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PHYTOCHEMICAL PROFILING AND STRUCTURAL CHARACTERIZATION OF BIOACTIVE CONSTITUENTS FROM THE ETHANOLIC LEAF EXTRACT OF CORDIA MACLEODII USING HIGH-RESOLUTION LC-MS ANALYSIS

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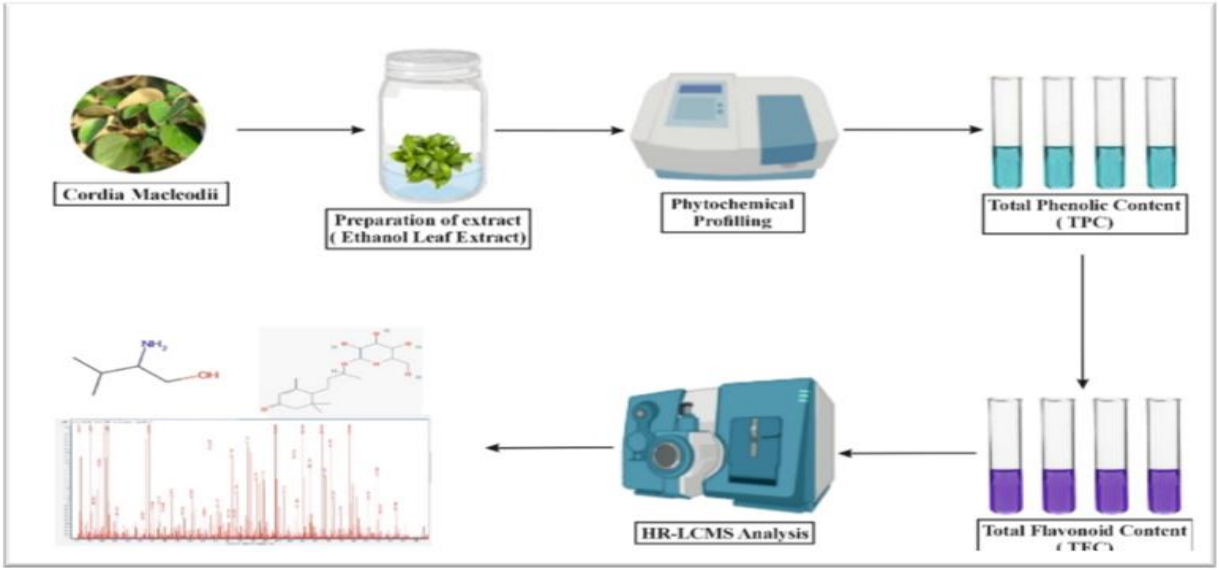
ABSTRACT

Background: *Cordia macleodii*, a critically endangered species recognized for its medicinal properties, possesses considerable ethnopharmacological significance; however, the comprehensive exploration of its phytochemical constituents remains limited. **Results:** The ethanolic leaf extract of *Cordia macleodii* underwent rigorous analysis via High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LC-MS/QTOF), which facilitated the identification of an array of bioactive constituents, encompassing phenolic acids, flavonoids, fatty acid derivatives, and glycosidic compounds. These identified compounds are associated with significant pharmacological activities, which include antioxidant, anti-inflammatory, and antidiabetic properties. **Conclusion:** The detailed phytochemical profiling of *Cordia macleodii* lays a critical groundwork for subsequent functional investigations, thereby integrating traditional medicinal practices with contemporary drug discovery methodologies, while underscoring its therapeutic potential as a reservoir of plant-derived bioactive compounds. **Highlights:** In-depth HR-LC-MS/QTOF analysis of the ethanolic leaf extract of *Cordia macleodii*. Identification of phenolic acids, flavonoids, and glycosidic compounds. Prospective therapeutic applications, encompassing antioxidant, anti-inflammatory, and antidiabetic activities. Integration of traditional medicinal applications with modern phytochemical validation.

KEYWORDS: *Cordia macleodii*, HR-LC-MS, phytochemical profiling, bioactive compounds, ethanolic extract, structural characterization, medicinal plant, metabolomics, anti-inflammatory, antioxidant, antidiabetic.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Medicinal plants have long served as a cornerstone for traditional remedies and modern drug discovery, offering a rich reservoir of bioactive compounds with diverse therapeutic properties. Among these, *Cordia macleodii*, commonly referred to as "Sanjivani" in local traditions of Chhattisgarh, India, has drawn increasing attention due to its reported ethnomedicinal uses in treating inflammatory disorders, infections, and chronic ailments. Despite its therapeutic potential, scientific investigations into its phytochemical composition remain limited, necessitating further research to elucidate its active constituents and pharmacological mechanisms. Understanding the bioactive compounds present in *Cordia macleodii* could pave the way for novel therapeutic applications and enhance its integration into modern medicine.[6-9]

Further research is essential to unlock the full spectrum of its bioactive constituents and validate its traditional uses through rigorous pharmacological studies. Such investigations could pave the way for developing novel therapeutic agents derived from this promising plant.[1]

Adding to the concern is the plant's declining natural population. *Cordia macleodii* is considered endangered in parts of its native range due to overharvesting, habitat degradation, and lack of cultivation initiatives. The dual importance of this species—both medicinal and ecological—necessitates urgent efforts not only toward its conservation but also toward understanding its bioactive chemical constituents at a molecular level.

The identification of phytochemicals through advanced analytical techniques is critical for decoding the therapeutic potential of lesser-studied medicinal plants. Previous studies on related species within the *Cordia* genus have revealed the presence of flavonoids, alkaloids, terpenoids, and phenolic acids—classes of compounds known for their antioxidant, anti-inflammatory, and anticancer activities. However, there is a notable lack of detailed chemical profiling of *Cordia macleodii* using high-resolution methods. By employing HR-LC-MS/QTOF analysis, this research aims not only to identify and structurally characterize bioactive constituents but also to lay the groundwork for future pharmacological evaluations.[4]

