

***In vitro* Antioxidant, Cytotoxic, Thrombolytic Activities and Phytochemical Evaluation of Methanol Extract of the *Ampelocissus Barbata* (Wall.) Leaves**

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Context: Oxidative stress and pertaining counterbalance mechanism are actively working in the living organisms. The overproduction of reactive oxygen species (ROS) in the ongoing equipoising process requires to be compensated by strong antioxidants. Plants as a rich source of antioxidants not only reduce oxidative stress but also possess cytotoxic, thrombolytic and phytochemical potentials. **Aims:** To find out the antioxidant, cytotoxic, thrombolytic and phytochemical capabilities of the methanolic extracts of *Ampelocissus barbata* (Wall.) leaves. **Methods and Material:** Assessment of the *in vitro* antioxidant activity of extract was carried out using DPPH radical scavenging assay, determination of reducing power capacity and total phenolic content. The thrombolytic activity was assessed by disintegration of clot and prospective phytochemical activities were by standard qualitative analysis such as Mayer's, Dragendroff's Wagner's and Hager's Reagent test for alkaloids; Libermann-Burchard and Salkowski Reagent tests for steroid and terpenoids; Molish Reagent, Benedict's Reagent, Fehling's Solution A & B reagent test for carbohydrates; Ferric Chloride (5%) Solution, Potassium Dichromate (10%) Solution tests for tannins; Shinoda test and Alkaline reagent test for Flavonoids; Froth tests & Haemolysis test for Saponins. **Statistical analysis used:** The statistical analysis was carried out using GraphPad Prism and Microsoft excel **Results:** Appreciable DPPH radical scavenging activity of the extract was observed with the IC₅₀ value of $107.47 \pm 1.46 \mu\text{g/ml}$. A significant correlation was found between the standard ascorbic acid (AA) and the plant extracts at the $p < 0.05$ for the reducing power assay where, the activity increased with the concentration of the extracts and the highest absorbance value was 3.025 ± 0.15 and 1.826 ± 0.006 for the AA and the extracts respectively. The plant also accommodates a considerable amount of polyphenols, reflected in the value of gallic acid equivalent $277.397 \pm 0.419 \text{ mg/ml}$. Finally, the percentage (%) of clot lysis for the thrombolytic activity was revealed to be 7.031 ± 0.697 , 35.297 ± 1.307 , and 75.083 ± 0.599 for the water (negative control), extract, and the standard Streptokinase respectively. **Conclusions:** The study disclosed the promising *in vitro* activity of the plant, which necessitates the further analysis for the isolation and evaluation of the active principles.

Keywords: *Ampelocissus barbata*; Antioxidant; Phytochemical Assay; Thrombolytic

The significance of oxidative metabolism is indispensable for the existence of living cells and is extensively acknowledged. By-product of these processes includes the generation of free radicals and other reactive oxygen species (ROS)¹. In the biological systems formation of ROS over the antioxidant capability results in oxidative stress²⁻⁴. Such species are associated with a large number of typical *in vivo* regulatory systems⁵. For instance in the plants as the signaling molecules, ROS regulates defence, growth, abiotic stress acclimation, and development thus plants have regulatory systems to maintain appropriate level of ROS in the cell⁵⁸. Similarly, imbalance of ROS may be harmful to the animals since ROS was identified as causative factors for diseases namely cancer, cardiovascular disease, rheumatoid arthritis, neurodegenerative disorders and diabetes⁵⁹. Redundant production of free radicals can beat defensive enzymes such as peroxidase, catalase as well as superoxide dismutase (SOD), bringing in devastating and fatal consequences for cells leading towards oxidation of the cellular proteins, DNA, and membrane lipids, ultimately shutting cellular respiration down⁶. In addition, the pathogenesis of different types of human diseases such as hypertension, atherosclerosis, inflammation diabetes mellitus, AIDS, and cancer have been found to be associated with free-radical mediated oxidative stress⁷. Moreover, the cell signalling pathways were also appeared to be influenced by reactive oxygen species in ways currently being unravelled⁸.

Thrombosis is the formation of a blood clot inside a blood vessel, obstructing blood flow in the systemic circulation⁹. Blood clots formed within a vein may dislodge from their origin and travel (embolus)¹⁰. Thrombolysis (the breakdown) of blood clots is carried out by stimulating plasminogen, which produces cleaved product plasmin, a proteolytic enzyme that disintegrates cross-links between fibrin molecules resulting insoluble degradation products from insoluble fibrin. Thrombolysis basically encompasses the application of thrombolytic drugs, also called "plasminogen activators" and "fibrinolytic drugs."¹¹ These drugs solubilize thrombin in accurately obstructed coronary arteries, thereby normalizing blood supply to ischemic myocardium, reducing necrosis and advance prognosis¹².

