Assessment of the Pharmacological Activities of *Ardisia solanacea* Roxb: An Ethnomedicinal Plant used in Bangladesh

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Abstract:

Objective: This study aims to uncover the anti-diarrheal, antioxidant, thrombolytic, and anthelmintic activities of methanol extract of *A. solanacea* (ASME) and its soluble n-hexane fraction in methanol (ASNH).

Materials and Methods: The phytochemical assessment of this plant was performed by using the standard method. The anti-diarrheal property was screened by castor oil induced diarrhea in Swiss albino mice and plant extract was administered into mice by oral gavage. The antioxidant property was being investigated by two different *in vitro* methods such as ferric reducing effect assay and superoxide scavenging activity assay. The thrombolytic activity was evaluated by *in vitro* clot lysis procedure, and the anthelmintic study was carried out on earthworm *Pheretima posthuma*.

Results: In castor-oil induced diarrhea, ASME and ASNH induced a significant decrease ($^{**}P<0.005$) in the total number of defecation within 4 hours of the testing period (200 and 400 mg/kg) when compared to the standard drug loperamide. During the evaluation of the antioxidant property, ASME showed promising reducing power with an IC_{50} value of 79.14 µg/mL when compared to the standard ascorbic acid in ferric reducing effect assay. After that, ASME displayed significant scavenging effect with the IC_{50} value of 154.36 µg/mL when compared to standard curcumin in superoxide scavenging activity assay. In the thrombolytic activity, different doses of ASME and ASNH were used. These extracts produced considerable clot lysis of human blood, showed significant ($P<0.001$) result in a concentration dependent manner. The crude ASME displayed anthelmintic activity in adult earth worm in a dose-dependent manner.

Conclusion: It can conclude that compared to standard drugs, *A. solanacea* have potential anti-diarrheal, antioxidant, thrombolytic, and anthelmintic activity.

Keywords: *Ardisia solanacea*, anti-diarrheal, antioxidant, thrombolytic, anthelmintic.

1. INTRODUCTION

Since ancient periods, a significant function was being played by different plant constituents or phytochemicals in the treatment and prevention of multiple human diseases [1]. Phytotherapy can be beneficial to treat life-threatening parasitic diseases [2]. Besides, herbal therapy can produce fewer side effects, cost-effective, and affordable [2]. The World Health Organization (WHO) concluded approximately about 20, 000 species of medicinal plants [3]. In most developing countries, the prime reason behind the mortality and morbidity is diarrhea that is most commonly caused by gastrointestinal infections. In every year, it is killing indiscriminately 4.6 million people, including 2.5 million children [4]. In 2015, Southern Asia and Sub-Saharan Africa were regions with the highest child death rates caused by diarrhea [5]. The use of lucid potable water is an important preventive measure against it. Eighty percent of people believe that medicinal plants can be used to treat diseases [6], including diarrhea. Traditional remedies are the most considerable and in some cases the only source of therapeutics in treating diarrhea and most of the preparations come from plant origin [7]. Alternative therapies are using to treat diarrhea in recent times. Diarrhea has also been a prominent disease treated by traditional medicine [8]. In recent times, it is encouraged to isolate and investigate the phytochemicals in the modern sophisticated laboratory for finding the novel constituents [9].

In the food industry and medical research, antioxidants from spicy, aromatic, medical, and other plants were also searched to formulate natural anti-oxidant preparations for the food, cosmetics, and other uses [10]. Among them, phenolic components are very crucial for dietary uses and the most widely used in research [11]. Plants which are containing phenolic compounds constitute phenolic acids, flavonoids, and polyphenols such as hydrolyzable and condensed tannins [12]. These compounds can practically terminate oxidative stress by the process of scavenging free radicals which in turn gives protection to plant parts, fruits, and vegetables as well as protect human DNA, proteins, and lipids from oxidative embezzlement [12]. Free radical scavenging activities may contribute to the prevention of the inflammatory processes [13].

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This is because active O_2 metabolites which are from phagocytic leukocytes that beset the tissues and cause injury to essential cellular components which lead to inflammatory tissue injuries [13]. Compounds that have scavenging properties towards these radicals are beneficial in inflammatory diseases [14, 15]. Herbal products have been applied to treat and prevent the incidence of cardiovascular diseases for a long time [16]. Many studies revealed that the natural compounds including medicinal herbs are most effective against atherosclerosis [17]. Plant extract decreased the level of serum lipid in rabbits fed with a high cholesterol diet [18]. Besides, recent studies have been revealed that the medicinal plants and their structural components possess antithrombotic activity [19]. The aerial parts of the plant also possess significant anthelmintic properties against different helminth parasites [20].

Our present investigation was done on a medicinal plant, named as *Ardisia solanacea* Roxb which belongs to Primulaceae family. In Bangladesh, it is locally called as Ban Jam and widely distributed in different regions of this country especially in hilly areas of Chittagong forest, Chittagong Hill Tracts, Sylhet and other evergreen forests. It is generally a small or glabrous tree that can reach a maximum height of 6 meters under the ordinary conditions [21]. This plant has potential antibacterial, neuropharmacological, oral hypoglycemic, cytotoxic, and antiviral properties were reported by the different research team [21, 22]. In this regard, the present study procedures mainly focused on to determine anti-diarrheal, antioxidant, antithrombotic, and anthelmintic properties of *A. solanacea* leaf extract.

