J Herbmed Pharmacol. 2026; 15(4): 516-524.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.53371

Journal of Herbmed Pharmacology

Phytochemical profiling and bioactivity assessment of crude methanolic extracts from different parts of *Merremia emarginata*: Antibacterial, antioxidant, thrombolytic, and cytotoxic evaluations

Arafath Rahman Akash[1,2], Most. Anika Naznin[1], Md. Tajmul Munshi[1], F. M. Al-Amin Kaisar[3], Md. Anwarul Haque[3], Monirul Islam[1*]

[1]Department of Pharmacy, Faculty of Science, Pabna University of Science and Technology, Pabna-6600, Bangladesh
[2]Department of Pharmacy, Faculty of Health Science, Atish Dipankar University of Science and Technology, Dhaka-1230, Bangladesh
[3]Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi-6205, Bangladesh

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 27 Aug. 2025
Revised: 8 Jul. 2026
Accepted: 9 Jul. 2026
epublished: 1 Oct. 2026

Keywords:
Anti-microbial
Cytotoxicity
Free radical scavengers
Thrombosis
Plant-derived bioactive compounds

ABSTRACT

Introduction: *Merremia emarginata,* a member of the Convolvulaceae family, is used to combat various maladies associated with infection, oxidative stress, and inflammation. Methanolic extracts prepared from the leaves, roots, and stalks of *M. emarginata* were evaluated for their phytochemical constituents as well as antioxidant, antibacterial, thrombolytic, and cytotoxic activities.

Methods: Total flavonoid content (TFC) and total phenolic content (TPC) were quantified using quercetin equivalent (QE) and gallic acid equivalent (GAE), respectively, using standard spectrophotometric procedures. Antioxidant capacity was quantified by the DPPH free radical scavenging method. The extracts were further screened for antibacterial effects against selected bacterial strains, thrombolytic activity using a clot lysis model, and cytotoxic potential against brine shrimp.

Results: Among the tested samples, the methanolic leaf extract showed the highest TPC (345±89 mg GAE/g) and TFC (243±28 mg QE/g). The leaf extract also demonstrated superior antioxidant capacity, with an IC_{50} value of 13.93±0.93 µg/mL. In tests for antibacterials, leaf extracts showed a considerable inhibitory effect against *Pseudomonas aeruginosa* (13.67 mm at 500 µg/disc), whereas stalk extracts significantly inhibited *Escherichia coli*. Thrombolytic assays revealed that the stalk extract produced the highest clot lysis activity (21 ± 3.3%) compared to the leaf and root extracts. The leaf extract had the highest cytotoxicity (LC_{50} = 13±6.1 µg/mL), whereas the stalk and root extracts were less effective.

Conclusion: *M. emarginata* extracts showed strong antioxidant and measurable antibacterial, thrombolytic, and cytotoxic activities *in vitro*, which suggest potential bioactivity, but further studies are needed to evaluate *in vivo* effects and isolated active compounds.

Implication for health policy/practice/research/medical education:
Relevant medicinal characteristics displayed by *Merremia emarginata* plant materials *in vitro* offer a way to isolate the plant's active ingredients in the future using bioassay guidance and possibly create new therapeutic substances.

Please cite this paper as: Akash AR, Naznin MA, Munshi MT, Kaisar FMAA, Haque MA, Islam M. Phytochemical profiling and bioactivity assessment of crude methanolic extracts from different parts of *Merremia emarginata*: Antibacterial, antioxidant, thrombolytic, and cytotoxic evaluations. J Herbmed Pharmacol. 2026;15(4):516-524. doi: 10.34172/jhp.53371.

Introduction

More than three-fourths of the total human population globally primarily depends on health care based on botanical extracts, and over one-third of the total plant flora have been used for the purpose of curing diseases since antiquity (1-3). The World Health Organization (WHO) advocates integrating herbal preparations with demonstrated efficacy into primary healthcare (4). There is an extensive history of drug candidates with medicinal plants, and some contemporary drugs are derived from

***Corresponding author**: Monirul Islam,
Email: phamonir21@pust.ac.bd

these natural products. Despite the challenges associated with phytomedicine-based drug development, plants are still being screened for new drugs. The bioactive molecules, which are active against multiple diseases, have long been used in ethnomedicine. There exists a treasure trove of potential therapeutics in nature that require isolation and characterization for use in conventional medicine. These therapeutics are categorized into plant-made biologics, small biomolecules, and phytopharmaceutical drugs, the latter of which are developed using an ethnobotany-driven research based on indigenous healthcare systems (5-7).

Merremia emarginata, also known as *Ipomoea reniformis* (8), is a tropical plant found in Africa, Malaysia, and India. Despite being considered a non-productive food crop, it is used as a leafy vegetable by economically disadvantaged populations in India. The creeping perennial herb has simple leaves with reniform to ovate-cordate morphology, yellow flowers, and sub-globose capsules with light brown seeds. *M. emarginata* has traditionally been used in ethnomedicine for treating various ailments, including abscesses, cough, fever, headache, migraine, rheumatism, diabetes, ear sores, epilepsy, helminth infections, wounds, and disorders affecting the skin, eyes, gums, kidneys, liver, and respiratory tract (8-13). Recently, two research studies on this plant were done by Rameshkumar et al and Purushoth et al. One study focused on the profiling of phenolic constituents using UPLC-Q-TOF-MS/MS along with the evaluation of nephroprotective effects, while the other investigated *in vitro* antioxidant and antibacterial activities using thioglycolic acid-capped cadmium telluride quantum dots (9,11,14). *M. emarginata*'s ethanolic extract's GC-MS analysis shows the existence of phytoconstituents that fall into the categories of acids, esters, alcohols, ethers and so on (14). Babu et al isolated scopoletin, cyanarin, tetrytol, and diacetyltetritol from ethyl acetate extract of *M. emarginata* (Burm.f.) and demonstrated their antioxidant, anti-inflammatory, and cytotoxic potentials (15). Angappan et al reported that chlorogenic acid from *M. emarginata* (Burm.f.) could act as a promising diuretic substance, with minimal adverse effects (12).

