Bangladesh Pharmaceutical Journal ***29****(1): 16-26, 2026 (January)*

Assessment of Antioxidant, Antidiabetic, Antidiarrheal, Analgesic and Antipyretic Activities and Phytochemical Screening on Different Fractional Methanolic Extract of *Ampelocissus barbata* (Wall.) Planch Fruits

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(Received: June 24, 2025; Accepted: September 07, 2025; Published (web): January 28, 2026)

Abstract

Ampelocissus barbata is a natural medicinal herb, regionally known as 'Jarila Lahari'. This research sought to examine the fruits of *A. barbata* for their phytochemical screening, analgesic, antipyretic, antidiarrheal, antidiabetic, and antioxidant properties. To determine the activities, the methanolic crude extract (CE), as well as ethyl acetate (EAF), carbon tetrachloride (CTF), chloroform (CF) and petroleum ether (PEF) soluble fractions of *A. barbata* fruits were evaluated using recognized chemical techniques. Alkaloids, tannins, flavonoids, terpenoids, glycosides, saponins, proteins, and xanthoproteins were identified in the extract. In the DPPH assay, the crude extract and the pet. ether fraction showed very strong IC_{50} values of about 14.832 µg/ml and 23.87 µg/ml, accordingly. The raw sample and pet. ether fraction showed significant values of approximately IC_{50} (36.83 µg/ml and 24.46 µg/ml), SC_{50} (42.29 µg/ml and 29.2 µg/ml) and SC_{50} (38.13 mg/l and 39.17 mg/l), significantly, in the NO• scavenging activity, hydrogen peroxide scavenging assay, and hydroxyl radical assay. In the antidiabetic test, both BGL (500 mg/kg) and BGL (250 mg/kg) demonstrated a notable decline in blood sugar levels at 60 and 90 minutes. Strong antidiarrheal action was shown by the extract, which decreased diarrheal episodes by 82.96% & 87.4% and decreased the writhing response in the analgesic test by 78% and 73% at 250 mg/kg and 500 mg/kg accordingly. It exhibited a statistically prominent ($p < 0.05$, 0.01, and 0.001) temperature decrease at same dosages. Fruits of *A. barbata* demonstrated significant pharmacological and antioxidant potential in both crude and fractionated extracts.

Key words: Antioxidant, antidiabetic, antidiarrheal, analgesic, antipyretic, phytochemical, DPPH, spectrophotometric.

Introduction

From prehistoric periods, People have used plants with medicinal properties for their curative purposes. Phytochemicals found in higher plants provide around 25% of the medications that are given. Half of the approximately 250,000 higher plants with promising phytochemicals are found in tropical forests; of these, 60% have biological activity developed, and 15% have plant components extracted and documented (Hamburger *et al*., 1991). Potent bioactive may be found in medicinal plants, primarily those with a widely recognized traditional use in folk remedies.

Ampelocissus barbata (Family: Vitaceae) is a climbing shrub and medicinal herb, locally known as Jarila-Lahari, Khoissang (Chakma) that grows in hilly forests. Local practitioners have long utilized the genus Ampelocissus to cure a range of ailments. *A. barbata* is utilized traditionally to relieve swelling, pain, jaundice, and boils (Morshed *et al*., 2013). In previous studies, the crude extracts and isolated metabolites have shown some phytoconstituents

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DOI: https://doi.org/10.3329/bpj.v29i1.87368

pharmacological effects, such as antioxidant, thrombolytic (Manik *et al*., 2022), and analgesic (Khandokar *et al*., 2021) properties. However, no study has been done before on fruits in this particular case of *A. barbata* in Bangladesh. Since the plant is used to treat a variety of clinical problems, its fruits were gathered, put through a phytochemical screening process, and the study assessed the plant's analgesic, antipyretic, antidiarrheal, antidiabetic, and antioxidant properties.

