Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Phytochemical and pharmacological investigation on hypoglycemia, sedation, antianxiety, free-radical scavenging, and cytotoxicity actions of *Mimosa diplotricha* C. Wright (Nila grass)

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Abstract

Background *Mimosa diplotricha* is an ethnic medicinal plant that has numerous ethnopharmacological applications. Here, tested the crude methanol extract (MDME), petroleum ether (MDPE), chloroform (MDCF), and n-hexane (MDNH) fractions of *M. diplotricha in vivo* to determine their bioactivities, specifically CNS and oral hypoglycemic, and evaluated their antioxidant and cytotoxicity in vitro.

Method Oral hypoglycemic activity was measured by an oral glucose tolerance test. Central nervous system activities were done by elevated plus maze (EPM), hole board, hole cross, and open field method. Antioxidant activity was measured by a superoxide scavenging and ferric-reducing effect assay. Cytotoxicity was determined by using a brine-shrimp lethality bioassay.

Result The test samples MDME and MDME markedly reduced the blood glucose levels after 90 min of the test interval, with values of 33.58% and 39.92%, respectively. In the open-field and hole-crossed tests, all the test samples exhibited reduced locomotor activities that preceded significant sedative activity. Among them, MDNH ($p < 0.001$) showed a marked sedative effect. During the EPM test, mice treated with MDME and its fractions preferred to remain in the open arm, indicating antianxiety-like behavior, whereas in the hole board model, MDME extract showed a reduction in head dipping compared to standard diazepam. In the antioxidant assay, the MDME extract demonstrated a significantly lower IC_{50} value in the superoxide scavenging and ferric reducing assays, respectively. In the cytotoxicity assay, we observed the lowest LC_{50} values for each extract when compared to the standard vincristine sulfate.

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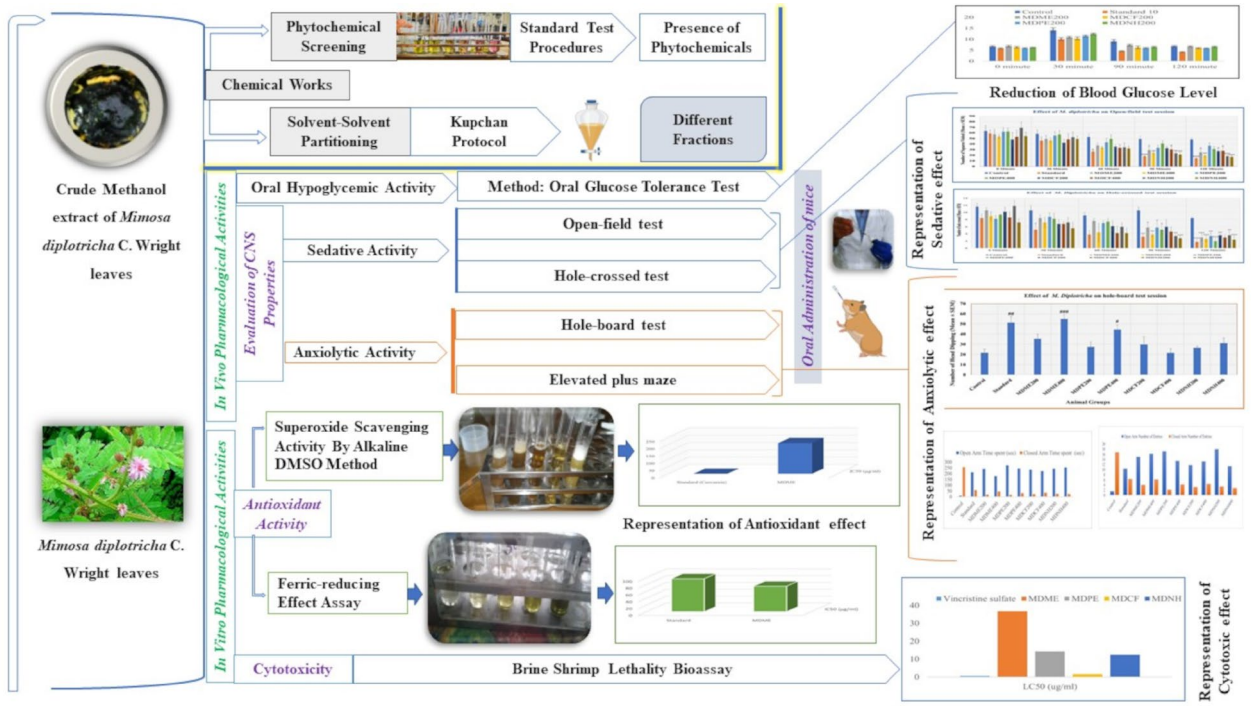
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Conclusion The current study indicates that the plant extracts have potential antioxidant, cytotoxic, hypoglycemic, sedative, and anxiolytic activities.

Keywords Antioxidant, Anxiolytic, Cytotoxicity, Hypoglycemic, Sedative and phytochemical screening

Graphical abstract



Background

Diabetes Mellitus (DM) is a multifaceted metabolic syndrome characterized by hyperglycemia, relative insulin inadequacy, and a gradual decline in insulin sensitivity [1]. Type 2 diabetes accounts for more than 90% of cases, and both inherited and environmental factors play a role in its etiopathogenesis. Type 1 diabetes affects five to ten% of people, and its main cause is the autoimmune destruction of pancreatic beta cells [2]. Recently, the exponential increase in diabetes has challenged the healthcare sector of the world which will affect 642 million people by 2040 [3]. The most widely consumed antidiabetic drugs are unidimensional in their mechanism of action and they do not concurrently adopt the multiple alterations implicated in the progression and development of diabetes [4]. Furthermore, due to a number of negative side effects, including severe hypoglycemia, liver damage, lactic acidosis, blood dyscrasias, colonic ulcers, and gastrointestinal intolerance, they reveal poorer efficacy with time, pitiful acceptance, and minimal compliance [5].

Anxiety and depression are estimated to affect 4.7% and 7.3% of the world's population, respectively [6]. Significant alterations in homoeostatic processes, such as immunological, endocrine, neurochemical, cognitive, and autonomic system dysregulation, can cause anxiety and sadness. Alterations in neuronal processes can lead to a variety of repercussions, including the breakdown of neuronal function, structural changes, disruption of neural networks and plasticity, and chemical imbalance in the brain [7]. Depression is the leading cause of disability, characterized by the prolonged presence of specific somatic and cognitive abnormalities in combination with a sad mood, loss of interest, feelings of low self-respect, interrupted sleep, and minimal energy [8]. Currently available antidepressants include tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), norepinephrine-dopamine reuptake inhibitors (NDRIs), selective norepinephrine reuptake inhibitors (SNRIs), norepinephrine reuptake inhibitor with serotonin receptor antagonism (NRISA), atypical, and NMDA-glutamatergic ionoceptor antagonists [9]. Despite being frequently prescribed for depression, current antidepressants have a number of drawbacks, such as low response rates and delayed therapeutic response, which make patient compliance problematic [10].

The most prevalent psychiatric disorders are anxiety disorders [11]. Treatment becomes more complicated due to the higher comorbidity between anxiety and depressive disorders or between anxiety disorders [12]. The available anxiolytic drugs for the treatment of anxiety include benzodiazepines, antidepressants, antihistamines, adrenergic agents, antipsychotics, serotonergic agents, glutamate modulators, neuropeptides, and neurosteroids [13]. However, all of the prevailing drugs exhibited severe adverse effects like respiratory depression, impaired coordination, slurred speech, confusion, coma, hypotension, seizures, dysarthria, lethargy, and dizziness [14]. Due to its wide range of brain targets, phytomedicine may be able to treat a variety of diseases [7]. Oxidative stress-mediated diseases are remarkable. Reactive oxygen species can seriously harm tissues and cells during infections and a variety of degenerative conditions, including cancer, aging, heart disease, and neurological diseases like Alzheimer's [15]. Despite the fact that our bodies have multiple antioxidant defense systems, in extreme situations we require outside assistance to maintain the equilibrium between reactive species and antioxidant systems [16]. Recently, there has been an increasing interest in plants that are potential sources of antioxidant compounds [17].

Another deadly condition, cancer is caused by several gene mutations that lead to uncontrolled and abnormal cell proliferation and the ultimate death of cells. Understanding the cellular growth regulation mechanism has helped identify drugs with particular antitumoral action and has also added to our understanding of carcinogenesis [18]. While a number of chemotherapeutic drugs are widely employed in contemporary clinical settings across the globe, efforts are still being made to discover novel chemopreventive medicines that can be safely and efficiently provided to human populations [19]. Natural cytotoxic agents may contribute to the treatment of cancer [20]. All of the therapeutic drugs that are currently on the market are chemically produced substances with good therapeutic action, but their long-term safety is still unknown and they have certain undesirable side effects [21]. So, there is a vital requirement for developing new therapeutic agents from an alternative source with higher efficiency, safety, and cost-effectiveness.

