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Phytochemical Screening and Pharmacological Evaluation of *Merremia vitifolia* (Burm.f.) Hallier f. Leaf Extract

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Abstract

The current investigation aimed to explore the various pharmacological properties of methanol extracts of *Merremia vitifolia* (Burm.f.) Hallier f. leaves (MEMV). Phytochemical analysis was performed following the established procedures. Using the hole board device, the anxiolytic action was measured. The oral glucose tolerance test (OGTT) was used to evaluate the hypoglycemic activity and the castor oil-induced diarrhea method was employed to explore the antidiarrheal activity in Swiss albino mice. Utilizing the brine shrimp lethality assay (BSLA), cytotoxicity was evaluated. In this study, it was observed that MEMV contained a number of active secondary metabolites such as triterpenes, alkaloids, glycosides, flavonoids, phenols and tannins. Compared to the control group, MEMV at 200 mg/kg and 400 mg/kg ($p<0.05$; $p<0.001$) significantly increased the number of head dips. However, MEMV showed potential as a dose-dependent anxiolytic medication. In the OGTT procedure, MEMV exhibited possible hypoglycemic effects that persisted for a shorter period. After 30 minutes of glucose load, the 200 mg/kg dose of MEMV significantly lowered blood glucose levels by 60.70% ($p<0.001$), in comparison to the control group. When compared to standard loperamide ($p<0.001$), the MEMV at both doses (200 mg/kg, 400 mg/kg) significantly ($p<0.001$) inhibited the total number of defecations during the testing period in the castor oil-induced antidiarrheal test. During the BSLA test, the LC_{50} values of MEMV were found to be 2.882 µg/mL. In comparison to the control group, MEMV demonstrated a good cytotoxic effect. To conclude, our research revealed that the plant extracts have potential anxiolytic, hypoglycemic, antidiarrheal, and cytotoxic properties.

Keywords: *Merremia vitifolia* (Burm.f.) Hallier f., phytochemicals, anxiolytic, hypoglycemic, antidiarrheal, and cytotoxicity.

Introduction

Natural products have long been a source of therapeutic agents with diverse medicinal properties that have been harnessed for centuries. Among those natural products, plants are particularly noteworthy and have been serving as reservoirs of bioactive compounds with potential pharmaceutical applications[1]. The World Health Organization approximated that over 80% of the global population relied on natural plant remedies and other traditional healing methods as their primary healthcare approach[2]. Moreover, the use of herbal remedies is increasing in developing regions[3]. *Merremia vitifolia*, commonly known as "Merremia" or "Grape-leaf Wood Rose," is a botanical entity that has attracted attention due to its traditional uses in folk medicine.

Diabetes mellitus, a metabolic disorder characterized by impaired insulin production or response, results in elevated blood glucose levels due to defects in carbohydrate metabolism[4,5]. The chronic hyperglycemia associated with diabetes induces oxidative stress, leading to pancreatic β-cell deterioration and subsequent complications[6]. With its prevalence affecting both the developed and developing nations, diabetes currently afflicts approximately 371 million

people globally and is expected to rise to 552 million by 2030 without intervention[7]. Lifestyle changes, notably decreased physical activity and increased calorie intake contribute to the predominance of Type 2 diabetes[8]. Conventional therapies for Type 2 diabetes have significant limitations, including adverse effects and non-compliance issues[9]. While initial management involves lifestyle adjustments, including exercise and dietary modifications, advanced cases often necessitate pharmaceutical interventions and medicinal food additives[10]. This underscores the need for alternative treatments such as plant extracts with hypoglycemic properties to overcome these limitations.

