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PHYTOCHEMICAL EVALUATION AND PHARMACOLOGICAL ACTIVITIES OF *ANTIDESMA MONTANUM* BLUME LEAF EXTRACT

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Abstract: The demand for medicinal plants and their derived substances is increasing day by day due to their relevance in the context of drug discovery and development. The goal of this investigation is to assess the pharmacological and phytochemical potentials of the grossly underexplored *Antidesma montanum* Blume (Family: Phyllanthaceae). The methanolic extract of the leave of this plant was fractionated and then followed by initial screening of phytochemical. The investigation of the pharmacological potential, which includes antioxidant, antidiarrheal, anti-inflammatory, analgesic, anti-pyretic, and anxiolytic evaluations, was accomplished using an *in vitro* free radical scavenging assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH), castor oil-induced diarrheal test, egg albumin test, acetic acid-induced writhing model, brewer's yeast induced fever test, swing test, open field, and light-dark test, respectively. The investigation o phytochemicals proposes that the methanol extract of *A. montanum* possesses flavonoids, tannins, terpenoids, saponins, amino acids, fixed oils, and sterols. Pharmacological evaluation suggests that *A. montanum* possesses significant antioxidant, anti-diarrheal, anti-inflammatory, and analgesic effects. The methanol and chloroform fractions exhibited better DPPH radical scavenging activities with an IC_{50}: 103 ± 0.05 and 108.7 ± 0.05 µg/ml, respectively. The methanol and chloroform fractions also showed anti-inflammatory capacities in the egg albumin (IC_{50} values: 89.10 ± 0.07 and 92.85 ± 0.07 µg/ml, respectively) model. The plant also showed anti-pyretic and anxiolytic activities in a dose-dependent manner. One of the possible sources of phytotherapeutic lead compounds is *A. montanum*. To extract and analyze the key bioactive components of this essential therapeutic plant, more research is required.

Keywords: *Antidesma montanum*; phytochemicals; pharmacological activities.

1. Introduction

Conventional medicines rely on phytochemical-rich plant extracts to cure different maladies because medicines obtained from natural sources are considered to be less toxic and free from undesirable effects as compared to synthetic ones. The genus *Antidesma*, for example, contains over 200 species, with *Antidesma montanum*

Blume is being the most common (Ismail et al., 2019).

A. montanum commonly referred to as mountain current tree or pani-helochis a shrub or trees belong to the family Phyllanthaceae. *A. montanum* species may be found in a broad range of environments from the coast to the highland, from profound forest shade to surrounding human habitations and open grassland, and at altitudes up to 2,000 m. It is distributed in East Asia – Bangladesh, India, China, Myanmar, Vietnam, Cambodia, Indonesia, Malaysia, and Thailand. The plant may reach a height of 20 m. For up to 7 m, the bole can be free of branches. Alternate, stipulate leaves with lateral stipules measuring 3-12 x 0.5-3 mm. Flowers are yellow and unisexual. The fruit is a drupe that is 3-6 x 2.5-4 mm and is ellipsoid to ovoid in shape (https://indiabiodiversity.org/species/show/7553). Internally, *A. montanum* roots in water have been used to cure malaria, chickenpox, and measles in Peninsular Malaysia, while the leaves have been used topically to treat thrush and headache in children. Stems and roots are used as a diuretic in Thailand. The roots are used in the treatment of stomach ache (Ismail et al., 2019). In South-East Asia, the leaves of other *Antidesma* species are also utilized medicinally. After giving delivery, moms drink a tea made from the leaves as a tonic. Topically, the leaves are used to treat lumbar aches and ulcers (http://tropical.theferns.info/viewtropical.php?id =Antidesma + montanum). The plant contains essential oils that is rich in n-hexadecanoic acid (Zhou et al., 2012a; Zhou et al., 2012b); alkaloids, steroids, anthquinones, saponins, phytophenols, flavonoids and tannins (Arbainand, 1993; Aguninaldoet al., 2004).

The methanol extract of *A. montanum* leaves has potential antioxidant activity capacity (Maehly, 1954). The current study examines the phytochemical and pharmacological evaluation of a leaf extract of *A. montanum* which has not been hitherto properly investigated.

2. Materials and methods

Collection, identification, and extraction

Zhengzhou Fresh leaves of *A. montanum* were taken from a forest at Kamalgonj in Bangladesh, for this study, and were recognized by a taxonomist at Jahangirnagar University's Department of Botany. The plants were placed on a herbarium sheet, and the Bangladesh National Herbarium in Mirpur, Dhaka, identified the sample (Accession number-46870, DACB). The leaves were gathered, sorted from unwanted plants or plant components, and washed in water. They were dried in the shade for one week at room temperature (25-35 ºC). With the aid of a competent grinder, the leaves were crushed into a coarse powder. Until the analysis, the powder was maintained in an airtight container in a dark, cool, and dry environment. 350 gm of energized material were soaked in 1800 ml of methanol (99-100%). For a period of 15 days, the container and its contents were sealed and preserved with some shaking and stirring in between. After that, a piece of clean white linen was used to roughly filter the entire mixture. Finally, the extract was filtered using filter paper. The methanol extract was obtained by centrifuging the filtrate with a rotary evaporator. The resulting extract was kept in a beaker covered with a perforated sheet of aluminum foil for several days to ensure complete evaporation of the residual methanol. It resulted in a sticky concentration with a greenish-black tint. Crude methanol extract was the name given to the

concentration. By following the same process, a chloroform extract of *A. montanum was* also prepared.

