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ORIGINAL ARTICLE

UNVEILING THE CHARACTERIZATION OF BIOACTIVE CHEMICAL COMPOUNDS FROM *RUELLIA PROSTRATA* LEAF PETIOLE EXTRACT USING SPECTROSCOPIC TECHNIQUES

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ABSTRACT : The present study focuses on the comprehensive characterization of bioactive phytochemical compounds present in the hydro-ethanolic extract of Ruellia prostrata leaf petiole using advanced spectroscopic and chromatographic techniques. Preliminary phytochemical screening confirmed the presence of diverse secondary metabolites, including flavonoids, alkaloids, terpenoids, saponins, polyphenols, glycosides and steroids. UV–Visible spectroscopy revealed characteristic absorption peaks corresponding to conjugated sypetioles and phenolic compounds, while FTIR analysis identified key functional groups such as hydroxyl, carbonyl, amine and aromatic groups, indicating structurally diverse bioactive constituents. Further, LC–MS analysis enabled the identification of fourteen compounds, including hydroxybenzoic acid derivatives, flavonoids (apigenin- and kaempferol-like compounds), quinoline-type alkaloids and several high molecular weight triterpenoid and steroidal saponins, with good database matching scores. The predominance of saponins and polyphenolic compounds suggests significant pharmacological potential, particularly in antioxidant, antimicrobial, and anti-inflammatory applications. The integration of multiple analytical techniques provided a robust and reliable approach for phytochemical profiling, enhancing the accuracy of compound identification. The findings highlight *Ruellia prostrata* as a promising natural source of bioactive compounds with potential applications in pharmaceuticals, nutraceuticals, and functional food development. This study contributes to the growing body of research on plant-based therapeutics and supports the use of advanced analytical tools for comprehensive phytochemical investigations.

Key words : *Ruellia prostrate*, phytochemical characterization, LC–MS, FTIR, UV–Visible spectroscopy, bioactive compounds, saponins, flavonoids, terpenoids, natural products.

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INTRODUCTION

Medicinal plants have long been recognized as invaluable reservoirs of biologically active molecules that underpin both traditional healing sypetioles and modern therapeutic innovations (Kumar and Lee, 2026). In recent decades, there has been a paradigm shift toward the exploration of plant-derived bioactive compounds due to their diverse pharmacological activities and relatively low toxicity profiles. These compounds primarily secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids, and glycosides which play crucial roles in plant defense mechanisms while simultaneously offering immense therapeutic potential for humans (Kumar *et al*, 2024). Their biological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective effects, have positioned medicinal plants at the forefront of drug discovery and nutraceutical development (Zhang and Li, 2025).

The increasing global burden of chronic diseases, coupled with the alarming rise in antimicrobial resistance, has intensified the search for alternative and sustainable therapeutic agents. Plant-based bioactive compounds

have emerged as promising candidates due to their ability to interact with multiple biological targets and pathways. Recent studies highlight that medicinal plants are being extensively investigated as potential sources of novel antimicrobial agents capable of combating drug-resistant pathogens (Alvarez *et al*, 2026; Singh *et al*, 2024). This growing interest is further fueled by consumer preference for natural products and the expanding role of phytochemicals in functional foods and preventive healthcare strategies (Chen and Park, 2026 and Patel and Shah, 2025).

Despite the immense therapeutic potential of medicinal plants, the complexity of their chemical composition poses significant challenges in the identification, isolation, and characterization of individual bioactive compounds. Plant extracts often consist of intricate mixtures of compounds present in varying concentrations, making their analysis a demanding task. Therefore, advanced analytical and spectroscopic and chromatographic techniques have become indispensable tools in phytochemical research. Spectroscopic methods such as ultraviolet-visible (UV–Vis) spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), and mass spectrometry (MS) provide detailed insights into the structural and functional properties of bioactive molecules. These techniques enable rapid, accurate, and non-destructive analysis, thereby facilitating the comprehensive characterization of complex plant matrices (Patel *et al*, 2026 and Chen *et al*, 2024). In recent years, the integration of spectroscopic techniques with chromatographic methods such as high-performance liquid chromatography (HPLC) and gas and liquid chromatography (GCMS and LCMS) has revolutionized the field of natural product chemistry. Such hybrid approaches enhance the sensitivity, selectivity, and resolution of compound identification, allowing researchers to elucidate molecular structures with greater precision (Gonzalez and Smith, 2026 and Rodriguez *et al*, 2025).