Diarrhea, the passage of three or more loose stools, involves increased gastrointestinal motility and secretion alongside reduced fluid and electrolyte absorption[11,12]. It is particularly prevalent in developing countries, which is a leading cause of preventable death, primarily affecting children and infants[13]. According to reports from WHO and UNICEF, there are approximately 2.5 billion cases of diarrheal disease annually worldwide, resulting in 1.9 million deaths among children under five years old with the majority occurring in developing countries especially in African and Southeast Asian regions[14]. While various pharmaceuticals like intestinal transit inhibitors, enkephalinase inhibitors, 5-HT3-receptor antagonists, calcium-sensing receptor ligands, and pro-absorptive, antisecretory, and intraluminal agents are available for diarrhea treatment, they often come with various adverse effects such as bronchospasm, constipation, abdominal pain, irregular heartbeat, vomiting, and intestinal obstruction[15]. Medicinal plants present a promising avenue for developing new antidiarrheal drugs. Consequently, the WHO encourages research into traditional medicinal practices to treat and prevent diarrheal diseases[16]. Cancer remains one of the leading causes of mortality worldwide. Treatment with conventional chemotherapeutic agents is often associated with debilitating side effects[17]. Hence, the cytotoxic activity of *Merremia vitifolia* extract was included in our investigation, which can offer a safer and more tolerable alternative to the existing chemotherapeutic agents. Anxiety disorders, including generalized anxiety disorder (GAD), are among the most prevalent mental health conditions globally, affecting quality of life and productivity. It has been estimated that 4% of the global population has anxiety disorders and it was 301 million in the world in 2019[18]. While conventional medications are often used to manage these conditions, their widespread use is limited due to numerous side effects. Consequently, herbal medicines are being explored increasingly as they offer a cost-effective alternative with fewer adverse effects.

Merremia vitifolia is a member of the Convolvulaceae family and a perennial vine characterized by the twining stem with a length of 2–5 meters. It is extensively distributed across Bangladesh, India, Sri Lanka, Myanmar, Thailand and Malaysia. In tribal regions, the leaves and rhizomes of this plant have been traditionally utilized to address various ailments, including fever, headache, eye inflammation, rheumatism, dysentery, jaundice, eczema, and urinary disorders[19,20]. It has been studied recently to search for its pharmacological potentials and it has shown its immense potential as antioxidant, anti-arthritic and anti-nociceptive[19], inhibitor of α-glucosidase[21] and as antibacterial[22]. A total of 27 chemical compounds, such as Spinasterol, Stigmastan-3,6-dione, Trichosanic acid, Pheophorbide a, and Methyl pheophorbide, were detected and characterized in the plant extract by employing LC-MS/MS and GC-MS methodologies. Furthermore, the plant extract exhibited significant potential in inhibiting Histamine effects[23]. Phytochemical tests conducted on *Merremia vitifolia* stems revealed the presence of steroid and alkaloid compounds, while testing of the leaves was positive for phenolic compounds, flavonoids, saponins, steroids, alkaloids and carotenoids[24].

Additionally, the total flavonoid content was measured at 163.4 mg/L in *Merremia vitifolia*[25]. In another study,

the total alkaloid content of *Merremia vitifolia* leaves was estimated at 9.51 mg/g using UV-Vis spectrophotometry[26]. The present study aimed to assess the cytotoxic, antidiarrheal, anxiolytic and hypoglycemic activities and qualitative phytochemical screening of methanol extract of *Merremia vitifolia* leaves. Through these comprehensive investigations, this research sought to elucidate the biological potential of *Merremia vitifolia* leaf methanol extract that could contribute to developing novel therapeutics for managing various health conditions.

Materials and Methods

Solvents and chemicals

Diazepam, Loperamide and Glibenclamide were obtained from Square Pharmaceuticals Ltd. (Gazipur, Bangladesh). Vincristine Sulfate was collected from Incepta Pharmaceutical Limited (Savar, Dhaka). Glucose solution (10%) was collected from Orion Pharma Ltd. (Tejgaon, Dhaka), which was commercially known as 10% DA of Orion Pharma Ltd. Methanol was purchased from Sigma-Aldrich (Humburg, Germany). Normal Saline, DMSO, Tween-80 and Castor Oil were sourced from the local suppliers. All chemicals and reagents used in this study were of laboratory grade.

Plant material collection and authentication

The matured leaves of *Merremia vitifolia* (Burm.f.) Hallier f. was collected from the hilly areas of Chittagong Division of Bangladesh in November 2018 and identified by a taxonomist from the Department of Botany, University of Chittagong, Bangladesh.

Preparation of the plant extract

After collecting *Merremia vitifolia* leaves, those were cleaned and dried under mild sunlight for a week. Using a high-capacity grinding machine, the dried leaves were crushed into a coarse powder in the Phytochemical Research Laboratory, Biological Faculty, University of Chittagong. Then, the leaf extract was prepared using cold maceration using pure methanol. About 570 gm of the powdered plant sample was taken into a clean, round-bottomed flask of 5 liters and soaked in 2.4 liters of methanol. Then, the container was sealed with aluminum foil and kept at room temperature for 15 days with intermittent shaking and stirring. Later, the whole mixture was filtered through a fresh cotton plug and finally with Whatman No.1 filter papers. Then, the filtrate was concentrated using a Buchii Rota evaporator at low temperature and pressure to yield the crude extract. The weight of the extract yielded was 17 gm. Then, the % yield of the methanol extract was calculated from the following simple mathematical equation[27]:

$$\%\ yield\ of\ extracts = \frac{weight\ of\ extracted\ material}{weight\ of\ original\ plant\ material\ used} \times \frac{100}{1}$$

The percentage of yield of crude methanol extracts of *Merremia vitifolia* leaves was 2.98%.