Earlier studies on *M. emarginata* have largely centered on the antioxidant, antimicrobial, and cytotoxic activities of its leaf extracts (11). In contrast, based on available literature, there are no investigations on the thrombolytic potential of this botanical species. Furthermore, no thorough investigations have explored the cytotoxic, antimicrobial, antioxidant, and thrombolytic potentials of the stem and root extracts distinctly. The present work explored the phytochemical characteristics along with the antioxidant, *in vitro* antimicrobial, cytotoxic, and thrombolytic properties of crude methanolic extracts prepared from the leaves, stalk, and root of *M. emarginata*. The outcomes of this investigation generate novel perspectives on the biopotential of the leaf, root, and stem, emphasizing their considerable therapeutic value. This investigation paves the way for future explorations focused on the identification and characterization of specific secondary metabolites underlying the health-promoting properties of the plant across diverse health conditions.

Materials and Methods

Materials

Aluminium chloride ($AlCl_3$), ethanol, methanol, and ascorbic acid were purchased from Merck Company. Folin–Ciocalteu reagent (FCR), 1,1-diphenyl,2-picrylhydrazyl (DPPH), sodium carbonate, sodium hydroxide, sodium nitrite, sulfuric acid, quercetin, and gallic acid (GA) were purchased from Sigma-Aldrich (Germany). Ferrous sulphate, Müeller–Hinton broth agar (MHA), and discs of antibiotics were obtained from HiMedia Laboratories Pvt. Ltd., India. Streptokinase was collected from Popular Pharmaceuticals Ltd., and vincristine sulphate was collected from Beacon Pharmaceuticals PLC.

Collection of samples and preparation of the methanolic extract

The *M. emarginata* plant was collected from Pabna, Rajshahi. The plant was identified in the herbarium of the Department of Botany, Rajshahi University (Reference no. 25). Leaves, stalks, and roots were cleaned, sun-dried, and pulverized into a coarse powder using a grinder (Model: WBL-15JM75N, Walton, Bangladesh), then preserved for future use. Active compounds from each plant part were extracted using a cold methanol extraction, and the extracts were eluted through filter papers. By using a rotary evaporator, the elute was condensed at 45 °C (16).

Qualitative analysis of phytochemical compounds

The methanolic extracts of *M. emarginata* leaves, roots and stalks were subjected to standard procedures for phytochemical analysis, as described in a previously documented method (10,17) with some modifications. The study analysed the extract's presence of alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, fats, and oils. Detection of alkaloids was performed using Mayer's and Wagner's reagents, while saponins were detected through emulsion and frothing tests. Ferric chloride solution was used for tannin detection, while flavonoids were detected using a NaOH (10%) and concentrated HCl solution. Proteins were detected by a purple hue using Biuret reagents. Reducing sugars were detected using Fehling solutions. The sample was sandwiched between filter paper and study paper for fats and oils, and glycosides were detected using diluted H_2SO_4, 20% KOH, and Fehling's solutions.

Quantitative analysis of phytochemical compounds

Determination of total phenolic contents

With a few modifications, a previously described method (18,19) was used to measure the total phenolic content

(TPC) of the crude extract. The oxidising agent was the FCR, and the standard was GA. For the experiment, each standard and sample test tube was treated with 10% FCR in water (v/v), followed by 0.4 mL of the methanolic extract. Upon the addition of 7.5% Na_2CO_3 solution, the mixture was thoroughly vortexed, covered, and maintained at ambient temperature for a specified period. Absorbance was subsequently monitored at 760 nm against a control sample. For calibration, a solution containing 1-10 µg/mL of GA was utilized.

Determination of total flavonoid contents

The total flavonoid content (TFC) of the crude extracts was determined using a slightly modified aluminium chloride colorimetric assay following previously reported procedures (19,20). Flavonoid content was calculated to be quercetin equivalent (QE) using quercetin as the standard. This led to the creation of a calibration curve for QE. Each QE dilution (500 µL) was mixed with 2.5 mL of distilled water, followed by the incorporation of 150 µL of 5% $NaNO_2$ solution. After a brief incubation period, 300 µL of 10% $AlCl_3$ was added, and 100 µL of NaOH solution was added in increments of 0.55 mL. At a wavelength of 510 nm, the reaction mixture was spectrophotometrically analysed.

Evaluation of antioxidant activity by DPPH free radical scavenging assay

Free radical scavenging activity was evaluated using the DPPH approach with slight modifications to previously reported procedures (18,19). Serial dilution was used to create various amounts of ascorbic acid (100-1.5625 µg/mL) and sample extracts (100-1.5625 µg/mL). In the assay, 2 mL of each sample solution was combined with 3 mL of DPPH reagent in separate test tubes. The mixtures were incubated for thirty minutes at room temperature in the dark to allow completion of the reaction. After incubation, absorbance was determined at 517 nm using a Shimadzu UV-Vis spectrophotometer (Japan) against a blank solution (21). Scavenging activity was estimated with the aid of equation (1), from which the IC_{50} value was calculated.

$$Percentage\ of\ (\%)\ Scavenging = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad (1)$$

where, A_1 = Absorbance of the Sample/Standard and A_0 = Absorbance of the DPPH (Control).