Medicinal plants are thought to be a plentiful source for researching potential safe, effective medications, with half of the drugs being natural items with commercial relevance [22]. Plants have been utilized as a source to provide humankind with medicines that have the high therapeutic potential to treat numerous complicated health disorders since ancient times [23]. Plants have healing properties that are widely employed in many traditional medical systems, such as Chinese, Ayurvedic, and Unani. Currently, scientists are drawn to investigate the safety and effectiveness of plants since they have played a significant role in the discovery and development of contemporary medications for medical purposes. Bioactive phytoconstituents in plants are mainly responsible for providing a specific pharmacological action on the biological system. With the progression of scientific research, researchers have been successful in detecting various phytochemical compounds in medicinal plants that are proven to exhibit different types of physiological activities [24]. About 80% of the population uses herbs and other traditional medicines for their primary healthcare [25]. Vinblastine, vincristine, morphine, digitoxin, quinine, cocaine, codeine, pilocarpine, paclitaxel, artemisinin, silymarin forskolin, nitisinone, galantamine, apomorphine, tiotropium, capsaicin, and cannabidiol are some remarkable medicinal drugs that have been developed from medicinal plants [26].

In folk medicine, other Mimosa species, such as M. tenuiflora, M. pudica, M. pigra, M. caesalipiniifolia, M. hamata, M. xanthocentra, M. invisa, M. somnians, M. arenosa, M. albida, and M. linguis, are used to treat conditions such as rheumatoid arthritis, depression, anxiety, gastrointestinal disorders, inflammation, bronchitis, menstrual colic, diarrhea, persistent dysentery, asthma, and leucoderma.

Mimosa diplotricha is a fast-growing, annual, or perennial leguminous woody subshrub, and scrambling climber with multibranched vines and commonly known as the giant sensitive plant, creeping sensitive plant, or nila grass [27]. This leguminous vine is widely distributed in areas of Africa, Nigeria, France, the Hawaiian Islands, northern Australia, South and Central America, the Pacific Islands, and South and Southeast Asia [28]. Alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, phytosterol, proteins, lipids, and glycosides were discovered to be present in *M. diplotricha's* qualitative phytochemical analysis, however tannins, gum & mucilages, fixed oils, volatile oil, and terpenes were not [29]. This plant has yielded some interesting chemicals recently, such as diplochalcolins A and B, diplomero terpenoids A-F, diplotrin A-C, and diplotasin D [30]. Previous research shown that the plant's extracts have a number of therapeutic benefits, including antidiarrheal, anti-inflammatory, antioxidant, thrombolytic [31], anticancer [32], antinociceptive [29], insecticidal, and antimicrobial activity [33]. Other *Mimosa* species, including *M. tenuiflora*, *M. pudica*, *M. pigra*, *M. caesalipiniifolia*, *M. hamata*, *M. xanthocentra*, *M. invisa*, *M. somnians*, *M. arenosa*, *M. albida*, *and M. linguis*, are used to treat conditions such as rheumatoid arthritis, depression, anxiety, gastrointestinal disorders, inflammation, bronchitis, menstrual colic, diarrhea, persistent dysentery, asthma, and leucoderma [34]. So, present study aims to evaluate hypolglycemic, sedative, anxiolytic,

free-redical scavenging, and cytotoxicity action of *Mimosa diplotricha.*

Materials and methods

Collection and authentication of plant material

For the investigation, a plant sample of *M. diplotricha* was collected from Rangamati, a hilly area of Chittagong, Bangladesh on 26th January 2018. Then it was authenticated by the renowned taxonomist Dr. Shaikh Bokhtiar Uddin, Professor, Department of Botany, University of Chittagong.

Plant extract preparation

The collected leaves of *M. diplotricha* were washed with distilled water and air-dried under shade at room temperature. The dried leaves were pulverized with the help of a grinding machine and stored in an airtight container. The dried and finely powdered plant leaves were taken in a clean, round-bottomed flask and soaked in 3.5 L of pure methanol. The container with its contents was sealed with foil and kept for 15–20 days accompanying occasional stirring and shaking. Whatman No.1 filter paper was used to filter the whole mixtures of plant extracts and the obtained filtrate was then concentrated using a rotary evaporator at low temperature and reduced pressure to obtain dried crude extract. The weight of the extract yielded was 28 g. The following equation was used to calculate the percentage yield of plant extracts [35].

Solvent-solvent partitioning

Protocol designed by Kupchan and Tsou and modified by Wagenen et al. was used for solvent-solvent partitioning of methanol extracts of *M. diplotricha* leaves. The solvent used for this partitioning is chloroform, n-hexane, and ethyl acetate consecutively [36]. All fractions were properly dried and used for the investigation.

Qualitative phytochemical screening

The initial phytochemical screening was performed to assess the qualitative determination of phenols, terpenoids, flavonoids, saponins, tannins, phlorotannins, steroids, anthraquinones, alkaloids, glycosides, cardiac glycosides, resins, carbohydrates, proteins, fat and oil, and coumarin by using standard procedure [37]. The color intensity or the precipitate formation was used as an analytical response to these qualitative tests.

Experimental animals

About 20–25 g of Swiss albino mice were procured from the Bangladesh Council of Scientific and Industrial Research Laboratories (BCSIR), Chittagong. We used a single clean plastic cage with a metal net-structured frame lid to comfortably house the animals. A 12-hour light-dark cycle at a temperature of 25 ± 2 °C with a relative humidity of 60–70% was used for the adaptation of mice to laboratory conditions for 14 days before investigation. Nutritionally, pellet diets and water ad libitum were provided to the mice throughout the study. Proper care was provided to the animals in compliance with the ethical guidelines of internationally accepted standards for using laboratory animals. All segments of this report adhere to the ARRIVE Guidelines for reporting animal research. All experiments have been examined and approved by the ethical committee of the University of Chittagong under approval no-cc98056. At the end of each experiment, all the mice were sacrificed by utilizing diethyl ether anesthesia.

Acute toxicity study

Guidelines developed by the Organization for Economic Co-operation and Development (OECD) were utilized for conducting acute toxicity studies. Each group comprised six Swiss albino mice and abstained from food overnight before the administration of the extract. Each group of animals received plant extracts at oral doses of 1000–4000 mg/kg body weight. They abstained from food for a further 3–4 h after the administration of plant extract. The mice were observed for any changes in the skin, mucous membrane, eye, respiration rate, fur, and central and autonomic nervous systems for one day. One-tenth of the median lethal dose (LD50) would be considered an effective dose and calculated using a valid mathematical formula [38].

Experimental design

During the evaluation of oral hypoglycemic activity, thirty fasted experimental animals were selected and categorized into six groups (Groups I–VI), consisting of five mice in each group. Group I was treated as control (1% Tween 80 and DMSO in saline); Group II received a standard hypoglycemic drug (glibenclamide 10 mg/kg body weight); and the remaining four groups received 200 mg/kg methanol extract and its different fractions, *M. diplotricha*. We randomly selected sixty experimental animals and divided them into twelve groups, each containing five mice, for both sedation and anxiolytic activities. Group I (negative control) was administered 1% Tween 80 and DMSO in saline at 0.10 ml/10 g of body weight, and Group II (positive control) was utilized for the standard group for administering the standard drug diazepam at a dose of 1 mg/kg. The remaining ten groups received methanol extract and different fractions of methanol extract at doses of 400 and 200 mg/kg, respectively.

Evaluation of oral hypoglycemic activity

Oral Glucose Tolerance Test (OGTT)

The most accredited OGTT was performed to examine the hypoglycemic activity. All groups were administered

a 10% glucose solution at a dose of 2 g/kg body weight after 30 min of extract administration. At 30, 90, and 120 min of glucose loading, blood samples of mice were taken from their tail vein. The blood sugar level was monitored using a glucometer. Before treatment, every mouse was weighed properly, and the doses of vehicle, glibenclamide, and test extracts were adjusted accordingly [39].

Evaluation of CNS activities

Sedative activity

Open-field test To evaluate the sedative action of the *M. diplotricha* extract, the method illustrated by Gupta et al. was followed [40]. The field is colored in black and white and made of small squares alternately. Then the number of square fields visited by mice was recorded for 3 min at 0, 30, 60, 90, and 120 min of the experimental phase. Before any treatment, the weight of each mouse was taken properly, and the number of test samples and controls were fitted carefully.

Hole-crossed test

This test is usually performed to analyze the locomotor and exploratory functions of the extract. The test was done following the process defined by Takagi. The hole cross apparatus has a size of 30 cm×20 cm×14 cm surrounded by wooden walls and it has no rooftop. The number of movements on the hole board made by mice was recorded for 3 min at 0, 30, 60, 90, and 120 min intervals respectively [41].

Anxiolytic activity

Hole-board test

The hole board technique is a commonly used predictive tool for determining anxiety levels. This test measures the frequency with which the head dips through holes in a wooden board. The wooden board measures 20 cm by 40 cm long, with 16 equally aligned holes in it. The tendency to dip the head of the test animal into the hole is taken as a natural and anxiety-free behavior. Their administration was done 30 min before commencing the experiment. Then how many times and how long the mice poked their heads through the holes were counted and noted for a 5-minute observation period [42].

