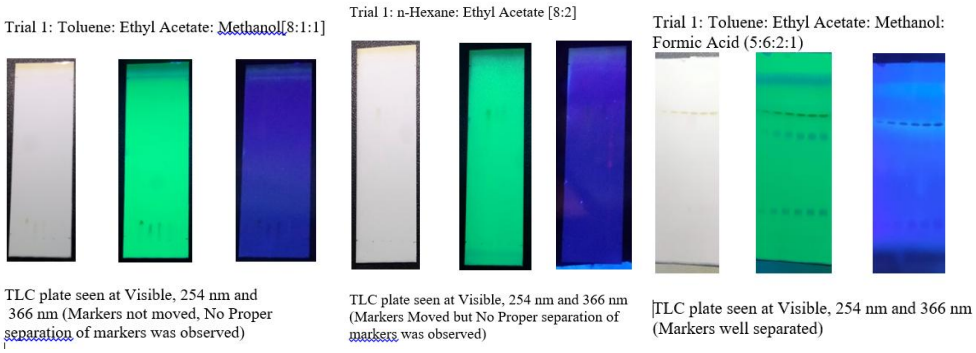

Figure 1. Optimization of Mobile Phase for HPTLC Separation of Phytoconstituents in *Tinospora Cordifolia* Leaf Extract

Table 1: Chromatographic parameters.

Sr. No.	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminium plate pre-coated with silica gel 60 GF_{254}
2.	Mobile phase	Toluene: Ethyl Acetate: Methanol: Formic Acid (5:6:2:1)
3.	Detection Wavelength	270 nm
4.	Saturation time	10 min
5.	Band width	6 mm

