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INTEGRATED PHYTOCHEMICAL PROFILING AND ANALYTICAL CHARACTERIZATION OF *CORDIA DICHOTOMA* G. FORST LEAVES USING SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT

Medicinal plants continue to serve as a vital source of bioactive compounds with significant therapeutic potential. The present study aims to systematically evaluate the phytochemical composition of *Cordia dichotoma* G. Forst. leaves through integrated analytical approaches. Preliminary phytochemical screening revealed the presence of major secondary metabolites including alkaloids, flavonoids, tannins, glycosides, and phenolic compounds across different solvent extracts. Quantitative estimation demonstrated that the hydroalcoholic extract possesses a high concentration of total phenolics (96.4 mg CE/g), flavonoids (99.2 mg QE/g), and tannins (163.1 mg TAE/g), indicating strong antioxidant potential.[1,2] FTIR spectral analysis confirmed the presence of functional groups such as hydroxyl, aromatic, and glycosidic linkages, supporting the occurrence of phenolic and flavonoid compounds. HPLC chromatographic profiling revealed a prominent peak at retention time 2.633 min, suggesting the presence of major phenolic constituents. These findings were consistent with reported literature emphasizing the phytochemical richness and pharmacological relevance of *Cordia dichotoma* leaves.[3-5] Overall, the study provides comprehensive analytical validation of bioactive constituents, highlighting the plant's potential as a natural source for pharmacological and therapeutic applications.

KEYWORDS: *Cordia dichotoma* G. Forst., Phytochemical analysis, Total phenolics, FTIR, HPLC, Flavonoids, Tannins, Medicinal plants.

1. INTRODUCTION

Natural products derived from medicinal plants play a crucial role in drug discovery and development due to their structural diversity and biological activities.[1-3] *Cordia dichotoma* G. Forst, a member of the Boraginaceae family, is widely recognized for its ethnomedicinal significance and therapeutic versatility. Traditionally, it has been used for the treatment of inflammation, respiratory disorders, gastrointestinal disturbances, and skin diseases.[4-7]

The pharmacological potential of this plant is attributed to the presence of diverse phytoconstituents such as flavonoids, tannins, phenolics, alkaloids, and glycosides, which exhibit antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities.[8-12] Recent advancements in analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared Spectroscopy (FTIR) have enabled precise characterization of these bioactive compounds. Therefore, the present study focuses on comprehensive phytochemical evaluation and analytical validation of *Cordia dichotoma* G. Forst., leaf extracts using qualitative, quantitative, and instrumental techniques.[13-15]

2. METHODOLOGY

2.1 PLANT MATERIAL COLLECTION, AUTHENTICATION, AND EXTRACTION

2.1.1 COLLECTION AND AUTHENTICATION: Fresh leaves of *Cordia dichotoma* G. Forst were collected from **Adibasaveshwara Nagara, Harapanahalli, Karnataka, India** during the appropriate vegetative season to ensure maximum phytochemical content. The plant material was taxonomically authenticated based on morphological characteristics such as leaf shape, venation, and bark texture, consistent with standard botanical descriptions.

2.1.2 DRYING AND POWDERING: The collected leaves were washed with distilled water to remove surface contaminants and remove adhering dust and debris and subsequently air-dried under **shade conditions at controlled room temperature (25 ± 2°C)** for **7–10 days** to prevent degradation of thermolabile compounds such as flavonoids and phenolics.

Figure 1: **Shade Drying of *Cordia dichotoma* G. Forst leaves.**

The shade dried material was pulverized using a mechanical grinder and sieved through sieve number 40 (425 µm**)** to obtain uniform particle size, ensuring improved solvent penetration and extraction efficiency.

Figure 2: **Pulverized powder of *Cordia dichotoma* G. Forst leaves.**

2.1.3 SUCCESSIVE SOXHLET EXTRACTION: Extraction was performed using a Soxhlet apparatus based on increasing solvent polarity to ensure exhaustive extraction of phytoconstituent. Approximately 100 g of powdered plant material was packed into a thimble. Sequential extraction was carried out using: Petroleum ether (40–60°C) – 6–8 hours, Chloroform – 6–8 hours, 70% Ethanol (Hydroethanolic solvent) – 8–10 hours. Each extraction cycle was continued until the solvent in the siphon tube became colorless, indicating complete extraction. The extracts were concentrated under reduced pressure using a rotary evaporator and stored in airtight containers at 4°C until further analysis.[16]

2.1.4 AQUEOUS EXTRACTION (MACERATION METHOD): The marc remaining after Soxhlet extraction was subjected to cold maceration: Powder was soaked in distilled water (1:10 w/v ratio) for 72 hours, Occasional stirring was performed to enhance diffusion.The extract was filtered and concentrated**.**

Figure 3: **Soxhlet Extraction of *Cordia dichotoma* G. Forst leaves.**

2.2 PRELIMINARY PHYTOCHEMICAL SCREENING: Qualitative phytochemical screening of various extracts was performed using standard chemical tests to detect major classes of secondary metabolites, including alkaloids, flavonoids, tannins, glycosides, saponins, carbohydrates, proteins, and steroids.