Materials and Methods

Sample preparation from the fruits: Fresh *A. barbata* fruits were collected from the Moulvibazar region of Bangladesh's Sylhet district in September 2023. The fruit's identification was guaranteed by specialists from the Bangladesh National Herbarium, which is situated in Mirpur, Dhaka. The same organization gave a voucher specimen number (94771) for correspondence and future use. After being cleaned, the gathered fruits weighed roughly 800 g. They spent 15 days in the sun to dry. 400 g of powder was discovered after the fruits were dried and processed into a fine powder through a crusher. The crushed substance was steeped in 1000 milliliters of methanol in a glass container. After being sealed, the container and its contents were kept for 16 days, shaking and stirring occasionally. After coarsely filtering the entire mixture with a small amount of fresh, white cotton, 100 g of the crude sample from *A. barbata* was separated. Ethyl acetate, carbon tetrachloride, pet ether and chloroform were used to partition roughly 5 g of the crude extract. Then, soluble fractions of pet ether, carbon tetrachloride, ethyl acetate, and chloroform were discovered.

Qualitative phytochemical screening: The crude sample and two soluble fractions, petroleum ether and ethyl acetate, were utilized in the qualitative analysis of phytochemicals using the standard protocols for alkaloids, tannins, flavonoids, terpenoids, glycosides, saponins, proteins, and xanthoproteins, including the Dragendrof, Molish, Benedict, Fehling, Alkaline, xanthoproteic, ferric chloride, potassium dichromate, sulfuric acid, Libermann-Burchard, and Froth tests (Shaikh *et al*., 2020).

In vitro studies

Antioxidant activity evaluation

DPPH radical scavenging assay: The free radical scavenging activity of the raw extract and its various fractions (carbon tetrachloride, chloroform, and pet ether) from *A. barbata* fruits was assessed by utilizing 1-Diphenyl-2-picryl hydrazyl (DPPH) (Endris *et al*., 2023). The raw sample and fractionated portions were mixed individually to 3 mL of a 0.004% w/v solution of DPPH at varying doses (1-100 µg/mL). The absorbances were assessed at 517 nm. The formula was

% inhibition = $[(A_O-A_S)/A_O]$ X 100.

A_O denotes the absorbance of control, and A_S denotes the absorbance of the sample extract. The linear regression analysis was employed to evaluate the IC_{50} values.

Nitric oxide (NO) scavenging assay: The crude extract and pet ether fraction's nitric oxide scavenging capabilities were assessed using the spectrophotometric method (Govindarajan *et al*., 2003). After mixing different amounts (5–100 µg/ml) of the raw extracts and fractions with sodium nitroprusside (5 mmol) in phosphate-buffered saline, the mixture was incubated for 30 minutes at 25°C. After diluting 1.5 milliliters of solution with 1.5 milliliters of Griess reagent, the absorbance at 546 nm was then measured using a UV/Vis spectrophotometer. The scavenging activity was computed as the inhibition percentage (I%) using the following formula:

$$I\ (\%) = [(A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}}] \times 100$$

A_{Blank} represents the absorbance of the control reaction (apart from the test component), whereas A_{Sample} represents the absorbance of the experimental sample with all reagents. The IC_{50} value, or the concentration required to scavenge 50% of nitric oxide free radicals, was evaluated using ascorbic acid as a reference.

Hydrogen peroxide scavenging assay: The assay used 0.136 gm/100 ml hydrogen peroxide solution in 40 mM phosphate buffer (pH 7.4). In phosphate buffer, hydrogen peroxide was combined with varying concentrations (6.25–800 mg/l) of the fruit

extract and pet ether fraction, respectively. After measuring the absorbance at 230 nm, it was contrasted to a blank solution which had phosphate buffer without hydrogen peroxide (Bulama *et al*., 2021). L-ascorbic acid was utilized as a reference to compare with samples. This formula was used to get the hydrogen peroxide scavenging percentage: (Figure 1)

$$I\ (\%) = [(A_{Blank} - A_{Sample}) / A_{Blank}] \times 100$$

The absorbance of the control reaction (without the test component) is represented by A_{Blank}, while the absorbance of the experimental sample with all reagents is represented by A_{Sample}.

Hydroxyl radical scavenging assay: With a small modification, the Halliwell (Halliwell *et al*., 1987), technique was used to assess the compounds' hydroxyl radical scavenging activity. 12.5 µL of sample extracts (crude and pet ether fraction) and standard (ascorbic acid) at varying concentrations (6.25 – 800 mg/l) were combined with 0.5 ml of a 2-deoxy 2-ribose solution (2.8 mM). The reaction mixture was prepared by adding 1 ml of 200 µM $FeCl_3$, 1 ml of 1.04 mM EDTA, 0.5 mL of 1 mM H_2O_2, and 0.5 ml of 1 mM ascorbic acid. 3.75 ml of 2.8% TCA and 3.75 ml of 1% TBA were added after an hour of incubation at 37°C, and the mixture was then maintained at 100°C for 20 minutes. At 530 nm, the absorbance was measured. With the exception of the standard and extracts, a blank was made with all the reagents. The scavenging percentage was computed as follows:

$$\%\ \text{Scavenged}\ (H_2O_2) = [(Ao\text{-}A_1)/Ao] \times 100.$$

Where, Ao represents the control's absorbance and A_1 represents the sample's absorbance.