Elevated Plus Maze (EPM) test

The EPM apparatus is composed of two open arms (30 × 5 cm, encircled by a 0.25-cm high border) and two closed arms (30 × 5 cm, enclosed by 25 cm high walls), with two pairs of identical platforms emerging from a central platform (5 × 5 cm), positioned oppositely. After one hour of post-treatments, we placed each animal from each group on the central platform of the maze, facing one of the open arms, and allowed them to move freely. The duration of each session was 5 min, during which we recorded the number of animals that entered open and closed arms and the amount of time they spent there [43].

In vitro evaluation of antioxidant activity

Superoxide scavenging activity by alkaline DMSO method

We assessed the superoxide scavenging capacity of the crude extract of M. diplotricha using Pandey's [44] alkaline DMSO technique. In this experiment, the concentration of oxygen extracted in DMSO correlates to the concentration of superoxide radicals in the alkaline DMSO solution. In summary, the non-enzymatic reaction mechanism produced this superoxide radical. The test samples were prepared by adding 0.3 ml of extract solutions (concentrations of 50, 100, 200, 400, and 800 µg/mL) in 0.1 ml of NBT solution (1 mg/ml) and finally by adding 1 ml of alkaline dimethyl sulfoxide (1 ml DMSO with 0.1 ml water and 5 mM NaOH) to make a final volume of 1.4 ml. Standard solutions (positive control) of different concentrations (five, ten, twenty, forty, eighty µg/ml) were also prepared, and curcumin was used as a standard in this assay. Negative control was made by adding 300 ml of plain DMSO to 0.1 ml of Nitroblue Tetrazolium (NBT) solution (1 mg/ml solution in dimethyl sulfoxide) and 1 ml of alkaline dimethyl sulfoxide (1 ml of DMSO with 5 mM sodium-hydroxide in 0.1 ml of water). The absorbance of all the controls and experimental test solutions was measured at 560 nm using DMSO as a blank. At 560 nm, antioxidants with low absorbance indicate that the generated superoxide is consumed [45]. The extract concentration that provided 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage versus the extract concentration on a graph.

Ferric-reducing effect assay

The reducing capacity of *M. diplotricha* methanol extract was measured using the modified *Sanja* method [46]. The yellow-colored test solution transforms to different shades of green and blue in this procedure, reflecting the sample's reduction capacity. Ascorbic acid of different concentrations (250,200, 150, 100, 50 µg/mL) was used in this assay as positive control or standard. In phosphate buffer saline (pH = 6.6), test samples were made at 27.5, 55, 110, 220, and 440 µg/ml concentrations. 2.5 ml potassium ferricyanide (1%), was combined with the standard and sample, respectively. This reaction mixture was incubated at 50 °C for 20 min. After incubation, the reaction liquid was cooled and 2.5 ml trichloroacetic acid (10%) was added and thoroughly mixed. This mixture was centrifuged for 10 min at 3000 rpm. The supernatant was collected and 2.5 mL of it was added to 2.5 ml of distilled water. Now, 1 mL of 0.1% ferric chloride was added to this reaction mixture and incubated at room temperature

for 10 min. The blank solution comprised the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the sample solution. Finally, the absorbance of blank, standard, and samples was measured at 700 nm. Higher reaction mixture absorbance indicated higher reducing power [38, 44, 47].

In vitro evaluation of cytotoxic activity by brine shrimp lethality bioassay

Preparation of seawater

About 38 gm of sea salt (pure NaCl) was weighed, dissolved in one liter of distilled water, and filtered off to get a clear solution. For adjustment of pH, 1 N NaOH solution was used and pH was set at 8.0 [48].

Hatching of brine shrimps

The test organism was brine shrimp eggs (*Artemia salina* leach), acquired from pet shops in Chittagong. We added the shrimp eggs to the seawater in a small tank. After 48 h of hatching, the eggs would mature as nauplii. The oxygen pump continuously supplied oxygen during the hatching time. The light (phototaxis) attracted the hatched shrimps, leading to the removal of nauplii free of eggshells from the lit section of the tank. We removed the nauplii from the fish tank using a pipette.

Study design

About 100 µl of DMSO in 5 ml seawater (negative control), 5 ml of each plant extract solution (test samples) of different concentrations (1000, 800, 500, 300, 100 µg/ml) in 5 ml seawater, and 5 ml of vincristine sulfate solution (positive control or standard) of different concentrations (10, 5, 2, 0.5, 0.25, 0.125 µg/ml) in 5 ml sea water were taken in different glass vials, and then 10 living nauplii were added to each of the experimental and control glass vials. After 24 h, the vials were inspected using a magnifying glass, and the number of survival nauplii in each test tube was counted and recorded. Using this information, the percent (%) of mortality of brine shrimp nauplii was computed for each concentration of the sample. The straight-line equation of the graph was used to compute the LC_{50} (median lethal concentration) of all test samples [49, 50].

Table 1 Qualitative analysis of the phytochemicals of methanol extract of *M. diplotricha*

Chemical Constituent	Results
Alkaloids	+++
Carbohydrates	+++
Glycosides	+
Saponins	+++
Tannins	-
Phenols	++
Gum and Mucilages	-
Flavonoids	++
Fixed oils and Fats	-
Terpenes	-
Phytosterols	+++
Lipids	+
Volatile oil	-
Proteins and amino acids	+

Bioavailability indicator '+++'indicates high concentration; '++' indicates moderate concentration; '+' indicates mild concentration, and '- 'indicates absence of phytochemicals

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed by one-way ANOVA Dunnett's t-test where $^{\#}P < 0.05$ was regarded as statistically significant. In addition, all data were analyzed using SPSS software (Version: 20, IBM Corporation, New York, USA). LC_{50} and IC_{50} values were estimated by linear regression equations through the usage of Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA).

Results

Qualitative phytochemical screening

The current study aimed to ascertain the qualitative existence or lack of preliminary phytochemicals in the examined extracts, revealing the existence of multiple phytochemicals in the extracts, as illustrated in Table 1.

Acute toxicity study

Any abnormalities, including convulsions, reduced motor activity, diarrhea, restlessness, coma, and lacrimation, were not observed at the experimental doses of the investigated extracts. At the experimental doses, no mice were killed. Therefore, we investigated the LD_{50} to be greater than 400 mg/kg.

Evaluation of oral hypoglycemic activity

Oral Glucose Tolerance Test (OGTT)

After 30 min of a glucose load, the glucose level was remarkably elevated as compared to the control. Then, after 90 min of a glucose load, all four test samples—MDME, MDCF, MDPE, and MDNH—showed a reduction in blood glucose level compared to a 30-minute glucose load, respectively. For the first session of the experimental period, 30 min after glucose load, MDME and MDCF exhibited prominent ($^{\#\#}p < 0.01$) antihyperglycemic activity after 90 min of the test interval (Table 2).

Evaluation of CNS activities

Sedative activity

Open-field test During this test, the squares visited by the test animals from different test groups decreased over the course of two hours. This indicates the sedative effect (e.g., reduction in spontaneous motor activity, exploratory behavior, and motor coordination), as shown in Fig. 1. The

Table 2 Oral hypoglycemic activity of methanol extract of *M. diplotricha* and its different fractions

Animal Group	Dose (mg/kg)	Mean ± SEM			
		0 min	30 min	90 min	120 min
Control (Normal Saline)		6.64 ± 0.44	14.08 ± 1.20 (+ 112.05)[a]	8.98 ± 0.90 (-36.22)[b]	6.76 ± 0.33 (-51.99)[b]
Standard (Glibenclamide)	10	5.82 ± 0.32	10.06 ± 0.72[##] (+ 72.85)[a]	4.60 ± 0.20[###] (-54.27)[b]	4.18 ± 0.24[###] (-58.45)[b]
MDME	200	6.76 ± 0.41	10.90 ± 0.46[#] (+ 61.24)[a]	7.24 ± 0.42[##] (-33.58)[b]	6.66 ± 0.31 (-38.90)[b]
MDCF	200	6.30 ± 0.40	10.42 ± 0.67[##] (+ 65.40)[a]	6.26 ± 0.62[##] (-39.92)[b]	5.92 ± 0.27 (-43.19)[b]
MDPE	200	5.90 ± 0.25	11.5 ± 0.46 (+ 94.92)[a]	5.94 ± 0.26[##] (-48.35) [b]	5.82 ± 0.26 (-49.39)[b]
MDNH	200	6.16 ± 0.18	12.44 ± 0.36 (+ 101.95)[a]	6.42 ± 0.34[##] (-48.39)[b]	6.54 ± 0.28 (-47.43)[b]

Figures in parentheses are the % increase (+) or decrease of glucose level; a = compared to the glucose at zero-time, b = compared to the glucose level at 30 min after the glucose load, MDME = Methanol extract of *Mimosa Diplotricha*, *MDCF* = Chloroform soluble fraction, MDPE = Petroleum ether fraction, MDNH = n-hexane soluble fraction. Each value represents the mean ± SEM ($n = 5$). One-way ANOVA followed by Dunnett's t-test after analysis. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to control