2.3 QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

2.3.1 DETERMINATION OF TOTAL FLAVONOID CONTENT: To determine the total flavonoid content, the stock solutions of *Cordia dichotoma* G. Forst leaves extract will be prepared with ethanol to a suitable concentration for analysis. Aliquots of each *Cordia dichotoma* G. Forst leaves extract will be pipetted out in series of test tubes and the volume will be made up to 1ml with distilled water. Sodium nitrite (5%; 0.3ml) will be added to each test tube and incubated for 5minutes at room temperature. Aluminium chloride solution (10%; 0.06ml) will be added and incubated for 5minutes at room temperature. Sodium hydroxide (1M; 0.25ml) will be added and total volume will be made up to 3ml with distilled water. Absorbance will be measured at 510nm against a reagent blank using U.V. spectrometer and concentration of flavonoids in the test sample will be determined and expressed as mg equivalent per gram of sample.[18]

2.3.2 DETERMINATION OF TOTAL PHENOLIC CONTENT: The total phenolic content of the *Cordia dichotoma* G. Forst leaves extract will be determined by taking aliquots of the extracts into 10ml glass tube and

the volume will be made up to 3ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with distilled water) and 2 ml sodium carbonate (20%) will be added subsequently in each test tube. A blue color will be developed in each test tube because the phenols will undergo complex redox reaction with phosphomolibdic acid in Folin-Ciocalteau reagent in alkaline medium. This results in a blue colored complex, molybdenum blue. The test solutions will be warmed for 1min, cooled and the absorbance will be measured at 650nm using known concentration of catechol. The concentrations of phenols in the test samples will be calculated from the calibration plot and expressed as mg catechol equivalent of phenol per gram of sample.[17]

2.3.3 DETERMINATION OF TOTAL TANNIN CONTENT: The tannins will be identified using $FeCl_3$ and gelatin tests. For this purpose, 0.1g of *Cordia dichotoma* G. Forst leaves extract will be transferred to a 100ml flask. 50ml of water will be added and boiled for 30min. After filtration with cotton filter, the filtrate will be transferred to a 500ml volumetric flask and the volume will be made up to the mark with distilled water. 0.5 ml aliquots will be transferred to the vials, 1ml 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ will be added and the volume will be made up to 10ml with distilled water. After 5 min the solution will be measured calorimetrically at 720nm. The total content of tannins present in the *Cordia dichotoma* G. Forst leaves extract will be obtained from standard calibration curve which will be made by taking the tannic acid as standard.[19]

2.4 FOURIER TRANSFORM INFRARED (FTIR) ANALYSIS: FTIR analysis of the hydroethanolic extract of *Cordia dichotoma* G. Forst leaves was carried out using an ATR-FTIR spectrophotometer (Alpha II) equipped with a ZnSe crystal. The sample was analyzed in semi-solid form over a spectral range of 4000–500 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans.[20]

2.5 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS[21]

HPLC analysis was performed using a Shimadzu LC-2030C Plus system equipped with a UV detector and C18 column (150 × 4.6 mm, 5 µm particle size).

CHROMATOGRAPHIC CONDITIONS

- Mobile phase: Acetonitrile : Buffer
- Flow rate: 0.9 mL/min
- Injection volume: 20 µL
- Column temperature: 28 °C
- Detection wavelength: 275 nm
- Run time: 15 min.

2.5.1 PREPARATION OF STANDARD AND CALIBRATION CURVE: Standard stock solutions (1000 µg/mL) of tannic acid, catechol, and quercetin were prepared in methanol and diluted to obtain concentrations ranging from 5–100 µg/mL.

2.5.2 SAMPLE ANALYSIS: The hydroethanolic extract was analyzed under optimized chromatographic conditions. The chromatogram exhibited a prominent peak at a retention time of 2.633 min with a peak area of 1838481, indicating the presence of a major phenolic compound.

Table 1: HPLC Analysis Instrumentation and Conditions.