Evaluation of *in vitro* antibacterial activity by the disc diffusion assay method

The antibacterial properties of crude methanolic extracts from leaves, roots, and stalks were tested against seven bacterial strains: four gram-positive species (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus licheniformis*, and *Staphylococcus aureus*) and three gram-negative species (*Escherichia coli*, *Shigella boydii*, and *Pseudomonas aeruginosa*), using a slightly modified disc diffusion technique as previously described (19). The prepared samples were dissolved in an appropriate solvent prior to analysis, impregnated onto sterile discs (5 mm in diameter), and subsequently dried before application. Kanamycin (30 µg/disc) and methanol were used as the positive and negative controls, respectively. Following incubation at 37 °C for 18–24 hours, antibacterial efficacy was assessed by measuring the visible zones of inhibition (21). The diameter of the zone of inhibition, measured in millimetres (mm), was used to quantify the test agent's antibacterial activity.

Evaluation of *in vitro* thrombolytic activity

The thrombolytic activity technique, as previously described (22,23), was slightly modified in order to assess the clot lysis of human blood using the crude extract. The analysis employed lyophilized 15,00,000 I.U. Streptokinase (STK) solution was combined with sterile saline to produce a suspension. 50 and 100 µL suspensions were utilized for *in vitro* thrombolysis. The weighted crude methanolic extract of *M. emarginata* parts was suspended in saline. No oral contraceptives or anticoagulants were administered to healthy individuals who gave venous blood samples. An aliquot of 500 µL blood was placed into previously weighed tubes and incubated at 37 °C for two hours. After clotting, serum was removed without disturbance, and the extract and STK solutions were added. Following clot lysis, each tube was reweighed, and the percentage of clot lysis was calculated from the difference in clot weight before and after treatment using equation (2).

$$\%\ Clot\ lysis = \frac{Weigt\ of\ the\ lysis\ clot}{Weigt\ of\ clot\ before\ lysis} \times 100 \quad (2)$$

Evaluation of cytotoxicity by brine shrimp lethality bioassay

Cytotoxic activity of the crude methanolic extracts obtained from the selected plant parts was evaluated using a slightly modified brine shrimp lethality bioassay with *Artemia salina* (Leach), following previously described methods (24,25). A conical jar was used to hatch brine shrimp eggs into nauplii. Subsequently, the tank received sterile artificial seawater containing 38 gL^{-1} sea salt, pH 8.5 was obtained using $NaHCO_3$ and NaOH, and it was aerated for 48 hours. After hatching, 10 nauplii were pipetted into each vial with 4.5 mL brine. After adding several amounts of crude extract (5–80 µg/mL), the volume was increased to 5 mL with brine solution. Incandescent lights were used to maintain a temperature of 35 ± 2 °C for 24 hours. Vincristine sulphate was the positive control. After 24 hours, each vial's surviving nauplii were counted using a magnifying lens. Based on this information, the LC_{50} was determined, and the average brine shrimp nauplii mortality at each sample concentration was calculated using equation (3).

$$\%\,Mortality = \frac{Number\ of\ dead\ nauplii}{Initial\ number\ of\ live\ nauplii} \times 100 \quad (3)$$

Statistical analysis

The results were shown as mean ± standard deviation (SD), with all tests performed in triplicate. Microsoft Excel 2019 and SPSS software were used for statistical analysis. One-way ANOVA was applied to compare group means, followed by Tukey's HSD post hoc test for pairwise comparisons. A *P* value less than 0.05 was regarded as statistically significant.

Results

Qualitative analysis of phytochemical compounds

Using pre-established standard procedures, a methanolic extract of *M. emarginata* was screened for phytochemical constituents for various secondary metabolites, and the results show that in leaf, root, and stalk extracts, alkaloids, flavonoids, tannins, and saponins were present, but glycosides, proteins, reducing sugar, lipids, and oils were absent.

Quantitative analysis of phytochemical compounds

TPCs and TFCs of different parts of *M. emarginata* were expressed as mg gallic acid equivalent (GAE)/g extract and mg QE/g extract, respectively. All extracts exhibited considerable amounts of phenolic compounds, ranging from 87 ± 43 to 345 ± 89 mg GAE/g extract. Among the tested samples, the methanolic leaf extract showed the highest phenolic content, whereas the root extract contained the lowest amount. Similarly, the leaf-derived extract showed the maximum flavonoid content (243 ± 28 mg QE/g extract), followed by the stalk extract (225 ± 54 mg QE/g extract) and root extract (193 ± 37 mg QE/g extract). In terms of flavonoid and phenolic contents, statistical analysis showed significant differences among the various extracts, with the leaf extract having the highest content, as shown in Table 1. Detailed data for TPC (mg GAE/g) determination and TFC (mg QE/g) determination are provided in Table S1 and Table S2, respectively.

