



ISSN (O): 2320-5407
ISSN (P): 3107-4928

Journal Homepage: www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/24243
DOI URL: <http://dx.doi.org/10.21474/IJAR01/24243>



RESEARCH ARTICLE

EXPLORING THE BIOACTIVE PHYTOCONSTITUENTS OF *MERREMIA VITIFOLIA*: A QUALITATIVE PHYTOCHEMICAL APPROACH

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Manuscript Info

Manuscript History

Received: 14 July 2026

Final Accepted: 16 August 2026

Published: September 2026

Key words:-

Merremia vitifolia; Convolvulaceae; phytochemical screening; methanolic extract; alkaloids; flavonoids; phenolic compounds; tannins; glycosides.

Abstract

Background:-*Merremia vitifolia* is a medicinal plant of the family Convolvulaceae that has attracted considerable interest because of its traditional therapeutic applications. Phytochemical screening is an important preliminary step in the investigation of medicinal plants because it provides information on the major classes of constituents that may contribute to their biological properties. However, qualitative information regarding the phytochemical profile of the methanolic extract of *M. vitifolia* remains limited.

Objective:-This study aimed to qualitatively evaluate the methanolic extract of *M. vitifolia* for major classes of phytochemicals and establish its preliminary phytochemical profile for subsequent pharmacological and phytochemical investigations.

Methods:-The methanolic extract of *M. vitifolia* was subjected to standard qualitative phytochemical screening procedures. Alkaloids were assessed using Mayer's, Wagner's, Dragendorff's, and Hager's tests; carbohydrates using Molisch's, Benedict's, and Fehling's tests; and glycosides using Modified Borntrager's and Legal's tests. Phenols, tannins, and flavonoids were evaluated using ferric chloride, gelatin, alkaline reagent, and lead acetate tests, respectively. Saponins, phytosterols, proteins, and amino acids were examined using Froth and foam tests, Salkowski's and Liebermann Burchard's tests, and Xanthoproteic and ninhydrin tests, respectively.

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Results:-The methanolic extract showed positive reactions for alkaloids, carbohydrates, glycosides, phenols, tannins, and flavonoids. Alkaloids were consistently detected by Mayer's, Wagner's, Dragendorff's, and Hager's tests, while carbohydrates produced positive reactions in Molisch's, Benedict's, and Fehling's assays. Glycosides

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were detected by both Modified Borntrager's and Legal's tests. Positive reactions for phenols, tannins, and flavonoids were obtained with ferric chloride, gelatin, alkaline reagent, and lead acetate assays, respectively. In contrast, saponins, phytosterols, proteins, and amino acids were not detected by the corresponding qualitative tests under the experimental conditions employed. The findings indicate that the extract possesses a diverse phytochemical profile comprising several important classes of secondary metabolites.

Conclusion:-The methanolic extract of *M. vitifolia* contains multiple phytochemical classes, particularly alkaloids, glycosides, phenols, tannins, and flavonoids, which may contribute to its reported medicinal potential. These preliminary findings provide a basis for further investigation through quantitative phytochemical analysis, chromatographic profiling, isolation and structural characterization of individual constituents, and targeted pharmacological studies.

Introduction:-

The intersection of traditional ethnomedicinal practices and modern pharmacological research has opened a vast, highly dynamic frontier in contemporary drug discovery, highlighting the indispensable role that plant-derived natural products play in global healthcare systems (Azad et al., 2024). Throughout human history, indigenous communities have relied heavily on botanical resources to manage complex arrays of ailments, utilizing whole plant matrices, crude extracts, and aqueous infusions to address infectious diseases, metabolic dysfunctions, and chronic inflammatory conditions (Olatunji et al., 2021). Plant-based therapeutics owe their biological efficacy primarily to the accumulation of secondary metabolites, biosynthetic compounds that do not participate directly in the normal growth or development of the organism, but instead function as critical ecological mediators and defensive agents against environmental stressors (Azad et al., 2024). Major secondary metabolite classes, including alkaloids, flavonoids, tannins, phenolic acids, saponins, and triterpenoids, provide a structurally diverse chemical reservoir that has historically served as the blueprint for numerous modern pharmaceutical agents (Olatunji et al., 2021; Lee et al., 2023).