PARAMETER	CONDITION
Column	C18 (150×4.6 mm, 5µm)
Mobile phase	Acetonitrile : Buffer
Flow rate	0.9 mL/min
Temperature	28°C
Detection	275 nm
Injection volume	20 µL

2.5.3. PREPARATION OF STANDARDS

- Stock: 1000 µg/mL (tannic acid, catechol, quercetin).
- Dilutions: 5–100 µg/mL.

3. RESULTS

3.1 PRELIMINARY PHYTOCHEMICAL SCREENING.

Table 2: Preliminary Phytochemical Screening of *Cordia dichotoma* G. Forst Leaves.

PHYTOCHEMICAL	PETROLEUM ETHER	CHLOROFORM	70% ETHANOLIC	AQUEOUS
Alkaloids	–	++	++	+
Carbohydrates	–	+	++	++
Glycosides (Cardiac)	–	+	+	–
Glycosides (Anthraquinone)	–	+	++	–
Glycosides (Saponin)	–	++	++	++
Flavonoids	–	++	+++	++
Tannins & Phenolics	–	+	+++	++
Proteins	–	+	++	+
Steroids	+	+	+	–

The hydroethanolic extract of *Cordia dichotoma* G. Forst leaves exhibited the richest phytochemical richness (+++), particularly for flavonoids and tannins, glycosides, and phenolic compounds indicating its superiority as an extraction solvent for bioactive compounds. Confirming its suitability for further analysis.

3.2 QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Table 3: Quantitative estimation of phytoconstituents in *C. dichotoma* leaf extracts.

Parameter	Wavelength	70% Ethanolic Extract	Aqueous Extract
Total Phenolics	650 nm	96.4 mg/g (Catechol eq.)	41.7 mg/g
Total Flavonoids	510 nm	99.2 mg/g (Quercetin eq.)	48 mg/g
Total Tannins	700 nm	163.1 mg/g (Tannic acid eq.)	67.7 mg/g

The hydroethanolic extract of *Cordia dichotoma* G. Forst leaves showed significantly higher phytoconstituent concentration, confirming its superior extraction efficiency for polyphenolic compounds**.**

3.3 FTIR SPECTROSCOPIC ANALYSIS: FTIR analysis of the hydro-alcoholic extract of *Cordia dichotoma* G. Forst leaves showed characteristic peaks indicating the presence of various functional groups. A broad peak at 3315 cm^{-1} represents O–H stretching vibration, confirming the presence of phenols and flavonoids. The peak at 2932 cm^{-1} corresponds to C–H stretching of alkanes, suggesting the presence of terpenoids and lipids. Peaks observed at 1565 cm^{-1} and 1516 cm^{-1} indicate aromatic C=C stretching, which is typical for polyphenolic compounds. The band at 1391 cm^{-1} corresponds to C–H bending vibrations, which may be associated with alkaloids or phenolic structures. Absorption peaks between 1247 cm^{-1} and 1029 cm^{-1} correspond to C–O stretching vibrations, indicating the presence of alcohols, glycosides, and carbohydrates. The peaks in the fingerprint region (926–519 cm^{-1}) represent complex molecular vibrations typical of plant secondary metabolites.