There are three main categories of fibrinolytic agents, namely tissue plasminogen activator (tPA) factors, streptokinase (SK), and urokinase (UK); amongst these, the UK and SK are widely used^{13,14}. But they are associated with high hemorrhagic risk¹⁵ and extensive anaphylactic reactions. Moreover, immunogenicity constrains treatment with SK¹⁶. Since all available thrombolytic agents suffer from significant deficits, for example, the requirement of large doses, definite specificity for fibrin, and bleeding tendency; therefore, better alternatives for the drugs with minimum side effects and maximum efficiency are badly needed¹⁷.

For thousands of years, plants acted as the source of various traditional medicines worldwide and still serve as the basis of new remedies for humankind. Initially, medications from plants are dispensed as crude drugs¹⁸.

In that connection, modern medicine is blessed with various active compounds isolated from plants, whereas for some of the essential drugs, the basic raw material continues to come from the plants¹⁹.

Moreover, phytochemistry abridges plant biochemistry and organic chemistry from the natural product. Generally, plant deals with a large variety of chemical substances, along with their biosynthesis, natural distribution, and biological metabolism function²⁰. Therefore, the plant extract with bioactive compounds such as tannins, alkaloids, phenolic compounds, and flavonoids is regarded as promising in terms of therapeutic activity²¹.

For many developing countries, plants are the principal source of the bioactive tenets and medicine used explicitly in the traditional system of medicine^{22,23}.

Thus, it is clear that many plant species still possess medicinally important compounds that need to be discovered²⁴.

As the plant remains an integral part in terms of the discovery of medicine thus the aim of the current work was to explore the phytochemical profiles of the plant *Ampelocissus barbata* for Antioxidant and thrombolytic activity.

Ampelocissus barbata is a species of liana that belongs to the grape family *Vitaceae*. Nathaniel Wallich described it from Sylhet (now in Bangladesh) and placed in the genus *Vitis*²⁵. The

Ampelocissus genus of *Vitaceae* was recognized by Planchon in 1884 and encompassed about 95 species that are dispersed in the tropical regions of Asia, Australia, Central America and Africa²⁶.

Taxonomy²⁷

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Vitales

Family: Vitaceae

Genus: *Ampelocissus* Planch.

Species: *Ampelocissus barbata* (Wall.) Planch.

Synonyms : *Vitis barbata* Wall. ; *Vitis latifolia*

Buch.-Ham.; *Vitis latifolia* Buch.-Ham. ex Wall.

Vernacular Name(s) : Jarila Lahari (Bangla);

Khoissang (Chakma); Dang Gyae (Marma); Kanai

Lak Mah (Tripura)²⁸

Flowering & fruiting: June-October.

Ecology: Hilly forests and bushy thickets of foot hills.

Use: Fruits are edible. Paste prepared from roots is applied to boils.

Distribution: Bandarban, Chattogram, Cox's Bazar, Khagrachari and Rangamati, Bangladesh.

MATERIALS AND METHODS

Plant material

Fresh Stem of *Ampelocissus barbata* was collected from Chakaria, Cox's Bazar; Chittagong, Bangladesh. Plant material was authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh, where voucher specimens have been deposited.

The novelty of the work and Literature search

The combined laboratory approaches to explore the plant in terms of phytopharmacology were not done before in the case of *Ampelocissus barbata* in Bangladesh. Therefore, this attempt was taken to evaluate the plant for active phytoconstituents with diverse implications as well as to widen the path for future screening of the plants for *in vivo* tests.

A previous study found dose-dependent analgesic activity and the presence of Phytoconstituents⁶⁰ Apart from that there are some traditional applications found in the literature. Such as in the Sikkim province of India mouth and tongue sores of the milk-sucking baby, as well as cattle, are treated with plant juice⁶¹.

Preparation of crude extract

The stem was sun-dried for one week. After proper drying, it was ground into a fine powder using a Stainless Steel Herb Grinder Pulverizer Machine machine (R.S.Industries; India) . The ground Stem (500 g) was soaked in a sufficient amount of 95% methanol (Merck, Germany, CAS 67-56-1) for ten days at room temperature with occasional shaking and stirring manually. Afterward, the whole mixture was filtered through a cotton plug, followed by Whatman filter paper No. 1. Then the solvent evaporation was executed under reduced pressure at room temperature to yield a semisolid (5.9 %) mass and preserved in a refrigerator for further use.

In vitro antioxidant assay

DPPH Radical Scavenging Assay

Free radical scavenging ability of the test sample was carried out by the method described by Brand-Williams *et al.*²⁹, with slight modifications

Table 1. Percentage of DPPH radical scavenging activity of ascorbic acid and methanol extract of *A. barbata* stem at different concentrations. Results are mean± SD of three measurements.