Evaluation of antioxidant activity by the DPPH scavenging method

A concentration-dependent increase in DPPH radical scavenging activity was observed for the tested extracts and standard compound (Figure 1 and Table S3). Among the evaluated samples, the leaf extract reflected the

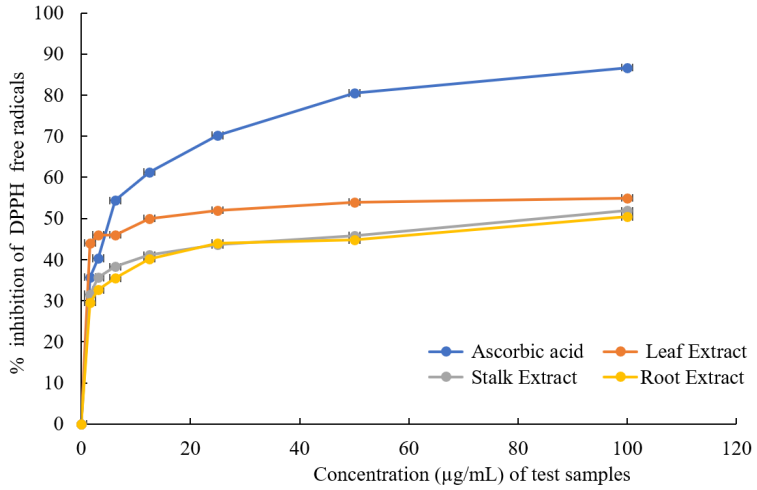

Figure 1. Dose-dependent inhibition of DPPH free radicals by methanolic extracts of the leaf, bark, and stem of *Merremia emarginata*. Antioxidant activity is presented as a percentage (%) inhibition at different concentrations. Data are presented as mean ± standard deviation (SD) from three independent experiments (n = 3), and error bars represent SD.

Table 1. The physical appearance, phytochemical contents, cytotoxic, and antioxidant activities of leaf, root, and stalk methanolic extracts of *Merremia emarginata*

Sample	Physical appearance	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	Antioxidant activity in IC_{50} (µg/mL)	Cytotoxic activity in LC_{50} (µg/mL)
Leaf extract	Dark green viscous	345±89[a]	243±28[b]	13.93±0.93[c]	13±6.1[d]
Stalk extract	Dark brown viscous	210±50[a]	225±54[b]	92±4.0[c]	21±8.9[d]
Root extract	Brown viscous	87±43[a]	193±37[b]	105±4.1c	27±9.1[d]
Ascorbic acid	-	-	-	5.19±0.07[c]	-
Vincristine sulphate	-	-	-	-	2.4±0.67[d]

Positive controls used in the antioxidant and cytotoxicity activity test were ascorbic acid and vincristine sulphate, respectively. The half-maximal inhibitory concentration of the extracts is denoted as IC_{50}, Median lethal concentration is denoted as LC_{50}, GAE: Gallic acid equivalent, QE: Quercetin equivalent. Statistically significant differences ($P < 0.05$) are indicated by different superscript letters (a-d) in the same column. Data represent mean ± SD of three independent determinations.

strongest scavenging potential, followed by the stalk and root extracts across all tested concentrations (Figure 1). The IC_{50} values ranged from 13.93 ± 0.93 to 105 ± 4.1 µg/mL, indicating significant antioxidant activity ($P < 0.001$), as presented in Table 1.

Evaluation of *in vitro* antibacterial activity by the disc diffusion assay method

The antibacterial potential of the extracts was evaluated based on the diameter of the inhibition zones, measured to the nearest millimetre (mm). The corresponding findings are presented in Table 2. Methanolic extracts of leaf, stalk, and root showed the lowest activity against *Bacillus cereus* (7 mm) and the highest activity against P*seudomonas aeruginosa* (13.67 mm) for leaf extract, and both stalk and root extracts showed the highest activity against *Bacillus megaterium* (11.67mm and 11.17 mm). The standard antibiotic, kanamycin, yielded inhibition zones ranging from 17.33 to 26.00 mm against all tested microorganisms.

Evaluation of cytotoxicity by brine shrimp lethality bioassay

Merremia emarginata plant's methanolic extracts of different parts were tested for cytotoxic action against brine shrimp nauplii. The outcome demonstrated that the plant extract's activity depended on the dose (Figure 2 and Table S4). The study found that standard vincristine sulphate had varying mortality rates (60-100%) at different concentrations. At 80 µg/mL, leaf, stalk, and root extracts showed significantly lower mortality rates of 76.7%, 76.7%, and 66.7%, respectively, as shown in Figure 2 and Table S4. The negative control DMSO showed minimal mortality. The LC_{50} values for leaf (13 ± 6.1 µg/mL), stalk (21 ± 8.9 µg/mL), and root (27 ± 9.1 µg/mL) were less harmful to brine shrimp than the standard medication, vincristine sulphate (2.4 ± 0.67 µg/mL). The leaf, stalk, and root extracts exhibited significantly lower mortality rates compared to the standard drug, as indicated by their LC_{50} values (Table 1).

Evaluation of *in vitro* thrombolytic activity

The comparison of the percentage of clot breakdown in each part of the plant extract with that of streptokinase, a standard thrombolytic medication, showed significant differences, as detailed in Table S5. In 50 and 100 µL solutions, the clot lysis values for STK (Streptokinase) were 37 ± 9.3 and 512 ± 6.1, respectively. The clot lysis values for the 50 µL solution containing leaf, root, and stalk extract were 9.3 ± 2.1, 14 ± 4.7, and 10 ± 3.0, respectively. In contrast, the clot lysis values for the 100 µL solution containing leaf, root and stalk extract was 19 ± 4.1, 14 ± 3.1,

Table 2. *In vitro* antibacterial activity of methanolic extracts of *Merremia emarginata* (leaf, bark, and stem) compared with kanamycin against selected bacterial strains