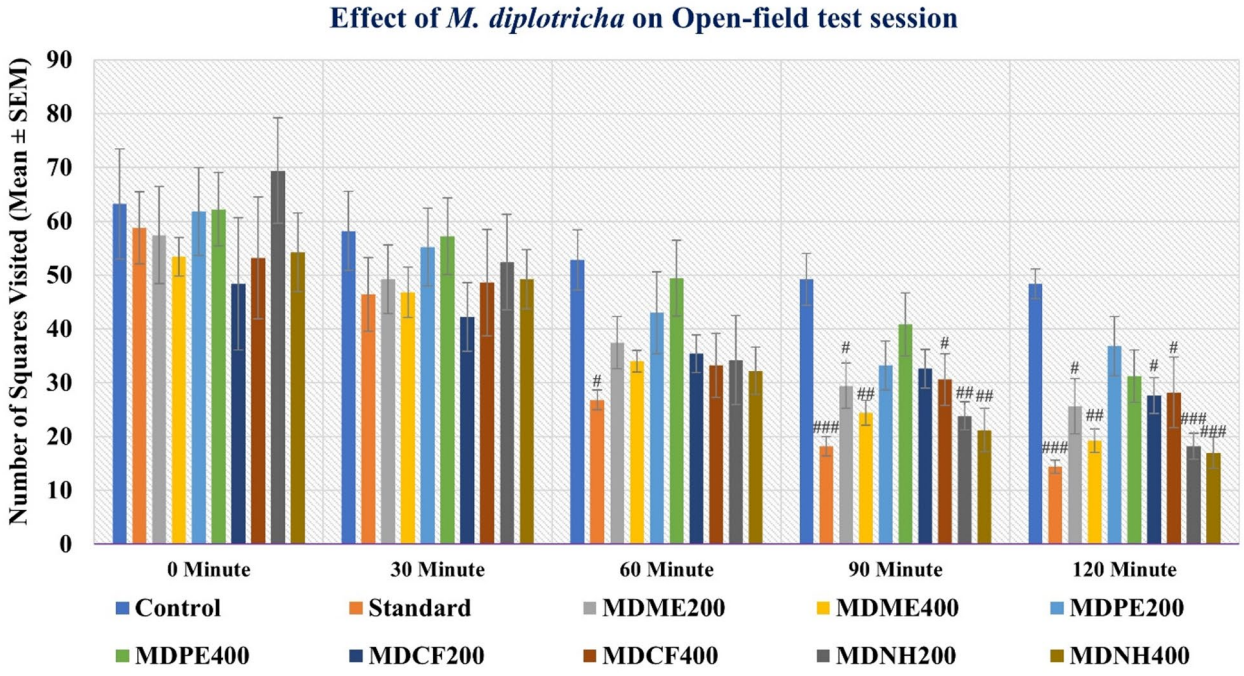


Fig. 1 Effect of *M. diplotricha* on Open-field test session. All values are expressed as mean ± SEM ($n = 5$). One-way ANOVA followed by Dunnett's test. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ significant compared to control. MDME = Methanol extract of *M. Diplotricha*, MDCF = Chloroform soluble fraction, MDPE = Petroleum ether fraction, MDNH = n-hexane soluble fraction

fractions MDME ($p < 0.05$; $p < 0.01$) and MDNH ($p < 0.01$; $p < 0.001$) showed significant sedative effects at doses of 200 and 400 mg/kg, respectively.

Hole-cross test As shown in Fig. 2, the hole cross test revealed decreased locomotor activity in MDME, MDPE, MDCF, and MDNH as the test animals showed less movement to cross chambers throughout the 2-hour study period. The number of crossings between the chambers was higher during the pre-treatment session. MDNH showed a significant ($p < 0.001$) decreased locomotor action or sedative effect in comparison to other test samples.

Anxiolytic activity

Elevated Plus Maze (EPM) test

Compared to the standard, the extracts of *M. diplotricha* significantly affected the number of entries in both the open-arms and closed-arms models. Almost all the

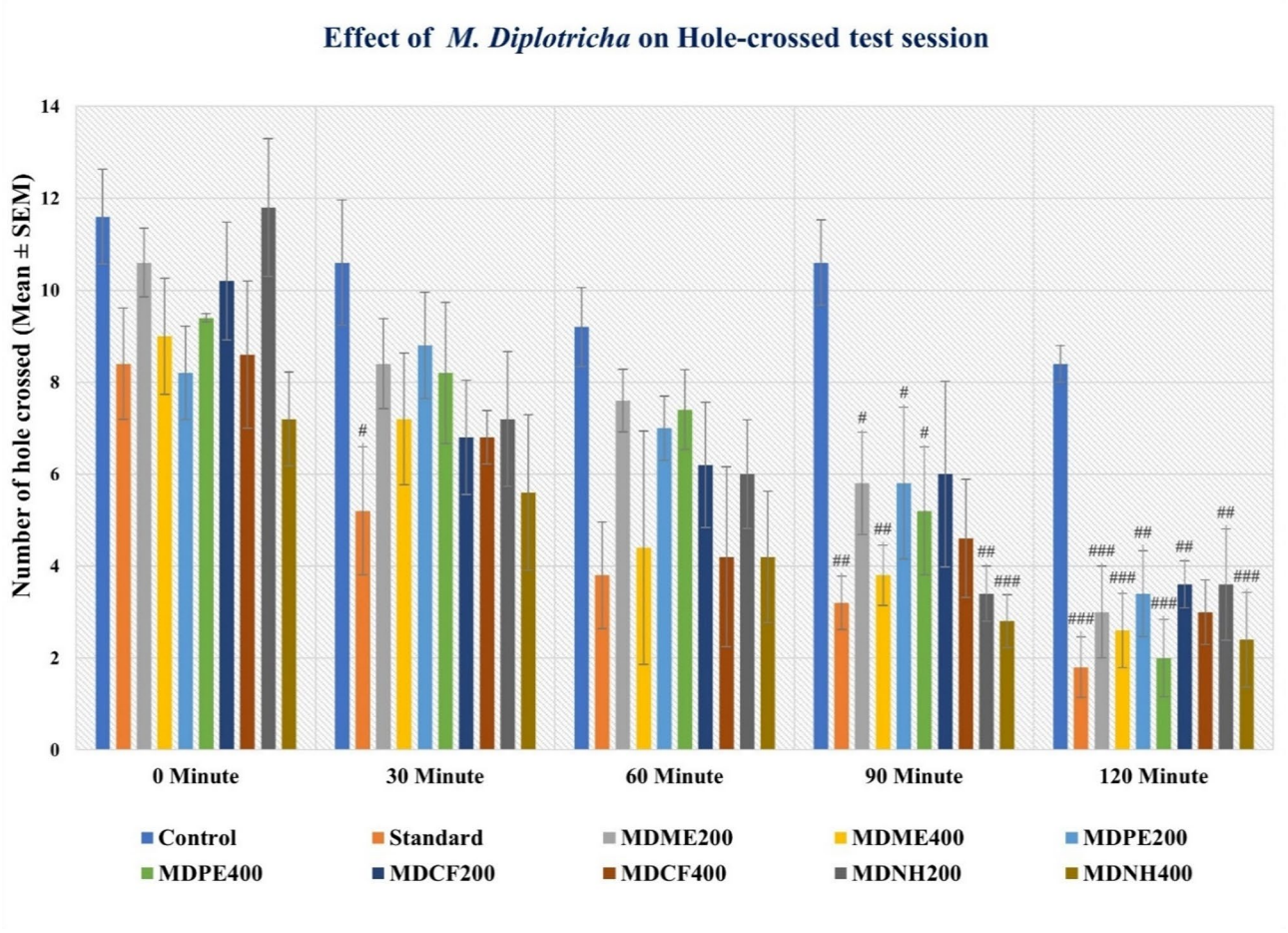


Fig. 2 Effect of *M. Diplotricha* on Hole-crossed test session. All values are expressed as mean ± SEM ($n = 5$). One-way ANOVA followed by Dunnett's test. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to control. MDME = Methanol extract of *M. Diplotricha*, MDCF = Chloroform soluble fraction, MDPE = Petroleum ether fraction, MDNH = n-hexane soluble fraction

Table 3 Effect of *M. Diplotricha* on elevated plus maze (EPM) test session

Group	(Mean ± SEM)			
	Open arm		Closed arm	
	Time spent (sec)	Number of entries	Time spent (sec)	Number of entries
Control	9.40 ± 3.20	1.6 ± 0.60	257.60 ± 13.59	16.80 ± 2.82
Standard	211.4 ± 13.00[a]	10.4 ± 1.99[a]	57.20 ± 10.8[a]	6.40 ± 1.57[a]
MDME200	242.80 ± 15.60[a]	15.00 ± 2.88[a]	19.6 ± 4.85[a]	4.00 ± 1.00[a]
MDME400	180.00 ± 17.11[a]	16.20 ± 2.92[a]	47.80 ± 5.17[a]	6.20 ± 0.66[a]
MDPE200	272.60 ± 4.13[a]	17.20 ± 1.65[a]	16.80 ± 3.50[a]	2.2 ± 0.58[a]
MDPE400	245.40 ± 9.21[a]	13.4 ± 1.43[a]	31.8 ± 7.05[a]	4.2 ± 0.86[a]
MDCF200	234.00 ± 11.69[a]	11.80 ± 1.50[a]	22.6 ± 8.48[a]	3.20 ± 1.28[a]
MDCF400	222.4 ± 10.26[a]	13.20 ± 1.65[a]	36.00 ± 5.32[a]	4.40 ± 0.93[a]
MDNH200	243.6 ± 10.66[a]	18.00 ± 1.14[a]	27.60 ± 4.47[a]	3.40 ± 0.5[a]
MDNH400	254.8 ± 8.72[a]	11.4 ± 2.06[a]	24.20 ± 8.21[a]	2.80 ± 0.86[a]

MDME = Methanol extract of *M. Diplotricha*, MDCF = Chloroform soluble fraction, MDPE = Petroleum ether fraction, MDNH = n-hexane soluble fraction. All values are expressed as mean ± SEM ($n = 5$). One-way ANOVA followed by Dunnett's test. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to control. Here, a = test groups are statistically significant

fractions provoked test animals to stay in the open arm as well as increased numbering entries into the open arm at doses of 200 and 400 mg/kg, respectively. They depicted a significant anxiolytic effect compared to the control. Table 3 tabulated all the test results.