Total tannin content determination: Total tannin content of the crude extract and its fractions (pet ether, ethyl acetate) was determined using the Folin–Ciocalteu method with gallic acid as standard (Ribarova *et al*., 2005). Samples were mixed with distilled water, diluted FC reagent, and 35% Na_2CO_3, then incubated for 30 minutes at room temperature. A UV spectrophotometer was used to detect absorbance at 725 nm, and the results were reported as milligrams of gallic acid equivalent (GAE) per 100 grams of dried extract.

Determination of flavonoid content: Total flavonoid content of the crude extract and its fractions (pet ether, ethyl acetate) was determined using quercetin as a standard (100–20 µg/l) (Meda *et al*., 2005). Absorbance of the sample measured at 510 nm after they had been diluted to 10 ml and combined with distilled water, 10% aluminum chloride, 5% sodium nitrite, and 1 M NaOH. Quercetin equivalent (QE) in milligrams per 100 grams of dry extract was used to express the results.

Determination of total phenolic content: Total phenolic content of the fruit extract and its fractions (pet ether, ethyl acetate, chloroform) was determined by the Folin–Ciocalteu method using gallic acid as standard (Ribarova *et al*., 2005). Samples (15.62–500 mg/L) were mixed with diluted FC reagent and 7% Na_2CO_3, incubated for 30 minutes, and absorbance was measured at 750 nm. Results were expressed as mg gallic acid equivalent (GAE) per 100 g dried extract (Javanmardi *et al*., 2003).

In vivo assays

Experimental animal: For the current investigations, young Swiss-albino mice that were 4-5 weeks old and weighed an acceptable 20–40 g on average were employed. Jahangirnagar University, Savar, Dhaka-1342, Bangladesh, is where the mice were purchased. They were maintained at room temperature under typical environmental circumstances. Before the experiment began, the animal was sent to the ethical review committee for approval (Ref No: CPP/DIU/EC/18).

Antidiabetic activity evaluation of the crude extracts

Oral glucose tolerance method: In the study, mice were placed into four groups (n=5), fasted for 12 hours, and given 250 and 500 mg/kg crude extracts orally, accordingly (Barik *et al*., 2008). The groups were group I (250 mg/kg), test group II (500

mg/kg), test group III (250 mg/kg), and group I (negative control). Glibenclamide (5 mg/kg) was employed as the positive control and Tween-80 (10 ml/kg) as the negative control. Glucose (2 mg/kg body weight) was given 30 minutes after the raw extract was administered, and blood glucose levels were analyzed at 0, 30, 60, and 90 minutes. Blood glucose levels were evaluated by measuring them in mmol/L using a glucometer and strips (Joy *et al*., 1999).

Anti-diarrheal activity evaluation of the crude extracts

Castor oil-induced diarrheal method: The crude extracts' antidiarrheal properties were evaluated using the reduction in stool frequency measurement (Tadesse *et al*., 2014). The selected mice were fasted (14 hours) but had open access to water and were split among four groups (n=5). The experimental design consisted of four groups: Group-I carried out the negative control with tween 80 at a concentration of 1%, Group-II acted as the positive control receiving loperamide at a dosage of 3 mg/kg, Group-III was the test group-1 (250 mg/kg), and Group-IV was the test group-2 with a dosage of 500 mg/kg. After 1 hour of dosing, each group received an oral administration of 0.5 ml castor oil. Particular mice of every group were housed in different cages with blotting paper for 4 hours and changed at the beginning of each hour (to count easily) after castor oil administration (Tadesse *et al*., 2014). The quantity of feces or liquid that discolored the paper was tallied, and the latent period was also noted. The following calculation was used for % inhibition of diarrhea:

% Inhibition = {Mean no. of wet defecation (control – test) / Mean wet defecation of control} × 100