Reagents and chemicals

ACI Pharmaceuticals Ltd. provided the diclofenac sodium and paracetamol, while ACME Laboratories Ltd. provided the diazepam and loperamide. Gonoshasthaya Pharmaceuticals Ltd. delivered the yeast. Merck Chemicals Ltd., Germany, provided the acetic acid and Tween-80, while sterile normal saline (0.9% NaCl) was provided by Beximco Infusions Ltd. The solvents and chemicals used were all of analytical quality. On the day of the experiments, all of the solutions were made.

Experimental animals

The animals utilized were *Swiss* albino mice measuring 20–25 gm. During the acclimation phase, the animals were maintained in regular laboratory settings (12 h light/dark cycle; room temperature 25 ± 2 °C; relative humidity 55–60%) and fed a standard diet (ICDDR, B prepared) and distilled water ad libitum. Prior to executing the studies, the animals were acclimatized to the laboratory environment for 7 days. Before the studies, the animals were starved for the night. All experimental animals were cared for in accordance with the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences' Ethical Principles and Guidelines for Scientific Experiments with Animals (1995). The Institutional Ethics Committee (SUB/IAEC/12.01) authorized all experimental protocols.

Preliminary phytochemical screening

The preliminary phytochemical investigations consist of testing distinct phytochemical classes contained in the extract. For this test, two types of extract solutions were prepared: a 5% w/v alcoholic extract solution (1.5 gm diluted in 30 ml ethanol) and a 5% w/v aqueous extract solution (In 2 ml of pure water, 0.1 gm was dissolved) (Harbopne et al., 1973; Evans et al., 1989; Edeoga et al., 2005).

Acute toxicity evaluation: Allium cepa assay

The crude methanol extract of the plant leaves was examined at 4.0, 3.0, 2.0, 1.0, and 0.5 mg/ml using distilled water as a control group. To stimulate root growth, the budding parenchyma and outer layers of the onions were carefully removed via the creation of a tiny circular incision. Generally, the tap water is applied for 20 min to clean the bulbs, while distilled water is used for soaking the root section in a cleaned container (15-20 ml capacity) at the condition of 25 ± 1 ^{o}C in the dark for the first 24 h. For a 24-hour exposure period, only the Alliums with adequate root development were replaced to the sample or controls containers containing five for each concentration. The roots were measured in mm after the exposure time. Root growth inhibition was determined to estimate the toxicity of the crude extract. The test sample's IC_{50} was also determined by applying following equation:

$$\log (LD_{50}) = 0.435 \times \log (IC_{50}) + 0.625$$

Antioxidant capacity

Total phenolic content assay

0.5 g dried material of plant was sonicated for 20 minutes with 50 ml methanol. The extract was centrifuged for 15 minutes after being divided into two liters. The standard, 30 mg of gallic acid, was combined with 250 ml of 99-100 percent methanol to make a 200 g/ml solution. Furthermore, using the right dilution process, four concentrations of solutions (150, 100, 50,

and 25 g/ml) were created, and volumetric flasks were taken and labeled. 1 ml of each concentration's standard solution was added to volumetric flasks, along with distilled water (9 ml) and 1 ml of Folin-Ciocalteu (FC) reagent, which was shaken and maintained for 5 minutes. The volume was adjusted with distilled water to reach a final amount of 25 ml after adding a 10 ml solution of 7% Na_2CO_3. They were maintained at room temperature for 90 minutes.

For each concentration, the UV absorbance was measured at 750 nm after 90 minutes against a blank. For accuracy, the absorbance was measured three times and the mean was chosen.

Total tannin content assay

0.5 g dried material of plant was sonicated for 20 minutes with 50 ml of 80% aqueous methanol. Two ml of the extract were centrifuged for 15 min. Gallic acid (30 mg) as a standard was mixed with 250 ml of methanol (99-100%) to prepare a 200 µg/ml solution. Another four concentrations of solutions were prepared by the proper dilution method. The concentrations were 150, 100, 50, and 25 µg/ml, respectively. 200, 150, 100, 50, and 25 µg/ml volumetric flasks were used, and standard solution (0.1 ml) was added to the flasks, followed by distilled water (7.5 ml), and 0.5 ml of FC reagent, which was held for 5 minutes. Then 1 ml of a 35% Na_2CO_3 solution was added, and 10 ml with distilled water was poured to adjusted the volume and maintained at room temperature for 30 minutes. For each concentration, the UV absorbance was measured at 725 nm against a blank. For accuracy, the absorbance was measured three times and the mean was chosen.