2. MATERIALS AND METHODS

2.1. Solvents and Chemicals

The solvents and chemicals were used to maintain the analytical and laboratory-grade (e.g., SIGMA, E. Merck, or BDH) solvents and chemicals were used in most of the experiments. Whitman No.1 (Sargent-Welch, USA), Tween-80, Loperamide (Square Pharmaceuticals Ltd., Bangladesh), Streptokinase (S-kinase, Popular Pharmaceutical Ltd., Bangladesh), Pure methanol (Merck KGaA, Darmstadt, Germany), Potassium ferricyanide (1%), Phosphate buffer saline, 0.1% ferric chloride, DMSO solution, and NBT (Nitro-blue-tetrazolium).

2.2. Plant Collection and Authentication

The plant specimen of *A. solanacea* leaves were plucked from the hillside of Chittagong during the time of July, 2017. The collected specimen then identified by a senior Taxonomist, Dr. Shaikh Bokhtiar Uddin, Department of Botany, Faculty of Biological Sciences, University of Chittagong., included a herbarium number RI-12609004.

2.3. Preparation of Plant Extract

The specimen of proposed plant leaves were washed, whack, and then dried for several days before the grinding process. The grinding of dried specimens was executed by employing a high-speed grinder in the Phytochemistry Research lab, Department of Pharmacy, University of Chittagong. The grounded materials were then transferred into an airtight container for long term storage.

2.4. Extraction of the Plant Material

The ground plant specimen of 300 g was held in a sacred, round-bottom flask (5 liters) in which two liters of pure methanol was poured. The flask along with its content was sealed by aluminum foil and concealed a minimum of 15-20 days. After this soaking period, the mixed content was then filtered through a renewed cotton plug and the filtered content was again purified and filtered by Whitman No.1 filter's paper. The solvent volume present in the filtrate was then removed at very low temperature and pressure using the Buchii Rota Evaporator, Manufactured by Cole-Parmer, UK. After the solvent evaporation, a greenish-black colored semisolid methanol extract was attained with 25 g of yield index. This index was represented as % yield, and calculation was done by using a simple mathematical approach [22].

2.5. Preliminary Qualitative Phytochemicals Screening

The qualitative existence and exclusion of different secondary plant metabolites in this plant material were investigated by using standard procedures [23, 24].

2.6. Experimental Animals

For the evaluation of *in vivo* activity, young males, aged between 3-4 weeks of Swiss albino mice, having a mean weight of 20-25 g. They were indiscriminately grasped from the Animal House of Pharmacy Department, Jahangirnagar University, Dhaka, Bangladesh. Then, they were sheltered in an especially engineered single clean plastic grate which was covered with a metal net on its top. These buckets of experimental animals were then shifted to the Animal House of the Department of Pharmacy, Faculty of

Biological Sciences, University of Chittagong. The house was maintained at an optimum temperature of 24 ± 1ºC, RH (Relative humidity) kept at 55%-65%, and 12 hours light-dark/ 12 hours dark circle. Then they were housed for one week and providing them with standard laboratory sustenance and water.

2.7. Experimental Design

To evaluate the antidiarrheal property induced by castor oil, thirty experimental animals were randomly elected, marked, weighed, and parted into six groups, each group consisting of five mice, and kept in a pre-cleaned container.

Group (I): Normal control (Normal saline with 1% Tween-80; 0.1 mL/10 g).

Group (II): Positive control (Loperamide; 3 mg/kg).

Group (III) and Group (IV): They were treated with 200 and 400 mg/kg of methanol extract of *A. solanacea*, respectively.

Group (V) and Group (VI): They were treated with 200 and 400 mg/kg of n-hexane soluble fraction of methanol extract.

2.8. Acute Toxicity Studies

An acute toxicity study was directed to determine the LD_{50} for the extracts. According to the Organization for Economic Co-operation and Development (OECD) guidelines state that the use of a 2000 mg/kg rodent limit dose can cause acute oral toxicity. In compliance with this guideline 2008: 425, a single female mouse was fasted for 3 hours and was charged with 2000 mg/kg of ASME & ASNH as a single dose by oral intubation. It was then examined for any sign of toxicity like as eyes, skin and fur, mucous membrane, circulatory and respiratory rate, and autonomic and CNS changes within the first 24 hours [25]. Based on the findings of the initially used mouse, the next four female mice were attained and fasted for 3 hours. Following that, they were treated with the same dose and again examined for any sign of toxicity or death in the next 14 days [26]. The effective therapeutic dose was calculated as one-tenth of the median lethal dose using the arithmetical model of Karber [27] in association with the Hodge and Sterner scale (LD_{50} > 2.0 g/kg) [28]. The following equation was used for the calculation of LD_{50}:

$$LD50 = LD100 - \sum (a \times b) / n$$

2.9. Evaluation of Antidiarrheal Activity in Animal Model

2.9.1. Castor-Oil Induced Diarrhea

To determine castor-oil induced diarrhea, we used a method which is described by Shoba and Thomas [29]. Here, a pure analytical grade castor oil of 1 mL was fed to rodents just after thirty minutes of oral administration of plant extracts. Then, they were kept under observation for the next four hours. The individual mouse had given defecation at each time been recorded.