Analgesic activity evaluation of crude extracts

Acetic acid-induced writhing reflex method: In this test, an intraperitoneal injection of acetic acid was used to induce pain (Khandokar *et al*., 2021). Four groups of mice (n=5) were employed in this investigation. Diclofenac sodium (25 mg/kg) served as the positive control in Group II, whereas Tween 80 (10 ml/kg) served as the negative control in Group I. The oral dosages of the plant extracts were 250 and 500 mg/kg. For optimal absorption, the animals were administered a 0.7% acetic acid solution intraperitoneally, and after 30 minutes, they were allowed to writhe for 15 minutes. Sometimes, the animal didn't give full writhing; each treatment group's writhing count was analyzed to the control and standard groups, with incomplete writhing being counted as half-writhing (Ripa *et al*., 2014).

Antipyretic activity of crude extract evaluation

Yeast-induced pyrexia method: The five animals were split up into four groups. After recording each mouse's normal body temperature, a 15% aqueous suspension of Brewer's yeast (10 ml/kg body weight) was subcutaneously injected into each mouse to cause pyrexia. Following a 24-hour fast with unrestricted access to water, each mouse's rectal temperature was measured. Saline (10 ml/kg) was given to Group I as a negative control, 150 mg/kg of paracetamol was given to Group II as a conventional medication, and 250 and 500 mg/kg of extract were given orally to Groups III and IV. Rectal temperature was once more taken at intervals of 1,2,3 and 4 hours following medication deliver (Patra *et al.*, 2009).

Statistical analysis: In order to generate the curve in the case of *in vitro* testing and to support the statistical significance of the data gathered from *in vivo* assays, statistical and mathematical calculations were carried out. In this context, Microsoft Excel (version 2010) was used. Statistical significance was established for *in vivo* test data when the p value was less than 0.05.

Results and Discussions

Phytochemical screening: A variety of bio-actives were found in *A. barbata* fruits by phytochemical screening. The crude extract along with different fractions (ethyl acetate, pet ether) showed eight phytoconstituents, including tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids, proteins, and xanthoproteins (Table 1).

The identified phytochemicals were more or less due to the polarity of the solvents.

Quantitative antioxidant activities

DPPH radical scavenging assay: The capacity of the crude filtrate and its different segmented portions (carbon tetrachloride, chloroform, pet ether) to lower DPPH has been used to assess their antioxidant activity. The DPPH radical reduction causes the hue to shift from purple to pale yellow, indicating scavenging action (Akar *et al*., 2017). At 517 nm, concentrations of the test material (crude extract, various fractions) and the standard (ascorbic acid) ranged from 1 to 100 µg/ml (Table 2). The crude sample and the fractions of pet ether, carbon tetrachloride, and chloroform have respective IC_50 values of 14.832 µg/ml, 326.73 µg/ml, 191.2 µg/ml, and 23.87 µg/ml. Although with each test sample, ascorbic acid had an IC_{50} value of 1.28 µg/ml, 33.932 µg/ml, and 40.79 µg/ml, accordingly. A lower IC_{50} value demonstrates strong antioxidant capacity.

Table 1. Phytoconstituents of *A. barbata* fruit extracts.

Phytochemical group	Chemical test	CE	EASF	PESF
Tannins	Potassium dichromate test	+	+	+
	Ferric chloride test	+	+	+
Saponins	Froth test	-	+	-
Alkaloids	Dragendrof's test	-	+	-
Reducing sugar	Benedict's test	-	-	-
	Fehling's test	-	-	-
Glycosides	Alkaline test	+	+	+
Flavonoids	Sulfuric acid test	+	+	+
	Alkaline reagent test	+	+	+
Terpenoids	Libermann-burchard test	-	+	-
Carbohydrates	Molish's test	-	-	-
Protein-xanthoproteins	Xanthoproteic test	-	+	+
Phenolics	Ferric chloride test	-	-	+

(+) sign and (-) sign show the presence and absence of the phytoconstituents. CE = Crude extract, EASF = Ethyl acetate soluble fraction, PESF = Pet ether soluble fraction.

Table 2. % Inhibition of the crude extracts and different fraction with different concentrations at 517nm.