Concentration (µg/ml)	% inhibition by ascorbic acid ± SD	% inhibition by <i>A. barbata</i> ± SD
25	22.21 ±1.035	18.58±0.782
50	44.92±1.398	37.80±0.253
100	58.97±1.567	49.98±1.049
200	69.59±0.942	60.56±0.624
400	84.49±1.437	75.66±0.810
	IC ₅₀ =73.97±03.37 µg/ml	IC ₅₀ =107.47±1.46 µg/ml

(modified in the university and standardized). The DPPH radical was used in the assay to quantify the ability of antioxidants to quench the DPPH radical. Upon scavenging the DPPH radical, the reaction mixture changes its colour from purple to yellow accompanied by decreasing absorbance at the wavelength 517 nm. One millilitre of the sample solution in methanol at various concentrations (25, 50, 100, 200 and 400 µg/ml) was mixed with three millilitres of 0.004% DPPH solution in methanol. After reaction for 30 min at room temperature in dark conditions, the absorbance values of the sample were measured by a UV spectrophotometer (Shimadzu, Kyoto, Japan) at 517 nm (ϵ_{\max}) against a corresponding blank. The calculation of Radical scavenging activity (%SCV) was accomplished by comparing the results of the test (sample/extract) with the control (not dealt with extract) applying the below formula:

$$\% \text{ SCV} = \frac{A_0 - A_1}{A_0} \times 100$$

Where SCV = Radical scavenging activity,

A_0 = Absorbance of the control (containing all reagents except the test compound)

A_1 = Absorbance of the test compounds (extracts / standard).

Table 2. Reducing power of ascorbic acid (Standard) & *A. barbata* at different concentrations. Results are mean± SD of three measurements

Concentration (µg/ml)	Absorbance (Mean ±SD)	
	Ascorbic acid	<i>A. barbata</i>
25	1.051±0.041	0.232±0.028
50	1.571±0.135	0.391±0.048
100	2.276±0.124	0.753±0.029
200	2.884±0.135	1.338±0.065
400	3.025±0.154	1.826±0.006

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph obtained by plotting % SCV versus concentration and subsequently verified using Graphpad prism. The assays were repeated three times, and ascorbic acid was used as standard.

Reducing power capacity

The reducing power of *Ampelocissus barbata* stem extracts was evaluated according to the method formerly described by Oyaizu³⁰ with slight modification (modified in the university and standardized)..

In the Reducing power assay, based on the reducing power of the sample, the test solution colour changes from yellow to several shades of green and blue³¹. The presence of antioxidant substances in the sample converts the Fe^{3+} to Fe^{2+} of the ferricyanide and thus the amount of Fe^{2+} complex monitored by taking absorbance at 700 nm.

Various concentrations of *Ampelocissus barbata* stem extracts (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml) were mixed with potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%) and phosphate buffer (2.5 mL, 0.2 M, pH 6.6). To complete the reaction, the mixture was incubated for 20 min at 50°C. Afterward, 2.5 ml

Table 3. Absorbance values of Gallic acid. Results are mean± SD of three measurements

Concentration (µg/ml)	Absorbance ±SD
25	0.181±0.008
500	0.386±0.012
100	0.824±0.051
200	1.647±0.059
400	2.921±0.054

Table 4. Data for the determination phenol content of *A. barbata* samples. Results are mean± SD of three measurements

Concentration of Sample solution (µg/ml)	Mass of the extract per ml in gm	Absorbance	Gallic acid equivalent c (mg/ml)	TPC as GAE (A)	Mean ±STD
400	0.0004	1.678	0.2221	277.397	277.397±0.419
400	0.0004	1.675	0.2215	276.884	
400	0.0004	1.681	0.2223	277.911	

Table 5. Thrombolytic Activity of *A. barbata*. Results are mean± SD of three measurements

Sl. Volunteer	Empty Weight of tube (gm)	Weight of tube without clot (gm)	Weight of Clot(gm)	Weight of Tube without clot after lysis (gm)	Weight of Lysis(gm)	% of clot Lysis	Mean% of clot Lysis ±SD
	A	B	C=B-A	D	E=B-D		35.2972±3.9196
01	0.8256	1.3211	0.4955	1.1321	0.1890	38.1433	
02	0.8197	1.0285	0.2088	0.9631	0.0654	31.3218	
03	0.8176	1.1321	0.3145	1.0285	0.1036	32.9412	
04	0.8235	1.2132	0.3897	1.1034	0.1098	28.1755	
05	0.8207	1.2662	0.4455	1.1087	0.1575	35.3535	
06	0.8023	1.2281	0.4258	1.0928	0.1353	31.7755	
07	0.8138	1.1284	0.3146	1.0054	0.1230	39.0973	
08	0.8311	1.0572	0.2261	0.9657	0.0915	40.4688	
09	0.7987	1.1243	0.3256	1.0071	0.1172	35.9951	
10	0.8138	1.3201	0.5063	1.1191	0.2010	39.6998	

of trichloroacetic acid (10%) was added to the mixture, followed by centrifugation at 3000 rpm for 10 min. 2.5 ml supernatant solution was withdrawn from the mixture and mixed with 2.5 ml of distilled water and FeCl₃ (0.5 mL, 0.1%), and finally the absorbance was measured at 700 nm against the blank, which contained the same solution mixture without plant extract or standard and was treated in the same manner as the samples solution. Ascorbic acid was used as standard. The correlation was determined between the absorbance of the Ascorbic acid and the plant extract. All analyses were carried out in triplicate, and results were averaged.