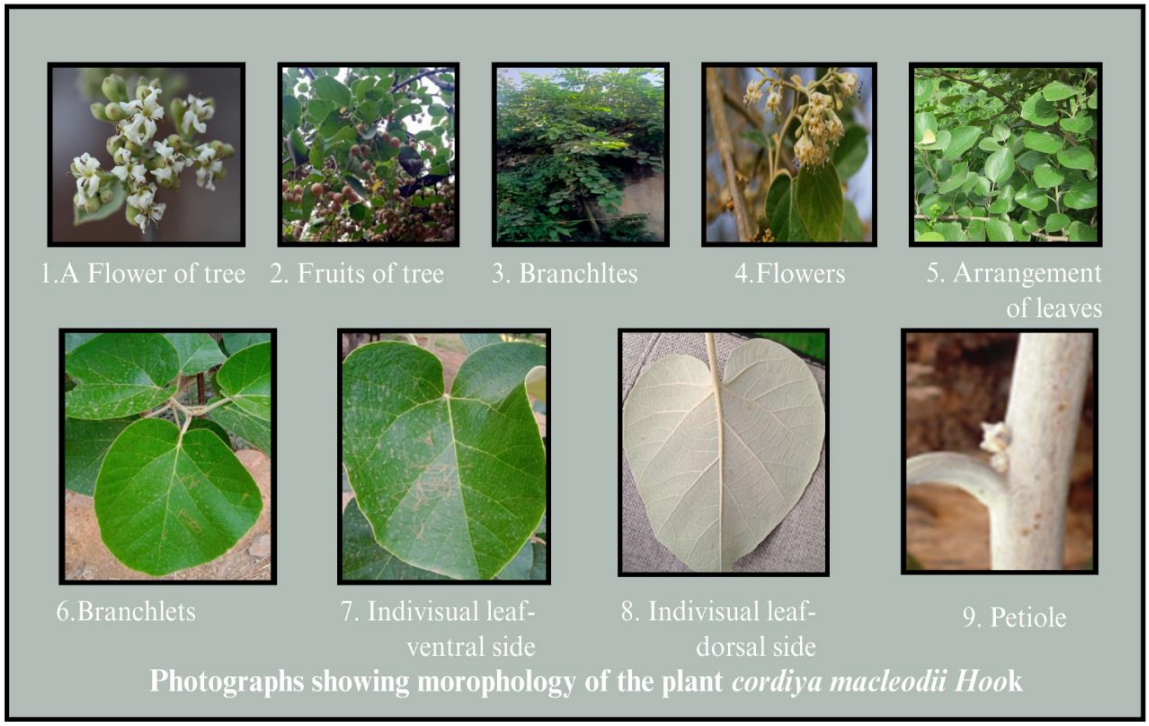


Figure 1: Morphological characteristics of *Cordia macleodii Hook*, depicting various anatomical features including (1) Flower of the tree, (2) Fruits, (3) Branchlets, (4) Flowers, (5) Leaf arrangement, (6) Branchlets, (7) Individual leaf (ventral side), (8) Individual leaf (dorsal side), and (9) Petiole.

2. METHOD AND MATERIAL

2.1 Chemicals and Reagents

All chemical compounds and reagents utilized in this investigation, encompassing methanol, ethanol, acetonitrile (HPLC and LC-MS grade), formic acid, and deionized water, were acquired from Merck (Mumbai, India) and Thermo Fisher Scientific (Waltham, MA, USA). Gallic acid, quercetin, and the Folin-Ciocalteu reagent employed for the determination of total phenolic content (TPC) and total flavonoid content (TFC) were sourced from Sigma-Aldrich (St. Louis, MO, USA). Aluminium chloride, sodium carbonate, and sodium nitrite were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Prior to application, all solvents and reagents were subjected to filtration through a 0.22 µm membrane filter to ensure the precision of analytical results.

2.2 Collection and Authentication of Plant Materials

In October 2023, viable specimens of *Cordia macleodii* were systematically collected by the Kanker Forest Department from the ecologically diverse region of Kanker District in Chhattisgarh, India. The sampling strategy was meticulously formulated to prioritize representative and pathogen-free specimens to ensure the validity of the subsequent analyses. The taxonomic identification and authentication were conducted by the Chief Botanist at Rashtrasant Tukadoji Maharaj Nagpur University, India. A voucher specimen (No. 371) was carefully prepared and archived in the university's herbarium for both archival and prospective reference purposes. The methodologies employed for collection and authentication were executed in alignment with the protocols delineated in our previous investigation.[5]

2.2.1 Preparation of extract

The fresh foliage of *Cordia macleodii* underwent a thorough washing process utilizing running water and was subsequently shade-dried over a duration of seven days to eliminate superficial contaminants. Approximately 1200 g of the dehydrated material was finely ground and securely stored in hermetically sealed containers to preserve its integrity. Sequential solvent extraction was performed on 120 g of this powdered material employing ethanol, methanol, and distilled water in an ascending order of polarity. The extraction procedure persisted until the solvent exhibited a colorless appearance in the siphon apparatus. The resultant filtrates were subjected to filtration using Whatman No. 1 filter paper, concentrated under reduced pressure at 40 °C within a vacuum oven, and preserved under cool, dark conditions for subsequent phytochemical and biological evaluations (Mohammed Golam Rasul, 2018). The extraction methodology was conducted in accordance with our established protocol, as previously described.[5]

2.3 Phytochemical analysis

2.3.1 Estimation of total phenolic contents (TPC)

The overall phenolic content of *Cordiya Macleodii* was quantified utilizing the protocol[6] with certain modifications. The standardization curve of gallic acid served as the reference control. A mixture comprising 100 µL of the test sample, 400 µL of distilled H2O, and 100 µL of Folin-Ciocalteu reagent was subsequently diluted with 500 µL of sodium carbonate (7%) and allowed to incubate at room temperature for a duration of thirty minutes. At a wavelength of 765 nm, the absorbance was recorded using a Jenway 7415 Scanning UV/Visible Spectrophotometer. The total phenolic content (TPC) was calculated employing the following formula and expressed in mg/g of gallic acid equivalent (GAE) based on dry weight (dw).

The equation for the standard curve was determined to be $y=0.0031x+1.4688$ ($R^2=0.9916$).

Total phenolic content (TPC) = cV/m

where 'TPC' denotes the total phenolic content in mg GAE/g dw, 'c' represents the concentration of gallic acid (mg/mL), 'V' indicates the volume of the extract (mL), and 'm' corresponds to the mass of the extract (g).

2.3.2 Estimation of total flavonoid contents (TPC)

The colorimetric assay utilizing aluminum chloride was implemented to ascertain the total flavonoid content inherent in *Cordiya Macleodii*.[4] The procedure involved the introduction of plant extracts (1 mL) into distilled H2O (4 mL), followed by the addition of sodium nitrite (5%; 0.3 mL) and aluminum chloride (10%; 0.3 mL), which were then subjected to incubation for a period of 6 minutes. Furthermore, 1 M NaOH (2 mL) was introduced into the mixture. The absorbance was subsequently recorded at a wavelength of 510 nm. The quantification of total flavonoid content (TFC) was conducted utilizing a calibration curve based on quercetin, expressed as mg/g Quercetin Equivalent (QE) on a dry weight basis. The equation representing the standard curve was established as $y=0.0294x+0.5003$ ($R^2=0.9866$).

Total flavonoid content (TFC) = cV/m

where ‘TFC’ signifies total flavonoid content in mg QE/g dry weight, ‘c’ denotes quercetin concentration (mg/mL), ‘V’ represents extract volume (mL), and ‘m’ indicates extract mass (g).