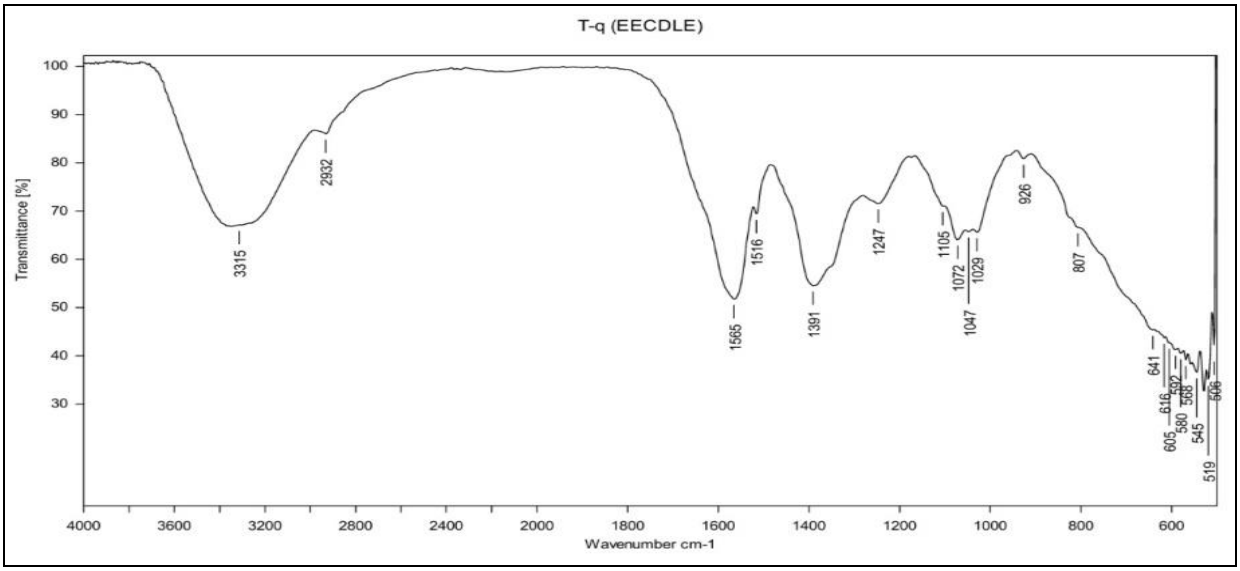

Figure 4: **FTIR spectrum of hydroethanolic extract *Cordia dichotoma* G. Forst leaves.**

Table 4: FTIR Functional Group Analysis and Peak interpretation of *Cordia dichotoma* G. Forst leaf extract.

WAVENUMBER (cm^{-1})	FUNCTIONAL GROUP	PHYTOCHEMICAL INDICATION
3315	O–H stretching	Phenols, Alcohols
2932	C–H stretching	Alkanes
1565	C=C stretching	Aromatic compounds
1516	Aromatic vibration	Flavonoids
1391	O–H bending	Phenolics
1247	C–O stretching	Esters, phenols
1105	C–O stretching	Alcohols
1072	C–O–C stretching	Glycosides
1047	C–O stretching	Carbohydrates
1029	C–O stretching	Secondary alcohols
926–641	Fingerprint region	Complex phytochemicals

3.4 HPLC ANALYSIS: HPLC analysis of *Cordia dichotoma* G. Forst leaf extract was conducted using Shimadzu LC-2030C Plus system with C18 column (150 × 4.6 mm, 5 µm) and UV detection at 275 nm**.** The HPLC chromatogram of the hydro-alcoholic extract of *Cordia dichotoma* G. Forst leaves revealed a prominent peak at a retention time of 2.633 minutes with an area of 1838481, indicating the presence of a major

phytochemical constituent. The chromatographic separation was carried out using detection at 275 nm, which is commonly used for detecting phenolic compounds and flavonoids. The presence of a sharp and well-defined peak with a tailing factor of 1.753 and 6811 theoretical plates indicates good chromatographic performance and column efficiency. The chromatographic profile suggests the presence of phenolic compounds such as quercetin, catechol, or tannic acid derivatives, which are known to occur in medicinal plants and contribute to antioxidant activity.

The results of HPLC analysis support the findings obtained from FTIR spectroscopy, confirming that *Cordia dichotoma* leaf extract contains bioactive phenolic and flavonoid compounds responsible for its pharmacological activities.

Table 5: Standard Calibration Curve (Tannic acid, Catechol, Quercetin).

CONC (µg/ml)	TANNIC ACID	CATECHOL	QUERCETIN
5 µg/ml	12499	71376	75471
10 µg/ml	27627	141387	154371
25 µg/ml	75165	345724	398483
50 µg/ml	155777	692429	828098
100 µg/ml	339224	1447790	1779731

3.4.1 Regression equations

- Tannic acid: y = 3439x − 8629
- Catechol: y = 14472x − 10178
- Quercetin: y = 17958x – 35177