Belonging to the Convolvulaceae family, the genus *Merremia* comprises numerous twining vines, herbs, and shrubs distributed widely across tropical and subtropical environments globally (Olatunji et al., 2021). Among them, *Merremia vitifolia*, commonly recognized by vernacular names such as grape-leaf wood rose or Bilajangbulu, occupies an important place in traditional and tribal pharmacopeias (Akter et al., 2021; 1.1.3). Traditional healers utilize various macroscopic parts of *M. vitifolia*, including its leaves, vines, and roots, to treat conditions such as intermittent fevers, persistent headaches, inflammatory eye disorders, rheumatism, urinary dysfunctions, and jaundice (Islam et al., 2025). Furthermore, fresh leaf juices and aqueous decoctions are frequently topically applied as cooling agents or poultices to alleviate local swellings, rheumatic joint stiffness, and dermatological eruptions (Akter et al., 2021). Despite widespread traditional utilization, establishing systematic chemical standardization remains vital to bridge the gap between folkloric claims and evidence-based clinical application (Azad et al., 2024).



Figure 1.1. *Merremia vitifolia* Flower



Figure 1.2. *Merremia vitifolia* Leaf

Unlocking the therapeutic potential of any medicinal plant requires a foundational, methodical qualitative phytochemical screening (Akter et al., 2021). Qualitative screening serves as the initial diagnostic step in pharmacognosy, allowing researchers to rapidly identify the presence or absence of major chemical groups across polar and non-polar extracts using systematic colorimetric assays and precipitation reactions (Shorna et al., 2024).

Previous exploratory studies on *M. vitifolia* indicate a rich profile of bioactive principles, including high concentrations of flavonoids, condensed tannins, carbohydrates, and diverse terpenoid structures (Akter et al., 2021). These active constituents drive vital physiological interactions; for instance, phenolic acids and flavonoids exhibit robust antioxidant capacities by scavenging reactive oxygen species (ROS), thereby mitigating oxidative stress and cellular damage (Azad et al., 2024). Similarly, triterpenoids and steroidal components are widely recognized for suppressing inflammatory pathways and stabilizing cell membranes against pathological denaturation (Akter et al., 2021).

Recent experimental evaluations have substantially validated the traditional applications of *M. vitifolia* through diverse in vitro, in vivo, and in silico investigations (Azad et al., 2024). Pharmacological assays of methanolic and aqueous leaf extracts have demonstrated marked antioxidant properties via DPPH and reducing power assays, significant thrombolytic activity, and prominent anti-nociceptive and anti-arthritic effects, such as the substantial inhibition of protein denaturation (Akter et al., 2021). Furthermore, recent studies examining its broader neuropharmacological, hypoglycemic, antipyretic, and antidiarrheal activities reveal that crude extracts can modulate physiological systems, successfully lowering yeast-induced pyrexia and reducing blood glucose levels in animal models (Islam et al., 2025; Ahmed et al., 2024; Shorna et al., 2024). Advanced computational methods, including molecular docking and molecular dynamics simulations, have further supported these findings by showing strong binding affinities of key phytoconstituents such as quercetin and pheophorbide A for cyclooxygenase-2 (COX-2) and other inflammatory targets (Islam et al., 2025). Despite these advances, the specific therapeutic profile of any botanical extract is inextricably linked to its multi-component matrix, necessitating rigorous qualitative profiling across varied solvent systems to identify active fractions (Akter et al., 2021).

Critical gaps remain in systematically mapping *M. vitifolia* secondary metabolites across diverse solvent polarities and plant organs (Olatunji et al., 2021). Comprehensive qualitative frameworks examining crude extracts alongside sequential organic fractions are essential to understand how solvent polarity governs the recovery of targeted active principles. This thesis, titled "Exploring the Bioactive Phytoconstituents of *Merremia vitifolia*: A Qualitative Phytochemical Approach," addresses this imperative by systematically mapping the qualitative metabolic profile of *M. vitifolia*, correlating these findings with traditional uses, and establishing a rigorous baseline for future quantitative estimations, compound isolation, and pharmaceutical development (Azad et al., 2024). Ultimately, this research contributes scientifically validated pharmacognostical data to support the therapeutic utility of indigenous flora in modern medicine (Olatunji et al., 2021).