2.4 High Resolution Liquid Chromatography – Mass Spectroscopy (HR-LCMS)

The comprehensive LC–MS/MS-QTOF investigation delineates the entirety of the phytochemical constituents present in the ethanolic extract of *Cordia macleodii*, employing a UHPLC-ESI-QTOF-MS apparatus (Agilent 6545XT Advance Bio LC/Q-TOF) which is operated using Mass Hunter software (version B.06.01) for the acquisition and analysis of data. The analysis was performed at the Central Instrumentation Facility, Indian Institute of Technology Bhilai, Chhattisgarh, India. The chromatographic separation was successfully accomplished utilizing a Zorbax Eclipse C18 column (2.1 × 150 mm, 5 µm) through a gradient elution comprising 0.1% formic acid in water (solvent A) and acetonitrile containing 10% water and 0.1% formic acid (solvent B) at a flow rate of 0.2 mL/min. The established gradient profile was as follows: from 2 to 20 minutes (A 95%: B 5%), from 20 to 25 minutes (A 5%: B 95%), and from 26 to 30 minutes (A 95%: B 5%). The mass spectrometric data were acquired in positive ion mode with capillary, source cone, and extraction cone voltages consistently held at 3.25 kV, 30 V, and 4 V, respectively. Nitrogen was employed as the desolvation gas at a flow rate of 900 L/h, with the source and desolvation temperatures calibrated at 120°C and 550°C, respectively. Mass spectra were documented over an m/z range of 100–1,200, achieving a resolution of 22,000 FWHM. The processing of data and identification of metabolites were undertaken using Progenesis QI software (version 2.2, Waters), leveraging a bespoke in-house database constructed from previously documented

metabolites of Cordia spp. sourced from Metlin, PubChem, HMDB, ChemSpider, ChEBI, and CHEMBL.

3. RESULTS AND DISCUSSION

Total Flavonoid Content (TFC) in Cordia macleodii Leaf Extracts

The total flavonoid content (TFC) in the ethanol, methanol, and aqueous extracts of *Cordia macleodii* foliage was assessed and is delineated in Table 1. The TFC values are quantified as milligrams of flavonoid per gram of dry extract (mg/g). The results reveal a significant heterogeneity in flavonoid concentrations among the extracts, as depicted in Figure 2 (Bar Graph) and Figure 3 (Line Graph). Among the various extracts, the ethanol extract manifested the highest flavonoid concentration at 76.90 ± 20.98 mg/g, thereby indicating that ethanol serves as an exceptionally effective solvent for the extraction of flavonoids from *Cordia macleodii* leaves.[7] This observation is consistent with prior investigations that have identified ethanol as a superior solvent for the extraction of polyphenolic compounds owing to its intermediate polarity, which facilitates the dissolution of both polar and nonpolar compounds efficiently. The methanol extract revealed a moderate flavonoid concentration of 56.15 ± 10.19 mg/g, which is significantly lower than that of the ethanol extract. Notwithstanding its efficacy in extracting certain phenolic compounds, the comparatively lower polarity of methanol relative to ethanol may elucidate its diminished extraction efficiency. The aqueous extract displayed the lowest flavonoid concentration, quantified at 35.39 ± 5.86 mg/g. This reduced concentration is likely attributable to the limited solubility of numerous flavonoids in aqueous solutions, which predominantly extract more polar compounds. The bar graph (Figure 2) proficiently illustrates the quantitative disparities in flavonoid content across the three extracts, accentuating the marked flavonoid yield in the ethanol extract. Correspondingly, the line graph (Figure 3) accentuates the trend in the variation of flavonoid content, further emphasizing the efficacy of ethanol as a solvent for the extraction of flavonoids from *Cordia macleodii* leaves.

Table 1: Total flavonoid content in different *cordia macleodii* leaf extracts.

S. no.	Different leaf extract	Total Flavonoid Content (mg/g ±S. E)
1.	Ethanol leaf	76.90 ± 20.98
2.	Methanol leaf	56.15 ± 10.19
3.	Aqueous leaf	35.39 ± 5.86

Line graph representation of Flavonoid content (mg/g) in ethanol, methanol, and aqueous leaf extracts.

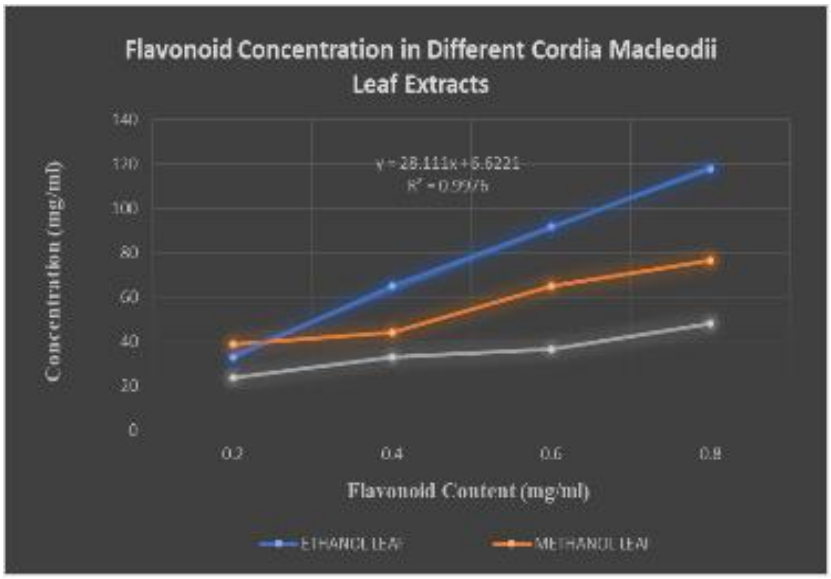


Figure 2: Total flavonoid content in different *cordia macleodii* leaf extracts.

Bar graph representation of Flavonoid content in ethanol, methanol and aqueous leaf extracts

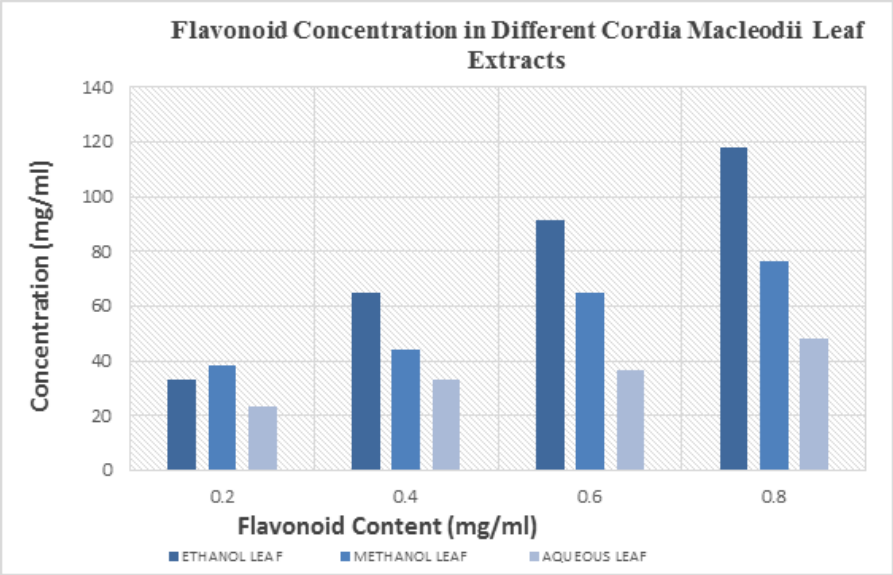


Figure 3: Total flavonoid content in different *cordia macleodii* leaf extracts.