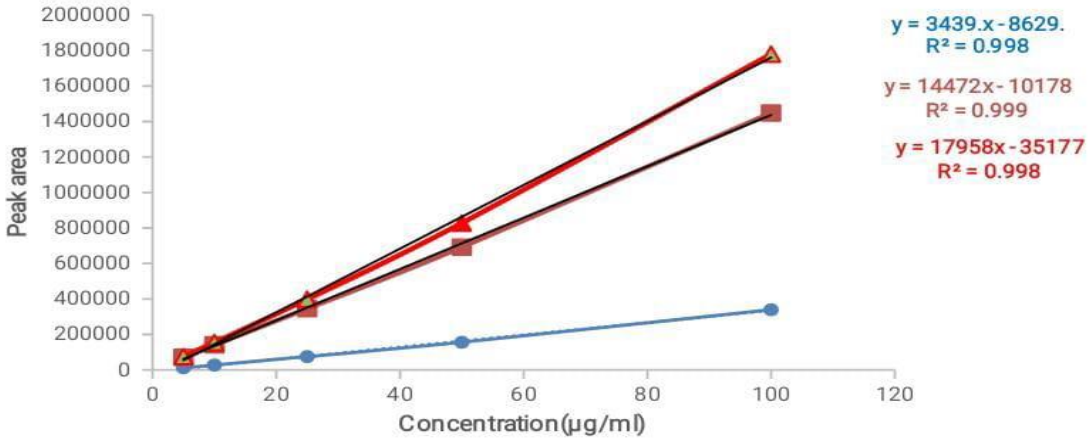

Figure 5: **Standard Calibration Curve (Tannic acid, Catechol, Quercetin).**

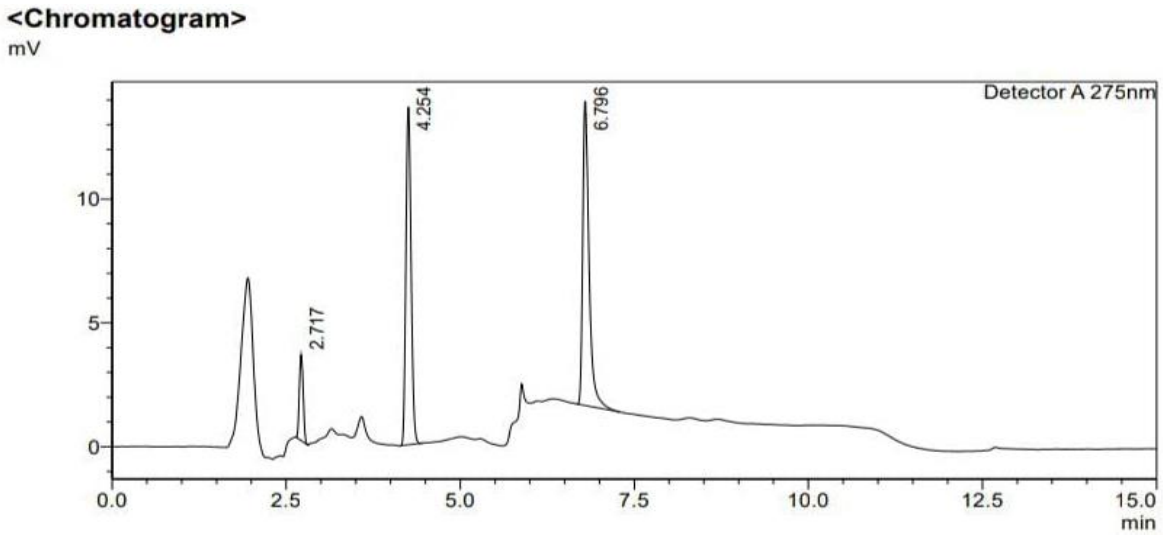

Figure 6: **Standarad Chromotogram of Tannic acid, Catechol, Quercetin at 5 mcg.**

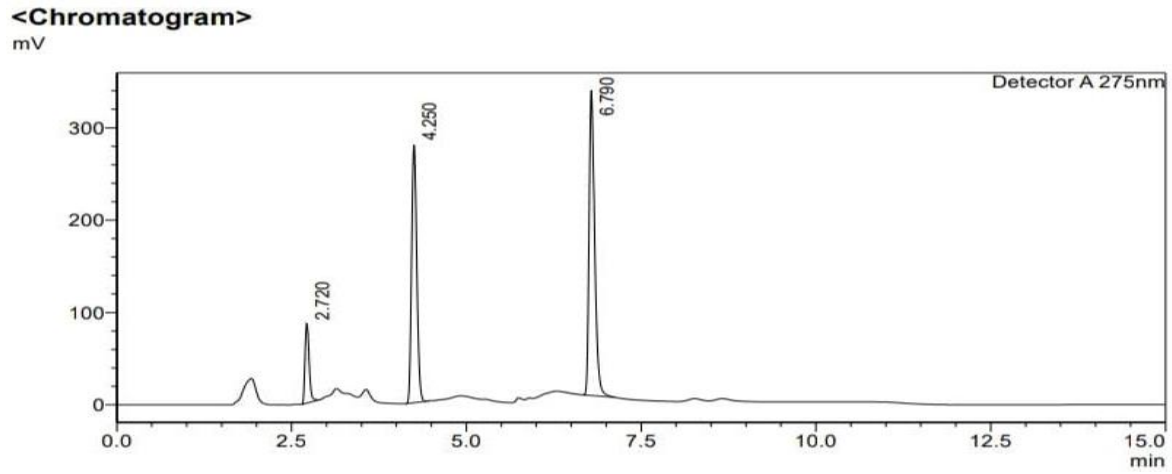

Figure 7: Standarad Chromotogram of Tannic acid, Catechol, Quercetin at 100 mcg.