Furthermore, emerging trends in green chemistry and sustainable extraction techniques have transformed the way bioactive compounds are obtained from plant sources (Ahmed *et al*, 2024). Cold maceration extraction is a widely employed technique for the isolation of bioactive compounds, particularly for preserving heat-sensitive phytochemicals. When combined with advanced spectroscopic with chromatographic analysis, it facilitates efficient recovery and precise characterization of phytoconstituents, enhancing the reliability of phytochemical investigations (Rahman *et al*, 2026; Patel *et al*, 2026).

Another significant advancement in this domain is the application of omics technologies and artificial intelligence in phytochemical research. Metabolomics, in particular, enables comprehensive profiling of plant metabolites, while AI-driven tools facilitate rapid data analysis and compound identification. These interdisciplinary approaches are transforming traditional phytochemical investigations into highly efficient and data-driven processes, paving the way for precision medicine and personalized therapeutics (Zhang *et al*, 2026; Wang *et al*, 2025). Such innovations are crucial for overcoming the limitations associated with conventional analytical methods and for unlocking the full potential of medicinal plants (Zhang *et al*, 2026 and Gupta and Verma, 2024).

In this context, the present study aims to unveil the characterization of bioactive chemical compounds from medicinal plant extracts using spectroscopic techniques with chromatographic methods. By employing a combination of modern analytical tools, this research seeks to provide a comprehensive understanding of the chemical constituents present in Ruellia prostrata leaf petiole extract and their potential biological activities. Such investigations are expected to contribute significantly to the fields of natural product chemistry, pharmacognosy, and drug discovery, ultimately supporting the development of novel therapeutic agents derived from plant sources.

MATERIALS AND METHODS

Collection of plant materials

The fully mature Ruellia prostrata leaf petiole was collected from Pappanadu, Thanjavur District, Tamil Nadu, India. Standard morphological characteristics were used to certify the plant species' identity. The plant were authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular sypetioleatics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (JV001) has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extract

The collected *Ruellia prostrata* leaf petiole were washed several times with distilled water to remove the traces of impurities from the plant. Then examined carefully old, infected and fungus damaged portion of the leaves were removed. Healthy leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The *Ruellia prostrata* leaf petiole extract was prepared using hydro-ethanol (70:30). Powdered *Ruellia prostrata* leaf petiole sample (1g) is added 50ml of hydro-ethanol, respectively. The extract

was shaken well for 1h and kept for 24 h. After 24 h, extraction was filtered using Whatman No.1 filter paper and the filtrate was further used for analysis.

Preliminary phytochemicals screening

Chemical tests were carried out on the aqueous extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The compounds present in the petioles were determined by literature methods viz phenol (Edeoga *et al*, 2006), Flavonoid (Boham and Kocipai-Abyazan, 1974), Total terpenoid (Ferguson, 1956) and Steroids (Attarde Daksha *et al*, 2010).

GC-MS and HPLC analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer sypetiole comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25mm ID × 1 µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 ìI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280ºC. Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dukes, 2013). Flavonoids were analyzed using HPLC by the method of Weerasak Samee (2007).