Total flavonoids content assay

A total of 0.5 gram of dried plant material was combined with methanol (50 ml) and sonicated for 20 minutes. Two (2) ml of the extract were centrifuged for 15 min. The standard, quercetin (30 mg), was mixed with 250 ml of methanol (99-100%) to prepare a 200 µg/ml solution. Another four concentrations of solutions (200, 150, 100, 50, and 25 µg/ml) were prepared by the proper dilution method. In volumetric flasks, 1 ml of standard solution of each concentration (200, 150, 100, 50, and 25 µg/ml) was taken, and then distilled water (4 ml) was added. In the next step, 5% sodium nitrite ($NaNO_2$) solution (0.3 ml) was mixed to every volumetric flask with shaking and kept for 5 minutes. Then it was mixed with a 0.3 ml solution of 10% $AlCl_3$. 2 ml of a 1 M sodium hydroxide (NaOH) solution was mixed at the sixth minute, and then distilled water is added to adjust the volume to a final amount of 10 ml using distilled water. For each concentration, the UV absorbance was measured at 510 nm against a blank. The absorbance was taken twice, and the mean was used for accuracy.

DPPH free radical scavenging assay

Extracts of each plant material (10 mg) were mixed with 20 ml of ethanol (99-100%) to prepare 500 µg/ml solutions of extract as a stock solution. Another five concentrations of solutions (200, 150, 100, 50, and 25 µg/ml) were prepared. In the same way, various concentrations of ascorbic acid solutions were prepared. A 20 mg DPPH powder was mixed with 500 ml of ethanol (99-100%) to prepare a 0.004% DPPH solution. Then, 2 ml of each concentration's solution was placed in test tubes, along with DPPH solution (6 ml), and each test tube was maintained in the dark for 30 minutes. In the same way, ascorbic acid

solutions were put into seven test tubes, the solution of DPPH was poured, and the tubes were maintained in the dark for 30 minutes. When simply ethanol was used as a blank, DPPH was also administered to the blank test tubes at the same time. A UV spectrophotometer set at 517 nm measured the absorbance of each test tube after 30 minutes. It was noted that the UV spectrometer reading was nullified with a blank solvent, i.e., ethanol, prior to taking absorbance measurements. The readings were noted down carefully. Then the percent inhibition was calculated. The IC_{50} was determined from the percentage inhibition *vs*. conc. graph (Badami et al., 2003).

Anti-inflammatory (Egg albumin) test

Ullah et al. (2014) performed this test with a little change. The 5 ml of reaction mixture contained 2.8 ml of phosphate buffered saline (PBS, pH 6.4), 0.2 ml of fresh hen's egg albumin, and various strengths of extract (2 ml), with final concentrations ranging from 0.12 to 0.36 % (v/v). As a negative control, a comparable volume of distilled water was used. The mixes were then heated at 70 °C for 5 minutes after being incubated at 37 ± 2 °C temperature with the help of a BOD incubator. Using the vehicle as a blank, the absorbance of it was used to measure at 660 nm after cooling. The reference medication, acetyl salicylic acid, was employed at a final concentration of 100 g/ml and was handled in the same way for the purposes of determining absorbance. The proportion of protein denaturation inhibition was estimated using the following formula:

$$\% \text{ Inhibition} = [(\text{Absorbance}_{\text{Control}} - \text{Absorption}_{\text{Test}}) / \text{Absorbance}_{\text{Control}}] \times 100$$

Anti-diarrheal (Castor oil-induced diarrheal) assay

Galvez et al. (1993) and Taufiq and Hossain et al. (2005) devised a systematic approach for studying castor oil-induced diarrhea. Animals (n = 9) were equally separated into three main groups (control, positive control (PC), and test treatment) after fasting them for 24 hours. Individual cage was used to place each mouse, which was lined with absorbent paper on the bottom. 0.1% Tween-80 was used to make aqueous solutions of the extract. The test groups received suspensions of *A. montanum* leaf extract at oral doses of 250 and 500 mg/kg body weight 40 minutes previous to the administration of 0.3 ml of castor oil to each mouse; the control group was given purified water with 0.1% Tween-80; loperamide was administered in the mice of the PC group at 3 mg/kg per body weight. After being administered castor oil, individual animals from each group were kept in separate cages with absorbent paper bottom and monitored for diarrhea every hour for 4 h. During the 4-hour period, the number of feces or any other fluid substance that discolored the adsorbent paper was counted and recorded for each mouse. Each mouse's latent duration was also recorded. Used papers were replaced with new ones at the start of each hour. The total number of feces expelled from the animals was recorded throughout a 4 h observation period.

Anti-pyretic (Brewer's yeast induced fever) test

The antipyretic test was carried out with the help of a method using Brewer's yeast to produce fever (Tomazetti et al., 2005; Turner, 1965). A clinical digital thermometer was used to record each rat's baseline rectal temperature at zero hour. The generation of

pyrexia in mice was produced after inducing a Brewer's yeast solution (20% w/v) in distilled water through subcutaneous injection at a dosage of 20 ml/kg body weight. The rectal temperature of each animal was measured after 18 h following Brewer's yeast injection, and only animals with a temperature increase of at least 1 °F were chosen for the experiment. The total 12 animals were equally divided into four groups randomly. Orally, 1% Tween-80 in normal saline was given in the control group. The conventional medicine paracetamol was orally administered to the PC group at a dosage of 100 mg/kg. Methanol extract was given to the test groups in oral doses of 250 and 500 mg/kg. The temperature of each animals was recorded at 0, 1, 2, 3, and 4 h after the therapy.