Tested bacteria	Plant part	Zone of inhibition (ZOI) in mm at different concentrations					
		Kanamycin (30 µg/disc)	Plant 100 µg/disc	Plant 200 µg/disc	Plant 300 µg/disc	Plant 400 µg/disc	Plant 500 µg/disc
Bacillus cereus	Leaf	17.67	0	0	0	0	7.00
	Stalk	17.67	0	0	0	0	7.00
	Root	17.67	0	0	0	0	7.00
Bacillus megaterium	Leaf	19.00	7.00	7.50	8.50	9.17	9.67
	Stalk	17.33	0	7.00	7.33	10.17	11.67
	Root	18.67	7.17	8.00	8.83	10.50	11.17
Bacillus licheniformis	Leaf	22.67	7.17	8.50	9.17	9.33	9.83
	Stalk	18.17	0	0	0	9.67	9.67
	Root	20.33	7.17	7.17	8.83	8.83	9.67
Staphylococcus aureus	Leaf	21.67	0	0	0	7.67	8.17
	Stalk	24.00	0	8.50	8.17	8.50	7.83
	Root	22.67	0	0	9.83	10.00	8.83
Escherichia coli	Leaf	18.00	0	7.83	8.67	8.83	10.17
	Stalk	18.33	9.83	9.83	10.50	9.17	10.00
	Root	18.50	9.00	10.17	10.83	10.50	11.50
Shigella boydii	Leaf	20.33	7.33	7.50	8.00	8.17	8.83
	Stalk	18.00	0	0	7.17	7.17	9.17
	Root	19.00	0	0	0	8.50	8.50
Pseudomonas aeruginosa	Leaf	23.67	10.50	10.83	11.0	11.67	13.67
	Stalk	26.00	9.33	8.50	8.33	8.33	8.33
	Root	21.67	0	0	6.83	7.50	8.17

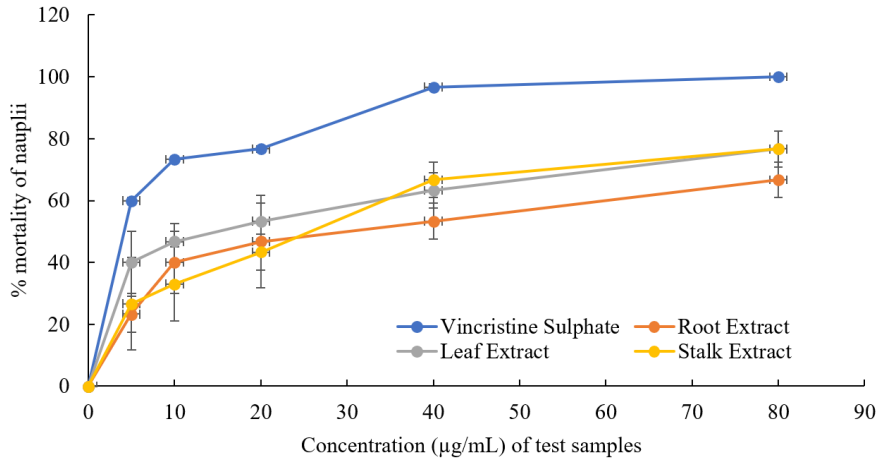

Figure 2. Dose-dependent cytotoxic effects of methanolic extracts of the leaf, bark, and stem of *Merremia emarginata*, shown as percentage (%) mortality. Data are presented as mean ± standard deviation (SD) from three independent experiments (n = 3), and error bars represent SD.

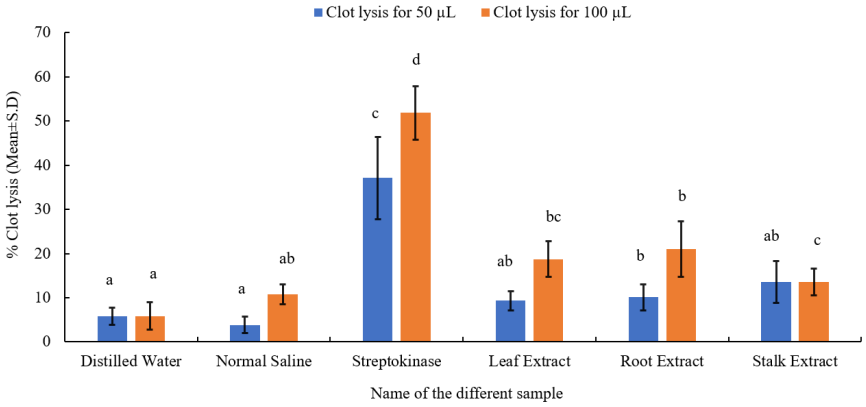

Figure 3. Thrombolytic activity of different sample solutions of Merremia emarginata at 50 µg (50 µL) and 100 µg (100 µL), expressed as percentage (%) clot lysis. Results are presented as mean ± standard deviation (SD) from three separate experiments (n = 3). Groups marked with different superscript letters differ significantly ($P < 0.05$), while those sharing the same letter are not significantly different. Error bars represent standard deviation (SD).

and 21 ± 3.3, respectively shown in Figure 3. Relative to the other extracts, root and stalk extracts exhibit the greatest percentages of clot lysis for 100 µL solutions and 50 µL solutions, respectively, as shown in Figure 3.

Discussion

Earlier research also reported that the leaves of *M. emarginata* from different extracts contained phytochemicals, namely saponins, alkaloids, tannins, and flavonoids (8,10), which aligns with the outcomes demonstrated in this investigation. In our study, these phytochemicals were also found in the methanolic extracts of the roots and stalks. The overall levels of phenolic compounds and flavonoids in extracts from the three different parts of the plants differed significantly, with leaf extract showing the highest diversity (Table 1), suggesting that leaves were a richer source of phenolic and flavonoid compounds compared to roots and stalks. The total flavonoid value varied, with leaf extract being the most constant and stalk extract showing the greatest variability. However, no notable differences in TFC were observed among the three groups, suggesting that different flavonoid types in the plant may exert distinct pharmacological actions. Although TPC and TFC values exhibited high SDs, triplicate assays confirmed consistent trends (Tables S1 and S2), likely due to slight variations in extraction efficiency.