Concentration (µg/ml)	CE	Std.	CTF	Std.	CF	Std.	PEF	Std.
1	3.04	14.99	2.61	52.43	10.04	14.98	3.05	14.99
10	4.11	20.85	3.28	55.71	13.32	20.84	3.57	20.85
20	5.69	62.79	3.62	79.17	16.34	62.78	4.00	62.78
50	6.53	68.01	4.05	82.10	27.09	68.01	4.78	68.01
100	10.42	71.29	4.29	83.94	29.36	71.30	7.93	71.29

CE= crude extract, CTF= carbon tetrachloride fraction, CF= chloroform fraction, PEF=pet ether fraction, Std. = standard (ascorbic acid).

Nitric oxide scavenging assay: The reduction in absorbance at 546 nm was used to calculate the sample and standards NO scavenging capacity (Adebayo *et al.*, 2019). Both the crude extract and pet ether fraction showed a strong IC_{50} value compared to the standard (ascorbic acid) (Fig.1). The pet ether

fraction demonstrated a more efficient IC_{50} value (24.46 µg/ml) than the crude extract (36.83 µg/ml). The standard had an IC_{50} value of 77.71 µg/ml. To calculate the IC_{50} value, a linear regression graph was used.

Hydrogen peroxide scavenging assay: The hydrogen peroxide scavenging activity of the pet ether fraction and crude extract of *A. barbata* fruits, as well as ascorbic acid at concentrations from 6.25 to 800 µg/ml, are shown in figure 2. Crude extract, pet ether fraction, and ascorbic acid (standard) had SC_{50} values of approximately 42.29 µg/ml, 29.2 µg/ml, and 96.4 µg/ml, respectively.

Hydroxyl radical scavenging assay: A powerful oxidant, the hydroxyl radical reacts with practically every biomolecule present in living cells (Sannigrahi S *et al.*, 2010). Both of the samples (crude extract and pet ether fraction) inhibited hydroxyl radical-mediated deoxyribose breakdown, which was compared to the standard (ascorbic acid) (Fig. 3). The SC_{50} values of crude extract, pet ether fraction, and standard were about 38.13 mg/L, 39.17 mg/L, and 138.8 mg/l, respectively.

Total polyphenolic contents estimation (Tannin, Flavonoid, and Phenolic): Polyphenolic substances are significant plant components with redox characteristics (Ayele *et al.*, 2022). The total polyphenolic content of the extract and fractions were shown (Table 3). The values were determined from the slope of the calibration curve and the correlation coefficient (R^2).

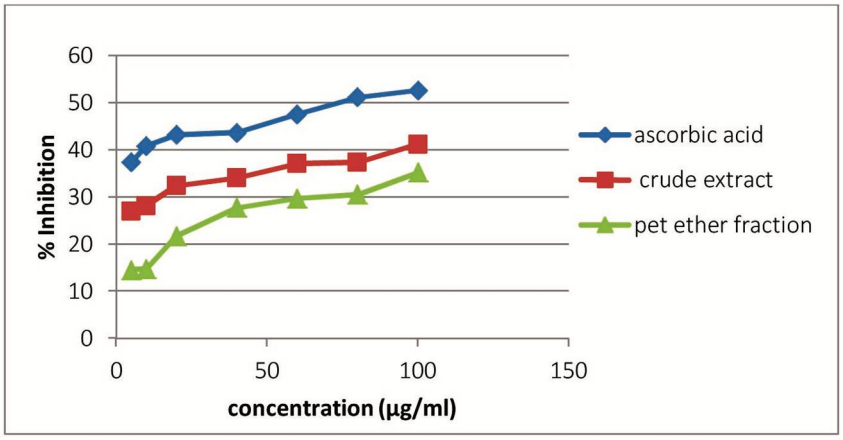

Figure 1. Comparison of % Inhibition Vs. concentration graph for standard and samples of NO scavenging assay.

