IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG
An International Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL PROFILING OF *CLERODENDRUM INFORTUNATUM* ETHANOLIC EXTRACT USING TLC AND LC–MS TECHNIQUES

[1]Raji M K, [2]Sunbee Prakash

[1]Reaearch Scholar, [2]Associate Professor
[1]Department of Pharmacy,
[1]Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India

Abstract: Clerodendrum infortunatum is an important medicinal plant, and in the present study its ethanolic extract was systematically evaluated using conventional phytochemical screening, thin-layer chromatography (TLC), and liquid chromatography–mass spectrometry (LC-MS) to establish its chemical profile. The plant material was extracted by macerating 100 g of dried powder in 500 mL of 95% ethanol for 72 h, yielding a concentrated crude extract that was used for all analyses. Preliminary phytochemical tests showed a strong presence of alkaloids and saponins (++), along with high levels of carbohydrates (++), while sugars, proteins, tannins, quinones, and phenols were detected in moderate amounts (+); in contrast, flavonoids, steroids, glycosides, terpenoids, and amino acids were not detected by conventional qualitative assays. TLC profiling on silica gel plates further confirmed the chemical complexity of the extract by displaying multiple well-resolved spots under UV light at 254 and 366 nm, indicating the presence of diverse classes of secondary metabolites such as alkaloids, tannins, and phenolic compounds. Advanced LC–MS/MS analysis using a C18 column and electrospray ionization provided detailed molecular information, revealing prominent ions at m/z 299 and 301 corresponding to flavonoids like kaempferol and quercetin, peaks at m/z 361 and 405 associated with luteolin-based glycosides, signals at m/z 449 and 491 indicative of iridoid and phenylpropanoid glycosides, and high-molecular-weight constituents at m/z 797 and 1013 representing saponins and complex glycosides. The combined analytical approach demonstrates that *C. infortunatum* contains a wide spectrum of bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial relevance, thereby scientifically validating its traditional medicinal use and supporting its potential for future pharmacological and therapeutic development.

***Index Terms* -** *Clerodendrum infortunatum*, LC–MS, Thin-layer chromatography, Phytochemical screening, Ethanolic extract.

I. INTRODUCTION

Medicinal plants have been used as the foundation of healthcare systems for thousands of years, particularly in developing countries where traditional medicine continues to play a major role in disease management [1]. Even in modern pharmaceutical science, a large proportion of drugs have originated from natural sources or have been developed based on plant-derived molecules. Plants synthesize a wide range of secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, and glycosides, which help them survive environmental stress and microbial attack. These same compounds often exhibit important biological activities in humans, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and metabolic regulatory effects. Therefore, systematic phytochemical and analytical evaluation of medicinal plants is essential to validate their traditional uses and to identify novel bioactive compounds for drug discovery.

Among the many medicinal plants used in traditional systems of medicine, *Clerodendrum infortunatum* has attracted increasing scientific interest. It belongs to the family Lamiaceae and is widely distributed across tropical and subtropical regions of Asia, including India, Sri Lanka, and Southeast Asia [2,3]. In Ayurveda and folk medicine, different parts of this plant are traditionally used for the treatment of fever, skin disorders, inflammation, respiratory problems, and digestive ailments. Leaves, roots, and stems of *C. infortunatum* are commonly employed in herbal formulations to relieve pain, treat infections, and promote general health. Despite its long-standing medicinal use, detailed chemical characterization of this plant remains incomplete, which limits its broader pharmaceutical exploitation.

The therapeutic effects of medicinal plants are largely determined by the type and concentration of their phytochemical constituents. Compounds such as alkaloids are known for their potent pharmacological actions, including analgesic, antimicrobial, and anticancer effects, while phenolic compounds and flavonoids are widely recognized for their strong antioxidant and anti-inflammatory properties. Saponins exhibit membrane-modulating, immune-stimulating, and cholesterol-lowering activities, whereas tannins contribute to antimicrobial and wound-healing effects. Understanding the phytochemical profile of *C. infortunatum* is therefore crucial to explain its traditional uses and to predict its potential therapeutic applications. However, conventional phytochemical screening alone is not sufficient to reveal the full chemical complexity of plant extracts [4].