The enhanced antioxidant potential observed in the methanolic leaf extract relative to the root and stalk extracts could be associated with its greater abundance of bioactive secondary metabolites, especially phenolics and flavonoids, which are recognised for their antioxidant-rich nature. The lower IC_{50} value of the leaf extract indicates greater ability to neutralise free radicals. The observed differences ($P < 0.001$) between extracts obtained from the leaves, roots, and stalks are attributed to the distribution of secondary metabolites within the plant. Previously reported IC_{50} value for the aqueous leaf extract (11) was

relatively higher (86.5 µg/mL) as compared to the finding in our study, possibly due to variations in solvent polarity and extraction efficiency.

The leaf extract showed the most potent antibacterial activity against *P. aeruginosa*, while the root and stalk extracts showed the strongest activity against *B. megaterium*. This variability in activity could be attributed to differences in the composition and concentration of bioactive compounds in various plant parts. Phytochemicals such as alkaloids, phenolics, tannins, and flavonoids have demonstrated inhibitory effects against bacterial strains (26) by breaking bacterial cell walls, affecting membrane permeability, and inhibiting key enzymes in microbial metabolism. The relatively higher activity observed in the leaf extract may stem from the greater abundance of these bioactive constituents. Prior studies revealed that various leaf extracts of this plant showed higher zones of inhibition in an *in vitro* antibacterial assay against different bacterial strains (10,27). An earlier study showed that the inhibition zones of 6-9 mm are generally considered to indicate slight activity, while those between 9-12 mm suggest moderate activity (28). The mostly observed inhibition zones in our investigation fall within the slight to moderate activity range, indicating minimal potential that warrants further investigation.

The study found that methanolic extract from *M. emarginata* showed minimal cytotoxic effects against the vincristine sulphate standard in the brine shrimp lethality test, supporting its use in traditional medicine. Post-hoc studies confirmed significant variations in LC_{50} values, with vincristine sulphate being more effective than root extract. Root extract was the least effective, with significant differences indicating cytotoxicity variation among the extracts (Table 1 and Table S4). In 2013, Babu et al evaluated the bioactivity of different *M. emarginata extracts*, with the ethyl acetate extract having a LC_{50} of 34.29 (15). An up-to-date investigation by Akano et al reported that the ethanol extract of *Irvingia gabonensis* seeds and its ethyl acetate fraction exhibited moderate cytotoxic activity against brine shrimp (29). The cell death attributes of medicinal plants are attributed to the existence of various chemicals such as carotenoids, phenolics, terpenoids, alkaloids, and nitrogen compounds (25). This investigation is a preliminary screening tool, and further cell-based studies are required to prove its anticancer potential.

The study examined the thrombolytic activity of methanolic extracts from different parts of *M. emarginata*, with streptokinase used as the positive control and normal saline as the negative control. The results indicated that water did not induce clot dissolution in either control group, and minimal thrombolytic activity was observed after treatment with methanolic extracts (Figure 3). The study revealed a significant difference in clot lysis between samples, with root extract showing a higher value, suggesting further research into their bioactive ingredients. The study found that clot lysis in leaf and stalk extracts increased with concentration, regardless of whether the root extract showed greater action at high concentrations (Table S5). At the same concentration, clot lysis varies, which may be caused by the volunteer's physiological state, obesity, or hemoglobin levels (30). Research carried out by Mehnaz et al highlighted that the methanolic extract of *Ficus religiosa* stem bark exhibited greater thrombolytic activity than its n-hexane extract (31). Similarly, Akter et al demonstrated that the aqueous fraction of *M. vitifolia* leaf extract produced 42.5% clot lysis compared to the negative control (32). Regarding our study, a clot lysis of 9.3-21% is relatively low compared to standard streptokinase (52%), which often exhibits higher efficacy. However, the presence of thrombolytic activity in the plant extract suggests that it may contain bioactive compounds with fibrinolytic properties, albeit at a lower potency (33). More detailed studies are needed to isolate and characterize the active chemicals accountable for this.

The phytochemicals, including flavonoids, tannins, and alkaloids, possess antioxidant and anti-inflammatory properties, potentially preventing diseases, reducing blood sugar levels, and having hypoglycemic characteristics. They also have antifungal and anti-diabetic properties, making them a potential source for the pharmaceutical industry (34). Phenolic molecules in plants are abundant and linked to antioxidant activity. A study found higher concentrations of phenolic and flavonoid compounds in plant components, indicating their high antioxidant capacity (9), as also seen in our study. Flavonoids, essential pigments in flower colors, attract pollinators, defend plants, and have anti-allergic, anti-inflammatory, antibacterial, and anticancer properties (35). Phytochemicals like tannins, which inhibit hydrolytic enzymes used by plant pathogens. Antioxidants and free-radical scavengers have antimicrobial properties, contributing to their antibacterial activity (36). Secondary metabolites have been used to develop antibiotics, anthelminthic agents, anticoagulants, antitumor substances, carcinogens, growth substances, hormones, hypotensives, insecticides, toxins and vitamins for medicine (37,38).

This study acknowledges limitations in its findings, particularly the collection of the plant *M. emarginata* from a single spot in Bangladesh, which may not represent the full diversity of phytochemicals found in other areas. Moreover, while the investigation verified the existence of several classes of bioactive substances, it didn't separate or recognize the specific constituents linked to the pharmacological consequences, leaving the exact mechanisms of action unclear.

Conclusion

The present study highlights the pharmacological potential of *M. emarginata*, demonstrating its antioxidant, antibacterial, thrombolytic, and cytotoxic activities.