2.10. Evaluation of Antioxidant Activity

2.10.1. Ferric Reducing Effect Assay

The reducing power of *A. solanacea* leaf extract was determined according to the modified method of Sanja [30]. In this method, the yellow-colored test solution changes to multiple shades of green and blue colours, indicating the reducing ability of the sample. Sample was prepared at the concentrations of 27.5, 55, 110, 220 and 440 µg/mL in phosphate buffer saline (p^H = 6.6). 2.5 mL of potassium ferric cyanide (1%) was mixed with standard and sample. This mixture of the reaction was incubated at 50ºC for 20 min. After a period of incubation, the reaction mixture was cooled and followed by the addition of 2.5 mL of trichloroacetic acid (10%) by proper mixing. This mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and 2.5 mL of it was added to 2.5 mL of distilled water. Now, 1 mL of 0.1% ferric chloride was added to this reaction mixture and incubated at room temperature for 10 min. Finally, the absorbance of this mixture was measured at 700 nm. The standard ascorbic acid was used in this assay.

2.10.2. Superoxide Scavenging Activity Assay by Alkaline DMSO Method

Superoxide scavenging activity of *A. solanacea* extract was evaluated by the alkaline DMSO method described by Pandey with slight modification [31]. In this assay, the concentration of oxygen is abstracted in DMSO corresponds to the superoxide radical concentration in the alkaline DMSO solution. In brief, the non-enzymatic system of reaction generated this superoxide radical. The mixture of reaction was containing 0.3 mL of sample (Concentrations of 50, 100, 200, 400 and 800 µg/mL) and 0.1 mL of NBT solution (1 mg/mL solution in DMSO) in DMSO. Finally, 1 mL of alkaline DMSO (1 mL DMSO containing 0.1 mL water with 5 mM NaOH) was added to make a final volume of 1.4 mL. Standard solution of different

concentrations (5, 10, 20, 40, 80 µg/mL) was also prepared in this same way and curcumin was used as standard in this assay. The absorbance was assessed at 560 nm. Plain DMSO of 300 mL, alkaline DMSO of 1 mL, and NBT (Nitro-blue-tetrazolium) solution of 0.1 mL was mixed to get control. Antioxidants with low absorbance at 560 nm indicate that the generated superoxide is consumed [32, 33]. The standard curcumin was employed in this test. In both methods, we only used crude methanol extract of *A. solanacea* to determine its antioxidant capacity.

2.11. Evaluation of Thrombolytic Activity

2.11.1. In vitro Clot Lysis Model

2.11.1.1. Blood Specimen

Whole venous blood (5 mL) was drawn from healthy volunteers (n= 10) without a history of oral contraception or anticoagulant therapy. An informed consent form was filled up by every subject participating willingly in this research project [34]. A lab technician from the Department of Pharmacy, University of Chittagong, who collected the blood from the volunteers, preserved the blood by the same lab personnel. The clot-bearing Eppendorf tubes were stored in the refrigerator of the Microbiology laboratory of the same Department.

2.11.1.2. Study Design

This experiment was conducted by the method described earlier by Prasad et al. [35]. In short, 5 mL of venous blood drawn from the participated healthy volunteers which, was distributed in different pre-weighted sterile eppendorf tubes (500 µL per tube) and the tubes were then incubated in an incubator at 37°C for 45 minutes to occur blood-clot. After clot formation, without disrupting the clot, the separated serum was completely removed, and then each tube containing the clot was again weighed to ascertain the clot weight (Clot weight= weight of clot-containing tube - weight of the tube alone). To each clot-containing eppendorf tube, different doses (5, 10, and 20 mg/mL) of methanol extract and its soluble n-hexane of *A. solanacea* leave was added separately. The amount of plant extract was 500 µl of each dose. Streptokinase (100 µL), positive control, and a negative non-thrombolytic control (100 µL of distilled water) were individually added to the numbered control tubes. After then, all the tubes containing extract, positive, and negative control were incubated at 37°C for the next 90 minutes and executed for clot lysis. After this incubation period, the released fluid was removed and the tubes were again weighed to observe dissimilation of the weight of the clot after breakdown. The observed dissimilation in the weight taken before and after clot lysis was expressed as the percentage of clot lysis.