Total Phenolic Content (TPC) in Cordia macleodii Leaf Extracts

The quantitative analysis of the total phenolic content (TPC) in the ethanol, methanol, and aqueous extracts derived from *Cordia macleodii* leaves is systematically presented in Table 2. The TPC values, denoted in milligrams of phenolic content per gram of dry extract (mg/g), exhibit significant variability among the three solvent systems, as illustrated in Figure 4 (Bar Graph) and Figure 5 (Line Graph). The ethanol extract exhibited the highest phenolic content, quantified at 116.52 ± 10.66 mg/g, signifying its superior efficacy in the extraction of phenolic compounds.[6] Ethanol is extensively acknowledged for its capability to solubilize both polar and non-polar phenolic compounds, thereby facilitating a greater recovery of polyphenols in contrast to methanol and aqueous solvents. The elevated phenolic content observed in the ethanol extract may suggest a substantial presence of bioactive phenolic acids and flavonoids, which are recognized for exhibiting robust antioxidant and cytotoxic properties. The methanol extract, presenting a TPC of 97.18 ± 14.24 mg/g, demonstrated a moderate level of phenolic content. Methanol, classified as a semi-polar solvent, is proficient in the extraction of specific phenolic compounds; nonetheless, its diminished efficacy relative to ethanol may be attributed to the preferential extraction of more polar phenolics, culminating in a comparatively lower yield. The aqueous extract recorded the minimal phenolic content, quantified at 94.82 ± 14.24 mg/g. This reduced phenolic content can be ascribed to the limited solubility of various polyphenolic compounds within an aqueous medium. Generally, water is more adept at extracting hydrophilic compounds such as tannins and glycosides, while being less effective for the extraction of lipophilic phenolics.

Table 2: Total phenolic content in different *cordia macleodii* leaf extracts.

S. no.	Different Leaf Extract	Total Content ±S.E. (mg/g)
1.	Ethanol Leaf	116.52 ± 10.66
2.	Methanol Leaf	97.18 ± 14.24
3.	Aqueous Leaf	94.28 ± 14.24

Line graph representation of Phenolic content (mg/g) in ethanol, methanol, and aqueous leaf extracts.

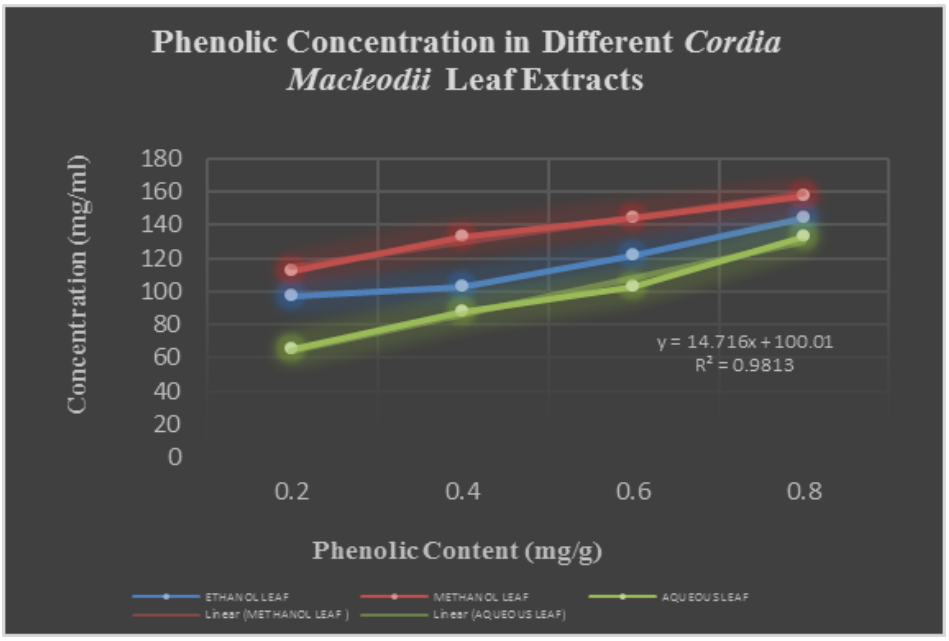


Figure 4: Total flavonoid content in different *cordia macleodii* leaf extracts.

Bar graph representation of Phenolic content in ethanol, methanol, and aqueous Stem extracts.

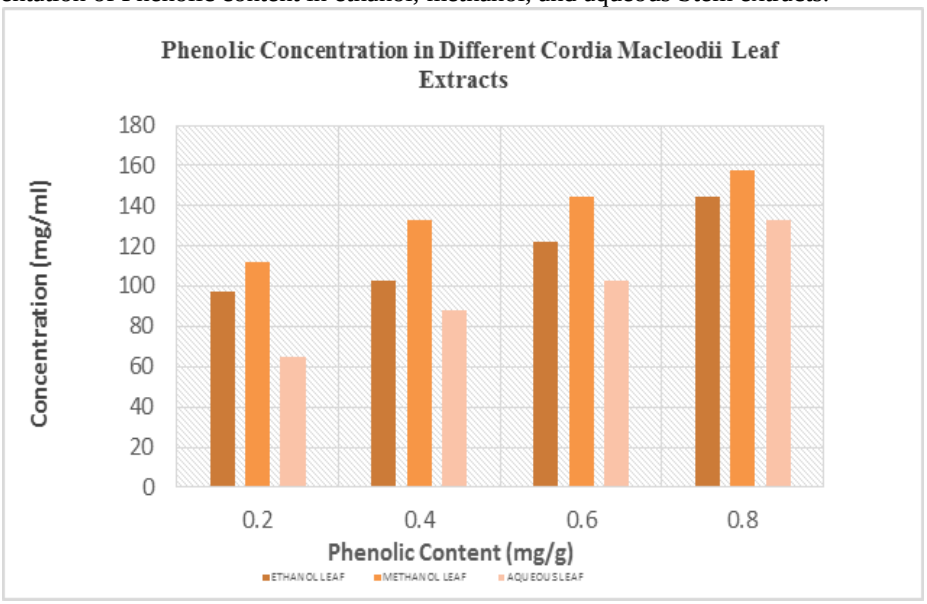


Figure 5: Total phenolic content in different *cordia macleodii* leaf extracts.