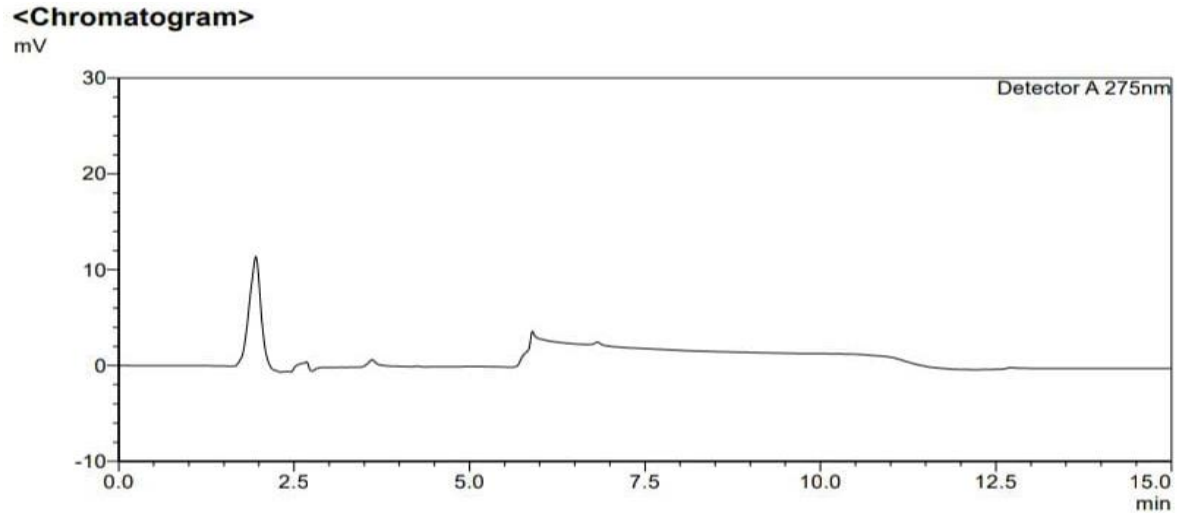

Figure 8: **Blank Chromotogram (Methanol).**

3.4.2 SAMPLE ANALYSIS: (***Cordia dichotoma*** **G. Forst leaf extract)**

The Hydroethanolic Extract Chromatogram Showed:

- Retention Time: **2.633 Min**
- Peak Area: **1838481**
- Tailing Factor: **1.753**
- Theoretical Plates: **6811**

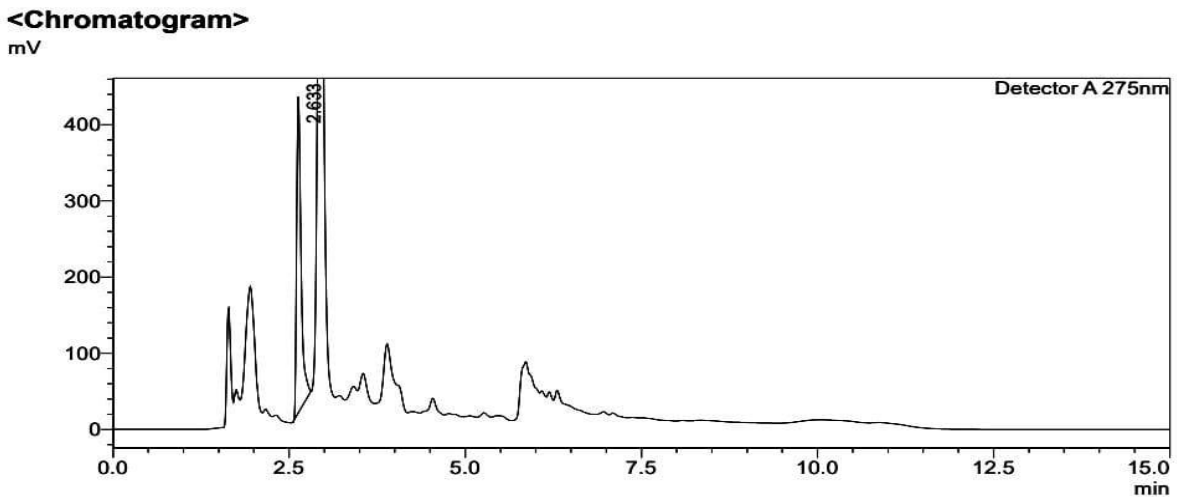

Figure 9: **HPLC Chromatogram of *Cordia dichotoma* G. Forst leaves extract.**

The hydroethanolic extract *Cordia dichotoma* G. Forst leaves was analyzed under optimized chromatographic conditions. The chromatogram exhibited a prominent peak at a retention time of 2.633 min with a peak area of 1838481, indicating the presence of a major phenolic compound.