2.12. *In vitro* Evaluation of Anthelmintic Activity

The anthelmintic property of plant extract was done by following the method of Ajaiyeoba [36]. This was done on adult earthworms *Pheretima posthuma*. These were collected from the waterlogged areas, near the campus of the University of Chittagong. Appraised by veterinary technicians, the collected earth is 3-5 cm long, 0.1-0.2 cm wide, and weighs 0.8-3.04 g. They were thoroughly washed with saline water. The different concentrations (10-50 mg/mL) of investigated plant extract were prepared. The final volume of each concentration was made to 10 mL, and then transferred to different Petri dishes. The marketed albendazole was used as a standard drug. These earthworms were delivered into Petri dishes each containing five earthworms. Continuous observations were made to look-out any physical changes (paralysis and death) in the earthworms.

2.13. Statistical Analysis

Data were represented as mean ± standard error (SE). All statistical analysis of both control and treated samples of *A. solanacea* leaf extract were performed by one-way analysis of variance (ANOVA) Dunnett's t test where $*P<0.05$ was regarded as statistically significant. In addition, all data were analyzed by using SPSS tools (Version: 20, IBM Corporation, New York, USA). IC50 values were estimated by using linear regression equations through the usage of Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA).

3. RESULT

3.1. Preliminary Qualitative Phytochemicals Screening

This study was conducted to screen for the presence of different phytochemicals in the plant. The investigation carried out revealed that Table **1** showed the presence of alkaloids, tannins, steroids, flavonoids, carbohydrates, glycosides, especially anthracene glycosides, phenols, proteins, terpenoids (triterpenoids).

3.2. Oral Acute Toxicity

The oral acute toxicity dose experiment was done by using of the limit dose of 2000 mg/kg B/W of the

Table 1: Presence of Phytochemicals in *Ardisia solanacea* Leaf Extract

Phytochemicals	Test	ASME	ASNH
Steroids	Salkowski's test	+	-
	Liebermann-Burchard test	+	-
Tannins	$FeCl_3$ test	++	-
Flavonoids	Zn-HCl reduction test	++	+
	Lead acetate test	+	-
Triterpenes	Salkowski's test	+	-
	Liebermann-Burchard test	+	-
Saponins	Shake or foam test or froth test.	++	++
	Olive oil test	+	++
Protein	Molisch's test	+	+
Phenols	Test with 1% of copper sulphate solution and NaOH	+	+
Carbohydrate	Fehling's test	++	+
Anthraquinones glycosides	Hydroxy anthraquinones	-	-
Resins	Test with acetone solution	++	+
Fats or fixed oils	Biuret test	++	+
Glycosides	NaOH test	++	+
Cardiac-glycosides	Keller- killiani test	+	-
Alkaloids	Dragendorff's test	++	+
	Mayer's test	+	++
	Hager's test	+	+
	Wagner's test	+	+

Note: ASME= methanol extract of *A. solanacea*, ASNH= soluble n-hexane fraction in methanol extract, Bioavailability Indicator: (++) ve= High presence, (+) ve = Medium presence, (-) ve= Absence.

mouse commenced safely since at this dose the experimental animals didn't show any apparent physical and behavioral changes, ascertaining that the LD_{50} of the extract is greater than 2000 mg/kg.

3.3. Evaluation of Antidiarrheal Activity

At both doses of 200 and 400 mg/kg, the methanol extract along with soluble n-hexane fraction induced a gradual decrease in the total number of defecation in 4 hours of the experiment while compared to the control. In these doses, ASME induced 49.27% & 63.77% of inhibition which was significant ($^{**}P$<0.005) whereas, ASNH resulted 40.58% & 56.52% of inhibition ($^{**}P$<0.005) respectively. This is almost equivalent to the standard drug loperamide (65.22% inhibition, $^{**}P$<0.005), as shown in Tables **2** & **3**.

3.4. Evaluation of Antioxidant Activity

3.4.1. Ferric Reducing Effect Assay

The methanol extract at a concentration of 440 µg/mL, produced the highest reducing power of 73.53 ± 3.44%. Yet, the standard ascorbic acid at a concentration of 250 µg/mL showed the highest reducing power of 89.35 ± 3.39%. Moreover, the methanol extract produced an IC_{50} value of 79.14 µg/mL whereas, ascorbic acid showed 71.03 µg/mL. All these results are shown in Table **4**.

3.4.2. Superoxide Scavenging Activity Assay

The results showed in Table **5**, revealed that the methanol extract induced scavenging property at multiple concentrations (5, 10, 20, 40, and 80 µg/mL). Here, the highest scavenging, i.e., 82.66 ± 0.30% was observed at 800 µg/mL. On the other hand, it was 96.43 ± 0.16% at 80 µg/mL in the case of standard curcumin. Yet, the methanol extract displayed IC_{50} value of 154.36 µg/mL, whereas, the curcumin showed 0.0000125 µg/mL.