The occurrence of bioactive secondary metabolites, including phenols, flavonoids, tannins, and alkaloids, supports its traditional medicinal use. Notably, the leaf extract exhibited the strongest antioxidant and minimal thrombolytic activities, indicating its potential role in preventing pathologies associated with oxidative imbalance and thrombosis. The antibacterial and cytotoxic properties suggest further exploration for novel drug development. However, comprehensive investigations using contemporary analytical tools and techniques, such as high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry, are required to isolate active compounds and elucidate their mechanisms of action. These findings may help lead to the scientific validation of *M. emarginata* and its potential inclusion in herbal pharmacopoeias.

Acknowledgments

The facilities provided by Pabna University of Science and Technology and the University of Rajshahi, Bangladesh, allowed the authors to conduct this work, for which they are really grateful.

Authors' contribution

Conceptualization: Monirul Islam.
Data curation: Arafath Rahman Akash, Most. Anika Naznin, and Md. Tajmul Munshi.
Formal analysis: Monirul Islam, Most. Anika Naznin, Md. Tajmul Munshi, F. M. Al-Amin Kaisar, and Md. Anwarul Haque.
Funding acquisition: Monirul Islam.
Investigation: Monirul Islam, Arafath Rahman Akash, Most. Anika Naznin, and Md. Tajmul Munshi.
Methodology: Monirul Islam, Most. Anika Naznin, and Md. Tajmul Munshi.
Project administration: Monirul Islam.
Resources: Monirul Islam and F. M. Al-Amin Kaisar.
Software: Arafath Rahman Akash and Monirul Islam.
Supervision: Monirul Islam.
Validation: Arafath Rahman Akash.
Visualization: Monirul Islam and Md. Anwarul Haque.
Writing–original draft: Arafath Rahman Akash and Monirul Islam.
Writing–review & editing: All authors.

Conflicts of interests

The authors declare no competing interests.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of AI-assisted tools in the writing procedure

The authors used the free version of QuillBot for minor paraphrasing during the preparation of the manuscript, and no other AI tools were used.

Ethical considerations

The present investigation received approval from the Institutional Ethics Committee (IEC), Pabna University of Science and Technology Pabna-6600, Bangladesh (Ethical clearance number: PUST/IEC/Memo/25/2023).

Funding/Support

This research was not funded by any institution. The corresponding author paid for the expenses.

Supplementary files

Supplementary file 1 contains Tables S1-S5.

References

1. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal Plants. In: Bose TK, Das P, Joy PP, editors. Tropical Horticulture. Calcutta: Naya Prokash; 2001. p. 449–632.
2. WHO. WHO traditional medicine strategy: 2014-2023. Geneva, Switzerland: WHO; 2013. p. 1–76.
3. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi:10.3389/fphar.2013.00177
4. WHO. Regulatory situation of herbal medicines: A worldwide review. Geneva, Switzerland: WHO; 1998. p. 1–5.
5. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582–614. doi:10.1016/j.biotechadv.2015.08.001
6. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (India). 2022;65(3):399–411. doi:10.1007/s13237-022-00405-3
7. Tanaka N, Kashiwada Y. Phytochemical studies on traditional herbal medicines based on the ethnopharmacological information obtained by field studies. J Nat Med. 2021;75(4):762–83. doi:10.1007/s11418-021-01545-7
8. Devadasu C, Naga JN, Srinivasa BP, Afzal BS. Biological evaluation of *Merremia emarginata* for antibacterial, antifungal and antioxidant activities. World J Pharm Pharm Sci. 2017;6(7):1509–25. doi:10.20959/wjpps20177-9571
9. Rameshkumar A, Sivasudha T, Jeyadevi R, Sangeetha B, Ananth DA, Aseervatham GSB, et al. In vitro antioxidant and antimicrobial activities of *Merremia emarginata* using thio glycolic acid-capped cadmium telluride quantum dots. Colloids Surf B Biointerfaces. 2013;101:74–82. doi:10.1016/j.colsurfb.2012.05.034
10. Sachithanandam P, Parkavi S, Ganesh P, Swaminathan C. Phytochemical analysis, antibacterial activity and antioxidant activity of leaf extracts of *Merremia emarginata* (Burm. F.). Int J Pharm Sci Res. 2020;11(10):5214–8.
11. Olatunji TL, Adetunji AE, Olisah C, Idris OA, Saliu OD, Siebert F. Research progression of the genus *Merremia*: A comprehensive review on the nutritional value, ethnomedicinal uses, phyto-chemistry, pharmacology, and toxicity. Plants. 2021;10(10):2070. doi:10.3390/plants10102070
12. Angappan R, Devanesan AA, Thilagar S. Diuretic effect of