Determination of total phenolic content

The determination of total phenolic content for the methanol extract of *Ampelocissus barbata* was carried out by employing the method described by Singleton et al. with slight modification(modified in the university and standardized)., involving Gallic acid as standard and Folin-Ciocalteu reagent (FCR) as the oxidizing agent³². The basis for this test lies in the oxidation of phenolic groups caused by phosphomolybdic and phosphotungstic acids (FCR).

In brief, a sample aliquot of 0.5 mL of extract (1 mg/mL) was added to a test tube containing 2.5 mL of Folin-Ciocalteu (Diluted 10 times with deionized water) reagent. Moreover, 2.0 mL of Na₂CO₃ (7.5% in water, w/v) was added, and the resulting solution was vortexed and left 30 minutes at 25^oC to complete the reaction. The absorbance of the consequential blue colour was taken at 760 nm against blank. Using gallic acid as standard, total phenolic content was calculated from a calibration curve using gallic acid and expressed as mg of gallic acid equivalent (GAE) per gram dry weight (dw) was calculated by the following formula:

$$A = (c \times V)/m$$

Where,

A = total content of phenolic compounds, mg/g plant extract, in GAE;

c = the concentration of gallic acid established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant extracts, gm.

Data are reported as mean (SD) for at least three replications.

In vitro Thrombolytic activity

In vitro thrombolytic activity of *Ampelocissus barbata* chloroform extract was determined following the method described by Prasad *et al.*³³

Streptokinase (SK)

For the *in vitro* thrombolytic analysis, lyophilized SK vial of 15,00,000 I.U (Commercially available from Square Pharmaceuticals Ltd. Bangladesh) was used. The suspension was made by adding 5 ml sterile distilled water with proper mixing. From the resulted suspension, 100 μ l (30,000 I.U.) was utilized for thrombolysis.

Specimen

Four (04) mL whole blood was drained from ten healthy human volunteers who have no history of anticoagulant therapy or an oral contraceptive. Before collecting the sample from volunteers, prior consent was taken as per the protocol approved by the Institutional Ethics Committee of Northern University Bangladesh. Approval number: DoP/RC/EC/2021/04/app/01.

From the collected blood, 500 μ l (0.5 ml) of the blood was transferred to individual Eppendorf tubes weighed beforehand to form clots.

Herbal preparation

100 mg *Ampelocissus babata* methanol extract was suspended in 10mL distilled water and then agitated using a vortex mixer. The suspension was kept for overnight and was poured to eliminate soluble supernatant. It was then filtered by using a 0.22 micron syringe filter, and the filtrate was prepared for the assay.

Clot lysis

The collected blood specimen was incubated at 37°C for 45 minutes followed by complete careful removal of serum after clot formation. To find out the weight of the clot, the tube was weighed (clot weight = weight of clot containing tube – the weight of tube alone).

The tubes were labelled correctly. Then, 100 μ l aqueous extract of *Ampelocissus barbata* was added to the Eppendorf tube with a pre-weighed clot, incubated for 90 minutes at 37°C to observe for clot lysis. The fluid discharged in incubation was eliminated, and the tubes were weighed one more time to detect the weight variation after disruption of the clot. The change in weight was stated as a percentage of clot lysis. 100 μ l of SK applied as positive control and 100 μ l of distilled water as a

Table 6. Clot lysis by water and *Ampelocissus barbata* compared with streptokinase. Results are mean \pm SD of three measurements

Treatment	% clot lysis \pm SD	P value when compared with negative control
Water	7.667 \pm 0.230	
<i>Ampelocissus barbata</i>	35.2972 \pm 3.9196	P < 0.05
Streptokinase	75.577 \pm 0.489	P < 0.05

Table 7. Phytochemical elements recognised in the extracts of *Ampelocissus barbata*.

Phytochemical class	Observation
Alkaloids	+++
Triterpenoids and Steroids	-
Carbohydrates	-
Tannins	+
Flavonoids	++
Saponins	+

+++ = very prominent; ++ = moderate; += Minor; - = Absent

+++ = very prominent; ++ = moderate; += Minor; - = Absent

negative non-thrombolytic control, where all the tubes were treated in the same manner as described above.