3.5. Evaluation of Thrombolytic Activity

In Table **6**, the methanol extract induced 26.02% (**P<0.001) of thrombolytic action whereas, soluble

Table 2: Evaluation of Antidiarrheal Activity of Crude Extract and its n-Hexane Fraction of *Ardisia solanacea*

Group	Number of mice	Number of defecation by each mouse in different hours				Total number of defecation	Average number of defecation
		1st	2nd	3rd	4th		
Control	M-1	2	4	3	3	12	13.80
	M-2	4	3	5	3	15	
	M-3	2	4	5	0	11	
	M-4	3	3	3	5	14	
	M-5	3	6	4	4	17	
Standard (Loperamide)	M-1	1	1	1	1	04	4.80
	M-2	0	1	1	3	05	
	M-3	0	2	2	2	06	
	M-4	1	1	2	0	04	
	M-5	0	1	2	2	05	
ASME 200	M-1	0	1	3	3	7	7.00
	M-2	1	2	2	2	7	
	M-3	1	1	2	2	6	
	M-4	1	2	1	2	6	
	M-5	1	2	3	3	9	
ASME 400	M-1	1	2	1	2	6	5.20
	M-2	0	1	2	1	4	
	M-3	1	2	1	2	6	
	M-4	0	1	2	2	5	
	M-5	0	1	3	1	5	
ASNH 200	M-1	1	3	2	3	9	8.20
	M-2	1	1	3	2	7	
	M-3	1	2	2	2	7	
	M-4	1	2	2	4	9	
	M-5	1	1	4	3	9	
ASNH 400	M-1	0	2	1	2	5	6.40
	M-2	1	3	2	2	8	
	M-3	1	1	1	2	5	
	M-4	1	1	2	3	7	
	M-5	1	1	3	2	7	

Here, M= Mice, ASME= methanol extract of *Ardisia solanacea*, ASNH= n- hexane soluble fraction.

Table 3: Evaluation of Antidiarrheal Activity of Methanol Extract and its n-Hexane Fraction of *Ardisia solanacea*

Animal group	Dose (Mg/kg)	Mean±SEM				
		1st hr (% of inhibition)	2nd hr (% of inhibition)	3rd hr (% of inhibition)	4th hr (% of inhibition)	Total (% of inhibition)
Control	0	2.80 ± 0.37	4.00± 0.54	4.00 ± 0.44	3.00 ± 0.83	13.80 ± 1.06
Standard	10	0.40 ± 0.24** 85.57%	1.20 ± 0.20** 70.00%	1.60 ± 0.24** 60.00%	1.60 ± 0.50 46.67%	4.80 ± 0.37** 65.22%
ASME 200	200	0.80 ± 0.20** 71.43%	1.60 ± 0.24** 60.00%	2.20 ± 0.34* 45.00%	2.40 ± 0.25 20.00%	7.00 ± 0.55** 49.27%
ASME 400	400	0.40 ± 0.24** 85.57%	1.40 ± 0.25** 65.00%	1.80 ± 0.34* 55.00%	1.60 ± 0.24 46.67%	5.00 ± 0.37** 63.77%
ASNH 200	200	1.00 ± 0.00** 64.28	1.80 ± 0.37** 55%	2.60 ± 0.40 35.00%	2.80 ± 0.34 6.67&	8.20 ± 0.49** 40.58%
ASNH 400	400	0.80 ± 0.20** 71.43%	1.60 ± 0.40** 60.00%	1.80 ± 0.37* 55.00%	2.20 ± 0.20 26.67%	6.00 ± 0.60** 56.52%

Note: Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by Dunnett's t test. $^{**}P < 0.005$, $^{*}P < 0.01$, $^{*}P < 0.05$ compared with control. ASME= methanol extract of *Ardisia solanacea*, ASNH= n- hexane soluble fraction.

Table 4: $FeCl_3$ Reducing Effect (%) of Methanol Extract of *Ardisia solanacea*

Samples Name	Conc. (µg/mL)	Mean ± SEM	% Increase of reducing power (Mean±SEM)	IC_{50} (µg/mL)
ASME	440	0.091± 0.00058**	73.52 ± 3.44	79.12
	220	0.087 ± 0.00058**	65.93 ± 3.32	
	110	0.084± 0.00088**	58.33 ± 3.20	
	55	0.076 ± 0.00058**	43.76 ± 2.64	
	27.5	0.069 ± 0.00088**	33.00 ± 3.24	
Standard (Ascorbic acid)	250	0.100 ± 0.00088**	89.35 ± 3.40	71.03
	200	0.095 ± 0.00088**	77.94 ± 3.00	
	150	0.089 ± 0.00058**	67.81 ± 2.7	
	100	0.082 ± 0.00088**	55.16 ± 2.66	
	50	0.075 ± 0.00058**	43.76 ± 2.64	
Control	0	0.053 ± 0.00088***	-	-

Data are shown as Mean ± SD for triplicate. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test (SPSS, Version 20.0, and NY) for multiple comparisons. Values with $^{**}P < 0.005$ were considered as significant. ASME= methanol extract of *Ardisia solanacea.*

Table 5: Superoxide Scavenging (%) Activity of Methanol Extract of *Ardisia solanacea*