Table 6: HPLC Chromatographic Parameters of *Cordia dichotoma* G. Forst leaves.

PARAMETER	VALUE
Retention time	2.633 min
Peak area	1838481
Tailing factor	1.753
Theoretical plates	6811

Table 7: Sapmle Retention time Comparision with Standards.

COMPOUND	RETENTION TIME
Tannic acid	2.720 min
Catechol	4.250 min
Quercetin	6.790 min

The observed peak closely matched the retention time of tannic acid, confirming its presence in the extract.[22-24]

4. DISCUSSION

The present study provides a systematic and comprehensive analytical evaluation of *Cordia dichotoma* G. Forst leaves, with particular emphasis on the interrelationship between extraction methodology, phytochemical composition, and instrumental characterization. The integration of qualitative screening, quantitative estimation, FTIR spectroscopy, and HPLC profiling collectively establishes that the plant is a significant source of bioactive secondary metabolites, predominantly polyphenolic compounds, which are known to contribute to its therapeutic potential.

The extraction process demonstrated a pronounced dependence on solvent polarity, which critically influenced the recovery of phytoconstituents. Among the various extracts, the hydroethanolic fraction exhibited the highest qualitative abundance of phytochemicals, especially flavonoids, tannins, and glycosides. This enhanced extraction efficiency can be attributed to the intermediate polarity of the hydroethanolic solvent system, which facilitates the solubilization of both polar and moderately polar compounds. In contrast, extraction with petroleum ether yielded negligible phytochemical content, indicating that non-polar solvents are less effective in isolating the major bioactive constituents of *Cordia dichotoma*. The chloroform extract demonstrated moderate presence of certain compounds such as alkaloids and glycosides, suggesting selective extraction of semi-polar constituents. Although the aqueous extract contained appreciable levels of carbohydrates and some glycosides, it exhibited comparatively lower concentrations of phenolic compounds. These observations underscore the importance of solvent selection in phytochemical investigations and are consistent with established extraction principles, where hydroalcoholic systems are widely recognized for their superior efficiency in extracting polyphenolic compounds.[25-26]

The quantitative analysis further reinforced these findings, revealing that the hydroethanolic extract contained significantly higher levels of total phenolics (96.4 mg/g), flavonoids (99.2 mg/g), and tannins (163.1 mg/g) compared to the aqueous extract. The predominance of these compounds is of considerable biological importance, as phenolics are well known for their ability to neutralize reactive oxygen species through electron donation, thereby reducing oxidative stress.[27-28] Flavonoids, which constitute a major class of polyphenols, are reported to regulate inflammatory pathways, inhibit lipid peroxidation, and provide vascular protection.[29-30] Similarly, tannins exhibit strong protein-binding capacity and antimicrobial activity, contributing to their role in wound healing and gastrointestinal protection. The markedly elevated levels of these bioactive constituents in the hydroethanolic extract highlight its enhanced therapeutic potential and justify its selection for further analytical investigation.

FTIR spectroscopic analysis provided molecular-level confirmation of the phytochemical composition of the extract. The presence of a broad absorption band around 3315 cm^{-1} is indicative of hydroxyl (–OH) groups, which are characteristic of phenolic compounds and flavonoids. The absorption peaks observed at 1565 cm^{-1} and 1516 cm^{-1} correspond to aromatic C=C stretching and ring vibrations, confirming the presence of conjugated aromatic systems typical of polyphenols. Additionally, bands in the region of 1247–1029 cm^{-1} were attributed to C–O and C–O–C stretching vibrations, suggesting the occurrence of alcohols, esters, and glycosidic linkages. The complexity observed within the fingerprint region further reflects the diverse structural nature of plant secondary metabolites. Collectively, the FTIR findings provide strong structural evidence supporting the presence of phenolic, flavonoid, and glycosidic compounds, thereby corroborating the results obtained from phytochemical screening and quantitative analysis.[31]

HPLC analysis offered definitive chromatographic validation and enabled precise identification of key phenolic constituents within the extract. The chromatographic profile revealed a prominent peak at a retention time of approximately 2.633 min, which closely aligns with the retention time of standard tannic acid, indicating its probable presence as a major component. Further comparison with standard chromatograms confirmed distinct retention times for catechol and quercetin, demonstrating effective separation and identification of individual phenolic compounds. The presence of a dominant peak with high peak area suggests that tannic acid or structurally related derivatives constitute a significant fraction of the extract.