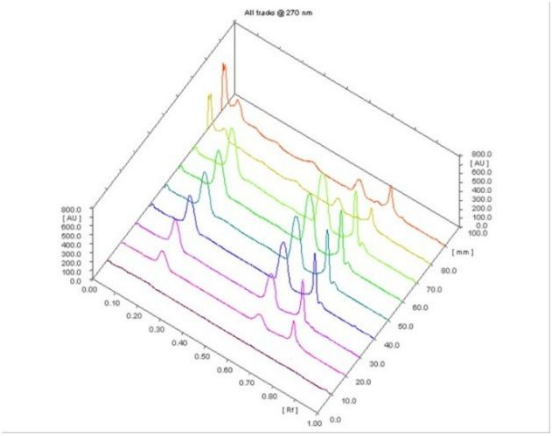

Figure 1: 3D Densitogram Plot of *Tinospora Cordifolia* Leaf Extract at 270 nm

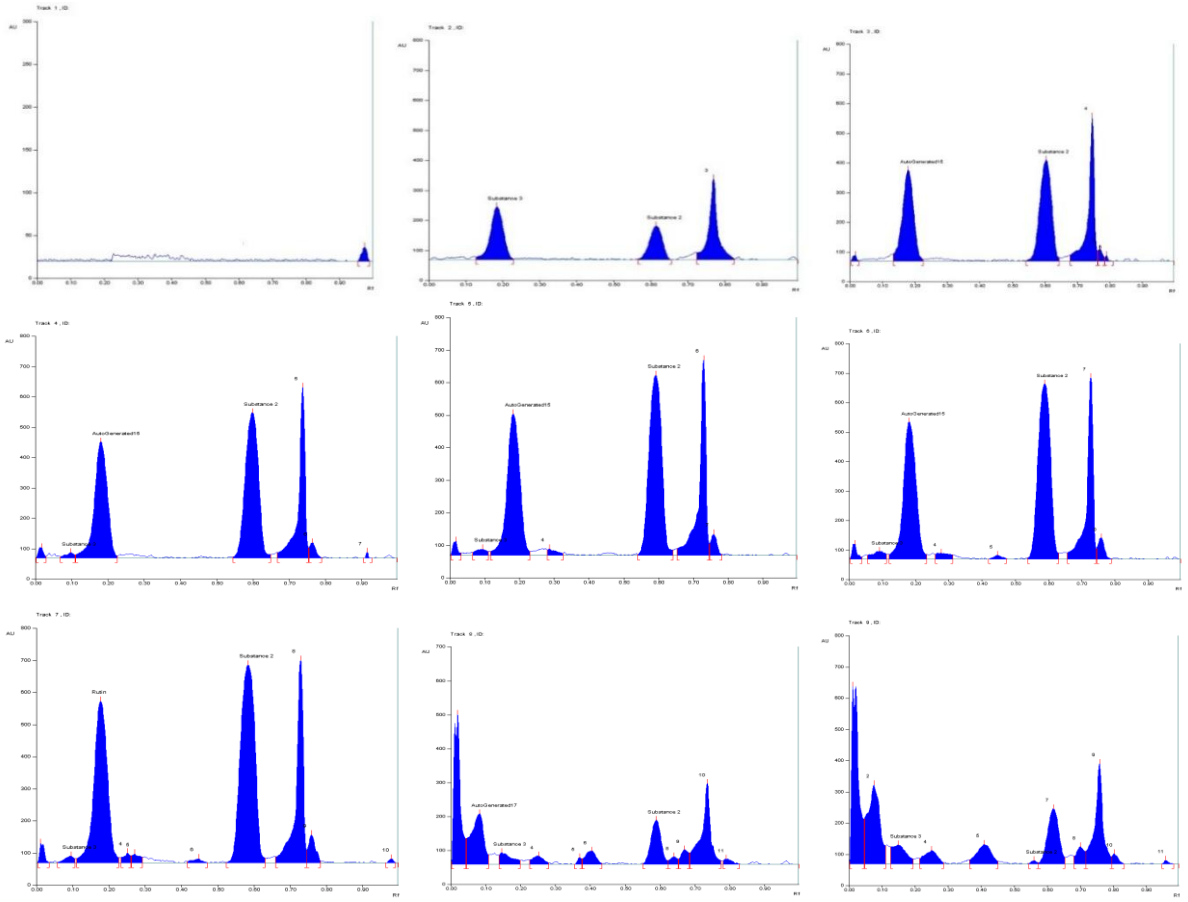

Figure 2: HPTLC Densitogram of *Tinospora Cordifolia* Leaf Extract for Phytochemical Screening

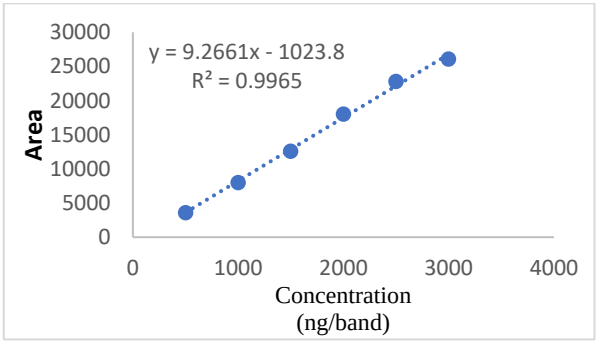

Figure 3: Calibration Curve of Gallic Acid

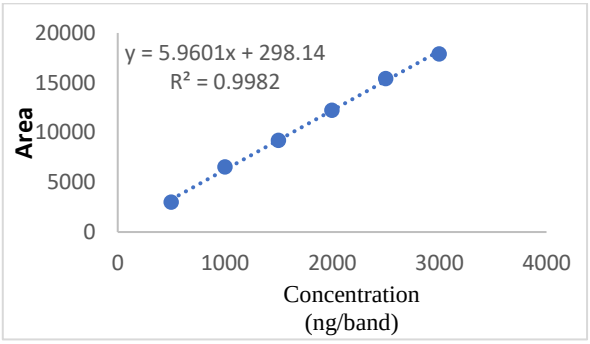

Figure 4: Calibration Curve of Quercetin

Table 2. Regression analysis for Calibration Curve of Gallic Acid and Quercetin

		Y	m	c	X		10 mg	10 mg	100mg
					10/20 µl	1 ml	10 ml		%
					ng	ng	µg	mg	mg
Gallic Acid	10 µl	10852.8	9.266	-1023.8	1281.74	128174	128.174	0.128	1.282
	20 µl	21852.8	9.266	-1023.8	2468.88	123444	123.444	0.123	1.234

Quercetin	10 µl	3993.8	5.9601	298.14	620.067	62006.7	62.007	0.062	0.62
	20 µl	8111.4	5.9601	298.14	1310.93	65546.4	65.546	0.066	0.655

The present study evaluated the phytochemical composition and HPTLC screening of *Tinospora cordifolia* leaf extract. The ethanolic extract obtained through Soxhlet extraction yielded 26% of the dry extract, demonstrating an efficient extraction process. The preliminary phytochemical screening of the extract indicated the presence of several bioactive compounds, including carbohydrates, alkaloids, flavonoids, phenols, proteins, and amino acids, while glycosides, steroids, and tannins were not detected. These findings suggest that Tinospora cordifolia is a rich source of potential therapeutic compounds.