Samples Name	Conc. (µg/mL)	Mean±SEM	% Increase of superoxide scavenging (Mean±SEM)	IC_{50} (µg/mL)
ASME	800	0.50 ± 0.004**	82.6 ±0.30	154.36
	400	0.28 ± 0.001**	69.40 ± 0.36	
	200	0.19 ± 0.001**	54.01 ± 0.54	
	100	0.15 ± 0.001**	40.96 ± 0.78	
	50	0.12 ± 0.001**	28.52 ± 0.83	
Standard (Curcumin)	80	2.51 ±0.002**	96.42 ± 0.16	0.00001
	40	1.80 ± 0.002**	95.23 ± 0.06	
	20	1.57 ± 0.001**	94.52 ± 0.075	
	10	1.32 ± 0.003**	93.51 ± 0.08	
	5	0.65 ± 0.003**	86.78 ± 0.20	
Control	0	0.053 ± 0.00088***	-	-

Data are shown as Mean ± SD for triplicate. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test (SPSS, Version 20.0, and NY) for multiple comparisons. Values with $^{**}P < 0.005$ were considered most significant. ASME= methanol extract of *Ardisia solanacea*.

n-hexane fraction induced 24.03% (***P<0.001) of human blood to dissolve at a dose of 10 mg/mL as compared to the control. Besides, standard drug streptokinase (30,000 I.U.) displayed 48.66% of clot lysis. Except for the highest dose (20 mg/mL), ASME and ASNH both showed more significant results, respectively 32.64% and 33.42%, as shown in Tables **6** and **7**.

3.6. Evaluation of Anthelmintic Activity

The significant activity of the methanol extract was observed at the highest dose (50 mg/mL) on adult earthworm while the standard drug albendazole showed significant activity at a dose of 10 mg/mL.

4. DISCUSSION

In this ethnopharmacological study, *A. solanacea* leaf extract was experimented for preliminary phytochemicals screening, anti-diarrheal, antioxidant, thrombolytic, and anthelmintic property. However, the leaves of this plant are usually applied for the dealing of various health problems [22].

In symphony with OECD guidelines, the LD_{50} was found to be >2000 mg/kg for the experimental extracts. Henceforth, the LD50 of experimental extracts found three times the minimum effective dose (100 mg/kg), it was taken as an appropriate candidate for the studies [37].

Table 6: Effect of Methanol Extract and its n- Hexane Soluble Fraction of *Ardisia solanacea* in Clot Lysis of Human Blood *In-vitro*

Extract	SIN	(A) mg	(B) mg	C(A-B) mg	(D) mg	(E) mg	% of clot lysis	% of clot lysis Mean±SEM
Control	1	795	1036	241	1025	11	4.56	5.30±0.38
	2	790	1064	274	1056	9	3.28	
	3	813	1073	260	1061	12	4.62	
	4	802	1003	201	990	13	6.47	
	5	801	1023	222	1011	12	5.40	
	6	800	1025	225	1010	15	6.67	
	7	768	1079	311	1067	12	3.86	
	8	778	1044	266	1030	14	5.26	
	9	803	1045	242	1030	15	6.19	
	10	814	1069	255	1052	17	6.67	
Streptokinase (100 µl)	1	778	1410	632	951	459	72.626	65.96±1.82
	2	773	1387	614	987	400	65.146	
	3	785	1364	579	993	371	64.076	
	4	789	1335	546	1001	334	61.173	
	5	801	1425	624	1008	417	66.267	
ASME (20 mg/ml)	1	788	974	186	916	58	31.18	32.64±1.99
	2	786	991	205	913	78	38.05	
	3	812	1002	190	927	75	39.47	
	4	808	1014	206	951	63	30.58	
	5	783	1091	308	1011	80	25.974	
	6	789	1062	273	967	95	34.79	
	7	804	1111	307	1031	80	26.06	
	8	798	1047	249	939	108	43.34	
	9	805	1040	235	983	57	24.25	
	10	787	1001	214	931	70	32.71	
ASME (10 mg/ml)	1	787	999	212	953	46	21.69	22.08±1.77
	2	803	1025	222	980	45	20.27	
	3	796	1017	221	975	42	19.01	
	4	802	1100	298	1043	57	19.13	
	5	786	1064	278	1012	52	18.71	
	6	785	1070	285	1009	61	21.41	
	7	806	1005	199	965	40	20.10	
	8	808	1004	197	955	49	24.87	
	9	788	985	197	925	60	30.46	
	10	786	981	195	932	49	25.13	
ASME (5 mg/ml)	1	785	984	199	935	49	24.63	26.02±1.75
	2	789	989	200	941	48	24.00	
	3	806	1003	197	957	46	23.35	
	4	804	1005	201	949	56	27.86	
	5	783	1050	267	953	52	19.47	
	6	784	1054	270	993	61	22.59	
	7	800	1094	294	1013	81	27.55	
	8	793	1007	214	956	51	23.83	
	9	801	1017	265	959	58	26.85	
	10	787	997	210	913	84	40.00	

(Table 6). Continued.