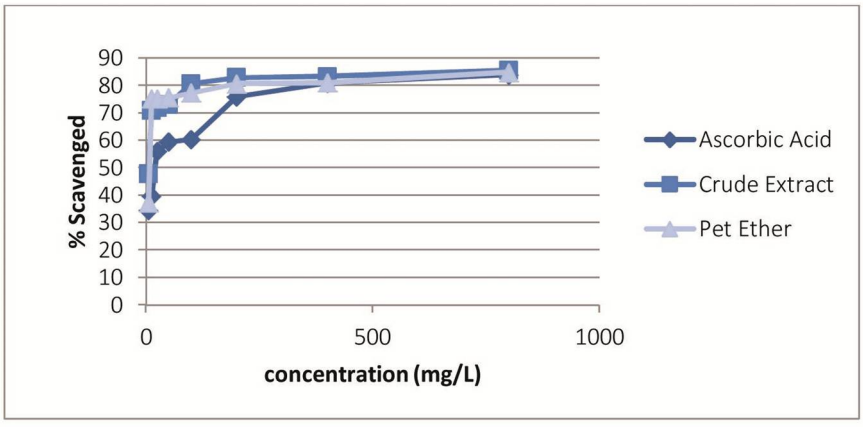

Figure 2. Comparison of % scavenged vs. concentration graph for standard (ascorbic acid), crude and pet ether fraction

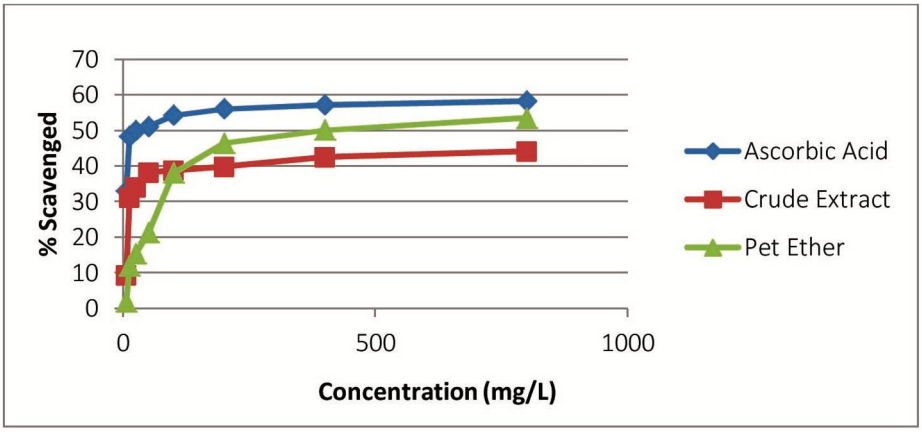

Figure 3. Comparison of % Scavenged vs. concentration graph for standard (ascorbic acid), crude extract and pet ether fraction.

Table 3. Total polyphenolic content using crude extract and different fractions.

Polyphenols mg (GAE)/100g	Standard	Crude methanol extract	Pet ether fraction	Ethyl acetate fraction
Tannin	Gallic acid	16.69 ±2.14	21.356±1.8	141.22±4.5
Flavonoid	Quercetin	8.87±0.35	3.50±0.23	31.73±0.30
Phenolic	Gallic acid	3015±18.75	1817.5±2.1	488±12.30

***In vivo* assays**

Antidiabetic activity: In the oral glucose tolerance test, the effect on blood glucose level (BGL) in mice after oral administration of the crude extract at different doses (250 mg/kg and 500 mg/kg) showed a reduction in BGL at different times (Table 4). Significant activity was observed, especially at 60 & 90 min (Figure 4) and that was comparable to that of the control (Glibenclamide).

Antidiarrheal activity: The crude extract of *A. barbata* fruits, at doses of 250 and 500 mg/kg, significantly ($p<0.0001$) reduced the total amount of stools and delayed the beginning of diarrhea in the castor oil-induced diarrheal mice. % Defecation was inhibited by 87.4% and 82.96% at 250 and 500 mg/kg, respectively. Additionally, the standard drug (loperamide) at a dose of 3 mg/kg reduced the overall quantity of feces and showed an 88.88% inhibition of defecation (Figure 5).

Analgesic activity: In a dose-dependent manner, the *A. barbata* crude extract at 250 mg/kg and 500 mg/kg considerably reduced pain perception within 15 minutes. At 25 mg/kg body weight, the standard medication (acetic acid) showed 65% inhibition, whereas the crude extracts at 250 mg/kg and 500 mg/kg showed 78% and 73% inhibition, respectively (Table 5).

Antipyretic activity: All test doses of the crude methanolic extract resulted in significant temperature decreases when compared to the negative control (Fig 6). The crude extract of *Ampelocissus barbata*, both 250 and 500 mg/kg, displayed antipyretic activity by inhibiting the pyretic action (reduction of rectal temperature of mice at the fixed time duration), where, the standard used was Paracetamol (150 mg/kg).