Phytochemical screening of the extract

A small quantity of the freshly prepared MEMV was subjected to the preliminary phytochemical evaluation to detect the presence of phytochemicals such as alkaloids, terpenoids, flavonoids, saponins, phenol and tannins, steroids, anthraquinones, glycosides, cardiac glycosides, resins, carbohydrates, proteins, fat and oil by employing a standard procedure[28-30].

In vivo pharmacological studies

Experimental animals

Young male Swiss Albino Mice weighing 25-35g were collected from the animal resources facility of BCSIR, Chittagong. They were housed in clean and dry propylene cages with 12 hours light-dark cycle at a temperature of 25±2°C and a relative humidity of 60%-70% in the animal house of the Pharmacy Department, University of Chittagong. The mice were provided a standard laboratory diet and water ad libitum throughout the study. Food was withdrawn 12 hours before and during the experiment. This study was approved by the Animal Ethics Review Committee (AERB), Faculty of Biological Sciences, University of Chittagong.

Experimental design for in vivo testing

For evaluation of the anxiolytic activity, four groups of mice were selected for each investigation, and 5 mice were assigned to each group. Group (I) was treated with control (1% Tween-80 and DMSO in Saline, 10 mL/kg body weight), Group ((II) was standard and received Diazepam 1 mg/kg orally for both hole board methods. Other groups received different doses (200 and 400 mg/kg) of MEMV. For oral hypoglycemic activity, 15 mice were selected randomly and divided into 3 groups with five mice in each group. Group (I) was treated as control (1% Tween-80 and DMSO in Saline, 10 mL/kg body weight), Group (II) received standard drug (Glibenclamide, 10 mg/kg) and the other group was used for administration of MEMV at the dose of 200 mg/kg. To assess the antidiarrheal property, a total of 20 mice were selected randomly and were divided into four groups having five mice in each group. Group (I) was treated as control (1% Tween-80 and DMSO in Saline, 10 mL/kg body weight), Group (II) received standard drug (Loperamide, 50 mg/kg) and other groups were used for administration of MEMV at the doses of 200 and 400 mg/kg.

Evaluation of anxiolytic potential

Hole-board method

The hole-board test is a test which determines the level of anxiety and response of animals to anxiety[31]. The apparatus is composed of a wooden chamber ($40\times40\times25cm^3$) with 16 holes (each of 3 cm diameter) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the mice could peep through the holes referred to as head-dipping. The frequency and duration of head-dipping are assumed to provide measures of neophilia or directed exploration that are independent of the general locomotor activity of the animal[32]. After 30 minutes of treatment with the control, standards and test samples, the mice were placed singly on the hole board apparatus and allowed to explore the apparatus freely for 5 minutes. The total number of head dipping of mice into the holes at the altitude of their eyes during a five-minute trial period was recorded[33].

Evaluation of oral hypoglycemic activity

One of the most reliable ways to assess hypoglycemic activity is an oral glucose tolerance test[34]. Prior to conducting the experiment, the test substances and the control materials were dosed accurately, and each mouse was weighed precisely. Upon 30 minutes of extract administration, all groups received treatment with a 10% glucose solution (2 mg/kg body weight). After 30, 90 and 120 minutes of glucose administration, blood samples were taken from the tail vein. Blood glucose level was measured by using glucometer. Test samples, control, and glibenclamide were administered orally.

Evaluation of antidiarrheal activity

Castor-oil induced diarrhea

To determine the antidiarrheal activity of the plant extract, castor-oil induced method was applied[35]. In this study, each experimental animal of predefined group received a particular treatment orally, i.e., control, standard, and methanol extract at doses of 200 mg/kg and 400 mg/kg, respectively. Then, they were placed in individual pre-cleaned cages with bloating paper floor lining, and the floor lining was changed every hour. After 30 minutes intervals of feeding, those mice received a pure analytical grade castor oil of 1 mL to induce diarrhea. Then, they were observed for the next 4 hours for defecation, and the number of feces for each mouse was recorded. The percentage of inhibition of diarrheal feces was calculated by the following formula[36]:

$$\%\ of\ inhibition\ of\ defecation = (1 - \frac{B}{A}) \times 100$$

Here, A= Mean number of defecations by castor oil; B= Mean number of defecations by drug or extract

Evaluation of in vitro cytotoxic activity

The technique of brine shrimp lethality bioassay was applied to study the cytotoxic activity of the experimental plant[37].