Extract	SIN	(A) mg	(B) mg	C(A-B) mg	(D) mg	(E) mg	% of clot lysis	% of clot lysis Mean±SEM
ASNH (20 mg/ml)	1	771	992	221	933	59	26.69	33.42±2.78
	2	823	1050	227	977	73	32.16	
	3	803	1013	210	897	116	55.24	
	4	806	1022	216	953	69	31.95	
	5	788	978	190	926	52	27.37	
	6	779	1026	247	925	101	40.89	
	7	785	1117	332	1017	100	30.12	
	8	800	1157	357	1050	107	29.97	
	9	786	1041	255	995	66	25.88	
	10	809	1057	248	973	84	33.87	
ASNH (10 mg/ml)	1	805	1049	244	997	52	21.32	24.03±2.16
	2	787	1022	235	983	39	16.59	
	3	799	1057	258	1013	44	17.05	
	4	784	1017	233	977	40	17.17	
	5	780	1023	243	967	56	23.05	
	6	789	988	199	936	52	26.13	
	7	803	1013	210	965	48	22.86	
	8	806	998	192	928	70	36.46	
	9	821	1019	198	968	51	25.76	
	10	775	999	224	923	76	33.93	
ASNH (5 mg/ml)	1	773	998	225	949	49	21.78	19.57±0.98
	2	819	1009	190	973	36	18.37	
	3	803	999	196	951	48	24.49	
	4	800	1003	203	967	36	17.73	
	5	788	997	209	948	49	23.45	
	6	779	1013	234	968	45	19.23	
	7	781	1007	226	959	48	21.24	
	8	800	1047	247	1011	36	14.57	
	9	784	1012	228	972	40	17.54	
	10	801	1038	237	997	41	17.30	

SIN= Sample Identification number, A= Initial weight of eppendorf tube, B= weight of tube after clot formation, C= Weight of clot, D= Weight the tube after application of sample, E= Weight of lysis, ASME= methanol extract of *A. solanacea*, ASNH = n-hexane soluble fraction.

Table 7: Effect of Methanol Extract and its n- Hexane Soluble Fraction of *Ardisia solanacea* in Clot Lysis of Human Blood *In-vitro*

Samples	% of Clot Lysis (Mean±SEM)
Control	5.30 ± 0.38
Streptokinase (100 uL)	65.96 ± 1.82***
ASME (20 mg/mL)	32.64 ± 1.99***
ASME (10 mg/mL)	22.08 ± 1.77***
ASME(5 mg/mL)	19.57 ± 0.98***
ASNH(20 mg/mL)	33.42 ± 2.78***
ASNH(10 mg/mL)	24.03±2.16***
ASNH(5 mg/mL)	19.57±0.98***

Each value represents the mean ± SEM, (n= 10). One- way ANOVA followed by Dunnett's t test. ****P*<0.001, ***P*<0.01 compared with control. ASME= methanol extract of *A. solanacea*, ASNH = n- hexane soluble fraction.

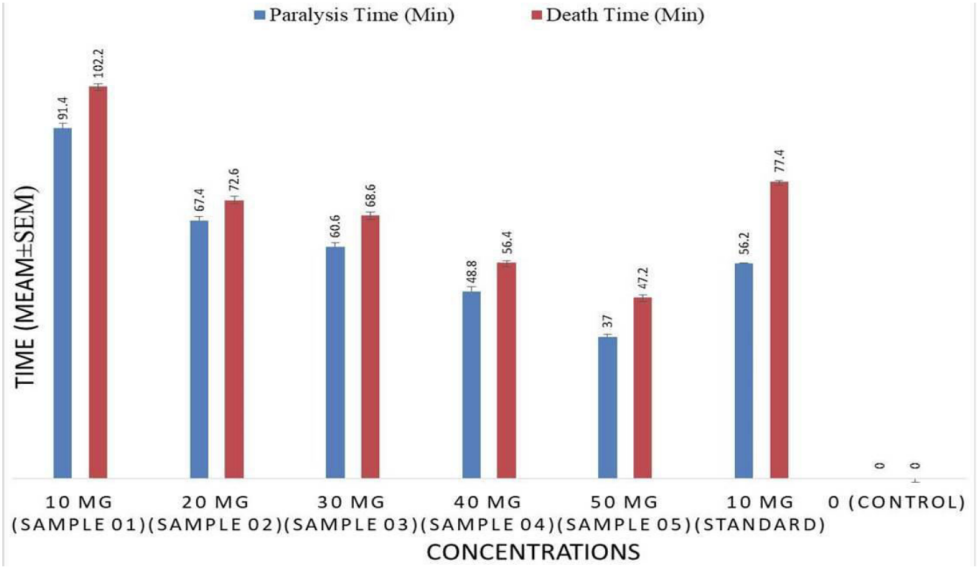

Figure 1: Time for paralysis and death of earthworms for extract and standard, ASME= Methanol extract of *Ardisia solanacea* leaves.