LC–MS Analysis method

Phytochemical profiling of the sample was carried out using a Shimadzu Corporation Liquid Chromatography–Mass Spectrometry (LC–MS) (LCMS-8045) sypetiole equipped with an electrospray ionization (ESI) interface. The LC–MS sypetiole performance was monitored regularly, with the rotary pump and turbomolecular pump run times recorded as 28,232 h and corresponding operational duration, respectively. Data acquisition and processing were carried out using Shimadzu LC–MS software. Compound identification was performed by comparing observed m/z values with spectral databases such as HMDB, METLIN, Mass Bank and mzCloud. Matching confidence was evaluated using MzCloud similarity scores and FISh scoring algorithms from ChemSpider. Identification of unmatched signals was re-attempted on the ChemSpider database (Venmathi Maran *et al*, 2021) using MS data and supported with a FISh scoring of above 50. Identification confidence was categorized based on matching score as follows: values greater than 90% were considered high confidence; values between 80–90% indicated a good match; values between 70–80% were regarded as tentative identification; and values below 70% were classified as low confidence, requiring MS/MS confirmation.

RESULTS

Phytochemicals are found naturally, physiologically active chemical substances found in plants that are beneficial to human health as nutrients and therapeutic substances. They provide to the color, flavor, and scent of plants in addition to shielding them from harm and illness. Tannins, steroids, terpenoids, flavonoids, triterponids, alkaloids, anthroquinone, saponins, polyphenol, glycoside, anthocyanins and emodins were present while coumarins was absent in hydro-alcoholic extract of Ruellia prostrata leaf petiole.

UV-VIS spectral analysis

UV-visible spectroscopy analysis confirms the presence of terpenoids, alkaloids, and phenols in the *Ruellia prostrata* leaf petiole extract. The UV spectra were used to identify the compounds containing σ-bonds, π-bonds, aromatic ring profiles, chromophores and lone pairs of electrons. The present study found the following peaks: 202.6 (4.000 nm), 250.1 (1.571 nm), 745.3 (0.168 nm), 843.9 (0.168 nm), and 988.2 (0.159 nm). Because the *Ruellia prostrata* leaf petiole extract includes about phenols and compounds related to their structure, the result shows the existence of peaks from 250 to 700 nm exposures.

Table 1 : Phytochemical analysis of different extract in *Ruellia prostrata* leaf petiole

Phytochemicals	Hydro alcoholic Extract
Tannin	+
Saponin	++
Flavonoids	+
Steroids	+
Terpenoids	++
Triterpenoids	++
Alkaloids	++
Anthroquinone	+
Polyphenol	++
Glycoside	+
Coumarins	-
Emodins	+
Anthocyanins	+