Materials and Methods:-

Plant Material Collection, Authentication, and Preparation:-

Fresh, disease-free whole plants of *Merremia vitifolia* (Burm.f.) Hallier f. (family Convolvulaceae) were collected from mature stands. Botanical experts confirmed taxonomic identification and authentication, and deposited voucher specimens in the institutional herbarium for future reference. The collected plant materials were thoroughly washed under running tap water, rinsed with distilled water, and shade-dried at ambient room temperature (25°C to 30°C) for several weeks to prevent the thermal degradation of thermolabile phytoconstituents (Azad et al., 2024). Once completely dried, the whole plant parts of *Merremia vitifolia* were coarsely powdered using a mechanical grinding machine and stored in airtight glass containers protected from light and moisture until extraction.

Extraction Procedure:-

One hundred grams of coarse *Merremia vitifolia* powder was macerated in 500 mL of analytical-grade methanol at room temperature in flat-bottomed glass containers with frequent agitation for 72 hours (Akter et al., 2021). The resulting mixture was filtered through sterile Whatman No. 1 filter paper. The filtrates were then concentrated under reduced pressure using a rotary evaporator at a controlled temperature to yield crude extracts, which were stored at 4°C for subsequent qualitative phytochemical screening (Azad et al., 2024).

Qualitative Phytochemical Screening Procedures:-

The freshly prepared methanolic extract of *M. vitifolia* underwent preliminary qualitative phytochemical screening to identify major classes of bioactive constituents. The extract was screened for alkaloids using Mayer's, Dragendorff's, Wagner's, and Hager's tests; carbohydrates using Molisch's, Benedict's, and Fehling's tests; glycosides using the modified Bornträger's and Legal's tests; saponins using the froth and foam tests; phytosterols using Salkowski's and Liebermann-Burchard's tests; phenolic compounds using the ferric chloride test; tannins using the gelatin test; flavonoids using the alkaline reagent and lead acetate tests; proteins using the xanthoproteic test; and amino acids using the ninhydrin test (Mannan, 2026).

Detection of Alkaloids:-

- **Mayer's Test:** A fraction of the extract was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution). The formation of a cream or dull-white precipitate indicated the presence of alkaloids (Tiwari et al., 2011).
- **Wagner's Test:** The extract was treated with a few drops of Wagner's reagent (iodine in potassium iodide). The formation of a reddish-brown precipitate confirmed the presence of alkaloids (Kokate et al., 2010).
- **Dragendorff's Test:** The extract was treated with Dragendorff's reagent (potassium bismuth iodide solution). The development of a prominent orange-red precipitate indicated alkaloids (Tiwari et al., 2011).
- **Hager's Test:** The extract was treated with saturated aqueous picric acid solution. The formation of a yellow-colored crystalline precipitate confirmed the presence of alkaloids (Hossain et al., 2013).

Detection of Carbohydrates:-

- **Molisch's Test:** The extract was treated with a few drops of alcoholic α -naphthol solution, followed by the careful addition of concentrated sulfuric acid (H_2SO_4) along the inner walls of the test tube. The formation of a purple or reddish-violet ring at the liquid junction confirmed carbohydrates (Kokate et al., 2010).
- **Benedict's Test:** The extract was treated with an equal volume of Benedict's reagent and heated in a boiling water bath for 5 minutes. The development of a red, yellow, or green precipitate indicated reducing sugars (Tiwari et al., 2011).
- **Fehling's Test:** The extract was heated with equal volumes of Fehling's solution A and B on a water bath. The formation of a brick-red precipitate indicated the presence of reducing sugars (Kokate et al., 2010).

Detection of Glycosides:-

- **Modified Bornträger's Test:** The extract was treated with dilute hydrochloric acid, boiled, filtered, and extracted with an organic solvent like chloroform. The organic layer was treated with dilute ammonia solution. The appearance of a pink, red, or violet color in the ammoniacal layer indicated the presence of glycosides (Tiwari et al., 2011).
- **Legal's Test:** The extract was dissolved in pyridine, treated with a few drops of sodium nitroprusside solution, and made alkaline with sodium hydroxide. The formation of a pink to blood-red color indicated cardiac glycosides (Kokate et al., 2010).