Hole-board test

In this test session, the number of head-dippings increased in all test groups in comparison to the control group. Among them, the result produced by MDME extract was statistically significant at a dose of 400 mg/kg. Figure 3 displays all the data sets.

In vitro evaluation of antioxidant activity

Superoxide scavenging assay by alkaline DMSO method

The results shown in Table 4 revealed that among the five different concentrations (5, 10, 20, 40, and 80 µg/ml), curcumin showed the highest scavenging activity, which was 96.66% at 80 µg/mL. At a concentration of 800 µg/mL, the ME (methanol extract) of *M. diplotricha* leaf showed the best scavenging activity at 69.57%. Methanol

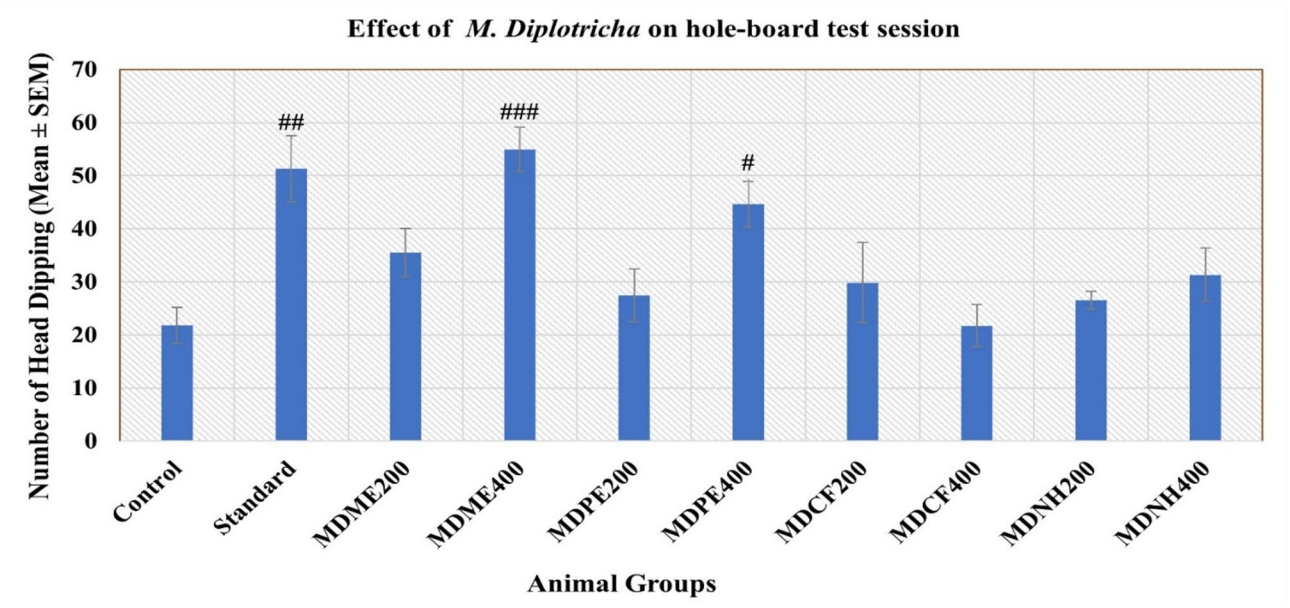


Fig. 3 Effect of *M. Diplotricha* on hole-board test session. All values are expressed as mean ± SEM ($n = 5$). One-way ANOVA followed by Dunnett's test. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to control. MDME = Methanol extract of *M. Diplotricha*, MDCF = Chloroform soluble fraction, MDPE = Petroleum ether fraction, MDNH = n-hexane soluble fraction

Table 4 Superoxide scavenging (%) activity of methanol extract of *M. Diplotricha*

Samples	Concentration (µg/ml)	Mean ± SEM	% of superoxide scavenging activity	IC_{50} (µg/ml)
MDME	800	$0.276 \pm 0.008^{\#\#\#}$	69.57	211.39
	400	$0.204 \pm 0.004^{\#\#\#}$	58.82	
	200	$0.162 \pm 0.008^{\#\#\#}$	48.15	
	100	$0.133 \pm 0.012^{\#}$	36.84	
	50	0.125 ± 0.010	32.80	
Standard (Curcumin)	80	$2.515 \pm 0.010^{\#\#\#}$	96.66	0.0000127
	40	$1.799 \pm 0.016^{\#\#\#}$	95.33	
	20	$1.565 \pm 0.020^{\#\#\#}$	94.63	
	10	$1.318 \pm 0.014^{\#\#\#}$	93.63	
	5	$0.644 \pm 0.011^{\#\#\#}$	86.96	
Control	-	0.084 ± 0.003		

Data are shown as mean ± SEM for triplicate. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. MDME = Methanol extract of *M. Diplotricha*

Table 5 Ferric reducing effect assay of methanol extract of *M. diplotricha*

Samples	Concentration (µg/ml)	Mean ± SEM	% increase of reducing power	IC_{50} (µg/ml)
MDME	440	$0.094 \pm 0.00709^{***}$	77.36	96.64
	220	$0.087 \pm 0.00306^{**}$	64.15	
	110	$0.083 \pm 0.00611^{**}$	56.60	
	55	0.075 ± 0.00306	41.51	
	27.50	0.065 ± 0.00666	22.64	
Standard (Ascorbic acid)	250	$0.100 \pm 0.00586^{***}$	88.68	74.49
	200	$0.094 \pm 0.00361^{***}$	77.36	
	150	$0.089 \pm 0.00404^{**}$	67.92	
	100	$0.082 \pm 0.00404^{**}$	54.72	
	50	0.075 ± 0.00624	41.51	
Control	-	0.53 ± 0.00603		

Data are shown as mean ± SEM for triplicate. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. MDME = Methanol extract of *M. Diplotricha*

extract showed an even lower IC_{50} value (211.39 µg/mL) than that of the reference antioxidant curcumin (0.0000127 µg/mL). The value of the IC50 of methanol extract is also very promising because the cutoff value of the IC50 is 1000 µg/mL.

Ferric-reducing effect assay

At a concentration of 440 g/mL, we obtained the methanol extract with the maximum reducing capacity (77.36%). On the other hand, ascorbic acid showed a maximum reducing capacity of 88.68% at a concentration of 250 g/mL. The IC_{50} values for ascorbic acid and methanol extract were 74.49 g/mL and 96.64 g/mL, respectively. Table 5 displays all these results.

In vitro evaluation of cytotoxic activity by Brine shrimp lethality bioassay

We tested the crude MDME extract and its various soluble fractions of *M. diplotricha* for cytotoxic activities on brine shrimp nauplii, as shown in Table 6. The standard vincristine sulfate showed an LC_{50} value of 0.622 (µg/ml), which is highly significant compared to the control. The crude methanol extract, MDME, MDPE, MDCF, and

Table 6 A brief overview of the cytotoxic activity of *M. diplotricha* extracts

Samples	Equation	R_2	LC_{50} (µg/ml)
Vincristine sulfate	y = 41.927x + 58.644	0.925	0.622
MDME	y = 37.12x − 8.094	0.9521	36.73
MDPE	y = 25.473x + 20.593	0.7649	14.27
MDCF	y = 18.264x + 46.681	0.922	1.52
MDNH	y = 28.04x + 19.27	0.8185	12.47

MDME = Methanol extract of *M. Diplotricha*, MDCF = Chloroform soluble fraction, MDPE = Petroleum ether fraction, MDNH = n-hexane soluble fraction

MDNH fractions had LC_{50} values of 36.73 g/ml, 14.27 g/ml, 5.41 g/ml, 1.52 g/ml, and 12.47 g/ml, respectively. Among the test samples, MDCF displayed significant cytotoxicity with the lowest LC_{50} value of 1.52 (µg/ml).

Discussion

In this study, we conducted phytochemical screening and examined the in vivo and in vitro pharmacological activities of *M. diplotricha* leaves. Qualitative phytochemical analysis exhibited that crude methanol extract from the leaves of *M. diplotricha* contains different phytochemicals that predict biological and therapeutic activities. Therefore, we expected this species to have numerous medicinal uses. The present investigation of this plant revealed that the methanol and fractionated extracts possess considerable hypoglycemic, sedative, anxiolytic, antioxidant, and cytotoxic properties in different experiment models.