(—) Absent, (+) Present and (++) Higher colour intensity

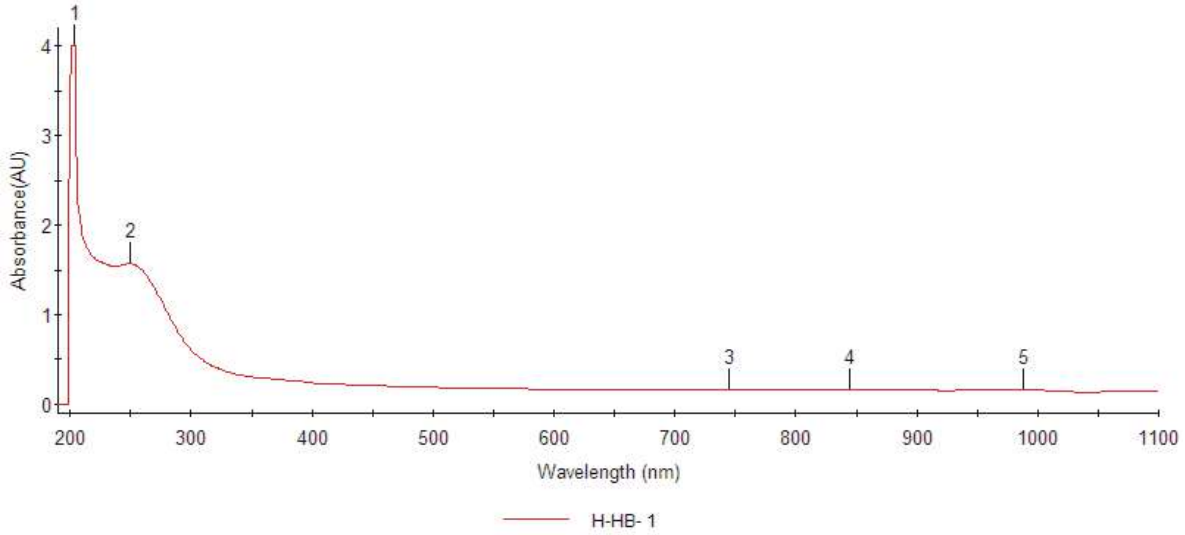

Fig. 1 : UV-Visible spectrum of *Ruellia prostrata* leaf petiole extract.

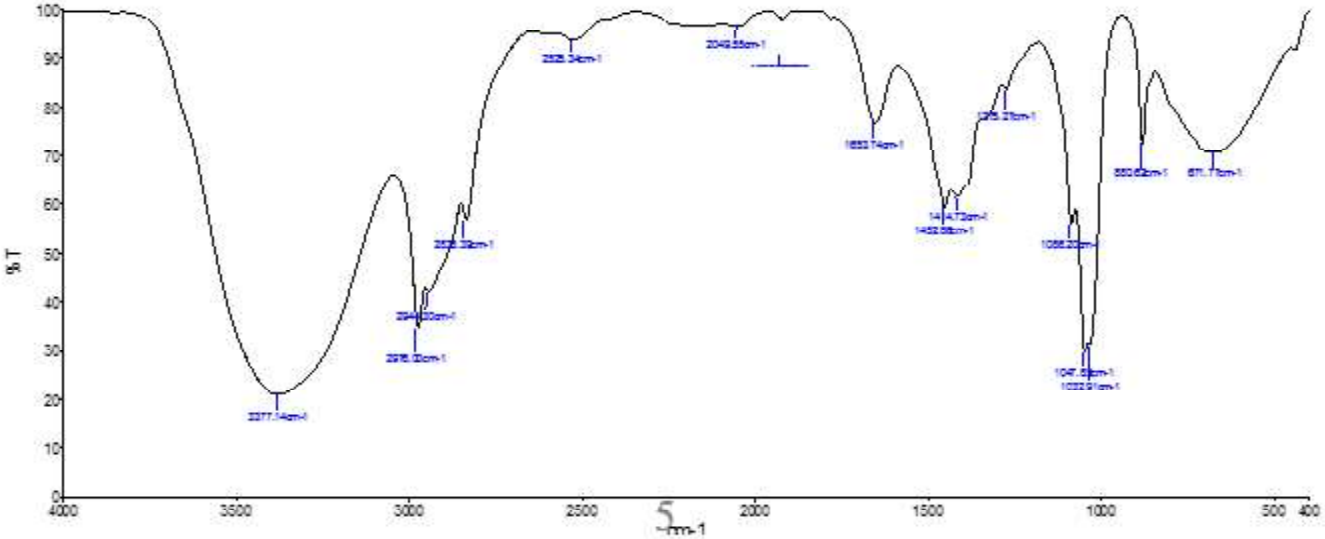

Fig. 2 : FTIR spectrum of *Ruellia prostrata* leaf petiole extract.

Fourier Transform Infra-Red Spectroscopy

The FTIR spectrum of *Ruellia prostrata* leaf petiole extract, recorded in the 4000–400 cm^{-1} region, showed strong absorption bands corresponding to a variety of functional groups. These include phenols, alcohols, alkanes, aldehydes, alkenes, aromatic compounds, and both aromatic and aliphatic amines (including primary and secondary amines). FTIR analysis works by measuring the absorption of infrared radiation by chemical bonds, enabling the identification of functional groups such as C=O, O–H, C–H, and N–H. This technique generates a unique molecular "fingerprint" that helps characterize bioactive compounds like phenols, flavonoids, and alkaloids. The analysis of peak positions in the infrared region confirmed the presence of these functional groups, indicating that the petiole extract contains multiple active chemical constituents..