Detection of Saponins:-

- **Froth Test / Foam Test:** The extract was vigorously shaken with distilled water in a test tube and allowed to stand for 10 minutes. The development of a stable, persistent honeycomb-like froth (greater than 1 cm in height) indicated the presence of saponins (Hossain et al., 2013).

Detection of Phytosterols:-

- **Salkowski's Test:** The extract was treated with chloroform and filtered. The filtrate was treated with a few drops of concentrated H_2SO_4 , shaken, and allowed to stand. A golden-yellow or red coloration in the lower chloroform layer indicated phytosterols/triterpenoids (Kokate et al., 2010).
- **Libermann-Burchard's Test:** The extract was dissolved in a few drops of acetic anhydride, boiled, cooled, and treated cautiously with concentrated H_2SO_4 along the sides of the test tube. The formation of a brown ring at the junction and a turning to deep green or violet in the upper layer indicated phytosterols (Tiwari et al., 2011).

Detection of Phenolic Compounds:-

- **Ferric Chloride Test:** The extract was treated with a few drops of neutral 5% ferric chloride ($FeCl_3$) solution. The appearance of a dark green, deep blue, or reddish-black color indicated phenolic compounds (Tiwari et al., 2011).

Detection of Tannins:-

- **Gelatin Test:** The extract was treated with a 1% gelatin solution containing sodium chloride. The formation of a bulky white or yellowish precipitate indicated the presence of tannins (Akter et al., 2021).

Detection of Flavonoids:-

- **Alkaline Reagent Test:** The extract was treated with an aqueous solution of sodium hydroxide (NaOH). An intense yellow coloration that became colorless upon the addition of dilute hydrochloric acid confirmed flavonoids (Azad et al., 2024).

- **Lead Acetate Test:** The extract was treated with a few drops of 10% lead acetate solution. The formation of a bulky yellow or white precipitate indicated the presence of flavonoids (Kokate et al., 2010).

Detection of Proteins:-

- **Xanthoproteic Test:** The extract was treated with a few drops of concentrated nitric acid (HNO_3) and heated. The formation of a yellow color, which turned orange upon the addition of excess sodium hydroxide (NaOH) solution, indicated aromatic amino acid-containing proteins (Hossain et al., 2013).

Detection of Amino Acids:-

- **Ninhydrin Test:** The extract was treated with 0.2% ninhydrin reagent solution and heated on a water bath for a few minutes. The development of a purple, pink, or bluish-violet color indicated the presence of free amino acids (Tiwari et al., 2011).

Results of Qualitative Phytochemical Screening of *Merremia vitifolia*:-

Qualitative phytochemical screening of the methanolic extract of *Merremia vitifolia* revealed a diverse and promising profile of secondary metabolites that underpin its traditional medicinal applications. The analysis confirmed the active presence of alkaloids, carbohydrates, glycosides, phenols, tannins, and flavonoids, as demonstrated by consistent positive outcomes across multiple confirmatory assays (such as Mayer's, Wagner's, Dragendorff's, and Hager's tests for alkaloids; Molisch, Benedict's, and Fehling tests for carbohydrates; Modified Borntrager's and Legal's tests for glycosides; Ferric chloride test for phenols; Gelatin test for tannins; and Alkaline reagent and lead acetate tests for flavonoids). Conversely, tests for saponins (Forth and foam tests), phytosterols (Salkowski's and Libermann Burchard's tests), and proteins and amino acids (Xanthoproteic and ninhydrin tests) were negative, indicating their absence or levels below the limit of detection in this methanolic extract. The robust presence of bioactive constituents such as flavonoids, phenolics, and alkaloids strongly correlates with the plant's known antioxidant, anti-inflammatory, and therapeutic properties, offering a solid biochemical rationale for its continued exploration in modern pharmacognosy.