The oral hypoglycemic activity was done by the oral glucose tolerance test (OGTT), a physiological method to induce diabetes mellitus. Numerous epidemiological studies and clinical trials suggest that hyperglycemia is considered the principal reason for diabetic complications [51]. Hence, sustained reduction in hyperglycemia or fruitful control of glucose is the vital function in preventing or reversing the disease. Therefore, we selected the glucose-loaded hyperglycemic model to assess the antihyperglycemic effects of *M. diplotricha* plant extracts [52].

This test artificially raised the blood glucose level without harming the pancreas. It is a widely used technique to evaluate the hypoglycemic activity of plant products [53]. In vitro OGTT test revealed that the MDME and MDCF fractions exhibited a significant reduction in blood glucose levels after 30 min of oral administration. In addition, *M. diplotricha* leaf extract displayed the presence of bioactive components such as tannin, alkaloid, saponin, phenol, flavonoid, and cardiac glycosides, implying that the blood glucose-lowering activity of the crude extract is possible due to the presence of these phytochemicals. Phytoconstituents like flavonoids and tannins induce insulin release from pancreatic beta cells [54]. Plant-derived phytochemicals influence glucose metabolism by increasing insulin secretion and action, decreasing gluconeogenesis, increasing glucose transporter expression and translocation, elevating pancreatic cell proliferation, inhibiting alpha-glucosidase and amylase, protecting pancreatic beta cells from oxidative stress and inflammation, and inhibiting apoptosis [55–57].

To observe the locomotion and exploration activities, the open field method and hole cross-test sessions were used, respectively. In open-field test sessions, the MDME and MDNH demonstrated reduced frequency and amplitude of mouse movements, while the MDNH demonstrated reduced exploration during hole cross-test sessions. These studies' findings demonstrated that the extract decreased locomotor activity, supporting its CNS depressive effects. These changes in spontaneous motor activity, which is a measure of the CNS's level of excitability, may be due to the plant extract's CNS-depressant effects [58]. Reduced peripheral and central locomotion counts support the fact that mice's motor activity has decreased. The conventional medication diazepam exhibits CNS depressive activity by activating ligand-gated chloride-selective ion channels triggered by GABA to block neurotransmitter release. Flavonoids abound in the leaves of *M. diplotricha*. Flavonoids that increase GABA can have a sedative effect, followed by a depressive effect [59]. The experiment's findings and reports of being high in flavonoids imply that they may involve GABA (the brain's GABA type A) activation and inhibition of neurotransmitter release.

To determine the anxiolytic effects, we used two animal models, namely, the elevated plus maze test and the hole board test. The Elevated Plus Maze (EPM) method is utilized as a reliable tool in determining the selective anxiolytic effect of the drug. It is mainly based on the observation that rodents consistently stay more in the closed arms when placed in mazes with both open and closed arms, and that exposing animals to an elevated maze alley triggers an approach-avoidance conflict [60, 61]. Here, the open arm is considered to be more aversive for experimental animals. So, any anxiolytic agent that can stimulate rodents to stay in open-arm exploration is beneficial [62]. In our study, the mice treated with the crude methanol extract of *M. diplotricha* leaves and its different fractions demonstrated a reduction in anxiety-like behavior by provoking mice into open arms. Similarly, the hole board test helps to measure the exploratory responses and multiple dimensions of the unconditioned behavior of a rodent in an obscure setting [63]. In this test, an increase in head-dipping actions may indicate an anxiolytic-like mood [64], while the indecision to visit the hole indicates a positive sign of anxiety [65]. On the contrary, our present plant extract produced considerable exploratory behavior in rodents exposed to hole poking aptitude by the higher number of head dippings.

Similarly, the positive control, diazepam, also exhibited an increase in head dipping when compared to the control.

The superoxide scavenging assay method was utilized for examining the antioxidant properties. This test looked at the extract's potential to scavenge the superoxide radical that was produced by the NaOH-alkaline DMSO-NBT system, which led to the creation of the blue formazan [66]. The produced superoxide is stable in solution at normal temperatures, converting nitro-blue tetrazolium to formazan color. A red dye called formazan cannot develop without the assistance of a superoxide scavenger that may react. The extract's IC_{50} value demonstrated how it inhibited formazan formation. Both curcumin and *M. diplotricha* extracts demonstrated dose-dependent superoxide scavenging efficacy. Here, the plant extract's IC_{50} value remained below the standard substance curcumin, indicating that the crude plant extract had a significant superoxide scavenging effect compared to the standard. In this case, *M. diplotricha* leaf extract can stop the superoxide radical from working without using an enzyme. The superoxide radical is a source of many ROS and damages many parts of cells. This is true even though the enzyme superoxide dismutase can help break down the superoxide radical [67]. In addition, the study of phytochemicals showed that the plant extract contains saponins, tannins, flavonoids, and terpenes, all of which can help with its scavenging effect [68–70]. Any compound's reducing capacity, a good indicator of antioxidant properties, can also determine its antioxidant potential. Therefore, a ferric oxide effect assay was used to identify the scavenging activity of crude plant extract. Both *M. diplotricha* methanol extract and ascorbic acid had high scavenging activity, indicating that high absorbance indicates high reducing power. The crude plant extract's reducing power increased as its concentration increased. The abundance of reductants in the samples, such as antioxidants, leads to the reduction of the Fe3+/ferricicyanide complex to the ferrous form [71]. The methanol extract, along with the standard drug ascorbic acid, showed their highest reducing capacity at concentrations of 440 µg/mL and 250 µg/mL. We found the IC_{50} values of ascorbic acid and methanol extract to be 74.49 g/mL and 96.64 g/mL, respectively. In our study, the extract's reducing power was lower than that of ascorbic acid, but the extract's IC_{50} value was very close to that of ascorbic acid, indicating that *M. diplotricha* has statistically significant reducing power.

To determine the cytotoxicity, an in vitro brine shrimp lethality bioassay was used. The assay involves the settling of shrimp larvae into a crude plant extract in saline solution, followed by the evaluation of lethality after a 24-hour testing period. Furthermore, this technique is a relatively cost-effective and feasible study design, making it an ideal benchtop tool for ethnopharmacology laboratories [72].

In this test, the positive control, when contrasted to the negative control, was fatal, with a very low LC_{50} value, resulting in considerable shrimp mortality. The lower the LC_{50} values, the more hazardous the drug will be, and vice versa. However, different dose levels in the present test samples caused varying degrees of mortality in *Artemia salina*. Here, the chloroform fraction of the methanol extract has significant cytotoxicity compared to the positive control. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, saponins, and phytosterols in the extracts, potentially contributing to their cytotoxic properties [50]. Flavonoids cause cytotoxicity by increasing the generation of cellular ROS [73]. Saponins cause cancer cells to die [74]. Previous research has revealed that alkaloids can inhibit the proliferation of various cancer cells [50, 75, 76].

Conclusion

The current investigation discovered that distinct extracts of *M. diplotricha* leaves possess a variety of phytochemicals, including the highest presence of alkaloids, flavonoids, tannins, and saponins, and evaluated significant hypoglycemia, sedative, anxiolytic, antioxidant, and cytotoxic properties. It has the potential to significantly contribute to medicinal chemistry and drug discovery research. However, we need to conduct further advanced research and analytics on this medicinal plant to determine its precise ethnomedicinal effect.

Limitation of the study

The phytochemical screening of *M. diplotricha* leaves provided a preliminary identification of phytochemicals related to pharmacological activities. The absence of a precise, structurally confirmed compound analysis hindered the progress of our fundamental research.

Abbreviations

MDME	Crude methanol extract of M. diplotricha
MDPE	Petroleum ether fraction
MDCF	Chloroform fraction
MDNH	n-hexane fraction
EPM	Elevated Plus Maze
HB	Hole-Board
OGTT	Oral Hypoglycemic Tolerance Test
CNS	Central Nervous System
DMSO	Dimethyl sulfoxide
DM	Diabetes Mellitus
IC	Inhibitory Concentration
LC	Lethal Concentration
TCA	Tricyclic Antidepressants
NMDA	N-methyl-D-aspartate
MAOI	Mono Amine Oxidase Inhibitors
SNRI	Serotonin-Norepinephrine Reuptake Inhibitors
SSRI	Selective Serotonin Reuptake Inhibitors
ROS	Reactive Oxygen Species
GABA	Gamma Amino Butyric Acid

Acknowledgements
The authors are grateful to Department of Pharmacy, University of Chittagong, Bangladesh for their cordial co-operation by providing laboratory support to carry out.

Author contributions
Mohammed Kamrul Hossain; Designed the experiments and conception, Jannatul Naima; Concept design, experiment leading, drafted manuscript and data interpretation. Mohammad Rashedul Islam; Experimental assistance, Data analysis, proofreading, critically reviews manuscript. Bakul Akter, Ainun Nahar, Nusrat Jahan, Ummah Tasnim Nisat; Manuscript editing. Mohammed Kamrul Hossain and Mohammad Rashedul Islam; gave final approval for the submission of revised version. All authors read and approved the final manuscript.