Analgesic (Acetic acid-induced writhing) test

This test was carried out with the help of a method defined by the Ahmed et al. (2004) and Ali et al. (2017). Animals were randomly separated into control, PC, and test groups after fasting for 18 hours with water.

Individual cage was used to place each mouse, which was lined with absorbent paper on the bottom. 0.1% Tween-80 was used to make aqueous solutions of the extract. A feeding needle was used to provide the test samples, control and diclofenac sodium orally. The writhing-inducing drug, acetic acid solution (0.7%), was then given to each animal group intraperitoneally. The number of writhing was measured for 15 minutes after a 5-minute gap to allow for acetic acid absorption.

Anxiolytic-like activity test

For a comprehensive evaluation of the anxiolytic-like potential, various protocols, which include swing, open-field, and light-dark tests, were adopted as mentioned below.

Swing test (SWT)

The swing test was adopted, which is a conventional, simple, and straightforward behavioral test that simply entails touching the animal by its tail and documenting the direction of swings it makes over a period of time. The animals were individually placed in a Plexiglas box (40 × 40 × 35.5 cm) and given two minutes to habituate and achieve a neutral stance. The animal was held near the base of its tail, about an inch away. It was then raised to a height of 1 inch above the surface it had been lying on. On either side, the animal was confined in the vertical axis, which was designated as no more than "lo." When the animal moved its head off of the vertical axis to either side, it was recorded as a swing. For the next swing to be tallied, the animal must return to the vertical posture before trying another swing. Only one swing was tallied when the animal swung and redoubled its efforts to migrate toward one side without returning to the upright posture. The animal was then re-suspended and time was reset once it was in a neutral posture. Because swings were frequently exhibited in fewer than one set, the frequency of swings was measured rather than the duration. When the animal was lifted for more than 5 sets, it did not start swinging. The behavior was produced by a mild squeeze on the tail. A hand counter was used to keep track of the swings. To calculate the percentages of left and right swings, the total number of swings on each side was divided by the total number of swings on both sides (Capaldiet al., 1972; Borlonganet al., 1995; Roghaniet al., 2002; Islam et al., 2014).

Open-field test (OFT)

A gray polystyrene box (45 x 45 x 60 cm) was split into two zones: the perimeter and the unpleasant core area of the device. Each mouse was put in the center of the box for the duration of the test, which lasted 5 minutes. The following were the computed standard measures: a) total distance traveled in centimeters; b) as a proportion of total time, time spent in the center zone. The experimental region of the equipment was cleaned with a 0.15% acetic acid solution after each test. Albino mice of either sex (20-25 g) were divided into four groups (Groups I-IV), each with four mice. Prior to the test, they were fasted for the night, although water was provided. Only Group I was kept as a typical control vehicle. Groups III and IV were given various amounts of test extracts, p.o., while Group II was given diazepam (1 mg/kg, i.p.). Each mouse was put in the center of the open field arena 30 minutes after receiving the vehicle, standard, and test extract, and the following parameters were collected during a 10-minute test session (Archer, 1973).

Light–dark test (LDT)

The light-dark test, an insensitive paradigm often employed to measure activity in anxiety disorders, was used. This device comprises of a 40 x 60 x 20 cm acrylic box separated into dark and light chambers. The white colored light chamber (40 x 40 cm) was linked to the dark chamber (40 x 20 cm) by a 7 cm aperture at floor level.

A 60-W white light lamp was suspended in the light chamber above 40 cm high. Animals (n = 12) of 20-25 gm weight of either sex were placed into four groups (Groups I-IV), each with three animals. They fasted for the night before the test, but were given water. The therapy was administered once a day. Each mouse was placed in the light chamber facing the opening into the dark chamber 30 minutes after administration of the vehicle, standard drug, and test extract to different groups, and the following observations were manually recorded during a 10-minute trial: duration of immobility, number of squares crossed, and time spent in the light compartment (Lister, 1990).

Statistical analysis

The data were examined statistically using one-way analysis of variance (ANOVA) across time, followed by Dunnett's post hoc multiple comparison test, using GraphPadPrism software (version: 6.0), with $p < 0.05$ at a 95% confidence interval.

3. Results

Phytochemical screening test

According to preliminary phytochemical research, the plant's methanol extract contains amino acids, fixed oils, flavonoids, glycosides, oleoresins, terpenoid, tannins, steroids, and other compounds (**Table 1.**).