Modern analytical techniques have significantly improved the ability to identify and characterize plant metabolites. Thin-layer chromatography (TLC) remains a simple and effective technique for preliminary separation and fingerprinting of phytochemicals, allowing rapid detection of multiple compounds in a single extract. It is widely used for quality control, authentication, and comparative analysis of herbal materials. On the other hand, liquid chromatography coupled with mass spectrometry (LC–MS) provides highly sensitive and accurate identification of compounds based on their mass-to-charge ratios. LC–MS enables the detection of both low- and high-molecular-weight constituents, making it one of the most powerful tools for phytochemical profiling and metabolite identification.

In this context, the present study was designed to carry out a comprehensive phytochemical evaluation of the ethanolic extract of *Clerodendrum infortunatum* using a combination of conventional screening, TLC, and LC–MS techniques. By integrating these methods, the study aims to generate a reliable chemical fingerprint of the plant and to identify key bioactive constituents responsible for its medicinal properties. Such systematic profiling not only supports the scientific validation of traditional herbal use but also provides a foundation for future pharmacological, toxicological, and formulation-based studies. Ultimately, this work contributes to the growing effort to bridge traditional herbal medicine with modern pharmaceutical science.

II. RESEARCH METHODOLOGY

2.1. Plant Material and Extraction

The plant material employed in this investigation was *Clerodendrum infortunatum*. Fresh samples were obtained from authenticated locations and taxonomically confirmed using standard botanical identification methods. The collected plant material was thoroughly washed to remove debris, shade-dried at room temperature, and pulverized into a fine powder using a mechanical grinder. About 100 g of the powdered material was macerated in 500 mL of 95% (v/v) ethanol for 72 hours with occasional agitation to improve extraction efficiency. The resulting mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain the crude ethanolic extract. The dried extract was preserved at −20°C until further experimental use [5, 6].

2.2. Phytochemical analysis of ethanolic extract of *Clerodendrum infortunatum* by conventional method.

The ethanolic extract of *Clerodendrum infortunatum* was subjected to qualitative phytochemical screening using standard chemical tests to identify the major classes of secondary metabolites. A measured quantity of the dried extract was dissolved in ethanol and filtered to obtain a clear test solution, which was used for all reactions. Alkaloids were detected using Dragendorff's reagent, while flavonoids were examined by the sodium hydroxide test. Carbohydrates were analyzed using Molisch's test, and reducing sugars were evaluated by standard sugar tests. Proteins were identified using the mercuric chloride test, whereas amino acids were assessed by Millon's test. Tannins were detected using both lead acetate and ferric chloride tests to differentiate their types. Saponins were identified based on their ability to form stable froth upon vigorous shaking with water. Quinones and phenolic compounds were determined using appropriate colorimetric reactions, and glycosides and steroids were tested using standard alkaline and specific reagent-based methods. The development of characteristic color changes or precipitates was taken as a positive indication for the presence of the corresponding phytochemical group. All tests were carried out in triplicate to ensure reproducibility, and the results were recorded qualitatively as absent (−), present (+), or strongly present (++), providing a comprehensive overview of the phytochemical composition of the ethanolic extract [7, 8].

2.3. Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was employed as an initial method for the separation of phytochemical constituents. The ethanolic extracts were applied as small spots onto pre-coated silica gel plates (Merck, 60 F254). Various mobile phase compositions were evaluated to achieve effective separation of the compounds. Following chromatographic development, the plates were examined under ultraviolet light at 254 nm and 366 nm. Additional visualization was carried out using detection reagents such as vanillin sulfuric acid spray and iodine vapour to facilitate the identification of separated phytoconstituents [9, 10].

2.4. Liquid Chromatography-Mass Spectrometry (LC-MS)

The phytochemical composition of the ethanolic extracts was analysed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) to detect and quantify the major bioactive constituents. Prior to analysis, the extracts were reconstituted in methanol and passed through a 0.45 µm membrane filter to remove particulate matter. Separation was carried out using an LC–MS system comprising an Agilent 1200 Series HPLC linked to an Agilent 6460 Triple Quadrupole mass spectrometer equipped with a C18 column (250 × 4.6 mm, 5 µm). The mobile phase was composed of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B), operated under a programmed gradient elution. Mass spectral data were recorded in both positive and negative ionization modes, and compound identification was performed based on the obtained MS/MS spectra [11, 12].

III. RESULTS AND DISCUSSION

3.1. Plant Material and Extraction

Clerodendrum infortunatum, a plant revered for its extensive medicinal value, was methodically collected with utmost care to ensure its integrity for research purposes. The collection process itself forms the foundational step in scientific studies, as the authenticity and quality of plant material directly influence the reliability of subsequent findings. Once harvested, the plant material underwent a rigorous surface sterilization process using ethanol. This sterilization phase is essential for eliminating potential contaminants, such as surface microbes or residual soil particles, which could otherwise compromise the purity and accuracy of later chemical analyses.