Each antioxidant experiment was independently carried out a minimum of three times, with standard deviations calculated based on the results. The

antioxidant activity increases with the amount of extract present. By applying several analytical techniques with different solvents in the antioxidant test, we have found a variety of test results. The raw elicit, ethyl acetate, and pet ether all displayed noteworthy levels in some evaluations. Besides, potential activity was also found in the in vivo tests.

Table 4. Effects of the crude extracts and control on mice after treatment.

Group	Treatment (mg/kg)	Blood glucose level (mmol/l)			
		0min	30min	60min	90min
I (negative control)	Tween 80 (10ml/kg)	3.36 ±0.407	17.62 ±0.799	14.88 ±0.216	14.58 ±0.223
II (positive control)	Glibenclamide (5mg/kg)	3.22 ±0.394	15.04 ±0.249	10.34 ±0.128	6.72 ±0.51
III (Test group-I)	Crude extract (250mg/kg)	2.48 ±0.282	16.42 ±0.193	14.3 ±0.298	11.62 ±0.22
IV (Test group-II)	Crude extract (500mg/kg)	2.44 ±0.185	14.88 ±0.104	11.36 ±0.497	9.92 ±0.197

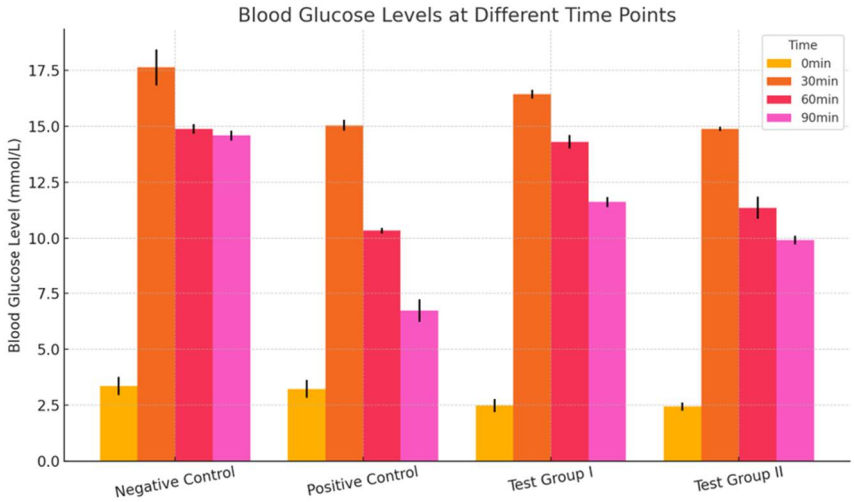

Figure 4. Comparison of blood glucose levels change within groups at different time points.

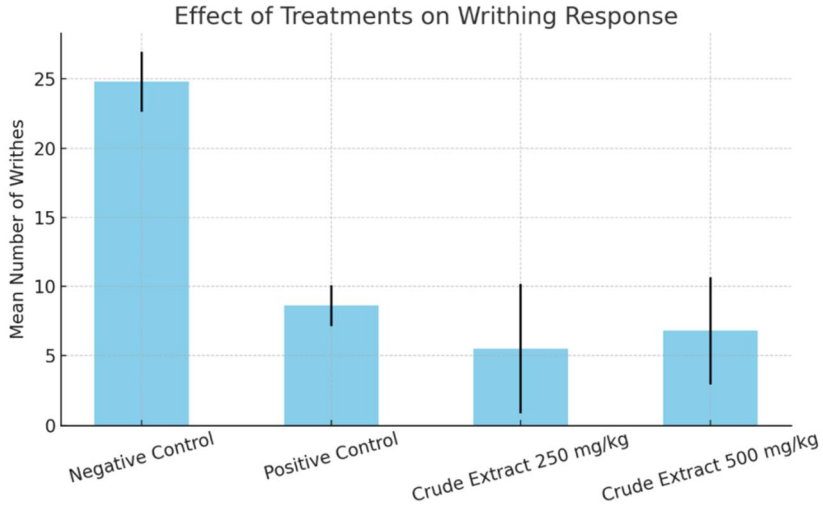

Figure 5. Effects of crude extract on mice's writhing response to acetic acid.

Table 5. Statistical evaluation of the experiment.