Moreover, the calibration curves for tannic acid, catechol, and quercetin exhibited excellent linearity, reflecting high analytical precision and method reliability. Chromatographic parameters, including acceptable tailing factor and adequate theoretical plate count, further confirmed the efficiency and suitability of the analytical system. These results substantiate the presence of important phenolic biomarkers and highlight the applicability of HPLC as a reliable tool for phytochemical standardization.[32-33]

A strong concordance was observed among all analytical techniques employed in the study. Qualitative phytochemical screening established the presence of major metabolite classes, quantitative estimation confirmed their abundance, FTIR analysis provided structural validation, and HPLC profiling enabled precise identification of specific bioactive compounds. This integrated analytical approach significantly enhances the robustness, reliability, and scientific validity of the findings, demonstrating that *Cordia dichotoma* leaves possess a complex and diverse phytochemical composition.

The high concentration of phenolics and flavonoids identified in this study suggests that *Cordia dichotoma* exhibits substantial antioxidant potential, which may underlie its traditionally reported pharmacological activities. These compounds are known to play a crucial role in modulating oxidative stress, inflammatory responses, and microbial activity, thereby supporting their therapeutic application in various chronic and degenerative conditions. Furthermore, the identification of specific compounds such as tannic acid, catechol, and quercetin reinforces the pharmacological significance of the plant, as these molecules are widely recognized for their antioxidant, anti-inflammatory, and protective biological effects.[34-35]

Overall, the findings of the present investigation provide strong scientific evidence supporting the phytochemical richness and therapeutic relevance of *Cordia dichotoma* leaves, establishing a solid analytical foundation for future studies aimed at drug discovery and phytopharmaceutical development.

5. CONCLUSION

The present investigation provides a comprehensive analytical evaluation of *Cordia dichotoma* G. Forst leaves, integrating qualitative phytochemical screening, quantitative estimation, and advanced instrumental characterization techniques. The findings clearly demonstrate that the plant possesses a rich and diverse phytochemical profile, which substantiates its traditional medicinal relevance.

Preliminary phytochemical analysis confirmed the presence of multiple classes of bioactive secondary metabolites, including flavonoids, tannins, phenolic compounds, glycosides, alkaloids, and saponins. Among the various solvent extracts, the hydroethanolic extract exhibited the highest phytochemical abundance, highlighting the critical role of solvent polarity in efficient extraction of pharmacologically active constituents.

Quantitative analysis further validated these observations, revealing significantly elevated levels of total phenolics (96.4 mg/g), flavonoids (99.2 mg/g), and tannins (163.1 mg/g) in the hydroethanolic extract. These compounds are well known for their potent antioxidant, anti-inflammatory, and therapeutic properties, thereby indicating the strong bioactive potential of the plant.

FTIR spectral analysis provided structural confirmation of functional groups associated with polyphenols, flavonoids, and glycosides. The presence of characteristic absorption bands corresponding to hydroxyl, aromatic, and glycosidic linkages strongly supports the phytochemical composition identified through qualitative and quantitative methods.

HPLC analysis offered precise chromatographic validation of key phenolic constituents. The observed retention time (~2.63 min) closely corresponded to that of tannic acid, while comparison with standard chromatograms of catechol and quercetin confirmed the presence of these compounds. The well-defined peaks, acceptable tailing factor, and adequate theoretical plate count indicate good chromatographic resolution and system suitability. Furthermore, calibration data demonstrated excellent linearity, ensuring the reliability and reproducibility of the analytical method.

Collectively, the integration of phytochemical screening, spectroscopic characterization, and chromatographic profiling provides robust analytical evidence supporting the phytochemical richness of *Cordia dichotoma* G. Forst leaves. The presence of significant levels of phenolic and flavonoid compounds suggests strong antioxidant potential and validates its traditional therapeutic applications.[36-37]

In conclusion, *Cordia dichotoma* G. Forst leaves represent a promising natural source of bioactive phytoconstituents with significant therapeutic potential. The study provides a strong scientific foundation for further exploration of this plant in the development of novel phytopharmaceuticals and evidence-based herbal formulations.

6. LIMITATIONS AND FUTURE PERSPECTIVES

The present study is limited to analytical characterization and does not include isolation and structural elucidation of individual bioactive compounds. Additionally, biological activity was not experimentally evaluated using *in vitro* or *in vivo* models. Future research should focus on bioactivity-guided fractionation, advanced characterization using LC–MS and NMR techniques, and

pharmacological validation to establish clinical relevance and therapeutic efficacy.

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CONFLICT OF INTEREST: The authors declare there is no conflict of interest.

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