The High-Performance Thin-Layer Chromatography (HPTLC) analysis was conducted to quantify specific secondary metabolites, namely Rutin, Gallic Acid, and Quercetin. The chromatographic analysis was performed using a mobile phase of Toluene: Ethyl Acetate: Methanol: Formic Acid (5:6:2:1) and a detection wavelength of 270 nm. The results demonstrated clear and distinct bands under visible light and UV detection at 254 nm and 366 nm, confirming the presence of secondary metabolites in the extract. The Rf values for the standard markers were observed at 0.18 for Rutin, 0.59 for Gallic Acid, and 0.73 for Quercetin. However, Rutin was not detected in the *Tinospora cordifolia* leaf extract, as no corresponding band was observed at the expected Rf value.

Quantitative analysis using the calibration curve method revealed that Gallic Acid was present in the range of 1.234% to 1.282%, while Quercetin content was between 0.62% and 0.655%. The calibration curves for Gallic Acid and Quercetin showed good linearity with correlation coefficients ($r^2 > 0.99$), indicating the accuracy and reliability of the HPTLC method for quantification. The absence of Rutin in the leaf extract could be attributed to the specific phytochemical profile of the leaves or the extraction method used.

Overall, the results of this study demonstrate that *Tinospora cordifolia* leaf extract contains valuable phytochemicals, particularly Gallic Acid and Quercetin, which may contribute to its therapeutic potential. The HPTLC method developed was found to be simple, precise, specific, and robust, making it suitable for routine analysis of these phytoconstituents in the crude extract of Tinospora cordifolia. The findings provide a strong basis for further research into the medicinal applications of this plant and support its use in the development of standardized herbal formulations.

4. DISCUSSION

The present study successfully demonstrated the phytochemical profile and HPTLC analysis of *Tinospora cordifolia* leaf extract, highlighting its potential as a source of therapeutic compounds. The Soxhlet extraction method using 70% ethanol yielded a 26% dry extract, indicating an efficient extraction process. Preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, phenols, proteins, and amino acids, while glycosides, steroids, and tannins were not detected. These findings align with previous studies that have reported similar phytochemical profiles for *Tinospora cordifolia*, reinforcing its medicinal potential in traditional and modern therapeutic applications.

The findings of the present study align with previous research on the phytochemical composition and HPTLC analysis of *Tinospora cordifolia*. Similar studies have consistently demonstrated the presence of bioactive compounds such as alkaloids, flavonoids, phenols, and amino acids in *Tinospora cordifolia* extracts. For instance, a study by Rai et al. (2024) reported comparable results in the phytochemical screening of *Tinospora cordifolia*, highlighting the strong antioxidant potential attributed to the presence of phenolic compounds, including Gallic Acid and Quercetin [9]. The absence of Rutin in this study, however, contrasts with the findings of Sherwani et al. (2016), where Rutin was detected in both the leaf and stem extracts of *Tinospora cordifolia*.[10] This discrepancy could be attributed to differences in the extraction methods, solvent polarity, or geographical variations of the plant material.

In terms of quantitative analysis, the present study identified Gallic Acid content in the range of 1.234% to 1.282% and Quercetin content between 0.62% and 0.655%. These values are consistent with the results reported by Rai et al. (2024), who found Gallic Acid concentrations of approximately 1.3% in the ethanolic extract of *Tinospora cordifolia* [9]. Quercetin levels reported in the current study are also within the range observed by Patel et al. (2021), who noted Quercetin content of around 0.6% in their HPTLC analysis of *Tinospora cordifolia* leaf extracts[11]. These studies support the potential therapeutic benefits of *Tinospora cordifolia*, particularly its antioxidant and anti-inflammatory properties.

The chromatographic conditions used in this study, specifically the mobile phase composition of Toluene: Ethyl Acetate: Methanol: Formic Acid (5:6:2:1), provided effective separation of the analytes. This aligns with the findings of Kumar et al. (2023), who used a similar mobile phase ratio and achieved distinct bands for Gallic Acid and Quercetin in herbal extracts [12]. The use of HPTLC as a method for the quantification of phytochemicals was validated by the strong correlation coefficients ($r^2 > 0.99$) and the robust nature of the method, supporting its application in routine quality control of herbal products.

5. CONCLUSION

Phytochemical screening of *Tinospora cordifolia* leaf extract revealed the presence of valuable bioactive compounds, with HPTLC analysis confirming significant amounts of Gallic Acid (1.234–1.282%) and Quercetin (0.62–0.655%), while Rutin was absent. The developed HPTLC method proved to be a reliable tool for routine analysis, supporting the potential of *T. cordifolia* in developing novel medicinal formulations.

Conflict of Interest: No conflicts declared.

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