$\% \text{ clot lysis} = \left(\frac{\text{Weight of the lysis clot}}{\text{Weight of clot before lysis}} \right) \times 100$.

The experiment was repeated 10 times with the blood samples of 10 volunteers.

Phytochemical screening

The presence of different chemical groups in the methanol extract of *A. barbarata* W. leaves was screened as the preliminary step in the phytochemical studies. The chemical group tests were performed following standard test procedures^{19,24, 34-40}. In each test, 10% (W/V) solution of

extract in Chloroform was taken unless otherwise mentioned in the individual test.

Statistical analysis

The statistical analysis (determination of IC_{50} values, the Pearson correlation coefficient at $P < 0.05$) was performed using GraphPad Prism version 9.0.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com

Calculation of mean, standard deviation, and the constructions of graphs was carried out using Microsoft Excel version Office 2016.

RESULTS

In vitro Antioxidants Activity

DPPH Radical Scavenging Assay

The complete procedure is previously delineated and the results obtained are given in Table 01 as well as elucidated in Figure 01. Test results exhibited concentration-dependent scavenging of the free radicals. The range of concentration for both the ascorbic acid (AA) and the plant extract was from 25-400 $\mu\text{g/ml}$,

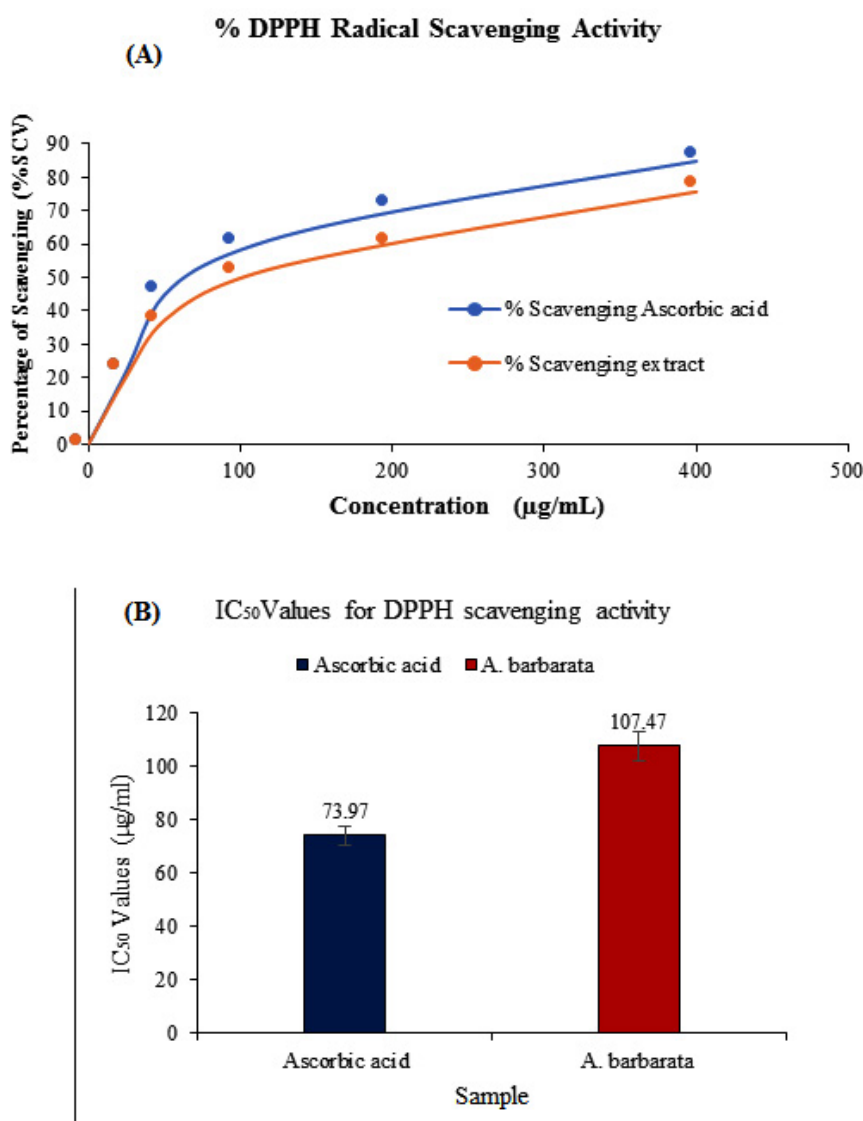


Fig. 1. (A) %-scavenging activity of Ascorbic Acid and methanol ether extract of *A. barbarata* (stem) at different concentrations. (B) IC_{50} Values of the standard and sample. Results are mean \pm SD of three measurements

where the % inhibition was lower 22.21% (AA); 18.58% (*A. barbarata*) at 25 $\mu\text{g/ml}$ and maximum 84.49% (AA); 75.66% (*A. barbarata*); at the highest concentration 400 $\mu\text{g/ml}$; for the standard and the extract respectively. The 50% inhibitory concentration of the antioxidant called IC_{50} was found as 73.97 ± 03.37 $\mu\text{g/ml}$ and 107.47 ± 1.46 $\mu\text{g/ml}$ for the standard and the plant extract, respectively.