3.2. Phytochemical analysis of ethanolic extract of *Clerodendrum infortunatum* by conventional method.

Phytochemical analysis of the ethanolic extract of Clerodendrum infortunatum provides crucial insight into the bioactive profile of this medicinal plant. The analytical screening, summarized through standardized qualitative tests, reveals a diverse chemical landscape within the extract. Notably, alkaloids demonstrated a strong positive reaction with Dragendorff's reagent (++) signifying their high abundance. Alkaloids are renowned for their potent pharmacological properties, including anti-inflammatory, analgesic, and antimalarial activities, which may underpin some of the plant's traditional medicinal uses. In contrast, tests for flavonoids (Sodium hydroxide test) and steroids registered negative results, indicating these classes of compounds are either absent or present in quantities below detectable thresholds. The absence of flavonoids is remarkable given their common role as antioxidants in plant extracts, suggesting the bioactivity of *C. infortunatum* is attributable to other components.

Sugars and proteins were both detected (with the sugar test showing a single + and protein by Mercuric chloride test also +), suggesting the presence of basic nutritional or structural components. The extract also tested positive for quinones (+), a class of compounds associated with antimicrobial and anticancer properties. High levels of carbohydrates evidenced by a double-positive result (++) with the Molisch test further contribute to the plant's biochemical profile, supporting its potential as a source of energy-rich compounds or as a raw material for fermentation and biotechnological applications. Tannins displayed varied results: Lead acetate test confirmed their presence (+), while Ferric chloride test was negative (-), indicating that only certain subtypes of tannins, possibly hydrolyzable rather than condensed tannins, are prevalent. Saponins, present at a high level (++), are noteworthy for their well-documented surfactant, anti-inflammatory, and immune-modulating properties; their abundance may be linked to the plant's traditional use in treating infections and gastrointestinal disorders. Conversely, glycosides were absent, as per the negative Sodium hydroxide test a finding that helps delineate the extract from other medicinal plants known for rich glycosidic content. Other phytochemical groups, including amino acids (Millon's test) and terpenoids, were also absent, suggesting a narrow biochemical adaptability in terms of nitrogenous and isoprene-derived secondary metabolites within this extract. Yet, phenols and quinones were both detected (+), underscoring the possible antioxidative and antimicrobial capacity inherent to the extract, as phenolic compounds are widely acknowledged for such roles. Tthe phytochemical profile of the ethanolic extract of *Clerodendrum infortunatum* reveals a rich source of alkaloids, saponins, tannins, sugars, carbohydrates, and selected proteins and phenolic compounds. These results emphasize the therapeutic significance of *C. infortunatum* and justify its traditional use in herbal medicine. The absence of flavonoids, steroids, glycosides, terpenoids, and amino acids narrows the spectrum of anticipated actions and side effects, potentially enabling targeted pharmacological exploitation. This analysis warrants further quantitative and bioactivity-guided investigations to isolate specific compounds responsible for clinical efficacy and explore their mechanisms of action within biological systems.