Preparation of seawater

38 gm of sea salt (pure NaCl) was dissolved in one liter of distilled water and filtered off to get a clear solution[38].

Hatching of brine shrimps

Brine shrimp eggs (*Artemia salina* Leach) were procured from the pet shop and used as test organisms. The prepared seawater was taken in a small tank and shrimp eggs were placed on one side of the tank, then covered with a lid. The eggs were incubated for 48 hours for hatching to give mature shrimps named nauplii. Constant oxygen supply was ensured by using an oxygen pump during hatching. The hatched shrimps were attracted to the lamp through the perforated dam and were taken for experiment with the help of a pipette.

Procedure

Plant extracts were prepared at different concentrations of 1000, 800, 500, 300, and 100 µg/mL with simulated sea water. Then ten living shrimps were picked out from the hatching tank and added to each concentrated extract preparation containing 5 mL of seawater. After 24 hours, the nauplii were observed using a magnifying glass for their survival, and the percent (%) of mortality was recorded for each concentration of the sample. In this study, vincristine sulfate solution was applied as a positive control. The median lethal concentration (LC_{50}) of all test samples was determined by the linear regression method by plotting the percentage of mortality against the correspondent concentration of the extracts, and percentage of mortality can be calculated by using the following equation[39]:

$$\%\ Mortality = \frac{\text{Number of dead nauplii after 24 hours of incubation}}{\text{Number of total nauplii transferred}} \times 100$$

Statistical Analysis

Data were presented as Mean ± SEM (Standard Error of Mean). The data were statistically analyzed using one-way ANOVA, followed by post-hoc Dunnett's "t" test and Tukey test with the Statistical Package for Social Science (SPSS, Version 16.0, IBM Corporation, NY). Statistical significance was determined at $^{***}p<0.001$, $^{**}p<0.01$ and $^{*}p<0.05$ compared to the control group.

Result & Discussion

Qualitative Evaluation of Phytochemicals

By carrying out the phytochemical screening of MEMV, a variety of secondary metabolites were identified. These results could guide investigations to quantify these secondary metabolites using sophisticated methodologies. The results are demonstrated in Table 1.

Table 1. Qualitative analysis of phytochemicals of crude methanol extract of *Merremia vitifolia* (Burm.f.) Hallier f.

Phytochemical tests	Reagent used	MEMV
Alkaloid	Wagner's test	+
Carbohydrate	Molish's test	+
Glycoside	Sodium hydroxide test	+
Saponin		–
Steroid	Liebermann Burchard's test	_
Phenol	$FeCl_3$ test	_
Tannin	$FeCl_3$ test	+
Flavonoid	Zinc Hydrochloric acid reduction test	+
Reducing sugar	Fehling's Reagent test	+
Protein	Biuret test	–
Tri-terpene	Liebermann-Burchard's test	+
Fixed oil & Fat	NaOH Reagent test	+
Cardiac Glycoside	Keller-Kiliani test	–
Anthraquinone Glycoside	Hydroxy anthraquinone test	_
Resin	Test with acetone solution	_

MEMV = Crude methanol extract of *Merremia vitifolia*; '+' means presence and '-'means absence of phytochemicals respectively.