Funding
The research work was partially supported by the Department of Pharmacy, University of Chittagong, Bangladesh. Besides, the authors have no other relevant affiliations or financial involvement with any organization.

Data availability
All data and materials are contained and described within the manuscript. The data set was deposited in publicly available repositories. The plant's materials for the study were identified and voucher specimens are deposited at Pharmacy Department of University of Chittagong. The dataset analyzed during our current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethical committee of University of Chittagong under approval no-cc98056.

Consent for publication
All authors have given their valuable consent to publish this article.

Competing interests
The authors declare that they have no competing interests.

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Received: 5 May 2023 / Accepted: 24 July 2025
Published online: 14 August 2025

References

1. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman Med J. 2012;27:269–73. https://doi.org/10.5001/omj.2012.68.
2. Suryasa IW, Rodríguez-Gámez M. Health and treatment of diabetes mellitus. Int J Health Sci (Qassim). 2021;5:I–V. https://doi.org/10.53730/ijhs.v5n1.2864.
3. Reed J, Bain S, Kanamarlapudi VA. Review of current trends with type 2 diabetes epidemiology, aetiology, pathogenesis, treatments and future perspectives. Diabetes Metab Syndr Obes. 2021;14:3567–02. https://doi.org/10.2147/DMSO.S319895.
4. Padhi S, Nayak AK, Behera A. Type II diabetes mellitus: a review on recent drug-based therapeutics. Biomed Pharmacother. 2020;131:110708. https://doi.org/10.1016/j.biopha.2020.110708.
5. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14:88–8. https://doi.org/10.1038/nrendo.2017.151.
6. Seabrook EM, Kern ML, Rickard NS. Social networking sites, depression, and anxiety: A systematic review. JMIR Ment Health. 2016;23:3:1–19. https://doi.org/10.2196/mental.5842.
7. Fajemiroye JO, Da Silva DM, De Oliveira DR, Costa EA. Treatment of anxiety and depression: medicinal plants in retrospect. Fundam Clin Pharmacol. 2016;30:198–15. https://doi.org/10.1111/fcp.12186.
8. Kaltenboeck A, Harmer C. The neuroscience of depressive disorders: A brief review of the past and some considerations about the future. Brain Neurosci Adv. 2018;2:1–6. https://doi.org/10.1177/2398212818799269.
9. Fasipe OJ. Neuropharmacological classification of antidepressant agents based on their mechanisms of action. Arch Med Heal Sci. 2018;6:81–9. https://doi.org/10.4103/amhs.amhs.
10. Richards D. Clinical psychology review prevalence and clinical course of depression: A review. Clin Psychol Rev. 2011;31:1117–25. https://doi.org/10.1016/j.cpr.2011.07.004.
11. Bystritsky A, Khalsa SS, Cameron ME, Schiffman J. Current diagnosis and treatment of anxiety disorders. P T. 2013;38:30–7. PMID: 23599668.
12. Penninx BWJH, Pine DS, Holmes EA, Reif A. Anxiety disorders. Lancet. 2021;397:1–14. https://doi.org/10.1016/S0140-6736(21)00359-7.
13. Garakani A, Murrough JW, Freire RC, Thom RP, Larkin K, Buono FD, et al. Pharmacotherapy of anxiety disorders: current and emerging treatment options. Front Psychiatry. 2020;11:1–21. https://doi.org/10.3389/fpsyt.2020.595584.
14. Fuentes D, Ray SD, Wayne F, Anxiolytics. Encycl Toxicol. 2014;1:280–86. https://doi.org/10.1016/B978-0-12-386454-3.00241-4.
15. Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant extracts for antioxidant activity. Life Sci. 2003;73:167–79. https://doi.org/10.1016/S0024-3205(03)00259-5.
16. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;56:1–13. https://doi.org/10.1155/2017/8416763.
17. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, et al. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci. 2017;18:20–31. https://doi.org/10.3390/ijms18010096.
18. Ruffa MJ, Ferraro G, Wagner ML, Calcagno ML, Campos RH, Cavallaro L. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J Ethnopharmacol. 2002;79:335–39. https://doi.org/10.1016/S0378-8741(01)00400-7.
19. Blackadar CB. Historical review of the causes of cancer. World J Clin Oncol. 2016;7:54–86. https://doi.org/10.5306/wjco.v7.i1.54.
20. Wang J, Jiang YF. Natural compounds as anticancer agents: experimental medicine. World J Exp Med. 2012;2:45–57. https://doi.org/10.5493/wjem.v2.i3.45.
21. Nisar B, Sultan A, Rubab SL. Comparison of medicinally important natural products versus synthetic Drugs-A short commentary. Nat Prod Chem Res. 2018;6:1–3. https://doi.org/10.4172/2329-6836.1000308.
22. Mathur S, Hoskins C. Drug development: lessons from nature (Review). Biomed Reports. 2017;6:612–14. https://doi.org/10.3892/br.2017.909.
23. Sofowora A, Ogunbodede E, Onayade A, Dentistry C. The role and place of medicinal plants in the strategies for disease. Afr J Tradit Complement Altern Med. 2013;10:210–29. https://doi.org/10.4314/ajtcam.v10i5.2.
24. Atanasov AG, Zotchev SB, Dirsch VM, International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021;20:200–16. https://doi.org/10.1038/s41573-020-00114-z.
25. Qadir SU, Raja V. Herbal medicine: Old practice and modern perspectives. Phytomed. Academic Press. 2021. pp. 149–180 https://doi.org/10.1016/B978-0-12-824109-7.00001-7
26. Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Tech Res. 2012;3:200–01. https://doi.org/10.4103/2231-4040.104709.
27. Pasiecznik P N. Mimosa Diplotricha giant sensitive plant. Asia-Pacific Invasive Species Netork CABI. 2013;1–3. https://doi.org/10.1079/cabicompendium.34196.
28. Uyi O. *Mimosa diplotricha*: a review and synthesis of its problem and control options. Cab Rev. 2020;15:1–8. https://doi.org/10.1079/PAVSNNR202015014.
29. Naima J, Islam MR, Proma NM, Afrin SR, Hossain MR, Hossain MK, et al. Phytochemical screening and antinociceptive activity of *Mimosa Diplotricha* leaves. Int J Pharm Sci Res. 2019;10:3679–84. https://doi.org/10.13040/IJPSR.0975-8232.10(8).3691-96.
30. Chiou CT, Shen CC, Tsai TH, Chen YJ, Lin LC. Meroterpenoids and Chalcone-Lignoids from the roots of *Mimosa Diplotricha*. J Nat Prod. 2016;79:2439–45. https://doi.org/10.1021/acs.jnatprod.6b00170.