Reducing Power Capacity

The plant's methanolic extract (MeOH) showed the potential reducing capacity, outlined in Figure 02 and Table 02. The result for reducing power capacity indicates that the extract's reducing power was enhanced in a concentration-dependent manner. The ascorbic acid (AA) was used as the standard reference compound. At the concentration of 25 $\mu\text{g/mL}$, the absorbance's

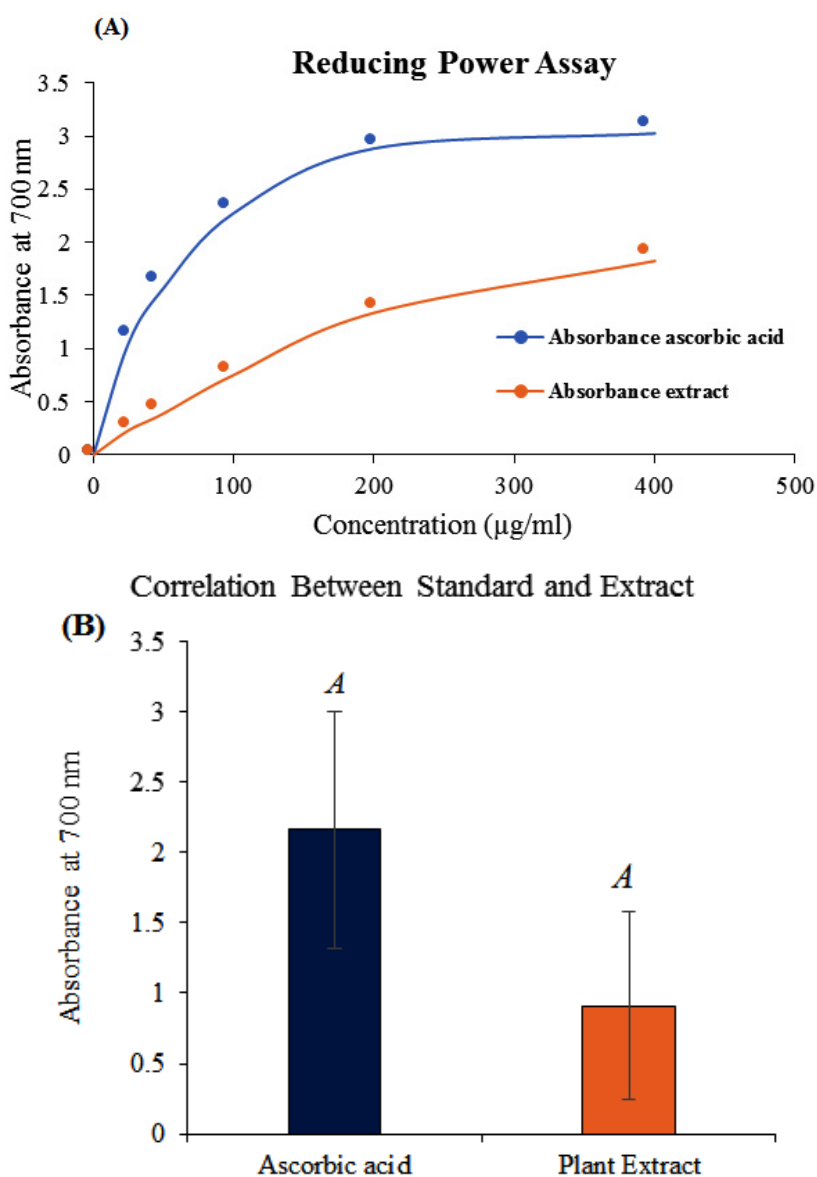


Fig. 2. (A) Reducing power of ascorbic acid and Methanolic extract of *A. barbata* (MeOH). Results are mean \pm SD of three measurements. (B) Correlation between Ascorbic acid and Plant extract. Means with similar letter (A) do not differ significantly ($P < 0.05$).

of the plant extract and AA were 0.232 ± 0.028 1.051 ± 0.041 , respectively. But it was significantly increased at $200 \mu\text{g/mL}$, depicting the absorbance as 1.338 ± 0.065 by the extract about which is almost about half of the AA at the same concentration. There was a significant correlation observed between the reducing power capacity of standard ascorbic acid and the plant extract at $P < 0.05$.