chlorogenic acid from traditional medicinal plant *Merremia emarginata* (Burm. F.) and its by product hippuric acid. Clin phytosci. 2018;4(1):29. doi:10.1186/s40816-018-0088-5
13. Gandhi GR, Sasikumar P. Antidiabetic effect of *Merremia emarginata* Burm. F. in streptozotocin induced diabetic rats. Asian Pac J Trop Biomed. 2012;2(4):281–6. doi:10.1016/S2221-1691(12)60023-9
14. Purushoth PT, Panneerselvam P, Suresh R, Selvakumari S, Shantha A. Phytochemical analysis of ethanolic extract of *Merremia emaraginata* Burm. F by GC-MS. Res J Pharm Biol Chem Sci. 2012;3(4):507–12.
15. Babu AV, Babu BH, Subbaraju G V. Isolation and bioactivity of diacetyltetritol from *Merremia emarginata* (Burm.f). Nat Prod Indian J. 2013;9(5):201–8.
16. Parvez GMM, Akanda MdKM, Karim R, Mehjabin S, Mou S, Zahan M, et al. Comparative phytochemical screening and antimicrobial evaluation of different varieties of Banana (*Musa sapientum*). Int J Innov Pharm Sci Res. 2016; 4(4):372–83. doi:10.13141/RG.2.2.36272.372383
17. Omar GA, Mohammed LY. Physiochemical standardization and phytochemical screening of *Urtica dioica* L. leaves growing in Zakho, Kurdistan region, Iraq. Sci J Univ Zakho. 2023;11(3):306–16. doi:10.25271/sjuoz.2023.11.3.1069
18. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022;27(4):1326. doi:10.3390/molecules27041326
19. Jain P, Haque A, Islam T, Alam MA, Reza HM. Comparative evaluation of *Ziziphus mauritiana* leaf extracts for phenolic content, antioxidant and antibacterial activities. J Herbs Spices Med Plants. 2019;25(3):236–58. doi:10.1080/10496475.2019.1600627
20. Khan MS. Determination of chemical composition, total flavonoid content, total phenolic content and antioxidant capacity of various crude extracts of *Manihot esculenta* Crantz leaves. Int J Res Appl Sci Eng Technol. 2018; 6(4):2433–44. doi:10.22214/ijraset.2018.4413
21. Peña-Rojas G, Escriba-Gutierrez E, Andía-Ayme V, Cordero-Clavijo LM, Lazo-Vélez MA. Bioactive potential of Tocosh supplemented with selenium-enriched *Saccharomyces Cerevisiae* biomass. Foods. 2025;14(18):3153. doi:10.3390/foods14183153
22. Zaman R, Parvez M, Jakaria Md, Abu Sayeed M, Islam M. In vitro clot lysis activity of different extracts of *Mangifera sylvatica* Roxb. leaves. Res J Med Plant. 2015;9(3):135–40. doi:10.3923/rjmp.2015.135.140
23. Vani M, Vijaya Rani P, Madhuri O, Vijaya Sai Sree M, Sai Ramya L, Gnana Chandrika M, et al. Phytochemical and in vitro thrombolytic activity evaluation of *Cassia siamea* L., Leguminosae leaf extracts, and pyrogallol. Int J Green Pharm. 2019;13(3):213–7.
24. Mclaughlin JL, Rogers LL, Anderson E, Lauder E, York N. The use of biological assays to evaluate botanicals. Drug Inf J. 1998;32:513–24. doi:10.1177/009286159803200223
25. Apu AS, Muhit MA, Tareq SM, Pathan AH, Jamaluddin ATM, Ahmed M. Antimicrobial activity and brine shrimp lethality bioassay of the leaves extract of *Dillenia indica* Linn. J Young Pharm. 2010;2(1):50–3. doi:10.4103/0975-1483.62213
26. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82. doi:10.1128/CMR.12.4.564
27. Elumalai E, Ramachandran M, Thirumalai T, Vinothkumar P. Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pac J Trop Biomed. 2011;1(5):406–8. doi:10.1016/S2221-1691(11)60089-0
28. Guarnieri A, Triunfo M, Scieuzo C, Ianniciello D, Tafi E, Hahn T, et al. Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect *Hermetia illucens*. Sci Rep. 2022;12(1):8084. doi:10.1038/s41598-022-12150-3
29. Akano O, Akinsomisoye O. Brine shrimp bioactivity and cytotoxic potential of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill seed. Jundishapur J Health Sci. 2024; 16:e142215. doi:10.5812/jjhs-142215
30. Bahar E, Ara J, Alam M, Nath B, Bhowmik U, Runi N. In-vitro antioxidant and thrombolytic activity of methanol extract of *Sida acuta*. J Pharmacogn Phytochem. 2013;2(2):125–33.
31. Mehnaz S, Hasan AHM, Ahmed S, Akanda MdKM, Hamiduzzaman M. In vitro and in vivo evaluation of *Ficus religiosa* stem bark extracts, including antioxidant, anti-inflammatory, antimicrobial, antidiarrheal, and antipyretic activities. J Herbmed Pharmacol. 2025;14:426–34. doi:10.34172/jhp.2025.52927
32. Akter S, Jahan I, Khatun MstR, Khan MF, Arshad L, Jakaria Md, et al. Pharmacological insights into *Merremia vitifolia* (Burm.f.) Hallier f. leaf for its antioxidant, thrombolytic, anti-arthritic and anti-nociceptive potential. Biosci Rep. 2021;41(1):BSR20203022. doi:10.1042/BSR20203022
33. Guguloth S, Malothu N, Kadiri S, Kunuru S. Thrombolytic property of herbal plants: A short review. Biomed Pharmacol J. 2022;15:837–46. doi:10.13005/bpj/2420
34. Shah MD, Hossain MA. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis*. Arab J Chem. 2014; 7(6):1034–8. doi:10.1016/j.arabjc.2010.12.033
35. Evans Charles W. Trease and Evans Pharmacognosy. 16th ed. London: Saunders, Elsevier Limited; 2009. p. 173–416.
36. Patra J, Dhal N, Thatoi H. In vitro bioactivity and phytochemical screening of *Suaeda maritima* (Dumort): A mangrove associate from Bhitarkanika, India. Asian Pac J Trop Med. 2011;4(9):727–34. doi:10.1016/S1995-7645(11)60182-X
37. Javanmardi J. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem. 2003;83(4):547–50. doi:10.1016/S0308-8146(03)00151-1
38. Khatiwora E, Adsul VB, Kulkarni MM, Deshpande NR, Kashalkar R V. Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. Int J Chemtech Res. 2010;2(3):1698–701.

Copyright © 2026 The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.