31. Islam MT, Ferdous J, Sultana I, Riaz TA, Sultana N. Non-clinical and pre-clinical Pharmacological investigations of *Mimosa Diplotricha*. Bol Inf Geum. 2015;6:72–8.
32. Madathil SR, Kannappan P, Muthusami S, Muneeswari P, Periyasamy L. Evaluation of anticancer potential of mimosa Diplotricha ethanolic leaf extract on N-Methyl-N-Nitroso Urea induced colorectal carcinogenesis in Wistar albino rats. Indian J Pharm Educ Res. 2022;56:479–88. https://doi.org/10.5530/ijper.56.2.69.
33. Uyi OO, Udeogwu CC, Rotimi J. Phytochemical constituents and insecticidal efficacy of the root and leaf powders of *Mimosa Diplotricha* and *Aspilia Africana* against *Callosobruchus maculates* (Fab.) (Coleoptera: Chrysomelidae). J Appl Sci Environ Manag. 2020;24:645–52. https://doi.org/10.4314/jasem.v24i4.16.
34. Majeed I, Rizwan K, Ashar A, Rasheed T, Amarowicz R, Kausar H, et al. A comprehensive review of the ethnotraditional uses and biological and Pharmacological potential of the genus *Mimosa*. Int J Mol Sci. 2021;22:7463. https://doi.org/10.3390/ijms22147463.
35. Islam MR, Naima J, Proma NM, Hussain MS, Uddin SMN, Hossain MK, et al. In-vivo and in-vitro evaluation of Pharmacological activities of *Ardisia solanacea* leaf extract. Clin Phytoscience. 2019;5:32. https://doi.org/10.1186/s40816-019-0128-9.
36. Emran TB, Rahman MA, Uddin MM, Rahman MM, Uddin MZ, Dash R et al. Effects of organic extracts and their different fractions of five Bangladeshi plants on in vitro thrombolysis. BMC Complement Altern Med. 2015;15:2015. https://doi.org/10.1186/s12906-015-0643-2
37. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer Dordrecht;1984. https://doi.org/10.1007/978-94-009-5570-7
38. Islam MR, Proma NM, Naima J, Uddin G, Afrin SR, Hossain MK. Assessment of the Pharmacological activities of *Ardisia solanacea* roxb: an ethnomedicinal plant used in Bangladesh. J Pharm Nutr Sci. 2020;10:302–14. https://doi.org/10.29169/1927-5951.2020.10.05.17.
39. Joy KL, Kuttan R. Anti-diabetic activity of picrorrhiza Kurroa extract. J Ethnopharmacol. 1999;167:143–48. https://doi.org/10.1016/s0378-8741(98)00243-8.
40. Gupta BD, Dandiya PC, Gupta ML. A psycho-pharmacological analysis of behaviour in rats. Jpn Pharmacol Sci. 1971;21:293–98. https://doi.org/10.1016/S0021-5198(19)36218-3.
41. Takagi K, Watanabe M, Saito H. Studies of the spontaneous movement of animals by the hole cross test; effect of 2-dimethyl-aminoethanol and its acyl esters on the central nervous system. Jpn J Pharmacol. 1971;217:797–10. https://doi.org/10.1016/S0021-5198(19)36179-7.
42. Perez RM, Perez JA, Garcia LM, Sossa H. Neuropharmacological activity of solanum nigrum fruit. J Ethnopharmacol. 1998;2:43–8. https://doi.org/10.1016/s0378-8741(98)00059-2.
43. Latha K, Rammohan B, Sunanda BP, Maheswari MS, Mohan SK. Evaluation of anxiolytic activity of aqueous extract of coriandrum sativum linn. In mice: A preliminary experimental study. Pharmacognosy Res. 2015;2015:47–51. https://doi.org/10.4103/0974-8490.157996.
44. Pandey MM, Govindarajan R, Rawat AK, Pushpangadan P. Free radical scavenging potential of saussarea costus. Acta Pharm. 2005;55:297–04.
45. Reddy BS, Reddy RK, Reddy BP, Ramakrishna S, Diwan PV. Potential in vitro antioxidant and protective effects of *Soymida febrifuga* on ethanol induced oxidative damage in HepG2 cells. Food Chem Toxicol. 2008;46:3429–42. https://doi.org/10.1016/j.fct.2008.08.034.
46. Sanja S, Sheth N, Patel N, Dhaval P, Biraju P, Patel D, et al. Characterization and evaluation of antioxidant activity of *Portulaca Oleracea*. Int J Pharm Pharm Sci. 2009;1:74–84.
47. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15–27. https://doi.org/10.1016/s0076-6879(99)99005-5.
48. Mosaddik MA, Haque ME. Cytotoxicity and antimicrobial activity of goniothalamin isolated from Bryonopsis laciniosa. Phytother Res. 2003;17:1155–57. https://doi.org/10.1002/ptr.1303.
49. Sathasivam P, Lakshmi T. Brine shrimp lethality assay of sesbania grandiflora ethanolic extract-in vitro study. J Adv Pharm Edu Res. 2017;7:28–30.
50. Afrin SR, Islam MR, Khanam BH, Proma NM, Didari SS, Jannat SW, et al. Phytochemical and Pharmacological investigations of different extracts of leaves and stem barks of *Macropanax dispermus* (Araliaceae): a promising ethnomedicinal plant. Futur J Pharm Sci. 2021;7:165. https://doi.org/10.1186/s43094-021-00313-4.
51. Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan CS. Antidiabetic effects of Panax ginseng berry extract and the identification of an effective component. Diabetes. 2002;51(6):1851–8. https://doi.org/10.2337/diabetes.51.6.1851.
52. Kasabri V, Afifi FU, Hamdan I. In vitro and in vivo acute antihyperglycemic effects of five selected Indigenous plants from Jordan used in traditional medicine. J Ethnopharmacol. 2011;133(2):888–96.
53. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012;166:877–94. https://doi.org/10.1111/j.1476-5381.2012.01911.x.
54. Mukhlesur M, Gadria MM, Satyajit D. Comparative bioactivity studies on two mimosa species. Bol Latinoam Caribe Plant Med Aromatics. 2008;7:38–43.
55. Den Hartogh DJ, Tsiani E. Antidiabetic properties of naringenin: A citrus fruit polyphenol. Biomolecules. 2019;9:4–9. https://doi.org/10.3390/biom9030099.
56. Choudhary N, Khatik GL, Suttee A. Saponins and their potential role in diabetes mellitus. Diabetes Manag. 2017;7:148–58. https://doi.org/10.2174/1573399816666200516173829.
57. Elekofehinti OO, Kamdem JP, Kade IJ, Rocha JBT, Adanlawo IG. South African journal of botany hypoglycemic, antiperoxidative and antihyperlipidemic effects of saponins from solanum Anguivi lam. Fruits in alloxan-induced diabetic rats. South Afr J Bot. 2013;88:56–61. https://doi.org/10.1016/j.sajb.2013.04.010.
58. Lakshmibai R, Amirtham D. Evaluation of free radical scavenging activity of mimosa pudica Thorns. Asian J Pharm Clin Res. 2018;11:153–56. https://doi.org/10.22159/ajpcr.2018.v11i11.27426.
59. Anisuzzman M, Hasan MM, Acharzo AK, Das AK, Rahman S. In vivo and in vitro evaluation of Pharmacological potentials of secondary bioactive metabolites of *Dalbergia candenatensis* leaves. Evid Based Complement Alternat Med. 2017;2017:5034827. https://doi.org/10.1155/2017/5034827.
60. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007;2:322–28. https://doi.org/10.1038/nprot.2007.44.
61. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and Anxiety-like behavior in mice. J Vis Exp. 2015;96:52434. https://doi.org/10.3791/52434.
62. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacol (Berl). 1987;92:180–85. https://doi.org/10.1007/BF00177912.
63. 62, Thoeringer CK, Erhardt A, Sillaber I, Mueller MB, Ohl F, Holsboer F, et al. Long-term anxiolytic and antidepressant-like behavioural effects of tiagabine, a selective GABA transporter-1 (GAT-1) inhibitor, coincide with a decrease in HPA system activity in C57BL/6 mice. J Psychopharmacol. 2010;24:733–43. https://doi.org/10.1177/0269881109103091.
64. Doukkali Z, Taghzouti K, Bouidida EL, Nadjmouddine M, Cherrah Y, Alaoui K, et al. Evaluation of anxiolytic activity of methanolic extract of urtica urens in a mice model. Behav Brain Funct. 2015;11:1–5. https://doi.org/10.1186/s12993-015-0063-y.
65. Sillaber I, Panhuysen M, Henniger MS, Ohl F, Kühne C, Pütz B, et al. Profiling of behavioral changes and hippocampal gene expression in mice chronically treated with the SSRI Paroxetine. Psychopharmacol (Berl). 2008;200:557–72. https://doi.org/10.1007/s00213-008-1232-6.
66. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, et al. High molecular weight plant polyphenolics (Tannins) as biological antioxidants. J Agric Food Chem. 1998;46:1887–92. https://doi.org/10.1021/jf970975b.
67. 66, Shirwaikar A, Shirwaikar A, Punitha I. Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. Nat Prod Res. 2007;13:40–5.
68. 67, Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of Carissa Opaca fruits. Food Chem. 2010;122:1205–11. https://doi.org/10.1016/j.foodchem.2010.03.120.
69. Lalhminghlui K, Jagetia GC. Evaluation of the free-radical scavenging and antioxidant activities of chilauni, *Schima wallichii* Korth *in vitro*. Future Sci OA. 2018;4:FSO272. https://doi.org/10.4155/fsoa-2017-0086.
70. Lalrinzuali K, Vabeiryureilai M, Jagetia GC, Lalawmpuii PC. Free radical scavenging and antioxidant potential of different extracts of *Oroxylum indicum in vitro*. Adv Biomed Pharm. 2015;2:120–30.
71. 70, Chung YC, Chang CT, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1. J Agric Food Chem. 2002;50:2454–8. https://doi.org/10.1021/jf011369q.
72. Bouzada ML, Fabri RL, Nogueira M, Konno TU, Duarte GG, Scio E, et al. Antibacterial, cytotoxic and phytochemical screening of some traditional

medicinal plants in Brazil. Pharm Biol. 2009;47:44–52. https://doi.org/10.1080/13880200802411771.
73. Matsuo M, Sasaki N, Saga K, Kaneko T. Cytotoxicity of flavonoids toward cultured normal human cells. Biol Pharm Bull. 2005;28:253–9. https://doi.org/10.1248/bpb.28.253.
74. Alam F, Najum Us Saqib Q, Waheed A. Cytotoxic activity of extracts and crude saponins from Zanthoxylum armatum DC. against human breast (MCF-7, MDA-MB-468) and colorectal (Caco-2) cancer cell lines. BMC Complement Altern Med. 2017;17:1–9. https://doi.org/10.1186/s12906-017-1882-1.
75. Lamchouri F, Zemzami M, Jossang A, Abdellatif A, Israili ZH, Lyoussi B. Cytotoxicity of alkaloids isolated from peganum Harmala seeds. Pak J Pharm Sci. 2010;6:699–706. PMID: 23811445.
76. Kabir MS, Mahamoud MS, Chakrabarty N, Ahmad S, Masum MA, Hoque MA, et al. Antithrombotic and cytotoxic activities of four Bangladeshi plants and PASS prediction of their isolated compounds. J Basic Clin Physiol Pharmacol. 2016;27:659–66.

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