In vivo pharmacological studies

Evaluation of anxiolytic activity

The most prevalent mental illness and the leading cause of disability worldwide are anxiety-related disorders, including post-traumatic stress disorder, obsessive-compulsive disorder, panic disorder, phobias, and generalized anxiety[40]. The Hole-board test is necessary to analyze head-dipping behavior to assess an animal's emotional state. A high anxiety state in the animal is indicated by its aversion to exploring new places, which is correlated with a low level of head dipping. Conversely, an increased number of head dipping represents neophilia or exploratory behavior[41]. A tabulation of the current investigation's outcomes is presented in Table 1. In this study, Diazepam was used as the standard, and it showed a significant (***$p<0.001$) increase in the number of head dipping (56.4 ± 1.63) compared to the control group at a low dose (1 mg/kg). MEMV at the doses of 200 mg/kg and 400 mg/kg significantly (*$p<0.05$ and ***$p<0.001$ respectively) increased the number of head dipping as compared to the control group. Based on the collected results, it has been observed that MEMV showed a noteworthy potential as a dose-dependent anxiolytic drug. Currently, several medications are available to treat anxiety disorders. Benzodiazepines such as Diazepam are CNS depressants, and it is available in the market as anxiolytics. It has a binding site on the gamma-aminobutyric acid receptor type-A ionophore complex ($GABA_A$)[42]. GABA is regarded as a critical neurochemical in the central nervous system. They may function by directly activating GABA receptors or potentiating the GABAergic inhibition in the central nervous system (CNS) system through hyperpolarization, which may increase the pace at which essential brain neurons fire. Preliminary phytochemical studies of the MEMV revealed the presence of alkaloids, tannins and flavonoids, which are ligands for GABA receptors in the central nervous system, suggesting that these phytoconstituents may be responsible for CNS depressant activity[43].

In this study, the anxiolytic action of MEMV at a high dose was comparable to that of Diazepam and it seemed possible that they functioned through the same GABA receptor complex. Because of their fewer side effects, natural compounds having GABA-mimetic activity may eventually replace synthetic drugs[44-46].

Evaluation of oral hypoglycemic activity

The oral glucose tolerance test (OGTT) assesses how well the body utilizes glucose, the primary form of sugar used by the body to produce energy. A useful and widely accepted test, the OGTT, was designed to support fasting plasma glucose concentration alone to streamline and expedite the diagnosis of diabetes[45,47]. Enhanced glucose tolerance in diabetic or normoglycemic mice fed with the plant sample confirmed its hypoglycemic properties. After 30 minutes of glucose loading, the crude methanol extract (at the dose level of 200 mg/kg) remarkably reduced the blood glucose level by 60.70% (***$p<0.001$) compared to the control. This hypoglycemic effect was comparable to that of the standard drug glibenclamide,

Table 2. Effect of MEMV on head dipping of Swiss Albino mice in Hole-board test.

Test Group	Dose (mg/kg)	Number of head dipping (Mean ± SEM)
Control	-	21 ± 1.30
Diazepam	1	$56.4 \pm 1.63^{***}$
MEMV	200	$28 \pm 1.30^{*}$
MEMV	400	$41 \pm 1.87^{***}$

Note: Each value represents the mean ± SEM (n = 5). One-way ANOVA followed by Dunnett's t test. Results were significant as $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ compared to the control; MEMV = Crude methanol extract of *Merremia vitifolia* leaves.

Table 3. Oral hypoglycemic activity of methanol extract of *Merremia vitifolia*

Animal Groups	Dose (mg/kg)	Mean ± SEM			
		0 minute	**30 minute (% of change)**	**90 minute (% of change)**	**120 minute (% of change)**
Control		6.86 ± 0.20	14.98 ± 0.54 (+118.37)[a]	9.66 ± 0.59 (-35.51)[b]	6.90 ± 0.15 (-53.94)[b]
Glibenclamide	10	5.92 ± 0.32	9.68 ± 0.61*** (+63.51)[a]	4.70 ± 0.19*** (-51.45)[b]	4.28 ± 0.28*** (-55.78)[b]
MEMV	200	6.26 ± 0.23	10.06 ± 0.50*** (+60.70)[a]	7.46 ± 0.42** (-25.84)[b]	6.44 ± 0.47 (-35.98)[b]

Note: Here the concentration of glucose in the plasma of mice is represented as (mmol/L); increase (+) or decrease (-) of blood glucose level; a = compared to the glucose level at zero min; b = Compared to the glucose level at 30 min after the glucose load. Each value represents the mean ± SEM (n = 5). Results were significant as ***p<0.001, **p<0.01, *p<0.05 compared with the control; MEMV = Crude methanol extract of *Merremia vitifolia* leaves.

where glibenclamide showed a 63.51% reduction compared to the control (***p<0.001) (Table 3). After 90 minutes of glucose loading, the sample showed a 25.84% reduction in blood glucose level compared to the 30 minutes of glucose loading for methanol extract, while the control exhibited a 35.51% reduction compared to the 30 minutes of glucose load for control (Table 3). So the extract showed no potential hypoglycemic actions after 90 minutes of glucose loading. The obtained result indicated that the methanol extract showed potential hypoglycemic actions sustained for a shorter duration. More research is needed to determine its therapeutic value.