Table 3.1. Results Qualitative Phytochemical Screening of *Merremia vitifolia*

Phytochemical Category	Screening Test	Result
Alkaloids	Mayer's Test, Wagner's Test, Dragendorff's Test, Hager's Test	Present
Carbohydrates	Molisch Test, Benedict's Test, Fehling Test	Present
Glycosides	Modified Borntrager's Test, Legal's Test	Present
Saponins	Forth Test, Foam Test	Absent
Phytosterols	Salkowski's Test, Libermann Burchard's Test	Absent
Phenols	Ferric Chloride Test	Present
Tannins	Gelatin Test	Present
Flavonoids	Alkaline Reagent Test, Lead Acetate Test	Present
Proteins & Amino Acids	Xanthoproteic Test, Ninhydrin Test	Absent

Detection of Alkaloids

Figure 3.1: Mayer's Test



Figure 3.2: Wagner's Test



Figure 3.3: Dragendorff's Test

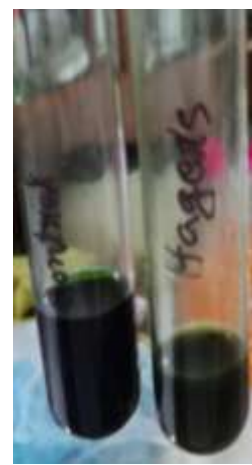


Figure 3.4: Hager's Test

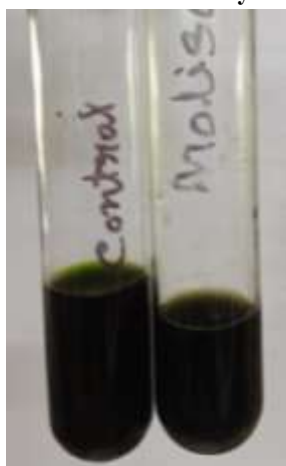
Detection of Carbohydrates

Figure 3.5: Molisch Test

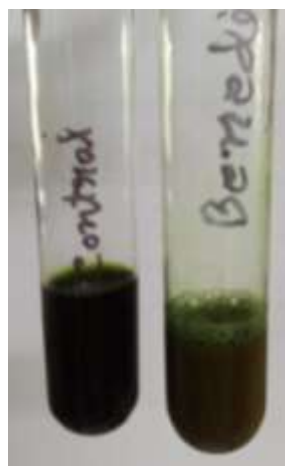


Figure 3.6: Benedict's Test



Figure 3.7: Fehling Test

Detection of glycoside

Figure 3.8: Modified Borntrager's Test



Figure 3.9: Legal's Test

Detection of saponins

Figure 3.10: Forth Test



Figure 3.11: Foam Test

Detection of phytosterol

Figure 3.12:
Salkowski's Test

Detection of phenol

**Figure 3.13: Libermann
Burchard's Test**



**Figure 3.14: Ferric Chloride
Test**

Detection of Tannins

Figure 3.15: Gelatin Test

Detection of Flavonoids

**Figure 3.16: Alkaline
Reagent Test**



Figure 3.17: Lead acetate Test

Detection of proteins and amino acids

Figure 3.18: Xanthoproteic Test

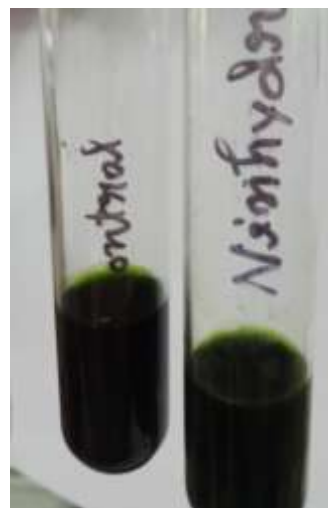


Figure 3.19: Ninhydrin Test

Discussion:-

The qualitative phytochemical screening of the methanolic extract of *Merremia vitifolia* revealed a rich and diverse profile of secondary metabolites, establishing a robust biochemical foundation for its traditional and therapeutic applications (Akter et al., 2021). The confirmation of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, and flavonoids highlights the plant's capacity to synthesize bioactive principles capable of interacting with human physiological systems (Azad et al., 2024). In pharmacognostical research, qualitative profiling serves as the primary diagnostic window into a plant's therapeutic potential, identifying active constituents that govern pharmacological efficacy (Olatunji et al., 2021). The simultaneous detection of multiple metabolite classes in *M. vitifolia* underscores a synergistic biochemical matrix, where individual constituents work in concert to elicit biological responses.