Determination of total phenolic content

A standard Gallic acid curve was constructed to estimate the phenolic content from the sample, and the Gallic Acid Equivalent (GAE) was obtained by reference formula. The absorbance values for the Gallic acid are represented in Table 03 and the standard curve in Figure 03. Using the data ($y = 0.0073x + 0.058$ $R^2 = 0.9948$) from the standard curve, the total phenolic contents of the sample were calculated and expressed as GAE. The average phenolic content of *A. barbata* was found to be 277.397 ± 0.419 mg Gallic acid/gm of extract, as shown in Table 04.

Thrombolytic activity

The assessed results for the thrombolytic property of the plant extract are presented in Table 05, 06 & Figure 04. In the thrombolytic activity test, the methanolic extract of *A. barbarata* demonstrated $35.2972 \pm 3.9196\%$ lysis of blood clot while $75.577 \pm 0.489\%$ for the positive control (streptokinase-SK) as well as $7.667 \pm 0.230\%$ lysis

were acquired for negative control (sterile distilled water).

Phytochemical analysis

The phytochemical screening indicates qualitative presence of carbohydrate, alkaloid, tannins, steroids, saponins and flavonoids (Table 07).

DISCUSSION

DPPH Radical Scavenging Assay

The free radical scavenging activity was evaluated by DPPH where ascorbic acid was used as the reference standard. The assay is carried out to find out the potential antioxidant activity of plant extract applying DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a radical donor. *In vitro* free radical scavenging activity assay of the methanolic extract for *A. barbata* (stem) demonstrated the presence of prospective antioxidant activity. The extract's efficiency in scavenging DPPH free radical with respect to a standard depicts the significant potential of the extract as a natural antioxidant⁴¹.

Reducing power capacity assay reveals the compound's reducing capacity as an important marker of its probable antioxidant activity. The reducing power of the plant extract components might serve as a significant indicator of its

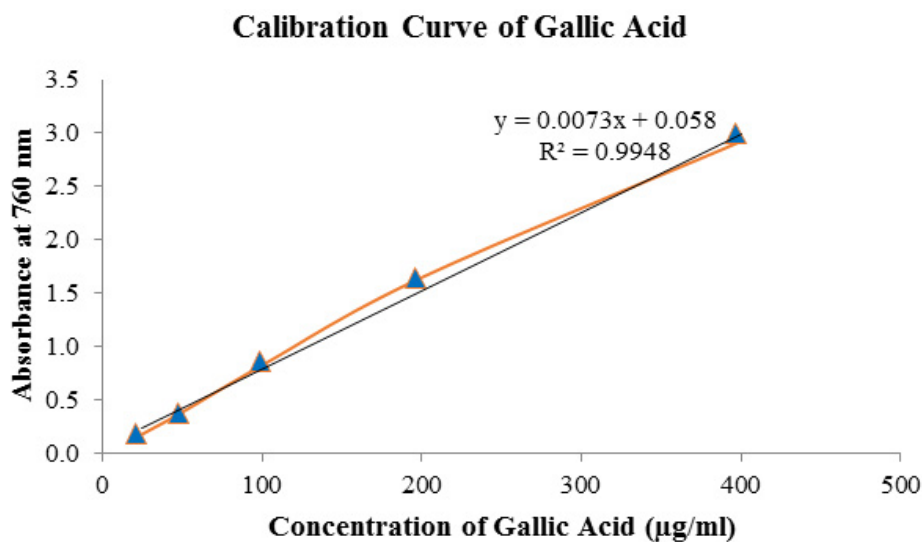


Fig. 3. Calibration curve of Gallic acid for total phenolic content determination. Results are mean \pm SD of three measurements.

potential antioxidant activity⁴². Previous studies found a direct correlation has observed between the reducing power of certain plant extracts and antioxidant activities.

Nevertheless, there are several types of mechanisms by which the antioxidants exert their activity, such as radical scavenging, prevention of continued hydrogen abstraction & chain initiation, binding of transition metal ion catalysts, reductive capacity, and decomposition of peroxides^{43,44}.

For a compound, the value of reducing capacity may be an important index of its significant antioxidant activity⁴⁵ as the compounds with reducing power are active electron donors and

consequently function as primary and secondary antioxidants.

Different studies have been indicated that the reducing properties of the compound are due to the presence of reductants⁴⁶, and the antioxidant activity is obtained by breaking the free radical chain through the donation of the hydrogen⁴⁷.