Evaluation of antidiarrheal activity

The active ingredient in castor oil is ricinoleic acid, which irritates and inflames the mucosa lining the intestines. Prostaglandins are released in response to this and change how mucous and electrolytes are transferred. Because of this action, the body cannot reabsorb water and NaCl, which causes a hypersecretory response that decreases the absorption of Na^+ and K^+ and encourages peristaltic activity and

Table 4. Evaluation of antidiarrheal activity of crude extract of *Merremia vitifolia*

Animal Group	Dose Mg/kg	Mean ± SEM				
		1st hr (% of Inhibition)	**2nd hr (% of Inhibition)**	**3rd hr (% of Inhibition)**	**4th hr (% of Inhibition**	**Total (% of Inhibition)**
Control	10 mL/kg of body weight	4.8 ± 1.07	4.4 ± 0.51	3.6 ± 0.40	3.0 ± 0.32	15.8 ± 1.53
Loperamide	50	0.4 ± 0.24*** 91.67%	1.2 ± 0.20*** 72.73%	1.4 ± 0.24*** 61.11%	1.4 ± 0.40* 53.33%	4.4 ± 0.40*** 72.15%
MEMV	200	1.6 ± 0.24* 66.67%	1.6 ± 0.40** 63.64%	1.0 ± 0.32*** 72.22%	1.0 ± 0.32** 66.67%	5.2 ± 0.86*** 67.09%
MEMV	400	1.6 ± 0.51* 66.67%	0.8 ± 0.58*** 81.82%	0.4 ± 0.24*** 88.89%	0.6 ± 0.40** 80.00%	3.4 ± 0.51*** 78.48%

Note: Each value represents the mean ± SEM (n = 5). One-way ANOVA followed by Dunnett's t test. Results were significant as ***p<0.001, **p<0.01, *p<0.05 compared with control; MEMV = Crude methanol extract of *Merremia vitifolia* leaves.

diarrhea. The prostaglandin synthesis inhibitors have been shown to postpone the diarrhea caused by castor oil[48]. The antidysenteric and antidiarrheal properties of medicinal plants are attributed to tannins, alkaloids, saponins, flavonoids, sterols, and triterpenes, as well as reducing sugars[49,50]. The alkaloids, flavonoids, and gggreducing sugars found in *Merremia vitifolia* methanol extract may aid this effect. In this experiment, defecation frequency was inhibited significantly (***P<0.001) by MEMV at both doses of 200 mg/kg (67.09% inhibition) and 400 mg/kg (78.48% inhibition), and the effect was dose-dependent. The results are listed in Table 4.

Evaluation of in vitro cytotoxic activity

The brine shrimp lethality bioassay (BSLA) against *Artemia salina* is an easy method to evaluate the cytotoxicity of the crude extracts. When the LC_{50} value was more than 1000 µg/mL, the toxicity level against the brine shrimp was classified as non-cytotoxic; when it was less than 1000 µg/mL, it was classified as cytotoxic[37]. The absence of brine shrimp death demonstrated the non-cytotoxic profile of the control group. When compared to the control (sea water), the reference standard vincristine sulfate showed a lethal LC_{50} value of 0.789 µg/mL. The LC_{50} value of MEMV was found to be 2.882 µg/mL. MEMV showed a good cytotoxic impact compared to the control group, with mortality being the highest at higher concentrations and the lowest at lower concentrations. The plant's phytochemical examination indicated the presence of flavonoids and tannins, both of which have anticancer qualities[51,52,53]. The results are listed in Table 5. With the help of modern separation and chemical identification techniques like chromatographic analysis, further studies could identify the specific ingredients responsible for those activities.

Conclusion

The current research concluded that *Merremia vitifolia* leaf extract possesses potential anxiolytic, hypoglycemic, antidiarrheal, and cytotoxic properties. These effects might be correlated with tannins, flavonoids, phenols and alkaloids in the crude extract of *Merremia vitifolia* leaves. It could be a valuable addition to future plant-based medication development approaches for future researchers.

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Table 5: A brief overview of the cytotoxic activity of the extract of *Merremia vitifolia*

Sample	Equation	R^2	LC_{50}(µg/mL)
Vincristine sulphate	y = 56.534x + 4.3789	0.9918	0.789
MEMV	y = 67.741x -145.2	0.4796	2.882

MEMV = Crude methanol extract of *Merremia vitifolia* leaves

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