LC–MS/MS-QTOF analysis

The liquid chromatography-mass spectrometry coupled with quadrupole time-of-flight (LC-MS/QTOF) analysis of the ethanolic leaf extract derived from *Cordia macleodii* facilitated the discernment of a vast array of bioactive constituents, encompassing phenolic acids, alkaloids, fatty acids, glycosides, and flavonoid derivatives, as illustrated in the chromatographic representation (Figure 6).[8] The compounds that were identified, along with their respective retention times (Rt), molecular formulas, and mass-to-charge (m/z) ratios, are meticulously cataloged in Table 3. Noteworthy phytochemicals that were recognized include 2-Methoxy-3-(2-propenyl) phenol (Rt = 3.92 min, m/z = 164.0996), a derivative of phenol, and 1,2,3-Propanediol diacetate (Rt = 5.63 min, m/z = 176.9783), classified as a glycerol ester. The mass spectra corresponding to these compounds are depicted in Figures 7 and 8,[9] respectively.

Fatty acids such as Hexadecanoic acid (Rt = 7.45 min, m/z = 256.2453) and 9,12-Octadecadienoic acid (Rt = 6.21 min, m/z = 280.9941) were observed, thereby indicating the presence of lipid-derived metabolites that possess established anti-inflammatory properties (Figures 9 and 10). The identification of C16 sphingosine (Rt = 5.12 min, m/z = 244.1738) accentuates the lipidic composition of the extract, as illustrated in Figure 11.A variety of alkaloids, including Affinine (Rt = 8.01 min, m/z = 297.0238) and Lochnerinine (Rt = 8.21 min, m/z =

371.0359), were detected, both of which are acknowledged for their neuroprotective and anticancer properties. The mass spectra for these compounds are presented in Figures 12 and 13.[10] Significantly, 14 β-Hydroxyyohimbine (Rt = 9.76 min, m/z = 343.2031) further substantiates the existence of alkaloidal derivatives with potential pharmacological significance (Figure 14).

The glycosides Blumenol C glucoside (Rt = 10.12 min, m/z = 355.0058) and Benzyl-O-(arabinofuranosyl-(-1->6)-glucoside (Rt = 7.65 min, m/z = 392.9414) were identified, demonstrating notable antioxidant capabilities (Figures 15 and 16). Moreover, Ganoderol A (Rt = 11.32 min, m/z = 411.0248), a triterpenoid recognized for its anti-inflammatory effects, was prominently detected (Figure 17). The identification of Tap Asp Glu (Rt = 10.87 min, m/z = 429.0182) and Luteolin 7-rhamnosyl (1->6) galactoside (Rt = 10.42 min, m/z = 447.0272) signifies the presence of bioactive peptides and flavonoid glycosides endowed with potential cytotoxic and antioxidant properties (Figures 18 and 19). Furthermore, an exceptional dihydrochalcone derivative, 2',4',6',3'-Tetrahydroxy-3'-geranyl-6",6"-dimethylpyrano-[2",3",4,5]-dihydrochalcone (Rt = 10.815 min, m/z = 505.01), was also discerned (Figure 20).[11]

In conclusion, the extensive phytochemical profiling of the ethanolic leaf extract of *Cordia macleodii* unveils a comprehensive spectrum of bioactive metabolites possessing significant pharmacological relevance, consistent with its traditional medicinal applications. These findings provide a foundational basis for subsequent pharmacological inquiries aimed at elucidating the therapeutic potential of the compounds identified.

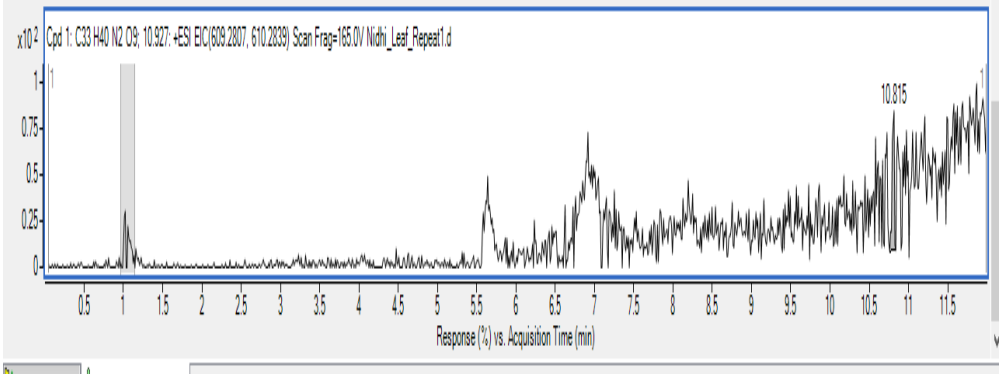


Figure 6: The chromatogram resulting from liquid chromatography of the ethanol leaf extract sourced from *Cordia macleodii*, acquired via ultra-high-performance liquid chromatography in conjunction with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS), delineates peaks that signify the presence of major compounds, particularly Palmitic acid and Oleic acid, as identified through their corresponding m/z fragmentation profiles.

Table 3: Enumeration of principal chemical constituents identified from ethanolic extracts of the foliage of *Cordiya macleodii* utilizing Ultra High Performance – High Resolution Liquid Chromatography Mass Spectrometry (UHPLC-HRMS).

S. No	Name of The Compound	Retention Time (Rt) (Min)	Molecular Formula	M/Z Value	Class
1	Phenol,2-Methoxy-3-(2-propenyl)	3.92	C10H12O2	164.0996	Phenol derivative
2	1,2,3, - Propanediol diacetate	5.63	C17H12O5	176.9783	Glycerol ester
3	9- Aminoacnidine	4.45	C13H10N2	223.0128	Aminoacridine derivative
4	Hexadecanoic acid	7.45	C16H32O2	256.2453	Fatty acid
5	9,12- Octadecadicnoic acid	6.21	C18H32O2	280.9941	Polyunsaturated fatty acid
6	(C16 sphingamine)	5.12	C16H35NO2	244.1738	Sphingolipid
7	Affinine (Alkaloid-G)	8.01	C20H24N2O2	297.0238	Alkaloid
8	Hydroxy-3,7-dimethyl 2,7-octadienyloxy)-7-methoxy coumarin	8.92	C20H24O5	327.2095	Coumarin derivative
9	Hydropenoxy-12,13-epoxy-9-octadecenoic acid	9.45	C18H32O5	337.0125	Epoxy fatty acid
10	(14 β-Hydroxyyohimbine)	9.76	C21H26N2O4	343.2031	Alkaloid derivative
11	(Blumenol C glucoside)	10.12	C19H32O7	355.0058	Glucoside

12	Lochnerinine	8.21	C22H26N2O4	371.0359	Alkaloid
13	Benzyl-O-(arabinofuranosyl-(-1->6)-glucoside	7.65	C18H26O10	392.9414	Glycoside
14	(Ganoderol A)	11.32	C30H46O2	411.0248	Triterpenoid
15	Tap Asp Glu	10.87	C20H24N4O8	429.0182	Peptide
16	Luteolin 7-rhamnosyl (1->6) galactoside	10.42	C27H30O15	447.0272	Flavonoid glycoside
17	2',4',6',3'-Tetrahydroxy-3'-geranyl-6",6"-dimethylpyrano-[2",3",4,5]-dihydrochalcone	10.815	C30H36O6	505.01	Dihydrochalcone derivative