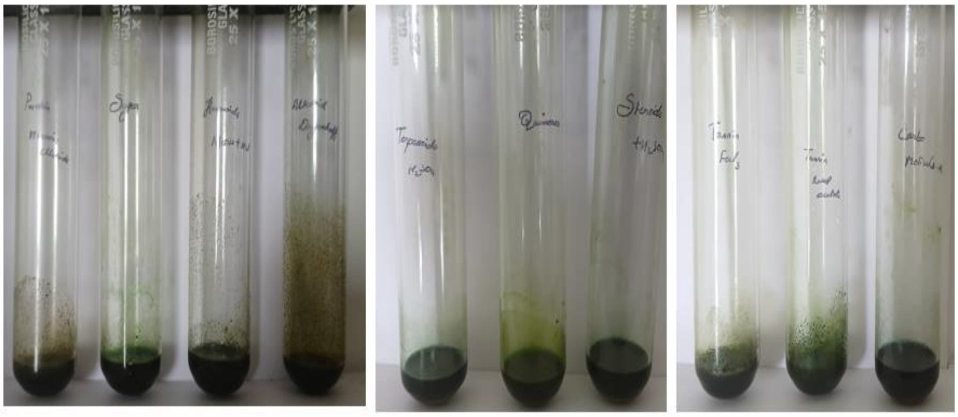

Figure 1: Phytochemical analysis of the ethanolic extract of *C. infortunatum.*

3.3. Phytochemical analysis of ethanolic extract of *Clerodendrum infortunatum* by TLC method.

Figure 3 illustrates the TLC profile of the ethanolic extract of *Clerodendrum infortunatum*, revealing multiple well-resolved spots corresponding to different classes of phytochemicals. The distinct Rf values observed for alkaloids, flavonoids, tannins, and phenols clearly demonstrate the chemical diversity of the extract. Such chromatographic fingerprints not only validate the presence of these phytoconstituents but also serve as a reference for future comparative and quality control studies involving this medicinal plant. Although TLC is primarily qualitative, it can also provide semi-quantitative information when combined with densitometric scanning or digital image analysis. These approaches enable estimation of the relative abundance of different compounds based on spot intensity. While less precise than instrumental chromatography, such semi-quantitative data are often sufficient for preliminary screening and comparative studies, especially during early stages of research or in settings where advanced instrumentation is unavailable. Thin-layer chromatography remains one of the most indispensable techniques in phytochemical analysis due to its simplicity, rapid execution, low operational cost, and broad applicability. It serves as a primary screening tool for the detection of diverse secondary metabolites, guides the selection of solvent systems for further purification, supports quality control and authentication of herbal products, facilitates chemotaxonomic studies, and can be effectively integrated with biological and spectroscopic methods for comprehensive analysis. As interest in plant-based medicines and natural products continues to grow, TLC will remain a cornerstone technique that bridges traditional botanical research with modern analytical science, ensuring both scientific reliability and practical feasibility.

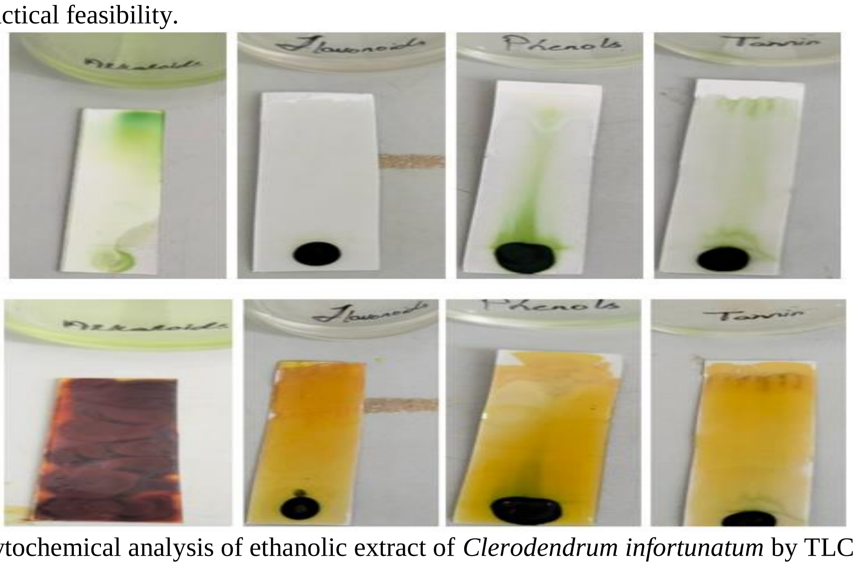

Figure 2: Phytochemical analysis of ethanolic extract of *Clerodendrum infortunatum* by TLC method.