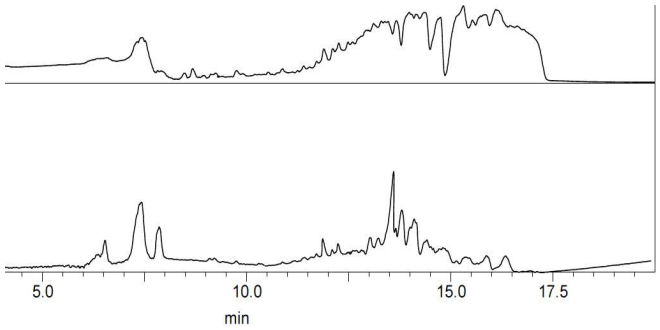

Fig. 3 : LC-MS chromatogram of hydro-ethanol extract of *Ruellia prostrata* leaf petiole.

LCMS Analysis of *Ruellia prostrata* leaf petiole

A total of 14 compounds were identified (06 in positive mode and 08 in negative mode) in the *Ruellia prostrata*

Table 2 : LC-MS screening and identiûcation of chemical compounds in hydro-ethanol extract of *Ruellia prostrata* leaf petiole.

S. no.	R.Time (min)	Observed Fragments m/z	Proposed compound	Class	Molecular Formula	Exact Mass (Da)	Database Reference	FISH Matching Score(Mz Cloud)/FISh Score (Chemspider)
1	1.117	226	Hydroxybenzoic acid derivative	Polyphenol	$C_{13}H_6O_4$	226.03	METLIN / HMDB	65%
2	1.125	108	Phenolic fragment	Polyphenol	$C_6H_4O_2$	108.02	MassBank	60%
3	7.356	721	Triterpenoid glycoside	Saponin	$C_{36}H_{58}O_{14}$	722.39	mzCloud	78%
4	7.857	270	Flavonoid (apigenin -like)	Polyphenol	$C_{15}H_{10}O_5$	270.05	METLIN	82%
5	10.887	278	Alkaloid (quinoline -type)	Alkaloid	$C_{18}H_{14}N_2O$	278.11	ChemSpider	70%
6	11.170	285	Kaempferol-like compound	Polyphenol	$C_{15}H_{10}O_6$	286.05	HMDB	85%
7	11.392	329	Glycosylated flavonoid fragment	Polyphenol	$C_{17}H_{14}O_7$	330.07	METLIN	75%
8	11.890	671	Steroidal saponin	Saponin	$C_{35}H_{58}O_{12}$	670.39	mzCloud	80%
9	11.721	458	Diterpenoid derivative	Terpenoid	$C_{22}H_{34}O_{10}$	458.21	MassBank	72%
10	14.211	746	Triterpenoid saponin	Saponin	$C_{40}H_{66}O_{13}$	746.45	mzCloud	84%
11	13.118	735	Glycosylated terpenoid	Terpenoid	$C_{39}H_{60}O_{13}$	736.42	ChemSpider	76%
12	13.626	741	Complex saponin	Saponin	$C_{38}H_{60}O_{14}$	740.41	METLIN	79%
13	14.550	822	Polyhydroxylated saponin	Saponin	$C_{42}H_{70}O_{16}$	822.48	mzCloud	86%
14	15.971	982	High MW triterpenoid saponin	Saponin	$C_{48}H_{78}O_{20}$	982.52	HMDB	88%

leaf petiole using the MS/MS spectra (Fig. 3 and Table 2). A few important classes of bioactive compounds such as flavonoids, phenolics, saponin, terpenoids, and steroids were detected in the Ruellia prostrata leaf petiole. Out of the 14 compounds, 6 saponin, 02 terpenoids, 05 polyphenolic and 01 alkaloids were detected. LC–MS analysis revealed the high abundance of saponins, and presence of polyphenolic, terpenoids and alkaloids, indicating significant bioactive phytochemical diversity. Compound identification was performed using METLIN, HMDB, mzCloud, ChemSpider and MassBank databases.