Acute toxicity (A. cepa) test

The largest root growth (RG) inhibition in A. cepa was 4.0 mg/ml after 24 hours, whereas the lowest was 0.5 mg/ml. A reduction in RG was seen in 0.5-1.0 mg/ml extract (CAM, HAM) at 24 h, while in 2.0-3.0 mg/ml extract (MAM) at 24 h. The half-minimal inhibitory concentrations (IC_{50}) calculated for the fractions of MAM, CAM, and HAM are 1.57 ± 0.07, 0.61 ± 0.07, and 1.25 ± 0.06, respectively, and the fraction of MAM, CAM, and HAM has shown an LD_{50} value of 5.13, 3.41, and 4.65 mg/ml, respectively (**Table 2.**).

Table 1. Phytochemical characterizationof the crude methanol extract of *A. montanum*

Phytochemical groups	Test results
Flavonoids	+
Glycosides	+
Tannins	+
Steroids	+
Saponins	+
Oleoresins.	+
Amino acid	+
Fixed Oils	+
Sterols	+

Table 2. Toxic effects of crude extracts and control on *Allium cepa*

Treatments		Root length in cm			% inhibition of root growth			IC_{50} [CI; R^2]
		MAM	CAM	HAM	MAM	CAM	HAM	
VEH		14.54 ± 0.96	19.34 ± 0.10	26.50 ± 0.13	-	-	-	-
Conc. (mg/ml)	0.5	3.21 ± 0.43	1.96 ± 0.22	3.21 ± 0.41	77.92	89.86	87.88	MAM: 1.57 ± 0.07 mg/ml [1.01- 2.43 mg/ml, 0.89] CAM: 0.61 ± 0.07 mg/ml [0.41-0.92 mg/ml; 0.93] HAM: 1.25 ± 0.06 mg/ml [0.86-1.82 mg/ml; 0.92]
	1.0	1.85 ± 0.26	1.44 ± 0.15	1.61 ± 0.27	87.27	92.55	93.92	
	2.0	1.64 ± 0.14	0.61 ± 0.55	1.51 ± 0.15	88.72	96.84	94.30	
	3.0	1.45 ± 0.26	0.41 ± 0.64	1.56 ± 0.27	90.02	97.88	94.11	
	4.0	0.82 ± 0.14	0.41 ± 0.42	0.86 ± 0.11	94.36	97.88	96.75	

Value are Mean ± SD (n = 5); VEH: Vehicle (distilled water); MAM: Methanol extract of *A. montanum* ; CAM: Chloroform extract of *A. montanum*; HAM: n- Hexane extract of *A. montanum*; IC_{50}: Concentration required to inhibit 50% of the root growth; CI: Confidence of interval; R^2: Coefficient of determination

Table 3. DPPH radical scavenging capacity of *A. montanum*

Parameters		% radical scavenge			
		MAM	CAM	HAM	AA
Conc. (µg/ml)	25	12.36 ± 0.01	12.25 ± 0.01	4.52 ± 0.01	40.58 ± 0.01
	50	21.74 ± 0.01	23.95 ± 0.01	13.35 ± 0.01	92.56 ± 0.01
	100	43.55 ± 0.01	36.97 ± 0.01	25.55 ± 0.01	96.10 ± 0.01
	150	60.63 ± 0.01	61.47 ± 0.01	48.45 ± 0.01	96.69 ± 0.01
	200	87.19 ± 0.01	80.13 ± 0.01	70.52 ± 0.01	97.48 ± 0.01
IC_{50} (µg/ml***)***		103 ± 0.05	108.7 ± 0.05	143.1 ± 0.03	27.44 ± 0.01
CI (µg/ml***)***		75.06-141.4	77.58-151.4	115.4-177.6	25.50-29.52
R^2		0.94	0.92	0.95	0.99

Values are Mean ± SEM (n = 3), MAM: Methanol extract of *A. montanum* ; CAM: Chloroform extract of *A. montanum*; HAM: n- Hexane extract of *A. montanum* ; IC_{50}: Half-minimal inhibitory concentration; CI: Confidence of interval; R^2: Coefficient of determination

Antioxidant assay

As mg eq. of quercetin and mg eq. of gallic acid, the plant's total tannin, total flavonoid, total phenolic, and content were estimated. The plant contains 80.3 mg GAE/100 g, 58.66 mg QE/100 mg, and 39.60 mg GAE/100 g of total phenol, total flavonoids, and total tannin, respectively.

The methanol leaf extracts of *A. montanum* (MAM) showed concentration-dependent DPPH radical scavenging capacities. At 200 g/ml, MAM had the highest capability in DPPH scavenging assay. The IC_{50} calculated for MAM, CAM and HAM were 103 ± 0.05, 108.7 ± 0.05, and 143.1 ± 0.03 µg/ml, respectively.

The standard drug, ascorbic acid (AA), also showed a concentration-dependent DPPH radical scavenging capacity. The IC_{50} value of AA was 27.44 ± 0.01 µg/ml (**Table 3.**).

Anti-inflammatory activity

The crude methanol extract of the plant demonstrated an anti-inflammatory activity in an egg albumin (*in vitro*) test in a concentration-dependent manner. At 0.36% (v/v), the extract showed the greatest prevention of albumin denaturation. The test sample had a greater anti-inflammatory impact than the usual ASA at 200 µg/ml. As indicated in **Table 4**, the EC_{50} values for the three fractions of MAM, CAM, and HAM were 89.10 ± 0.07, 92.85 ± 0.0 7 and 117.7 ± 0.0 7 µg/ml, respectively, whereas the normal EC_{50} of ASA was 93.86 ± 0.08 µg/ml. In contrast to ASA, a multiple evaluation of the standards and concentrations for each test reveals that the fractions at 92.85 ± 0.07 and 89.10 ± 0.07 µg/ml had significant impacts (**Table 4.**).