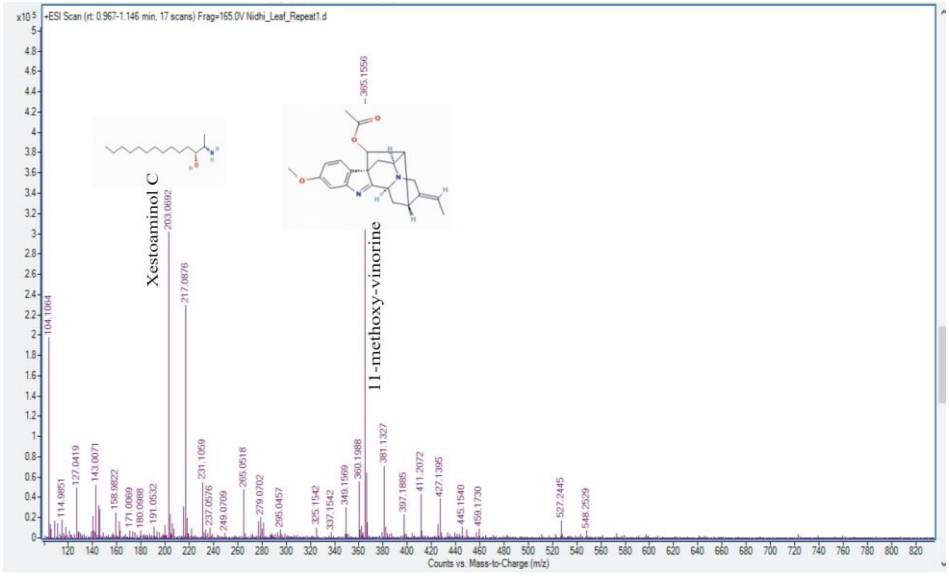


Figure 7: Mass spectrum at the retention time 0.967.

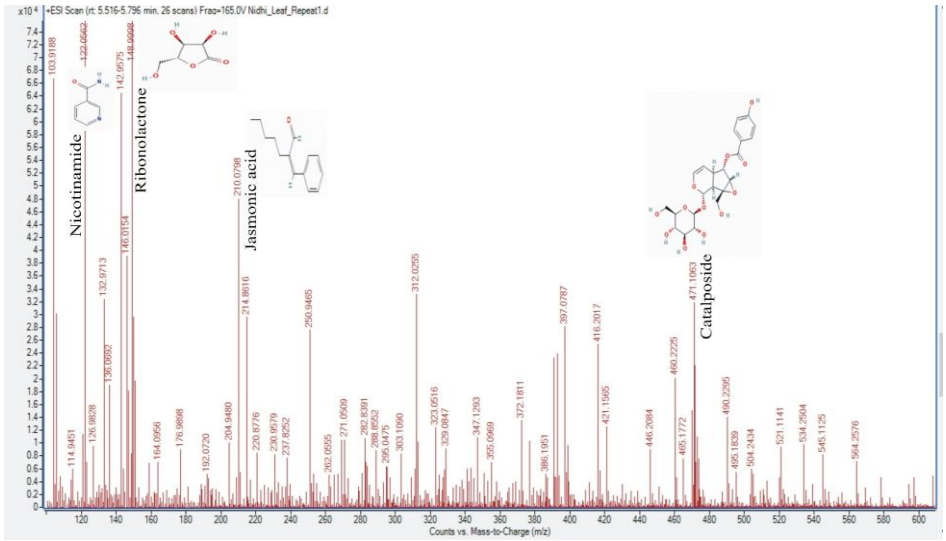


Figure 8: Mass spectrum at the retention time 5.516.

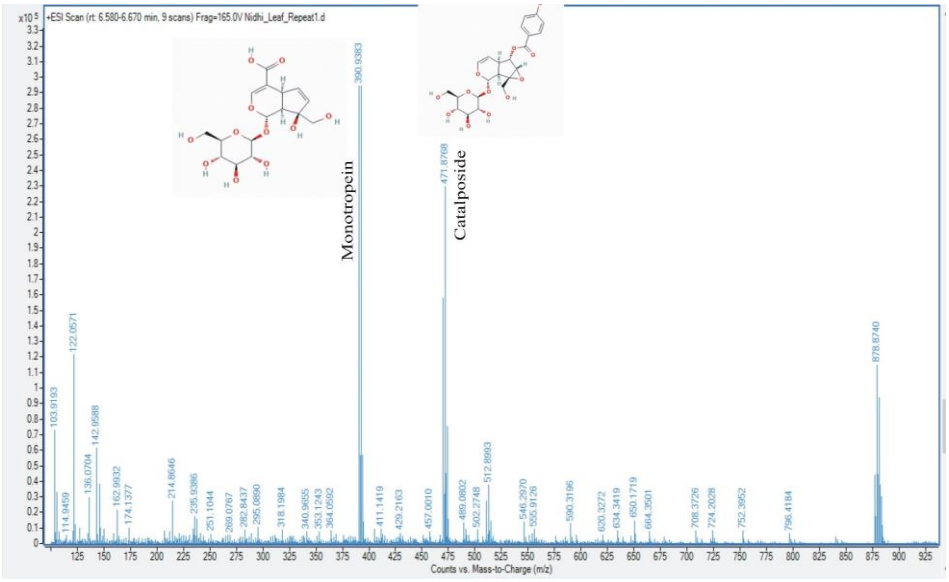


Figure 9: Mass spectrum at the retention time 6.580.

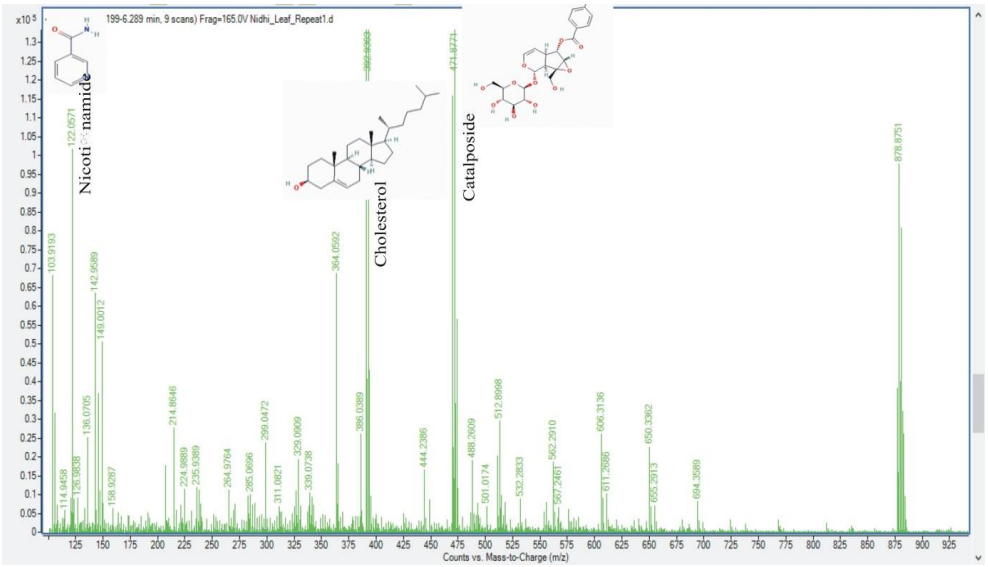


Figure 10: Mass spectrum at the retention time 6.199.