DISCUSSION

Traditional medicine, which has been developed over hundreds of years of trust and observation, uses medicinal herbs with a lengthy history. Herbal medicine and medicinal herbs have long been used, and recent advancements in contemporary therapeutics have encouraged the use of natural products for illness prevention and health maintenance all around the world. Numerous biological and therapeutic properties of phytochemicals have been documented. Pharmacists are interested in these components because to their low toxicity and therapeutic usefulness. Because it offers a balanced, effective, and safe way to extract a variety of bioactive substances while maintaining their biological activity, hydroalcoholic extraction is regarded as the best. A hydroalcoholic solvent dissolves both polar and semi-polar/non-polar substances by combining the polarity of water with the semi-polar character of alcohol. This makes it ideal for both industrial and research uses.

Because of their many biological roles, low toxicity, and possible therapeutic uses, secondary metabolites have attracted the attention of researchers (Zaki *et al*, 2012). A study that used hydroalcoholic extract to analyze the phytochemical content of *Ruellia prostrata* leaf petiole identified numerous compounds, including tannins, saponins, flavonoids, steroids, triterpenoids, terpenoids,

anthraquinones, alkaloids, glycosides, polyphenols, anthocyanins, and emodins. Flavonoids, total phenols, terpenoids, and alkaloids were found in significant amounts, with phenols being the most common, followed by terpenoids and flavonoids in accordance with previous findings (Patle *et al*, 2020; Nhon Hoang *et al*, 2023). The majority of secondary chemicals found in plants are phenol molecules, especially flavonoids and polyphenols. These substances exhibit pharmacological activity against disorders linked to oxidative stress, including cancer, ulcers, inflammation, and cardiovascular issues. They also have cytotoxic, depressive, antispasmodic, and antioxidant qualities. Fruits, vegetables, and drinks like coffee and tea all contain flavonoids, which are common polyphenol chemicals. Antioxidant activity, enzyme inhibition, anti-inflammatory effects, antibacterial activity, and anti-tumor capabilities are only a few of their numerous pharmacological and biological characteristics (Bitwell *et al*, 2023; El-Beltagi *et al*, 2022). Terpenoids are a significant class of naturally occurring chemicals produced from isoprene units that have a variety of therapeutic uses. Their hepatoprotective properties make them useful as diuretics, antibacterials, anti-carcinogens, anti-ulcer agents, and malaria medications. Artemisinin is one of the diterpenoids and sesquiterpenoids having anti-cancer effects (Soleimany *et al*, 2016).

UV-VIS spectral analysis

A useful method for identifying secondary metabolites and doing quantitative and qualitative research on biological and pharmacological materials is spectroscopy. UV spectroscopy has long been used in the study of flavonoids. Different flavonoid classes may be distinguished using the UV spectra of certain flavonoids, which show the influence of the kind of aromatic acyl groups, glycosidic substitution pattern, and amount of aglycone hydroxyl groups (Markham, 1982). The components that displayed different peaks in UV-visible spectra included chromophores, σ-bonds, aromatic rings, and lone pairs of electrons. Neha and Dhingra (2006) state that the existence of peaks between 250 and 700 nm suggests the presence of phenols and related chemicals in the petiole extract.

FTIR analysis

Chemical bonding in molecules may be identified using FTIR, a rapid and non-invasive analytical method that generates an infrared absorption spectrum that resembles a molecular fingerprint (Sadeghi et al, 2024). It has shown promise for categorizing and differentiating plants, related species, and microbial strains (Deepak Pawar *et al*, 2020). Many national pharmacopoeias acknowledge this method as essential for medication identification, and it is commonly used to identify chemical components and clarify structural structures. FTIR has shown to be a very helpful method for detecting compounds or functional groups in unknown plant extract combinations (Herrero *et al*, 2023).