Group	Animal group	Mean of writhing	SD (Standard deviation)	% Inhibition of writhing
I	Negative control Tween 80 (10ml/kg)	24.8	2.17	0
II	Positive control Diclofenac sodium (25mg/kg)	8.6	1.48	65
III	Crude extract (250 mg/kg)	5.5	4.65	78
IV	Crude extract (500 mg/kg)	6.8	3.85	73

Table 6. Effects of rectal temperature on different time intervals.

Group	Normal	After 24 hr	1 hr	2 hr	3 hr	4 hr
Negative control	93	97	101	97	93	89
Positive control	94	98	100	96	92	88
A. barbata (250 mg/kg)	95	99	99	95	91	87
A. barbata (500 mg/kg)	96	100	98	94	90	86

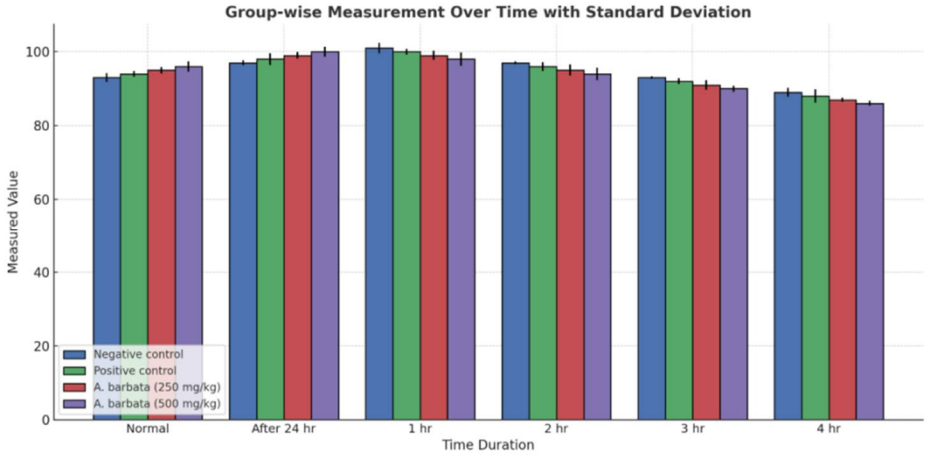

Figure 6. Variations in rectal temperature over time for various samples.

Conclusion

A. barbata fruit extract was tested through various *in vitro* antioxidant activities and *in vivo* assays in the current study since prior research on the plant has demonstrated that several components of the plant have medicinal properties. Significant action was seen when this extract was statistically assessed at various concentrations. The fruit's low IC_{50} and SC_{50} concentrations demonstrated strong antioxidant potential. This result proves that *A. barbata* fruit is a perfect source of antioxidants and they could be used as natural antioxidants. Besides, it also possesses significant pharmacological values. These findings also emphasize the necessity of

adopting the appropriate antioxidant activity measuring method. To obtain a potent drug from *A. barbata* fruit with notable activity, toxicological studies with deeper research are needed for the compound's isolation and characterization.

List of Abbreviations

DPPH: 2,2-Diphenyl-1-picrylhydrazyl, CE: Crude extract, EASF: Ethyl acetate soluble fraction, PESF: Pet ether soluble fraction, IC_{50}: Half maximal inhibitory concentration, BGL: Blood glucose level, SC_{50}: Half maximal scavenging concentration, EASF: Ethyl acetate soluble fraction, PESF: Pet ether soluble fraction, TCA: Trichloroacetic acid, TBA: Thiobarbituric acid

Conflicts of Interest

The authors affirm that there were no financial or commercial ties that might be interpreted as a potential conflict of interest during the research.

Author Contributions Statement

Shahenul Islam: Conception, *In vivo* experimentation, Supervision, Writing- Original draft, Writing- Review, Formal analysis; Sabrina Sultana: Phytochemical screening tests, *In Vitro* experimentation, Writing- Original draft, Referencing; Mohammad Mimkul Islam: Phytochemical screening tests, *In Vitro* experimentation; Badrunnahar Binty: Phytochemical screening tests, *In Vitro* experimentation; Nazmul Hossan: Phytochemical screening tests, *In Vivo* experimentation; Hasan Mahmud: Methodology, *In Vivo* experimentation; Md. Akbar Hossain: Writing- Review and proof reading, Formal Analysis.

Funding Information

This research received no external funding.

Acknowledgements

Authors would like to thank to all the participants who contributed to the studies with their contributions.

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