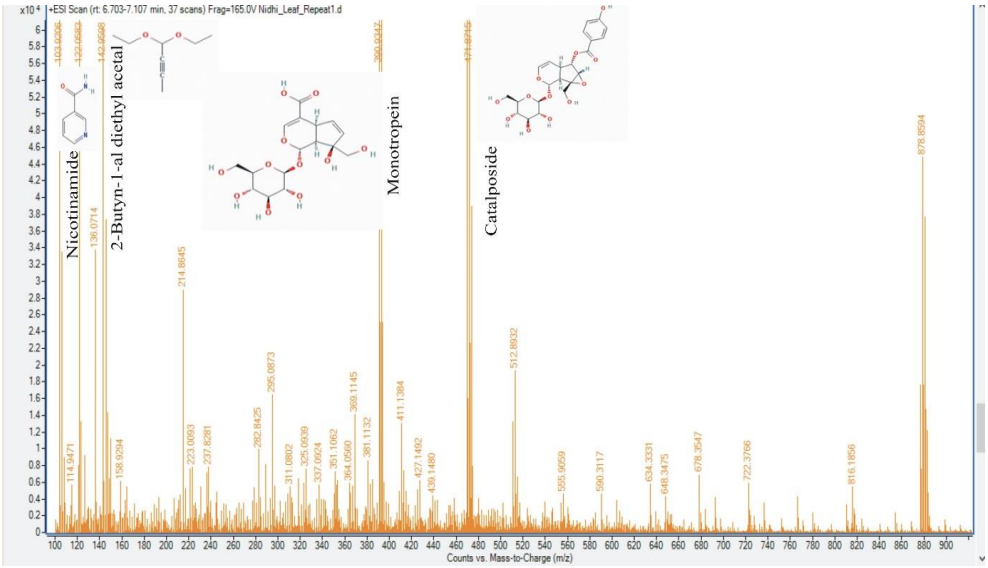


Figure 11: Mass spectrum at the retention time 6.703.

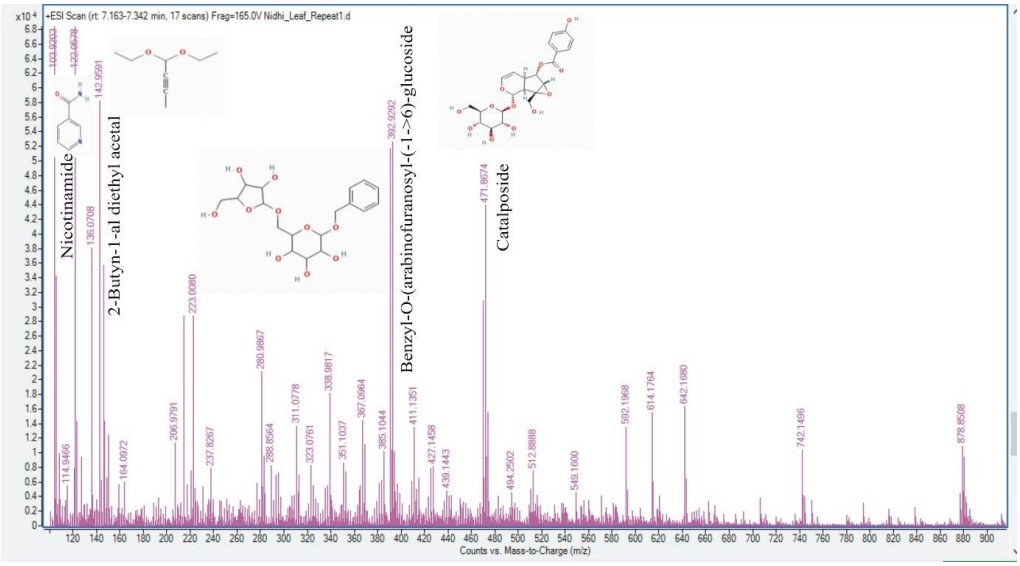


Figure 12: Mass spectrum at the retention time 7.163.

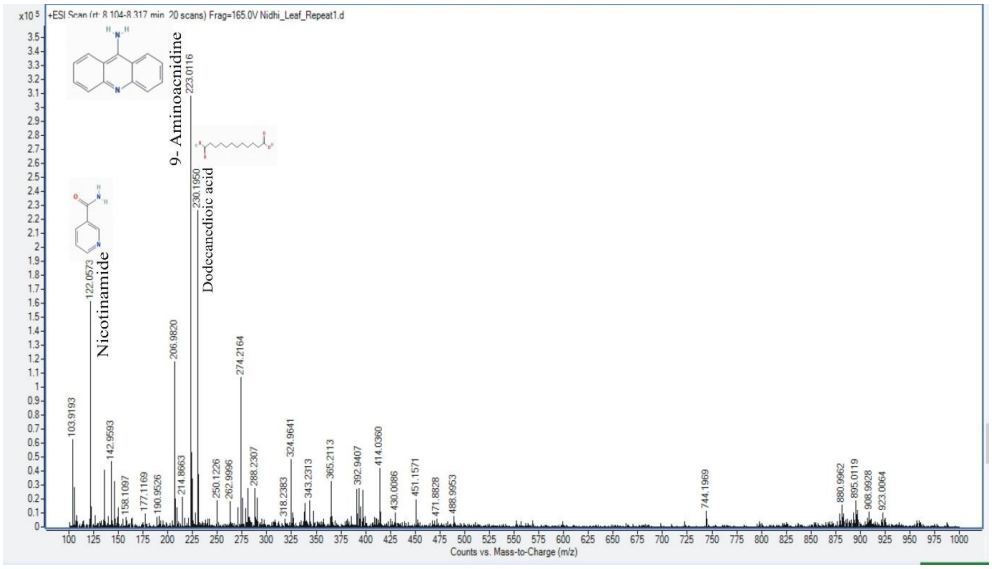


Figure 13: Mass spectrum at the retention time 8.104.