As a class of antioxidants, the phenolic compounds act as free radical scavengers. Phenols are one of the most important plant constituents because of their scavenging ability due to their hydroxyl group⁴⁸. The prominent amount of GAE of this plant indicates the high antioxidant profile of this plant. The presence of hydroxyl

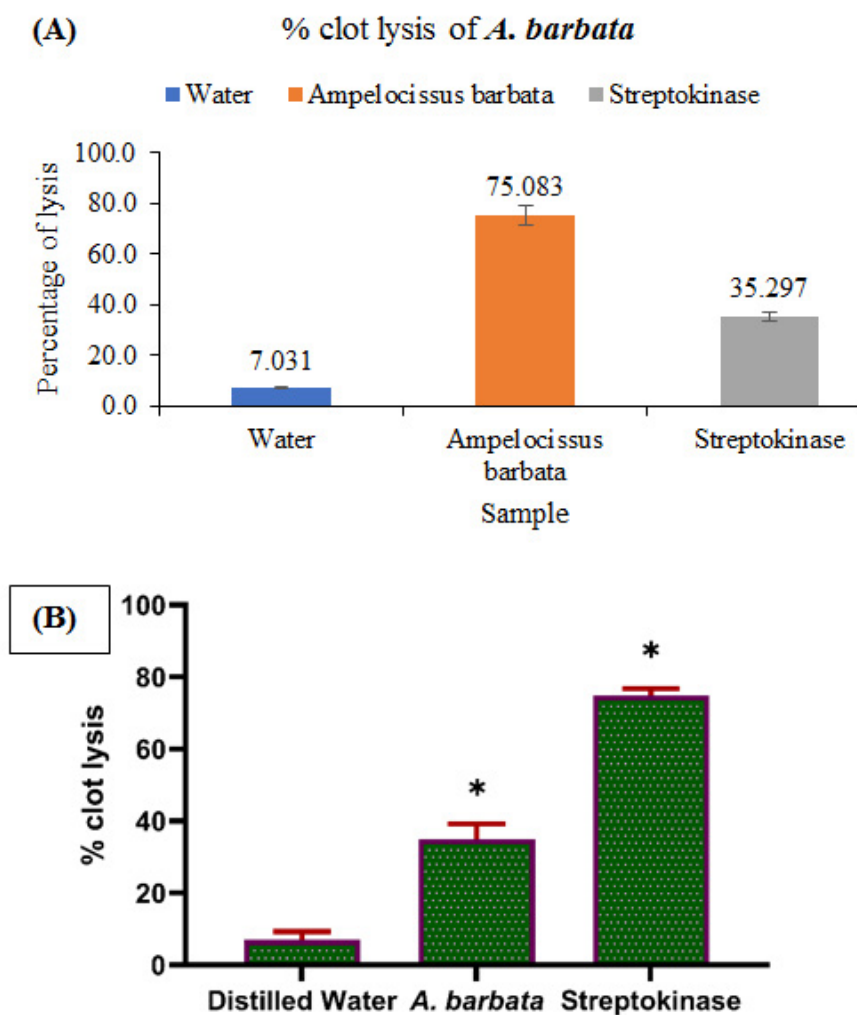


Fig. 4. (A) Clot lysis by water and *Ampelocissus barbata* compared with streptokinase (B) Significance of clot lysis. Results are mean \pm SD of three measurements.

groups confers scavenging ability to the phenolic compounds, ultimately making them important plant constituents⁴⁹. In general, the phenolic compounds are treated as solid chain-breaking antioxidants⁵⁰. However, the connection between the antioxidant activity and phenolic content has been reported by many authors^{51,52}, indicating that the antioxidative action is attributed directly to the phenolic compounds⁵³.

As compared to the lysis percentage of SK and water, the thrombolytic activity of *A. barbarata* was incredibly significant. The effective decrease in the percentage of fat of clot by the extract solution is indicative of promising thrombolytic potential. The plant extracts can be used for the development of anti-thrombotic agents for the healing of related cardiovascular diseases due to their promising thrombolytic activity.

CONCLUSION

From the above discussion, it can be concluded that the methanolic extract of the plant has DPPH radical scavenging, reducing power and antioxidant activity as compared to standard ascorbic acid. Furthermore, significant thrombolytic activity was also demonstrated with respect to SK. Phenolic compounds are present in plants, have antioxidant activity due to their redox properties, and therefore play a vital role in counterbalancing the free radicals^{54,55}.

With regards to reducing capacity, higher reducing powers might be attributed to higher amounts of total phenolic and flavonoid, and the reducing power of a compound may reflect its antioxidant potential⁵⁶.

However, this result shows the plant *A. barbarata* has appreciable thrombolytic activity and may become a useful thrombolytic agent with respect to further processing, furnishing a sound cardiovascular system⁵⁷.

Further comprehensive pharmacological and phytochemical study for the isolation and characterization of the specific compound is required to get a more potent agent with significant activity. Since the polyphenol compounds as well as other components with potent antioxidant activity are not known, thus advanced level of work should be performed for the isolation and

identification of the antioxidant components in *A. barbarata*.

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Conflict of interest

All authors declare no conflict of interest.

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Informed Consent

No such information was applied in the paper.

Ethics of Human and Animal Experimentation

Before collecting the sample from volunteers, prior consent was taken as per the protocol approved by the Institutional Ethics Committee of Northern University Bangladesh. Approval number: DoP/RC/EC/2021/04/app/01.

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