Anti-diarrheal (castor oil-induced diarrheal) test

In this experiment, the extract at a dose of 500 mg/kg per body weight provided a significant effect compared to the standard drug (loperamide), while a 250 mg/kg dose of body weight provided a moderate effect.

Table 4. Anti-inflammatory activitiy of crude extracts and standard drug in egg albumin

Parameters		**The percent inhibition of protein denaturation (%IPD)**			
		MAM	**CAM**	**HAM**	**ASA**
Conc. (µg/ml)	25	22.5 ± 0.03	21.5 ± 0.02	16.6 ± 0.01	22.5 ± 0.01
	50	28.5 ± 0.03	27.5 ± 0.02	21.3 ± 0.01	27.6 ± 0.02
	100	43.5 ± 0.03	42.5 ± 0.02	35.8 ± 0.01	39.7 ± 0.03
	150	66.7 ± 0.03	65.9 ± 0.02	55.7 ± 0.01	62.9 ± 0.01
	200	81.5 ± 0.03	77.6 ± 0.02	67.6 ± 0.01	87.5 ± 0.02
EC_{50} (*µg/ml*)		89.10 ± 0.07	92.85 ± 0.07	117.7 ± 0.07	93.86 ± 0.08
CI (*µg/ml*)		57.25 - 138.7	60.05 - 143.6	77.48 - 178.9	57.45 - 153.4
R^2		0.89	0.89	0.87	0.86

Values are mean ± SEM; MAM: Methanol extract of A.montanum; CAM: Chlorofrom extract of *A. montanum*; HAM: n-Hexane extract of *A. montanum* ; ASA: Acetyl salicylic acid; EC_{50}: Half-maximal inhibitory concentration; CI: Confidence of interval; R^2: Coefficient of determination

Table 5. Diarrheal secretions of mice in different treatment groups at 1st, 2nd, 3rd and 4th hr

Treatment groups	Dose (Route of admin.)	Latency (min)	1st hr	2nd hr	3rd hr	4th hr
NC	10 ml/kg (p.o)	25 ± 0.01	7.58 ± 0.02	4.33 ± 0.02	4.78 ± 0.02	2.09 ± 0.02
LOP	3 mg/kg (p.o.)	75 ± 0.01	3.29 ± 0.02	3.17 ± 0.02	0.00 ± 0.02	0.00 ± 0.00
MAM	250 mg/kg (p.o.)	55 ± 0.01	5.18 ± 0.02	4.23 ± 0.02	3.08 ± 0.02	2.58 ± 0.02
	500 mg/kg (p.o.)	65 ± 0.01	5.09 ± 0.02	3.18 ± 0.02	1.00 ± 0.00	0.00 ± 0.00
Values are mean ± SEM (n = 3); MAM: Methanol leaf extract of *A. montanum*; LOP: Loperamide.						

The crude extract reduced the stool count in both test groups. Four hours after the administration of castor oil, there was no more excretion of diarrheic feces in any of the groups of animals (**Table 5.**).

Anti-pyretic (Brewer's yeast induced fever) test

Table 6 shows the antipyretic activities of several dosages of the test drug (250 and 500 mg/kg), standard (Paracetamol, 100 mg/kg), control, and negative control (NC) in the brewer's yeast produced fever test. When compared to the control, the MAM at concentrations of 500 and 250 mg/kg of body weight had a mild impact. At the 4th hour, the mouse model in group-IV (500 mg/kg) demonstrated a decrease in body temperature.

Analgesic activity (Acetic acid-induced writhing) test

Table 7 shows the impact of the methanolic extract of *A. montanum* leaf on mice in the acetic acid-induced writhing test.

Table 6. Antipyretic effect of *A. montanum* in Brewer's yeast-induced pyrexia in mice

Treatment and dose	Rectal temperature (°F)					
	Basal temp.	0 hr(after 18 hr)	1 hr	2 hr	3 hr	4 hr
Control (10 ml/kg, normal saline)	33.33 ± 0.002	35.12 ± 0.02	35.11 ± 0.01	35.13 ± 0.01	35.15 ± 0.02	35.12 ± 0.01
PARA (100 mg/kg)	33.66 ± 0.01	35.55 ± 0.02	34.65 ± 0.01	33.75 ± 0.01	33.66 ± 0.02	33.66 ± 0.01
MAM (250 mg/kg)	34.89 ± 0.01	35.85 ± 0.02	35.71 ± 0.01	35.64 ± 0.01	35.61 ± 0.02	35.48± 0.01
MAM (500 mg/kg)	34.52 ± 0.01	35.19 ± 0.02	35.13 ± 0.01	35.03 ± 0.01	34.96 ± 0.02	34.27 ± 0.01
Values are mean ± SEM (n = 5); MAM: Methnolic extract of *A. montanum*; PC: Paracetamol (PARA)						