An alteration happens in active ion transport by decreased sodium absorption, and increased chloride ion secretion which can enhance luminal osmolarity, variation in intestinal rapidity, or in increased tissue hydrostatic pressure is an index of diarrhea [38]. These mechanisms revealed that the lipases act on castor-oil in which ricinoleic acid is liberated, and this ricinoleic acid initiates inflammatory, and irritating actions on the intestinal mucosa commencing to the release of prostaglandins [39]. The antidiarrheal agent acts on these pathophysiologic processes and causes them to inhibit. To evaluate the antidiarrheal property, a castor-oil induced method was applied in this study which is well documented [40, 41]. In this experiment, defecation frequency, and fluid content present in stool was inhibited and reduced, respectively by ASME, and ASNH at both doses of 200 and 400 mg/kg, in a dose-dependent way. The antidiarrheal activity of *A. solanacea* leaf in a dose-dependent process equips it more parallel to other plant extracts [42]. Nevertheless, there are numerous phytochemicals (such as tannins, flavonoids, alkaloids, and terpenes) present in plant extracts. The anti-diarrheal property of plant extract has been revealed if they possess flavonoids and tannins in their chemical profile [43]. The preliminary phytochemical screening of *A. solanacea* revealed the existence of these substances.

The plant extract containing phenolic compounds can significantly promote plant defense mechanisms. In pathophysiological processes both in animals and humans, they play a vital role. To identify the scavenging effect of this plant extract, ferric oxide effect assay was used in this study. The reducing power of a compound may tend to indicate potential and significant antioxidant activity [44]. The methanol extract along with the standard drug ascorbic acid showed their reducing capacity at concentrations between 50 and 250 µg/mL. They produced high absorbance at these ranges and exhibited high reducing activity [45]. As it is happened, because this plant extract may contain antioxidant molecules that render the conversion of ferricyanide (Fe^{3+}) complex to the ferrous form [46]. Again, the methanol extract was representing promising ($^{**}P$<0.005) IC_{50} value as the cut-off value of IC_{50} corresponds to 1000 µg/mL and this indicates the extract can initiate radical-scavenging reactions [47]. Again the same property was being examined by another method named superoxide scavenging assay technique. Plant extract that was treated with NaOH-alkaline DMSO-NBT system, an artificially created system results in the formation of the blue formazan [48]. The inhibitory effect of the extract on the formation of formazan is reflected by the IC_{50} value.

Here, the IC_{50} value of plant extract remained below the standard substance curcumin. The results showed that the plant extract produced a significant superoxide scavenging effect ($^{**}P$<0.005) as compared to standard curcumin. It means that the leaves of *A. solanacea* are capable of inhibiting non-enzymatic superoxide radical, which is simultaneously generated in the biological system. Superoxide-radical is a precursor of many ROS and is speculated to be harmful to various cellular components, however, both anaerobic and aerobic

organisms contain an enzyme superoxide dismutase that can catalyze the superoxide radical [49]. Again phytochemical analysis of our plant extract identified the existence of several phytochemicals (saponins, tannins, flavonoids, and terpenoids) that they may scavenge free radicals [50] as well as inhibit lipid-peroxidation [51, 52].

Now, the antithrombotic effect of this plant was evaluated by the *in vitro* clot lysis method and no clot lysis observed inside the eppendorf when distilled water was added to it. As usual, the standard drug streptokinase produced significant ($^{**}P<0.001$) thrombolytic clot lysis. In comparison to standard, ASME and ASNH showed that the antithrombotic effect was lower. As compared to control (Distilled water), they produced the effect was significant ($^{**}P<0.001$). Literally streptokinase is proved to be a bacterial plasminogen activator liquefy clots and plays a role to annihilate fibrin and the extracellular matrix that endure cells together [53, 54]. The phytochemical glycoside was found in the plant extract may play thrombolytic action in it [55].

Finally, the anthelmintic effect action of this plant extract was investigated on an earthworm. The methanol extract displayed its anthelmintic action by creating paralysis and consequently producing the death of earthworm in a dose-dependent manner. The plant contains alkaloid, phenols, tannins which are secondary metabolite can cause paralysis on earthworm by producing the effect on central nervous system [56].

5. CONCLUSION

This study implicates antidiarrheal, antioxidant, thrombolytic, and anthelmintic properties of *A. solanacea* leaves extract. Among them, antioxidant and antidiarrheal properties were significantly distinguished. Further research interventions are required to expand on the leaves of this plant to find out which compounds are exactly responsible for these pharmacological activities.

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AUTHOR'S CONTRIBUTION STATEMENT

MKH designed the research experiment & conception. MRI collected, processed the plant material. MRI, NMP and JN carried out the laboratory work. Data analysis and interpretation were aided by the MRI, MGU and SRA. MGU and MRI made the indispensable corrections in the write-up and critically revised the manuscript, gave final endorsement for the submission of a revised version. Finally, all authors read the final version and gave their confirmation to submit.

COMPETING INTEREST STATEMENT

All authors declare that they have no competing interests.

ETHICS CONSIDERATION

All authors hereby declare that "Principle of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All proposed research protocols have been examined and approved by the Ethical Committee of the University of Chittagong, Bangladesh under the approval no- cc98056.

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ADDITIONAL INFORMATION'S

No additional information is available for this paper.

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