When contextualized against previously published literature on the genus *Merremia* and related Convolvulaceae taxa, the findings of this study demonstrate notable phytochemical consistency while exhibiting distinct profile variations. For instance, investigations into *Merremia borneensis* reported comparable accumulations of flavonoids, carbohydrates, and phenolic compounds within tropical organic extracts, emphasizing structural conservation within the genus (Shah & Hossain, 2014). Similarly, qualitative and quantitative evaluations of *Merremia Emarginata* confirmed the wide spread presence of carbohydrates, tannins, phenolics, and flavonoids across whole-plant and leaf matrices (Prabhu et al., 2011). Furthermore, broader regional evaluations of *Merremia vitifolia* stem and leaf extracts have consistently documented robust titers of alkaloids, flavonoids, and condensed tannins, reinforcing that the metabolic profile observed in this study aligns with established chemotaxonomic markers of the species (Akter et al., 2021; Azad et al., 2024).

However, variations remain evident when comparing specific solvent fractions and regional plant collections. While studies by Shorna et al. (2024) and Islam et al. (2025) highlighted diverse biological properties such as antipyretic, anti-nociceptive, and thrombolytic activities correlated directly with phenolic and flavonoid content, the absence of saponins and phytosterols in the current methanolic extract contrasts with certain broader genus-level reviews that identify trace or fraction-dependent steroidal components (Olatunji et al., 2021). These discrepancies primarily reflect differences in solvent polarity, geographical distribution, soil composition, climatic conditions, and the physiological age of the harvested plant material (Lee et al., 2023). Methanol, being a highly polar protic solvent, efficiently extracts polar secondary metabolites such as glycosides, phenolic acids, and polar flavonoid glycosides, while non-polar constituents like free sterols or triterpenoids may require non-polar or intermediate organic solvents (such as petroleum ether or chloroform) for optimal recovery (Hossain et al., 2013).

The robust detection of flavonoids, phenolic compounds, and tannins in *M. vitifolia* holds profound pharmacological implications. Flavonoids and phenolics are well-documented natural antioxidants that scavenge reactive oxygen species (ROS), mitigate oxidative stress, and protect cellular macromolecules from oxidative damage (Priyadarsini et al., 2009; Azad et al., 2024). These mechanisms directly substantiate the plant's traditional use in managing inflammatory disorders, arthritic joint pain, and pyrexia, as polyphenolic antioxidants frequently inhibit key inflammatory mediators such as cyclooxygenase-2 (COX-2) and suppress protein denaturation pathways (Akter et al., 2021; Islam et al., 2025). Furthermore, the positive results obtained for alkaloids and glycosides align with modern pharmacological findings demonstrating central nervous system modulation, smooth muscle relaxation, and antimicrobial properties (Rahmani & Aly, 2015; Azad et al., 2024).

Conversely, the negative outcomes for proteins, amino acids, saponins, and phytosterols in this methanolic extract indicate that these classes are either absent or present below the threshold of qualitative detection under the experimental conditions used. This specificity helps guide future quantitative isolation and chromatographic fractionation protocols, ensuring subsequent research focuses on targeted purification of the verified bioactive constituents (Kokate et al., 2010).

Conclusion and Future Directions:-

In summary, the qualitative phytochemical screening of *Merremia vitifolia* successfully validates its rich ethnomedicinal heritage through systematic chemical profiling. By corroborating findings with broader phytochemical literature on the Convolvulaceae family, this study reinforces the chemotaxonomic and pharmacological importance of *M. vitifolia*. Future investigations should expand on these qualitative insights by employing advanced chromatographic techniques such as High-Performance Liquid Chromatography and Gas

Chromatography-Mass Spectrometry, alongside quantitative estimations and targeted in vivo biological assays, to isolate, characterize, and fully harness the therapeutic lead molecules present in this botanical resource.

Conflicts of interest:-

The authors do not disclose any conflicts of interest.

Acknowledgment:-

The authors acknowledge Sumon Kanti Datta assistance with the experiments.

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