LC–MS analysis

The LC–MS analysis of the extract revealed a diverse range of bioactive compounds belonging to polyphenols, flavonoids, alkaloids, and saponins, indicating the rich phytochemical profile of the sample. A total of fourteen compounds were tentatively identified based on their retention time (RT), mass-to-charge ratio (m/z), molecular formula, and database matching scores . At early retention times (RT ≈ 1.1 min), low molecular weight phenolic compounds such as hydroxybenzoic acid derivatives (m/z 226) and phenolic fragments (m/z 108) were detected. These compounds are well known for their antioxidant properties and play a significant role in free radical scavenging activity. The presence of such compounds suggests that the extract possesses potential antioxidative capacity (Zhang *et al*, 2023).

Flavonoids were prominently observed at RT values between 7.8 and 11.4 min, including apigenin-like (m/z 270), kaempferol-like (m/z 285), and glycosylated flavonoid derivatives (m/z 329). These compounds are widely reported for their anti-inflammatory, antimicrobial, and anticancer properties. The high matching scores (up to 85%) further confirm the reliability of their tentative identification. Flavonoids are known to contribute significantly to plant defense mechanisms and therapeutic efficacy (Wang *et al*, 2024). Alkaloids, represented by quinoline-type compounds (m/z 278), were also detected, indicating the presence of nitrogen-containing bioactive molecules. Alkaloids are pharmacologically important due to their antimicrobial and analgesic activities. Their presence enhances the medicinal value of the extract (Kumar *et al*, 2023).

A major portion of the detected compounds belongs to terpenoids and saponins, particularly in the higher retention time range (RT ≈ 10–16 min). Compounds such as triterpenoid glycosides (m/z 721), steroidal saponins (m/z 671), diterpenoid derivatives (m/z 458), and several high molecular weight saponins (m/z 746, 741, 822, and 982) were identified with high confidence scores (up to 88%). These compounds are known for their surface-active properties and biological activities, including anti-inflammatory, antifungal and cytotoxic effects. The dominance of saponins in the chromatographic profile suggests that the extract is particularly rich in amphiphilic

glycosides, which can enhance membrane permeability and facilitate interaction with biological sypetioles. High molecular weight triterpenoid saponins (m/z 982) indicate complex glycosylation patterns, which are often associated with enhanced bioactivity and stability (Li *et al*, 2024).

Furthermore, the increasing retention time correlates with increasing molecular complexity and hydrophobicity of the compounds, which is consistent with LC–MS separation principles. The combination of mzCloud, METLIN, HMDB, and ChemSpider databases provided reliable compound annotation, supported by good matching scores ranging from 60% to 88%. The LC–MS results clearly demonstrate that the sample contains a complex mixture of phytochemicals, predominantly Polyphenols and flavonoids shows antioxidant and therapeutic properties, Alkaloids act as pharmacological activity and Terpenoids and saponins revealed the major bioactive constituents with diverse biological functions. The abundance of saponins and flavonoids suggests strong potential for applications in pharmaceuticals, nutraceuticals, and environmental remediation.

CONCLUSION

The present study successfully elucidates the phytochemical composition of *Ruellia prostrata* leaf petiole extract using a combination of spectroscopic and chromatographic techniques. The preliminary phytochemical screening confirmed the presence of diverse secondary metabolites, including polyphenols, flavonoids, alkaloids, terpenoids, triterpenoids, and saponins, indicating a rich reservoir of bioactive compounds. Spectroscopic analyses (UV–Vis and FTIR) revealed characteristic absorption patterns and functional groups such as hydroxyl, carbonyl, and amine groups, which are associated with biologically active molecules. These findings validate the presence of structurally diverse phytoconstituents and support their potential role in therapeutic applications. Furthermore, LC–MS profiling identified fourteen compounds with varying degrees of confidence, highlighting the predominance of saponins, flavonoids, and terpenoids. The detection of high molecular weight triterpenoid saponins and flavonoid derivatives suggests enhanced pharmacological potential, including antioxidant, antimicrobial, anti-inflammatory, and cytotoxic activities. Overall, the study demonstrates that Ruellia prostrata is a promising source of biologically active compounds with significant potential for applications in pharmaceuticals, nutraceuticals and functional foods.

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