Table 7. Mean writhing and percentage protection in the treatment groups

Treatment groups	Dose (Route of admin.)	Mean of writhing	% writhing	%protection
NC	10 ml/kg (p.o)	36	100	0
Diclofenac-Na	25 mg/kg (p.o.)	6.00	16.66	83.34
MAM	500 mg/kg (p.o.)	6.67	18.22	81.88
	250 mg/kg (p.o.)	13.33	37.02	62.98
Values are mean ± SEM (n = 3); MAM: Methanolic extract of *A.montanum.* PC: Diclofenac-sodium				

Table 8. Anxiolytic-*like* effects of crude extracts and controls in *Swiss* mice

Treatment and dose	OFT			HCT	SWT	LDT
	Number of Field Cross	Number of Grooming	Number of Rearing	Number of Hole Cross	Number of swings	Residence in dark (Sec)
Control (10 ml/kg, p.o.)	145 ± 0.01	46 ± 0.02	48 ± 0.01	25 ± 0.02	17 ± 0.01	125 ± 0.02
DZP (2 mg/kg, i.p.)	111 ± 0.01	19 ± 0.02	15 ± 0.01	12 ± 0.02	10 ± 0.01	155 ± 0.02
MAM (250 mg/kg, p.o.)	109 ± 0.01	33 ± 0.02	23 ± 0.01	15 ± 0.02	10 ± 0.01	132 ± 0.02
MAM (500 mg/kg, p.o.)	98 ± 0.01	18±0.02	13 ± 0.01	11 ±0.02	06 ± 0.01	145 ± 0.02
Values are mean ± SEM (n = 3); DZP: Diazepam; LDT: Light-dark test; HCT: Hole cross test; MAM: Methanol extract of *A. montanum*; OFT: Open field test; SWT: Swing test.						

The crude extract generated the writhing inhibition (81.88%) in test animals at a dosage of 500 mg/kg of body weight, whereas the crude extract at a dose of 250 mg/kg of body weight produced 62.98% inhibition of writhing. When compared to the negative control, the findings were statistically significant ($p < 0.005$).

Anxiolytic-like activity test

SWT

The number of swings in the test groups (Gourp-III and IV) (250 and 500 mg/kg, p.o.) mouse model was lower than in the control group in the swing test. The outcome was statistically significant ($p < 0.05$) and equivalent to the reference medication diazepam (2 mg/kg).

OFT

The number of squares occupied of each mouse in the open field test was dramatically reduced in the test groups (Gourp-III and IV) throughout the research period. The extract had stronger CNS depressant action than the conventional medication, and the difference was statistically substantial.

HCT

MAM (250 and 500 mg/kg, p.o.) reduces locomotion in selected mice by lowering the number of holes crossed comparing to the control group in the hole cross test (**Table 8.**). The outcome was statistically significant ($p < 0.05$) and equivalent to the reference medication diazepam (2 mg/kg).

LDT

MAM significantly increased the dark present rate in the test groups (groups III and IV) comparing control group.

4. Discussion

The project work presented here was done to analysis some pharmacological and phytochemical features of the mangrove plant, *A. montanum*. Several portions of this plant are traditionally applied in the treatment of measles, chickenpox, malaria, headaches, stomach aches, ulcers, lumbar pains, eye diseases, relieving chest pain, headaches, and thrush in children, for diuretic and kidney stone removal, and for anti-dermatitis and skin disease curing effects.

This work was based on phytochemical and pharmacological assessment to investigate the metabolites and bioactivity of the plants.

The plants were subjected to drying under the shade, followed by pulverization. After proper grinding and cold extraction with methanol (99-100%), the extraction afforded an 8.00% yield. To obtain a sense of the active

ingredients in the extract, phytochemical assessments revealed the attendance of chemical constituents which include amino acids, fixed oils, flavonoids, glycosides, oleoresin, terpenoids, tannins, steroids, saponins, and sterols.

The total phenolic, total flavonoid, and total tannin content of the plant were calculated as mg eq. of gallic acid and mg eq. of quercetin, and the plant contains 80.3 mg GAE/100g, 58.66 mg QE/100 mg, and 39.60 mg GAE/100 g of total phenol, total flavonoids, and total tannin, respectively. The leaf extract of *A. montanum* showed moderate DPPH radical scavenging in comparison with ascorbic acid. Here, the IC_{50} of three fractions, MAM, CAM, and HAM, was 103 ± 0.05, 108.7 ± 0.05, and 143.1 ± 0.03 µg/ml, respectively. But in overall observation, it has been decided that segments of plant extract MAM and CAM showed moderate antioxidant activity while the HAM showed mild activity.

Furthermore, flavonoids (Pietta, 2000), glycosides (Lee et al., 2005; Katsube et al., 2006), tannins (Zhang and Lin, 2008), terpenoid (Grassmann, 2005), and saponin (Guelcin et al., 2004) exhibit antioxidant activity by inducing enzymatic antioxidant activity and itself as a non-enzymatic antioxidant and scavenging free radicals. From phytochemical screening of *A. montanum*, this plant contains terpenoids, glycosides, flavonoids, tannins, saponins, so the antioxidant activity was inhibited because of these phytochemicals.