3.4. Liquid Chromatography-Mass Spectrometry (LC-MS)

LC–MS profiling of the ethanolic extract of *Clerodendrum infortunatum* demonstrated the presence of a wide spectrum of biologically active constituents based on the detected m/z values. Signals observed at m/z 299 and 301 indicate flavonoids such as kaempferol and quercetin, compounds recognized for strong antioxidant and anti-inflammatory activities. Peaks corresponding to m/z 361 and 405 are characteristic of luteolin-based glycosides, further enhancing the therapeutic relevance of the extract. Additionally, iridoid and phenylpropanoid glycosides were identified at m/z 449 and 491, while higher molecular weight saponins and complex glycosides appearing at m/z 797 and 1013 reflect the plant's broad and diverse pharmacological potential. Together, these compounds support the traditional medicinal uses of *C. infortunatum* and provide a scientific basis for its continued pharmacological exploration [13]. Based on the LC–MS mass spectral data, the ethanolic extract of *Clerodendrum infortunatum* was found to contain a diverse range of bioactive phytochemicals, as indicated by several characteristic m/z values. Ions detected around m/z 299–303 correspond to flavonoids such as quercetin and kaempferol, while peaks in the region of m/z 331–361 suggest the presence of apigenin and luteolin derivatives. Signals observed at m/z 361 and 405 are indicative of flavonoid glycosides, particularly luteolin-based conjugates, and the prominent ion at m/z 449 supports the presence of quercetin glycosides such as isoquercitrin. Iridoid glycosides like loganin and aucubin were tentatively identified from ions near m/z 491–493, whereas peaks between m/z 523 and 537 are characteristic of phenylpropanoid glycosides, including verbascoside-type compounds. The detection of higher molecular weight ions in the range of m/z 637–659 reflects the occurrence of acylated flavonoid glycosides, while intense signals around m/z 797–875 and m/z 1013 correspond to triterpenoid and steroidal saponins and their glycosylated derivatives. Very high-mass ions above m/z 1200 further indicate the presence of complex poly-glycosylated saponins. Altogether, this mass spectral profile confirms that *C. infortunatum* is rich in flavonoids, iridoids, phenylpropanoid glycosides, and saponins, which collectively contribute to its broad spectrum of pharmacological activities.

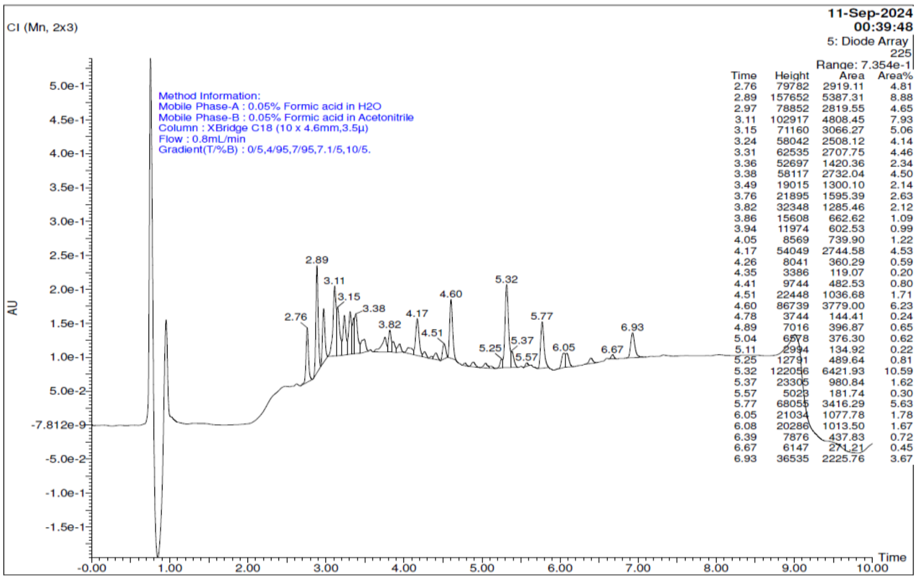

Figure 3: LC-MS analysis of ethanolic extract of *Clerodendrum infortunatum*.

IV. CONCLUSION

The findings of this study clearly demonstrate that the ethanolic extract of *Clerodendrum infortunatum* contains a wide range of pharmacologically important secondary metabolites, as evidenced by systematic phytochemical tests, TLC profiling, and LC–MS analysis. The notable abundance of alkaloids, saponins, and carbohydrates, together with the presence of tannins, phenolic compounds, quinones, sugars, and proteins, reflects the strong medicinal potential of the plant. The TLC fingerprint produced distinct and reproducible bands, highlighting the chemical complexity of the extract and providing a useful tool for its identification and quality evaluation. Furthermore, LC–MS investigation revealed ions corresponding to key bioactive molecules such as quercetin, kaempferol, luteolin derivatives, iridoid glycosides, phenylpropanoid glycosides, and saponin-related compounds, offering molecular confirmation of the plant's therapeutic relevance. These compounds are well known for their antioxidant, anti-inflammatory, antimicrobial, and protective biological activities, which explains the long-standing use of *C. infortunatum* in traditional medicine. Taken together, the results establish a reliable scientific basis for the medicinal value of this plant and highlight its promise as a natural source of biologically active molecules. The study also provides essential baseline data that can support future research aimed at isolating specific compounds, evaluating their biological mechanisms, and developing standardized herbal or pharmaceutical formulations.

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