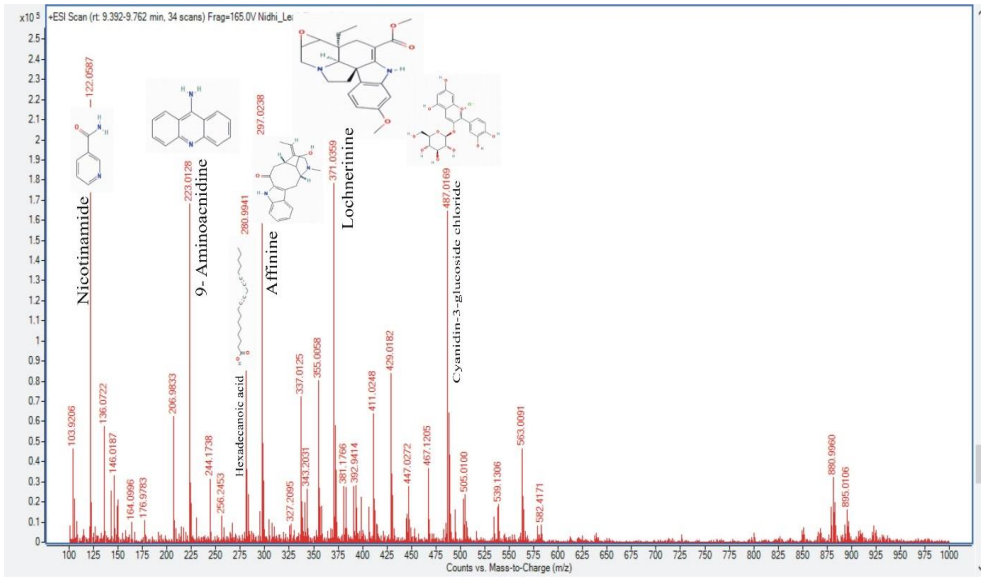


Figure 14: Mass spectrum at the retention time 9.392.

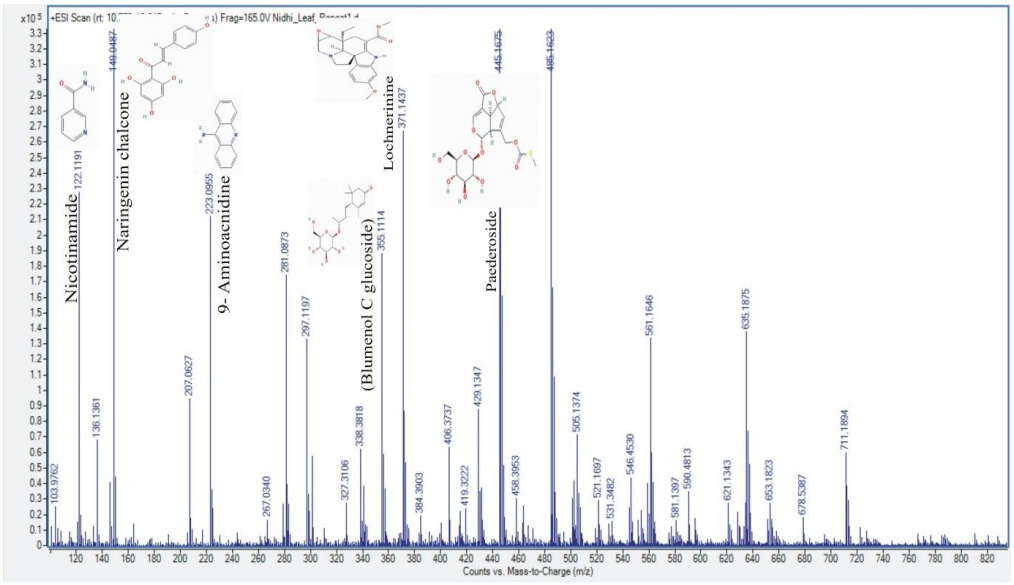


Figure 15: Mass spectrum at the retention time 10.770.

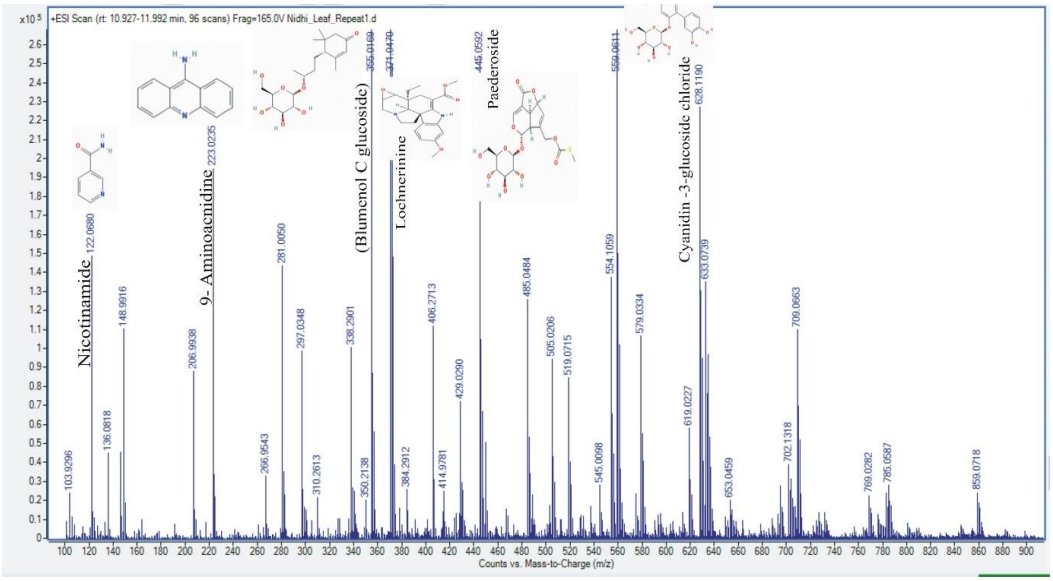


Figure 16: Mass spectrum at the retention time 10.927.

4. CONCLUSION

The extensive phytochemical analysis and structural elucidation of bioactive metabolites present in the ethanolic leaf extract of *Cordia macleodii*, conducted via LC-MS/QTOF methodology, unveiled a heterogeneous assortment of compounds encompassing phenolic acids, alkaloids, fatty acids, glycosides, and flavonoid derivatives. The identification of specific compounds, such as Hexadecanoic acid, Affinine, Ganoderol A, and Luteolin 7-rhamnosyl galactoside, highlights the medicinal significance of *Cordia macleodii*, which is in concordance with its traditional ethnomedicinal applications for anti-inflammatory, antioxidant, and anticancer purposes. Moreover, the identification of glycosides and coumarin derivatives indicates the existence of compounds that may exhibit neuroprotective and antimicrobial properties. The outcomes of this investigation furnish critical insights into the phytochemical composition of *Cordia macleodii*, thereby establishing a foundation for subsequent pharmacological assessments aimed at substantiating its therapeutic efficacy and investigating its potential as a source of plant-derived bioactive compounds for pharmaceutical development. Additionally, these findings accentuate the necessity of preserving Cordia macleodii as a repository of therapeutically relevant phytochemicals, thereby reinforcing the imperative for sustainable exploitation and further bioprospecting initiatives.

Conflicts of interest

The authors declare no conflicts of interest.

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