In this study, *A. montanum* was found to exhibit a significant role in anti-inflammatory activity. In the egg albumin (*in vitro*) test, the crude extract of the plant exhibited the highest inhibition of albumin denaturation at 200 µg/ml with fractions of MAM and CAM compared to the same standard (ASA) concentration. The extract at 200 µg/ml showed significant anti-inflammatory capacity because of the chemical constituents, which included flavonoids, glycosides, terpenoids, tannins, saponins, and steroids (Takagi et al., 1980; Dıazet al., 2004; Loke et al., 2008; Liang et al., 2010).

In the castor oil-induced antidiarrheal test, *A. montanum* exhibited substantial ($p < 0.005$) effects. The findings of this study demonstrated that the MAM showed significant anti-diarrheal activity. The extract's phytochemical investigation confirmed the presence of a variety of bioactive compounds. Phytosterols and flavonoids are among the secondary metabolites discovered to alter the synthesis of lipooxygenase (LOX), cyclooxygenase 1 and 2 (COX-1, COX-2) and hence decreasing the production of prostaglandin (Awad et al., 2005). The extract at 500 mg/kg dose provides a significant consequence on the reference drug loperamide, while a 250 mg/kg dose provides a moderate effect. The inclusion of phytochemicals such as phytosterols, flavonoids, saponins, and tannins, which act individually or synergistically, may be responsible for the extract's antidiarrheal properties.

A. montanum includes flavonoids, according to phytochemical study. Flavonoids are polyphenols that may be found in various amounts in almost all plants. Flavonoids have also been discovered to block the transcription and formation of prostaglandin synthase (COX-2) (O'Leary et al., 2004; Hamalainen et al., 2011). As a consequence, the existence of flavonoids might explain *A. montanum*'s antipyretic activity. With increasing concentrations of *A. montanum* extract, the efficacy of the antipyretic action was seen to rise. This can be attributed to a higher concentration of the extract component that has antipyretic properties. The results of this investigation show that MAM have mild antipyretic effects at dosages of 250 and 500 mg/kg of body weight, and that suppression of inflammatory mediator production and/or

release may be the principal mechanism of action. The antipyretic properties of MAM justify its use by traditional medicine practitioners in the treatment of fever.

The approach of testing peripherally active analgesics using an acetic acid-induced writhing model is helpful due to the fact that it focuses on assessing the medication-induced reduced number of pain-inducing paramers in the test animals. Acetic acid, used in this model, works by inducing prostaglandin production in animals, which can be inhibited by the test sample through peripheral mechanisms (Ferdous et al., 2008).

The presence of analgesic principles operating through the prostaglandin pathways might explain MAM's considerable pain reduction. The presence of bioactive chemicals is thought to be responsible for the reported analgesic effect. Flavonoids are thought to play a function in analgesic action through interacting with prostaglandins (Rao et al., 2008; Vanu et al., 2006).

Extracts at doses of 250 and 500 mg/kg inhibited the writhing reflex by 62.98 and 81.88%, respectively, in an analgesic activity test using the acetic acid induced writhing method, whereas the standard drug diclofenac-Na inhibited the writhing reflex by 83.34% at a dose of 25 mg/kg body weight.

The current investigation found that an oral administration of a MAM to mice generated a strong anxiolytic effect in four well-established anxiety experimental systems (e.g., HCT, OFT, LDT, and SWT). Anxiety-related behaviors in mice were dramatically reduced in various animal models, demonstrating that extract administration reduced anxiety in mice. Flavonoids, phenolic compounds, tannins, saponins, steroids, glycosides, and alkaloids were discovered during the phytochemical screening of EEAPR. It's probable that these compounds are involved in the title plant's anxiolytic activity.

Conclusions

The leaf extract of *A. montanum* demonstrated the existence of flavonoids, terpenoids, steroids, saponins, tannins, and oleoresin. The extract exerted a concentration-dependent toxic effect on *A. cepa*. The extract inhibited egg albumin denaturation depending on concentration. The extract exhibited significant antioxidant capacity. The MAM showed anti-diarrheal, anti-inflammatory, and analgesic effects. In a dose-dependent way, the MAM also showed antipyretic and anxiolytic properties in *Swiss* mice. It's worth noting that *A. montanum* might be a source of phytotherapeutic lead compounds. Further studies are necessary to isolate and investigate the responsible bioactive substances of this hopeful medicinal herb.

Conflict of interest

None declared.

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Authors` contributions

F. Zaman- laboratory studies, data collection, preliminary drafting; R. Hossain- laboratory studies, data collection; S. Molla, A.K. Das and M.H. Bappi- laboratory studies, data collection; M.T. Islam- data manipulation, data analysis, final drafting; C. Sarkar and O. Atolani - final drafting; R. Rouf- work design, data manipulation, data analysis, final drafting.

Consent for publication

The authors declare their consent for publication.

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